Distribution Agreement

In presenting this thesis or dissertation as a partial fulfillment of the requirements for an advanced degree from Emory University, I hereby grant to Emory University and its agents the non-exclusive license to archive, make accessible, and display my thesis or dissertation in whole or in part in all forms of media, now or hereafter known, including display on the world wide web. I understand that I may select some access restrictions as part of the online submission of this thesis or dissertation. I retain all ownership rights to the copyright of the thesis or dissertation. I also retain the right to use in future works (such as articles or books) all or part of this thesis or dissertation.

Signature:

_____ _____

Sarah Hope Yi Date

The impact of docosahexaenoic acid status and phenylalanine control on cognitive performance in females of reproductive age with phenylketonuria

By

Sarah Hope Yi
Doctor of Philosophy

Graduate Division of Biological and Biomedical Sciences
Nutrition and Health Sciences

_____
Rani H. Singh, PhD, RD, LD
Advisor

_____
Julie A. Kable, PhD
Committee Member

_____
Kevin M. Sullivan, PhD, MPH, MHA
Committee Member

_____
Ngoc-Anh Le, PhD, FAHA
Committee Member

_____
Thomas R. Ziegler, MD
Committee Member

_____
Usha Ramakrishnan, PhD
Committee Member

Accepted:

_____
Lisa A. Tedesco, PhD
Dean of the James T. Laney School of Graduate Studies

_____
Date

The impact of docosahexaenoic acid status and phenylalanine control on cognitive performance in females of reproductive age with phenylketonuria

By

Sarah Hope Yi
BA, Mount Holyoke College, 1999
MS, Framingham State College, 2002

Advisor: Rani H. Singh, PhD, RD, LD

An abstract of
A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
in Graduate Division of Biological and Biomedical Sciences
Nutrition and Health Sciences
2010

Abstract

The impact of docosahexaenoic acid status and phenylalanine control on cognitive performance in females of reproductive age with phenylketonuria

By Sarah Hope Yi

Subtle cognitive deficits are reported in people treated early for phenylketonuria (PKU). This dissertation examines the impact of phenylalanine (Phe) and docosahexaenoic acid (DHA), one known factor and one hypothesized factor, on cognitive status in females of reproductive age with PKU.

First, we assessed the impact of a 1-week camp intervention on cognitive processing speed in females with PKU. Second, we examined the association between biomarkers of DHA status and performance on tasks of verbal ability, processing speed, and executive function in a cross-sectional study. Third, we tested the effect of supplemental DHA on processing speed and executive function in a 4.5-month parallel, randomized, placebo-controlled, double-blinded study.

In the first study, a positive effect of the camp intervention was seen on measures of cognitive processing speed and plasma Phe control at the end of the camp. Improved performance exceeded a practice effect in both measures when compared with the test-retest sample; however, a larger sample size is needed to reach statistical significance. Changes in the processing speed task requiring sustained attention corresponded with changes in plasma Phe after controlling for verbal ability. In the second study, we confirmed low levels of DHA in plasma and red blood cell (RBC) total lipid percent DHA and in the diet of participants. We found a significant relationship between RBC total lipid percent DHA and verbal ability before and after controlling for concurrent plasma Phe. Associations between DHA and measures of processing speed and executive function were not seen. In the third study, supplementation with DHA appeared to be safe and effective in increasing biomarkers of DHA; however, we failed to find an effect of DHA on measures of processing speed and executive function.

Improved DHA status in addition to adequate Phe control may benefit aspects of cognitive performance in this population. Further research is needed to clarify which domains are affected by changes in DHA status, and the length and amount of DHA that is required to see these changes. Future investigations assessing the cognitive effect of DHA should measure domains more likely to be affected including verbal ability, memory, and learning.

The impact of docosahexaenoic acid status and phenylalanine control on cognitive performance in females of reproductive age with phenylketonuria

By

Sarah Hope Yi
BA, Mount Holyoke College, 1999
MS, Framingham State College, 2002

Advisor: Rani H. Singh, PhD, RD, LD

A dissertation submitted to the Faculty of the
James T. Laney School of Graduate Studies of Emory University
in partial fulfillment of the requirements for the degree of
Doctor of Philosophy
in Graduate Division of Biological and Biomedical Sciences
Nutrition and Health Sciences
2010

Acknowledgments

Thank you to the research participants and their families for participation in these studies and for teaching me so much during my study under Dr. Singh. I am also grateful to the metabolic community when we needed to conduct off-site study visits closer to the participant's home. I sincerely hope this research benefits the PKU community.

I would like to acknowledge and thank my advisor, Dr. Singh, for the opportunity to do and experience every aspect of clinical research. She offered remarkable support, guidance, and resourcefulness and introduced me to the fascinating world of inborn errors of metabolism. I also thank my committee members for being willing to evaluate my dissertation and for their guidance throughout this process. Thank you to Dr. Kable, who has gone above and beyond in her guidance, training, and discussions throughout these projects which allowed me to conduct interdisciplinary research. Thank you to Dr. Le, Dr. Ramakrishnan, Dr. Sullivan, and Dr. Ziegler for support, guidance, and insight; each of you has contributed to different aspects of support for which I am grateful. Thank you also to Marian Evatt, MD, MSc for being a great research collaborator and medical supervisor of my clinical research activities at Emory University Hospital.

The research was made possible through much appreciated financial support of the organization Unified to Support Metabolic Disorders, private donations for research, and support from the Atlanta Clinical and Translational Science Institute (PHS Grant UL1 RR025008 from the Clinical and Translational Science Award program and PHS Grant M01 RR0039 from the General Clinical Research Center program, National Institutes of Health, National Center for Research Resources). We thank Martek Biosciences for donating the DHA and placebo capsules. We also thank the Bionutrition,

Biostatistics, Inpatient nursing, and Outpatient nursing units and Core Lab within the ACTSI's Emory University Hospital Clinical Interaction Site for their technical support.

We appreciated the services of the Emory Investigational Drug Services, Emory Genetics Laboratory's Biochemical Laboratory, and the Peroxisomal Diseases Laboratory at Kennedy Krieger Institute throughout the studies. I thank the members of the Peroxisomal Diseases Laboratory at Kennedy Krieger Institute for welcoming me into the lab to learn how to analyze plasma and RBCs total lipid fatty acid profiles, and I especially thank Ann Moser for being a warm host during my visit.

Thank you to the staff and research assistants of the Emory Metabolic Camp and to the psychologists who assisted with the cognitive testing during the camp. Nyssa Davis, RD, LD, Debra Hamilton, MA, PsyS, Jeannie Visootsak, MD, MSc were especially helpful at critical points in the implementation and/or conduct of the trial.

I enjoyed working with members of the Emory Genetics Metabolic Nutrition Program and Emory Genetics—with the variety of perspectives and backgrounds I was never far from someone who could answer a question or delve into a conversation about clinical aspects, research etiquette, regulatory issues, Southern culture, etc. I truly appreciated and enjoyed this community. Thank you also to my practice defense audience members at work (Rosalynn Borlaza, MA, Teresa Douglas, MS, Meghan Quirk) and at home (JY) for great feedback and energetic patience. I also appreciate the support of classmates and faculty of the Nutrition & Health Sciences Program and at Emory, and discussions with and encouragement of my initial advisor, Dan Sellen, PhD.

Finally, and most importantly, thank you to my family and friends for indescribable support.

Table of Contents

List of Tables

List of Figures

List of Abbreviations

ALA	alpha-linolenic acid
AA	Arachidonic acid
ACTSI	Atlanta Clinical & Translational Science Institute
BMI	Body Mass Index
CWIT	Color-Word Interference Test
DHA	Docosahexaenoic acid
EGL	Emory Genetics Laboratory
EGMNP	Emory Genetics Metabolic Nutrition Program
EPA	Eicosapentaenoic acid
LA	Linoleic acid
LC-PUFA	Long chain polyunsaturated fatty acid
MF	Medical food
n-3 PUFA	a polyunsaturated fatty acid containing the first instance of unsaturation (i.e., double bond) at the third carbon from the methyl end of the fatty acid
PAH	Phenylalanine hydroxylase
Phe	Phenylalanine
PKU	Phenylketonuria
PPVT	Peabody Picture Verbal Test
PUFA	Polyunsaturated fatty acid
RBC	Red blood cell
Tyr	Tyrosine
W-J III	Woodcock-Johnson III

Chapter 1. Introduction

Phenylketonuria (PKU) is most commonly caused by a deficiency in the enzyme phenylalanine hydroxylase (PAH). Without early, appropriate, and lifelong diet treatment, people with the autosomal recessive disorder PKU exhibit delays in development and mental retardation. Even with early and lifelong treatment consisting of a low phenylalanine (Phe) diet and an amino acid-based Phe-free medical food, however, people with PKU reportedly still display minor cognitive deficits.

DHA is a fatty acid important to brain development and function. Fatty fish, egg yolks, and human milk, all high in Phe, are the main dietary sources of preformed DHA in the US; therefore, children and adults with PKU adhering to a Phe-restricted diet have no expected dietary intake of DHA outside of supplementation. DHA can be manufactured endogenously from alpha-linolenic acid which is found in some low Phe foods and medical foods; however, this process is generally inefficient. Several observational studies have shown decreased proportions of blood DHA in people with PKU not supplemented with DHA compared with controls without PKU.

The causal factor leading to cognitive deficits in PKU is thought to be related to increased concentration of Phe in the brain, particularly in early infancy and childhood; the biochemical mechanism is still uncertain, however, and may be multifactorial. Because decreased dietary intake and lower proportions of docosahexaenoic acid (DHA) have been found in the blood lipids of people with PKU, it has been hypothesized that DHA may play a role in cognitive status in PKU.

Supplemental DHA has been shown to improve measures of visually evoked potentials and motor skills in children with PKU. The impact of supplementation has not

yet been studied in adolescents and adults with PKU. As part of a long term goal to optimize cognitive outcomes of and nutrient recommendations for patients with PKU receiving diet treatment, the research presented in this dissertation aimed to investigate the relationship between biomarkers of DHA status and cognitive performance in adolescent and adult females with PKU.

Three studies are presented in this dissertation and focus on cognitive processing speed and executive function, two cognitive domains in which individuals treated early for PKU have shown deficits. Study 1 assessed the association between short-term improved plasma Phe concentrations and change in performance on cognitive processing speed tasks in adolescent and adult females with PKU attending a week-long camp intervention. Study 2 examined the relationship between biomarkers of DHA status and cognitive outcomes in adolescent and adult females with PKU, accounting for plasma Phe control, in a cross-sectional study. Study 3 evaluated the impact of DHA supplementation on cognitive processing speed and executive function task performance in a randomized, placebo-controlled, double-blinded trial.

Theoretical Underpinnings

The study hypotheses are based on the following findings from observational and intervention studies involving animals and/or humans. These studies are described further in the Background. It should be noted that some of the findings are preliminary and/or inconsistent between studies and thus deserve further investigation, particularly those involving the relationship between DHA and cognitive status.

1. Subtle deficits are reported in adolescents and adults treated early for PKU in domains including cognitive processing speed and executive function[1-4].
2. Brief interventions have shown the reversibility of the effects of blood Phe on performance on cognitive processing speed and executive function tasks in individuals with PKU with varied treatment histories[1-5].
3. Adolescents and adults treated for PKU display lower proportions of plasma and red blood cell (RBC) DHA compared with controls without PKU[6-7].
4. Children concurrently treated for PKU and supplemented with DHA for 12 weeks or longer have shown improved measures of visual evoked potentials[8-10] and motor skills[11].
5. Reported dietary intake of DHA is positively related to blood concentrations of DHA[12-13], and supplementation with pre-formed DHA increases proportions of DHA in plasma, serum, RBC, and platelet lipids[10, 14-18].
6. Dietary intake[19-25] and blood proportions[26-29] of DHA are positively related to proportions of DHA in brain phospholipids in animals and humans without PKU.

7. Supplemental DHA and biomarkers of DHA status are associated with cognitive processing speed[30] and the slowing of cognitive decline[31-32] in aging populations without PKU.

Specific Aims & Hypotheses

The goal of this dissertation is to contribute evidence for the relationship between DHA status and cognitive outcomes in females of reproductive age with phenylketonuria (PKU). The following specific aims and null hypotheses guide the three studies:

Study 1

Specific Aim: Evaluate the changes in measures of cognitive processing speed and plasma phenylalanine (Phe) in a 1-week Metabolic Camp intervention in adolescent and adult females with PKU.

Hypothesis: Improved plasma Phe will not be associated with improved cognitive processing speed.

Study 2

Specific Aim: Examine the associations between blood docosahexaenoic acid (DHA) status and cognitive status in adolescent and adult females with PKU in a cross-sectional study, accounting for plasma Phe.

Hypothesis: Higher DHA status will not be associated with cognitive performance in the domains of verbal ability, processing speed, and executive function accounting for plasma Phe.

Study 3

Specific Aim: Assess the effect of DHA versus placebo supplementation taken for 4.5-months on cognitive function in adolescent and adult females with PKU in a randomized, placebo-controlled, double-blinded trial.

Hypothesis: Supplementation with DHA will not improve cognitive performance in the domains of processing speed and executive function.

Main outcome measures

The main outcomes and measurement tools used in these studies include:

- Verbal ability
    - Peabody Picture Vocabulary Test—Third Edition.
- Cognitive processing speed
    - Woodcock-Johnson III timed tests of Decision Speed, Pair Cancellation, Math Fluency, and Reading Fluency;
    - Delis-Kaplan Executive Function System Color-Word Interference Test Color Naming and Word Reading conditions.
- Executive function: cognitive inhibition
    - Delis-Kaplan Executive Function System Color-Word Interference Test Inhibition/Switching condition.
- Executive function: cognitive flexibility
    - Delis-Kaplan Executive Function System Color-Word Interference Test Inhibition/Switching condition.
- Plasma Phe concentrations
    - Quantitative ion-exchange chromatography.
- Plasma and red blood cell total lipid DHA concentrations
    - Capillary gas chromatography-electron-capture negative-ion mass spectrometry.

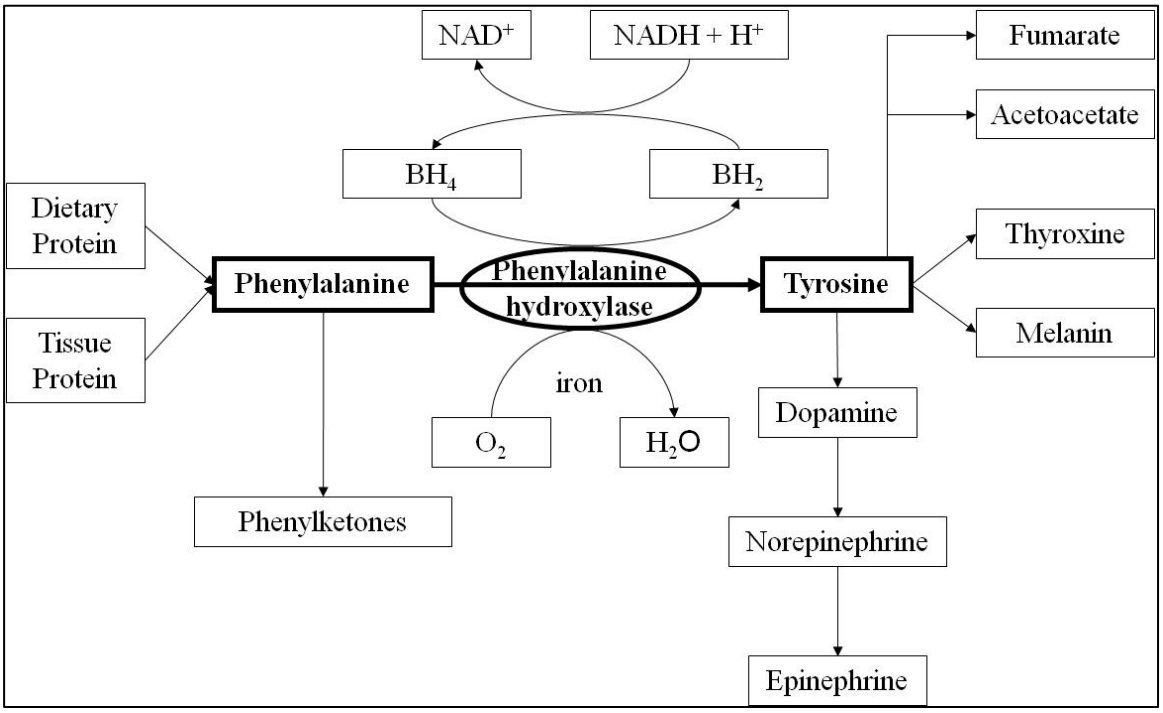
Chapter 2. Background

Phenylketonuria (PKU; OMIM 261600) is an autosomal recessive disorder with an incidence of approximately 1 in 19,000 to 1 in 13,500 newborns screened in the US[33]. PKU is managed through strict, lifelong dietary treatment aimed at reducing dietary phenylalanine (Phe) intake and plasma Phe concentrations while maintaining adequate nutritional status[34]. Without early and appropriate diet treatment, patients exhibit delays in development which can result in mental retardation[35]. If treatment is initiated early enough, mental retardation can be prevented[36]. Subtle deficits in cognitive status, however, are still seen in early treated people with PKU.

PKU: the disorder and the diet

PKU is characterized by a deficiency of the enzyme phenylalanine hydroxylase (PAH; EC 1.14.16.1). With normal activity, PAH metabolizes the essential amino acid Phe primarily into the amino acid tyrosine with a small amount converted to phenylketones[37] (Figure 1). Tyrosine is the antecedent to important compounds including dopamine, thyroxine, and melanin. With reduced PAH activity and unrestricted Phe intake, Phe and phenylketones rise to potentially harmful concentrations in blood and tissues with limited conversion to tyrosine[38]; tyrosine becomes an essential amino acid. To maintain target concentrations of Phe, phenylketones, and tyrosine in the blood and body tissues, the current mode of treatment for PKU consists of a diet restricted in Phe with tyrosine and essential amino acids provided by a Phe-free medical food.

Figure 1. Abbreviated pathway of phenylalanine metabolism



The average daily intake of Phe of Americans is estimated to be 3400 (SE: ±30) mg[39] which exceeds the typical Phe allowance for people with PKU. For people with PKU, the Phe allowance depends upon the severity of the disorder. In people with more severe forms of PKU, dietary Phe intake must be reduced to less than 500 mg/day to achieve recommended plasma Phe concentrations of 120-360 μmol/L (2-6 mg/dL). Patients with less severe forms of PKU generally need to restrict Phe intake between 500 and 1000 mg/day[38]. A more specific approach to estimating dietary Phe restriction was proposed by Guldberg and colleagues based on children with PKU aged three years and older as shown in Table 1.

Table 1. Dietary Phe restriction based on phenotypic PKU severity

PKU Severity	Phe Restriction (mg/kg/day)[40]
Classical PKU	<20[a]
Moderate PKU	20-25[a]
Mild PKU	25-50[a]
Mild Hyperphenylalaninemia	unrestricted[b]

Abbreviations: Phe, phenylketonuria; PKU, phenylketonuria.

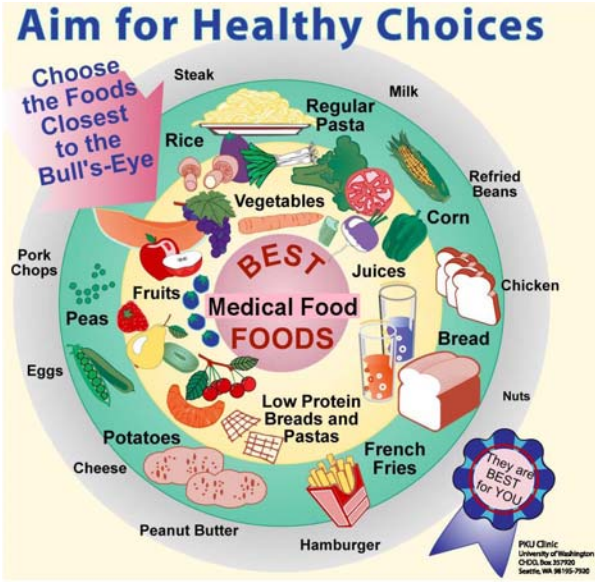
[a] To maintain a plasma Phe concentration of 300 μmol/L (5 mg/dL).

[b] To maintain a plasma Phe concentration below 600 μmol/L (10 mg/dL).

Phe is present in most protein-containing foods, with higher amounts in meats, beans, milk, nuts, and eggs, moderate amounts in grains and starchy vegetables, and lower amounts in fruits and other vegetables. In order to achieve a Phe intake below 500-1000 mg/day, foods containing higher amounts of Phe are eliminated from the diet and foods containing moderate and low amounts of Phe are consumed in controlled portions.

Figure 2 shows a simple pictorial example of patient education materials used to describe the recommended diet in food groupings based on Phe content. The center grouping consists of the Phe-free medical food, the second ring consists of foods lower in Phe content (<50 mg Phe/serving), the third ring consists of foods with moderate Phe content (50-175 mg Phe/serving), and the fourth (outside) ring consists of foods containing higher amounts of Phe (>175 mg Phe/serving). The foods in the outside ring are generally eliminated from the Phe-restricted diet.

Figure 2. Ranking of foods recommendations based on Phe content



Modified image used with permission from the Cristine M. Trahms Program for Phenylketonuria[41].

Due to the restrictive nature of the low Phe diet, proper treatment recommendations and adherence to the recommendations are critical for optimal health outcomes. By eliminating entire food groups (e.g., milk, meat, and beans) and tightly restricting other food groups (e.g., grains and starchy vegetables), it is necessary to supplement the low-Phe diet with essential nutrients to prevent deficiencies[42-43]. The Phe-free medical food provides essential amino acids (except Phe) plus tyrosine, and in practice provides 52-80% of total dietary protein equivalent[42, 44-45]. Medical food historically contained additional nutrients such as fats, carbohydrates, vitamins, and minerals[46], sometimes referred to as "complete" medical foods. For marketing reasons, in part to improve taste, convenience, and/or nutrient profile, many new varieties of medical food may not contain one or more of these additional nutrients, and are thus referred to as

"incomplete" medical foods. It must be noted, however, that neither "complete" nor "incomplete" medical foods are appropriate as the sole constituent of the Phe-restricted diet. Because Phe is an essential amino acid, measured amounts of Phe-containing foods must be consumed alongside the Phe-free medical food.

Omega-3 (n-3) long chain polyunsaturated fatty acid (LC-PUFA) status is of concern for people consuming a Phe-restricted diet due to the lack of animal products in the diet and the lack of n-3 LC-PUFAs docosahexaenoic acid (DHA; PubChem ID 445580) and eicosapentaenoic acid (EPA) in most medical foods. The omega-3 fatty acid DHA is virtually absent from the Phe-restricted diet[47], thus it is not surprising that children and adults with PKU have shown decreased concentrations of DHA when compared with children and adults without PKU[6]. Moseley and colleagues found significantly decreased plasma and red blood cell (RBC) DHA proportions in adolescents and adults with PKU compared with adult controls in the US[6]. In the Netherlands, people with PKU ranging in age from infancy through adulthood had significantly decreased plasma and RBC phospholipid DHA compared with age-matched controls[7]. Interestingly, although DHA status in PKU is generally lower when compared with controls, not all studies show significant differences compared with controls. Acosta and colleagues showed children in the US taking fat free medical foods, but not those taking fat containing medical foods, had significantly lower concentrations of RBC but not plasma DHA concentrations compared with sibling controls[48]. Pöge and colleagues showed younger but not older children with PKU in Germany had low DHA concentrations in multiple blood fractions[47]. A summary of these and other findings are presented in Table 45 in the Appendix.

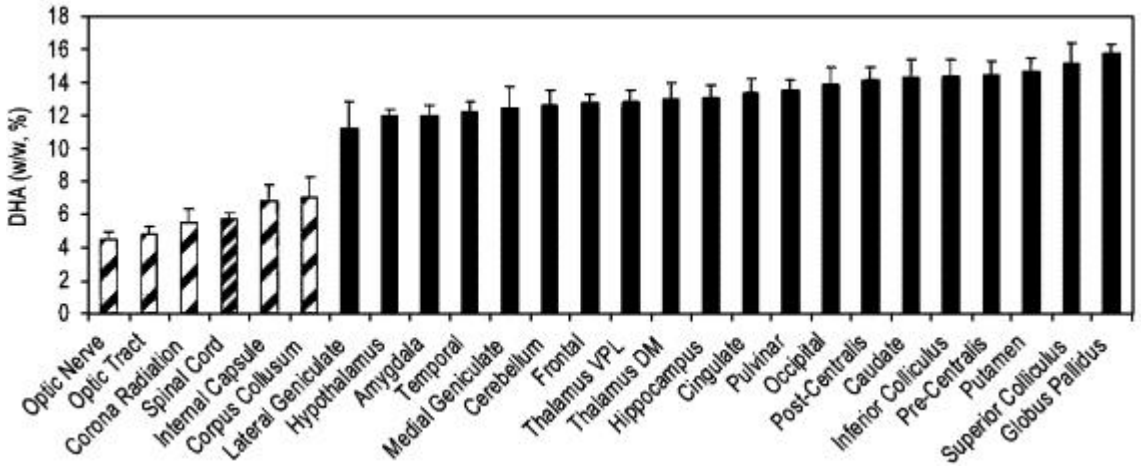
Adolescents and adults treated early for PKU are expected to have had decreased or no exposure to pre-formed DHA after birth. The potential exposure during infancy to pre-formed DHA would have been from a restricted amount of breast milk[49], if permitted by the treating metabolic clinic. DHA and arachidonic acid (AA) were only recently added to commercial infant formulas starting in 2002 in the US[50]. Not until 2008 was DHA and AA added to one of the medical foods for infants with PKU. In 2009 the first medical food with pre-formed DHA for children and adults with PKU became available.

The implications of reduced concentrations of DHA are currently unknown, but as shown below, evidence suggests a positive relationship between biomarkers of DHA status and cognitive outcomes.

DHA is a major polyunsaturated fatty acid in the brain

An integral component of neural tissues[51], DHA promotes neuronal survival and differentiation[52] and is distributed throughout the brain in structures including myelin, astrocytes, neural membranes, synaptosomes, and mitochondria[53,54]. As shown in Figure 3, DHA comprises 4.5-7% of fatty acids in white matter and 11.2-15.8% of fatty acids in gray matter of breastfed infant baboons[54] and consists of 15% of the total fatty acids in the prefrontal cortex[53].

Figure 3. Distribution of DHA in central nervous system of 4-week old breastfed baboons



Legend: hatched bars indicate white matter; black bars indicate gray matter; spinal cord is a mix of white and gray matter.

Reprinted from Prostaglandins, Leukotrienes, and Essential Fatty Acids, Volume 77, JT Brenna and G-Y Diau, The influence of dietary docosahexaenoic acid and arachidonic acid on central nervous system polyunsaturated fatty acid composition, page 248, Copyright 2007, with permission from Elsevier.

Umhau and colleagues recently estimated the adult human brain has a mean ± SD incorporation rate of 3.8 ± 1.7 mg DHA per day[55]. The authors estimated a 2.5 year half-life of whole brain DHA assuming a total of 5 g DHA in the brain.

Humans are capable of synthesizing DHA endogenously from omega-3 fatty acid precursors; however the process is relatively inefficient[56]. In a study of 8 healthy adult humans given an oral dose of 1 g deuterated ALA, an isotope tracer, an estimated 0.2% of ALA was converted to EPA, 63% of EPA converted to docosapentaenoic acid (DPAn-3), and 37% of DPAn-3 converted to DHA. Overall, only 0.05% of dietary ALA was ultimately converted to DHA[57].

ALA and LA utilize and compete for the same desaturases and elongases in the liver during endogenous synthesis of their longer chain derivatives DHA and AA, respectively[58]. It is hypothesized that high intake of linoleic acid (LA) relative to alpha-linolenic acid (ALA) inhibits endogenous synthesis of DHA from ALA due to this competition[59-61]. Improving the dietary LA:ALA ratio, however, has shown either a small change [62] or no change in blood DHA concentrations [18] in children with PKU.

Furthermore, while human supplementation trials of ALA generally result in increased concentrations of blood ALA, EPA, and n-3 DPA, concentrations of DHA usually remain unchanged[63-65]. Intake of preformed DHA is considered to be more efficient than ALA supplementation in increasing blood DHA concentrations[66]. Intake of preformed DHA, therefore, is the primary focus of the remainder of this background.

Dietary intake of docosahexaenoic acid (DHA) is positively related to blood concentrations of DHA

Moderately strong correlations are seen between reported intakes of dietary DHA and concentrations of DHA in plasma, RBCs, and adipose tissue[12-13]. With a consistent increase in DHA intake, plasma phospholipids show a maximal change within 4 weeks while RBC phospholipids continue to increase over a period of 16 weeks (Table 2)[14-16].

Table 2. Plasma and RBC DHA response to DHA supplementation

Reference	Population	Design	Plasma DHA	RBC DHA
Harris 2007[14]	N=21 premenopausal, non-pregnant women, ages 21-49 years, BMI<30 kg/m^2	16 weeks supplementation; 485 mg EPA + DHA/day from fish or capsules	≥50% increase in phospholipids (week 0 vs. week 4)	≥45% increase (week 0 vs. week 16)
Cao 2006[15]	N=20 (12 women), age (mean±SD): 48.8±8 years	8 weeks supplementation; 864 mg DHA/day from fish oil (n=9)	73% increase in phospholipids ($P < .01$)	42% increase ($P < .001$)
Katan 1997[16]	N=58 men, age (mean ± SD): 56.2±16.5 years	52 weeks supplementation; dosage range: 0-490 mg DHA/day from fish oil	1.12±0.13% increase in cholesteryl esters per gram dietary DHA	1.09±0.21% increase per gram dietary DHA

Likewise, when DHA intake is decreased, blood concentrations of DHA also decrease[15, 67]. Once supplementation is discontinued, plasma phospholipid and RBC membrane concentrations of DHA return to baseline concentrations within eight and sixteen to more than eighteen weeks, respectively (Table 3)[15, 67].

Table 3. Plasma and RBC DHA response to cessation of DHA supplementation

Reference	Population	Design	Plasma DHA	RBC DHA
Cao 2006[38]	n=20 (12 women), age (mean±SD): 48.8±8 years	24 weeks following 8 weeks supplementation; 864 mg DHA/day from fish oil (n=9)	Plasma phospholipids returned to baseline concentrations by 8 weeks post supplementation	Returned to baseline values 16 weeks post supplementation
Brown 1991[39]	n=12 men, 18-40 yrs, 3x3 cross-over trial, 30 weeks total	18 weeks following 6 weeks fish (410 mg DHA/day) and 6 weeks fish + oil (990 mg DHA/day) (n=4)	n/a	Not returned to baseline values 18 weeks post supplementation

Dietary intake of DHA is positively related to regional brain DHA

The amount of DHA intake has been shown to impact brain DHA concentrations. Table 4 shows the results of Chung and colleagues, who found a clear impact of varying levels of DHA intake on DHA concentrations in the rat brain[21].

Table 4. Regional brain content of DHA of rats fed different amounts of omega-3 PUFAs

Brain region[21]	Deficient n=9	Deficient + Fish Oil n=11	Sufficient n=10	Sufficient + Fish Oil n=10
Frontal cortex	25.2%	61.1%	100%	107.6%
Visual cortex	32.0%	64.6%	100%	92.9%
Cerebellum	38.8%	73.6%	100%	99.4%
Hippocampus	40.1%	91.5%	100%	126.9%
Olfactory bulb	49.5%	90.8%	100%	128.6%

Similarly, the turnover rate of DHA in brain phospholipids is slowed by dietary restriction of n-3 fatty acids. As shown in Table 5, DeMar and coworkers showed that the half-lives of DHA in rat brain phospholipids more than double after being on an n-3 deficient diet for 15 weeks[24].

Table 5. Half-life of brain phospholipid DHA according to dietary DHA sufficiency

Brain phospholipid	Rats fed an n-3 deficient diet[24]	Rats fed an n-3 sufficient diet
Phosphatidylethanolamine	77 days	32 days
Phosphatidylcholine	51 days	23 days
Total phospholipids	90 days	33 days

Blood concentrations of DHA are positively related to brain concentrations of DHA

Plasma and RBC DHA concentrations are commonly used as markers of brain concentrations of DHA in humans. Supporting this use, plasma and RBC long chain polyunsaturated fatty acid (LC-PUFA), specifically DHA, composition has been shown to change relative to neural membrane LC-PUFA composition in humans, neonatal pigs, and rats[26-29,], although not consistently[68].

Potential links between DHA and cognitive outcome

Functional impact of diet and brain DHA on cognitive outcomes

Several studies have shown improved learning ability in rats fed diets containing omega-3 fatty acids compared with rats deficient in omega-3 fatty acids[69-72]. A number of the human studies have therefore been conducted to investigate the role of exposure to DHA during the fetal and infancy periods on cognitive development. Studies have also been conducted to investigate the relationship between DHA status and cognitive decline in older adults. Fewer studies have been conducted to evaluate the status of DHA and the impact of DHA supplementation in children and adults. The following is a summary of

findings in humans and animals on the relationship between DHA status and cognitive outcome grouped by age category.

Third trimester fetus (approximately 26-42 weeks gestational age)

Accretion of DHA in the human forebrain occurs rapidly between the third trimester and first two years after birth[73]. DHA may be used in part to assemble synapses and dendrites, which also rapidly grow and develop during the third trimester[73]. During the third trimester the fetus may have decreased capacity to synthesize the Δ5 and Δ6 desaturases needed to convert precursor essential fatty acids (ALA and LA) to long-chain polyunsaturated fatty acids (DHA and arachidonic acid (AA)); maternal supplies of preformed DHA and AA are preferentially transported across the placenta during this period[74].

Adequate provision of DHA to the fetus during the third trimester may facilitate improved cognitive outcomes after birth. In a recent randomized controlled trial, Dunstan and colleagues show evidence for better eye-hand coordination at 2.5 years in children whose mothers received high dose fish oil supplementation (2.2 g DHA, 1.1 g EPA) compared with children whose mothers received olive oil from 20 weeks gestation through delivery[75].

Infants born before completion of the third trimester (early term) demonstrate the impact of prenatal DHA exposure. Infants born early term and/or who have low birth weight have been shown to have decreased concentrations of blood DHA[76]. With adequate postnatal DHA intake, early term infants may still have comparable brain accretion of DHA, as shown in neonatal baboons[77]. Tanaka and colleagues showed very low birth weight infants born early term who were breastfed had better performance on

tasks of executive function at 5 years of age compared with those fed unsupplemented formula. This study showed a significant, positive correlation between RBC DHA concentrations at age 4 weeks and two measures of executive function at age 5 years[78]. In addition, Henriksen and colleagues found early term, very low birth weight infants receiving breast milk who were supplemented with DHA and AA from birth through hospital discharge scored higher on tests of problem-solving and recognition memory but not communication skills at age 6 months than those who were breastfed but received placebo[79]. Amounts of DHA currently added to infant formulas may be adequate, however, according to a recent report finding no difference in language development at 26 months and behavior at 4.5 years in children given 0.3% versus 1% fatty acids as DHA from breast milk or formula during the early term period[80].

Infants

Provision of DHA to infants in the form of breast milk or supplemented infant formula has been an area of focus after evidence revealed a strong relationship between diet intake and brain concentrations of DHA. Upon post-mortem examination, infants fed human milk had significantly higher concentrations of RBC and cerebral cortex DHA compared with infants fed formula not supplemented with DHA[81]. Cerebral cortex DHA concentrations correlated positively with length of breastfeeding ($r^2 = .62$, $P < .01$) and RBC DHA concentrations[81].

Experts recommend consumption of pre-formed long-chain polyunsaturated fatty acids during infancy provided through breast milk or formula supplemented with at least 0.20-0.35% of total fatty acids as DHA and at least as much AA[82-84]. Human milk is a good source of preformed DHA, although actual content widely varies. Brenna and

colleagues conducted a meta-analysis of 65 international studies (n=2474 women), and found the DHA content in human milk ranged between 0.06 and 1.40% of total fatty acids (mean ± SD: 0.32 ± 0.22%). The authors speculated that the populations with the highest proportions of DHA in the milk tend to have the highest intakes of fish compared with the populations with the lowest proportions of DHA in the milk.

A recent review compiling the results of observational and randomized controlled trials of infant feeding found the majority of studies showed a positive impact of feeding human milk and/or formula supplemented with DHA and AA over formula without DHA and AA on a variety of cognitive outcomes[85]. Birch et al found infants fed unsupplemented formula (n=19) had significantly lower verbal IQ at 4 years than those who were breastfed (n=32). Infants fed formula supplemented with DHA (0.36% fatty acids) and AA (0.72% fatty acids; n=17) had verbal IQ similar to breastfed infants[86].

Auestad et al, on the contrary, showed no difference in visual, language and cognitive outcomes at 3 years in children given unsupplemented formula (n=37), formula supplemented with DHA (0.23%; n=35), DHA (0.12%) and AA (0.43%; n=35), or human milk during the first year of life (n=50)[87]. Previously, in 1998, Morley suggested four possibilities for inconsistencies in these studies: small sample size, varying amounts of ALA in control formula, current DHA status, and appropriateness of the utilized cognitive test(s)[88]. Another possibility may be differences in dosage of supplemental DHA[89]. The amount of DHA in the formulas in the Auestad study, for example, was 0.23% or 0.12% of total fatty acids, respectively, both of which are lower than the aforementioned mean content in human milk estimated by Brenna and coworkers of 0.32% fatty acids.

Children

Little is known about the impact of dietary DHA on cognitive outcomes in healthy children. A cross-sectional analysis of data taken from the Third National Health and Nutrition Examination Survey found a positive relationship between estimated polyunsaturated fatty acid intake and measures of short-term and working memory in children ages 6-16 years[90]. The report did not include information about specific types of polyunsaturated fatty acids, though. Ryan and Nelson found no changes in cognitive outcomes after supplementation for 4-months with 400 mg DHA in healthy 4 year olds (DHA Group, n=85; Placebo Group, n=90)[91]. They did, however, see a positive correlation between measures of verbal ability (percentile rank score) and blood DHA concentrations ($r^2 = .14$, $P = .018$, $n = 93$)[91]. A study of children living in Indonesia, Thailand, and South Africa found that those who were supplemented with fish oil had increased attendance at school; this result, however, was inexplicable to the investigators[92].

Children with peroxisomal disorders

Children with peroxisomal disorders (classic Zellweger syndrome and milder variants) display biochemical and brain deficiency of DHA[93] and provide insight into the functions of DHA in the body. Patients commonly experience sensitivity to light, impaired vision, gliosis, neuronal migration disorders, and myelin abnormalities[94]. In Spain, eight infants and children (baseline ages: 0.4 to 6.6 years) with Zellweger's Syndrome were supplemented with DHA at 100-600 mg/day to normalize blood DHA levels and had baseline and follow up magnetic resonance imaging (MRI) evaluations of myelination. The length of supplementation ranged from 6 weeks to 7 years, with

normalization of RBC DHA concentrations within several weeks after initiation of supplementation. Along with clinical improvements in visual and psychomotor status, seven participants showed improvements in myelin as detected by MRI; the eighth had normal MRI results at baseline and normal myelin progression at follow up[94]. It should be noted that this study did not include a control or comparison group.

Adults

Similar to healthy children, very little research has focused on the impact of dietary DHA on cognitive outcomes in healthy adults. Most of this research has focused on women during or after pregnancy due to significant changes in plasma and RBC concentrations of DHA seen during pregnancy and postpartum periods[95-96].

de Groot and colleagues investigated the relationship between blood DHA and cognitive outcomes in two studies of women of childbearing age. In the first study, 56 pregnant women were supplemented with 25 g of margarine daily from gestation week 14 through postpartum week 32. The experimental margarine contained 2.82 g ALA and 9.02 g LA per 25 g and the placebo margarine contained 0.03 g ALA and 10.94 g LA. The intervention did not result in changed blood DHA or cognitive outcomes. The investigators did, however, find a negative association between one measure of selective attention (reaction time) and plasma DHA at gestation week 14 and 32 weeks after delivery[97]. In a prospective, observational study of 54 non-pregnant women in the same age group, de Groot and colleagues found a negative relationship between one measure of simple processing speed (average speed on the Stroop Color-Word Interference Test, conditions one and two) and plasma DHA status[98]. There was no relationship, however, between plasma DHA and performance on two other tasks of simple processing speed. In

addition, in the previous study of pregnant women, no relationship was seen between DHA and simple processing speed as assessed using the same task (average speed on the Stroop Color-Word Interference Test, conditions one and two)[97].

In a postpartum study of breastfeeding women, women taking placebo (n=12) starting at the beginning of the post partum period had significantly lower plasma phospholipid DHA concentrations compared with women supplemented with 200 mg DHA/day (n=15) at four months postpartum. The phospholipid DHA in the placebo group dropped 31% while in the DHA group it increased 8% compared with baseline measures. Despite the preservation of DHA in the supplementation group, significant group differences were not seen in the mean completion times for a task assessing inhibition (color-word interference task) controlling for the word reading performance[99]. The small sample size may have resulted in a type II error, however. As seen in Table 6, 210 participants would be needed to assess differences between the placebo and DHA groups in order to minimize potential errors in accepting (type I error) or rejecting (type II error) the null hypothesis. An adequate sample size, therefore, may reveal positive effects of preserved DHA status on measures of cognitive processing speed (Color Naming) and cognitive inhibition (Interference) in this population.

Table 6. Estimated sample size needed to minimize type I and type II errors in a postpartum study of lactating women

Test name	DHA Group n=15 Mean (SD)	Placebo Group n=12 Mean (SD)	Change Mean	Sample size
Color naming (s)	52.8 (6.8)	55.5 (7.8)	-2.7	210
Word reading (s)	45.5 (6.4)	45.5 (3.8)	0.0	NC
Interference (s)	75.4 (11.5)	79.8 (11.9)	-4.4	176

Abbreviation: s, seconds.

[a] Sample size estimates calculated assuming $\alpha < .05$, $\beta \geq .80$, and equal numbers in each treatment group.

[b] NC: not calculated.

It has been suggested that people treated early and lifelong for PKU may have suboptimal exposure to DHA beginning early in life similar to infants born preterm[100]. Since rapid brain development and accretion of DHA occur early in life, it is important to characterize the effectiveness of DHA supplementation in this population if supplementation does not begin until in adolescence or adulthood.

Rhesus monkeys (n=3) deficient in omega-3 fatty acids given fish oil (1.35 g DHA + 2.01 g EPA) starting between ages 10 and 24 months showed significant increases in plasma, RBC, and cerebral cortex phospholipid DHA and corresponding decreases in docosapentaenoic acid (n-6) within a period of 12 weeks[25]. In addition, male

rats deficient in omega-3 fatty acids given supplemental fish oil starting at age 2 months showed improved working and spatial memory and improved brain fatty acids (shown previously in Table 4) after 80 days of fish oil supplementation (0.3% energy from n-3 PUFAs; 12 mg DHA + 18 mg EPA). Notably, rats (n=10) fed a diet sufficient in n-3s supplemented with fish oil during brain development (fetal and infant periods) and starting at age 2 months (adulthood) showed enhanced working and spatial memory and increased brain fatty acids in certain regions compared with unsupplemented rats (n=10) on a sufficient diet[21]. Therefore, it appears that supplementation of DHA may be effective in partially or almost fully recovering regional brain fatty acids and some cognitive outcomes in previously deficient adult rats.

Older adults

There is a growing body of research investigating the connection between DHA and cognitive outcomes in older adult humans, particularly in relation to memory and cognitive impairment. In a nested case-control study, RBC DHA concentrations in 120 participants in Scotland at age 64 years positively correlated with IQ and indicators of information processing speed and constructional ability[30]. At the end of a French 4-year longitudinal study of 246 men and women aged 63-74 years, the 27 participants exhibiting moderate cognitive decline had significantly decreased RBC DHA content compared with 219 participants who exhibited no decline (5.89 ± 1.0 vs. 6.34 ± 1.10% of total fatty acids, $P = .042$)[31]. Additionally, Japanese researchers found a positive effect of supplementation of 240 mg of DHA and AA for 90 days on performance in the cognitive domains of attention, immediate memory, and delayed memory in older patients with mild cognitive impairments (n=12) or organic brain lesions (n=10), but not in those with

Alzheimer's disease (n=8) or those with mild cognitive impairments receiving placebo (n=9)[32]. Manzato, however, saw no difference in plasma phospholipid DHA content between Italian veterans ages 65 years and older who scored low (n=93) or high (n=98) on a global cognitive function task[101].

A recent randomized controlled trial conducted in the Netherlands found no differences in processing speed, executive function, memory, and attention tasks in 302 men and women without dementia aged 65 years and older taking placebo, 226 mg EPA + 176 mg DHA, or 1903 mg EPA + 847 mg DHA for 26 weeks. Differences were seen in two sub-groups, however. Participants who were carriers of at least one apolipoprotein E4 (ApoE4) allele in both supplemental groups and men in the lower dose group had significantly greater improvements on the attention tasks compared with the placebo group[102]. Carriers of ApoE4 may have minor declines in cognitive performance and increased risk for Alzheimer's disease compared with noncarriers[103].

Twenty-two month old (aged) mice given 200 (low dose, n=10) or 400 (high dose, n=10) mg/kg/day DHA for 30 days showed increased lipid DHA levels in the hippocampus, frontal cortex, and striatum versus aged controls (n=10); the DHA levels in the low dose group were comparable to those in young (3-months old; n=10) and significantly higher in the high dose group. Both supplemented groups demonstrated improved memory performance, escape latency, and learning on behavioral tasks over the control group. Supplementation resulted in increased dopamine, norepinephrine, serotonin, and brain-derived neurotrophic factor in the hippocampus, frontal cortex, and striatum and increased nerve growth factor in the hippocampus and frontal cortex compared with that found in the aged control mice[104]. This study clearly shows an effect

of DHA on multiple measures, but the doses are very large in both supplemented groups. If neurochemical and cognitive improvements occur with supplemental DHA, it would be important to study the minimum dosage required for these improvements.

Potential confounding factors and effect modifiers

Factors including cigarette smoking, parity, current hormonal status, socioeconomic status, plasma Phe control, age, and overall nutrition status may be confounders (independently related to both DHA and cognitive status) or effect modifiers (directly impact the relationship between DHA and cognitive status).

Cigarette smoking

Cigarette smoking appears to be associated with both blood concentration of DHA and cognitive status. Due to the oxidative capacity of cigarette smoke and the susceptibility of polyunsaturated fatty acids to free radical oxidation[105-106], decreased blood concentrations of DHA are expected, and accordingly decreased concentrations of DHA have been shown in serum and RBCs of smokers[107-108] and whole blood of newborn offspring of smokers[109]. In a cross-sectional study, Simon and colleagues calculated the predicted effect of heavy smoking (>40 cigarettes/day) as a 30% decrease in serum DHA concentrations in men at risk for coronary heart disease (n=186)[107].

In a seemingly contradictory study, however, Pawlosky and colleagues observed significantly higher concentrations of plasma DHA in male smokers compared with male nonsmokers (mean ± SD: 40.8 ± 8.0 vs. 24.8 ± 7.4 μg/mL, $P = .04$); there were no differences between female smokers and nonsmokers. Following infusion with labeled ALA, female smokers had an increased synthesis rate of plasma DHA compared with female nonsmokers (5.8 vs. 2.9%, $P < .01$). Both male and female smokers retained

increased amounts of labeled DHA in plasma longer than the non-smokers[110]. The authors hypothesized that the increased concentrations of DHA in smokers may partially offset the effects of smoking on lipid peroxidation.

Little evidence is available for the relationship between cigarette smoking and cognitive function. One study suggests a positive relationship, with chronic administration of nicotine to rats resulting in improved attention and processing speed[111].

Pregnancy

Recent or current pregnancy appears to have a negative relationship with DHA and cognitive status in the female. Studies show decreased brain, liver, and RBC content of DHA in female rats that have completed at least one birth cycle compared with virgin female rats consuming similar diets[22, 112]. Humans also show changes in blood DHA content; in one study serum DHA decreased a mean of 11.9% between the 28th week of gestation and delivery ($P = .003$)[113]. A meta-analysis revealed that women during pregnancy and postpartum periods may have poorer memory than non-pregnant women when executive function is involved[114].

Hormonal status

Estrogen may have a positive impact on blood DHA status. Human females have higher proportions of blood DHA and slightly increased rates of synthesis of EPA, DPA, and DHA from precursor ALA than males[115-116]. Giltay and others show this increase is possibly due to the impact of estrogen, since male-to-female transsexuals displayed increased concentrations of blood DHA after being given estrogen therapy and female-to-male transsexuals displayed decreased DHA after testosterone therapy[116].

Performance on cognitive tasks may also vary according to hormonal status, or phase of menstrual cycle, in females. Specifically, during the mid-luteal (pre-menstrual) phase of the menstrual cycle, a period of increased estrogen and progesterone, compared with the phase during menstruation (a period of decreased estrogen and progesterone), females have improved performance on tasks of memory, concentration, fine motor-skills, semantic processing, speed and motor coordination, verbal memory, attention, visual memory, fine motor dexterity, and verbal fluency and poorer performance on tasks of mental rotation, perception, and spatial ability[117]. Correspondingly, women in early menopause not receiving estrogen replacement therapy have worsened performance on cognitive tasks of memory, abstract reasoning, and reaction times compared with those receiving estrogen replacement therapy[117].

Socioeconomic status

Socioeconomic status, including educational attainment, income, and occupation may be positively related to both DHA and cognitive status. In a cross-sectional study of 987 adults living with coronary artery disease, those in the highest tertiles of household income, education level, and grade of occupation had significantly higher content of RBC DHA compared with the lower two tertiles[118].

PKU management

The relationship between management of PKU and DHA status is currently speculative. In 2001, Infante and Huszagh hypothesized that increased concentrations of plasma Phe inhibits the production of DHA[119]; however, this hypothesis has not been tested.

The other probable reason for decreased concentrations of blood DHA is the lack of pre-formed DHA in the Phe-restricted diet. People consuming a vegetarian or vegan diet also show lower proportions of DHA in plasma and RBC total lipids (Table 7).

Table 7. Proportions of plasma and RBC lipid DHA in consumers of vegetarian and vegan diets

Author	Year	Diet	n	Blood fraction[a]	units	Mean	Variation
							95% CI
Rosell[120]	2005	Vegan	232	Plasma	%	0.7	0.61, 0.79
		Vegetarian	231	Plasma	%	1.16	1.07, 1.24
		Omnivore	196	Plasma	%	1.69	1.59, 1.79
							SEM
Geppert[121]	2005	Vegetarian	52	RBC	wt%	4.4	0.2
							SD
Agren[122]	1995	Raw food vegan	8	RBC	mol%	3.3	1.6
		Omnivore	11	RBC	mol%	6.7	0.9
							SE
Sanders[123]	1978	Vegan	18	RBC	mg/g	19	2.3
		Omnivore	18	RBC	mg/g	58	3.8

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

[a] Total lipids.

Age

Concentration of DHA in the brain appears to be related to age. In a postmortem study of 58 humans, DHA concentration in the cerebral cortex significantly increased with age between the ages of 2 and 18 years, however cerebral cortex and RBC DHA concentrations were not correlated. In adults between the ages of 19 and 88 years, DHA concentration in the cerebral cortex did not markedly change whereas cerebral cortex and RBC DHA concentrations were correlated[27].

Cognitive changes are also observed with age. Each cognitive test used in the studies described in this dissertation has associated age-effect assessments using data from the respective normative samples. Below is a brief description of the observed relationships between age and performance on the tests. Because of the differences seen by age, the standard scores for each test adjust for age.

Verbal ability, as assessed in the standardization sample for the Peabody Picture Vocabulary Test Form III, showed a steep improvement in mean raw score between individuals aged 2.5 and 22 years, a relative flattening until age 56, then a slight decline in performance through age 90[124].

Cognitive processing speed is hypothesized to be a fundamental component of cognition, and individual differences in processing speed may be seen starting in infancy[125]. For the Woodcock-Johnson III Tests the processing speed tasks were combined into a processing speed factor (Gs) through principal components analysis[126]. This factor peaked at approximately age 25 years then steadily declined through 85+ years. On the Color Naming condition of the Delis-Kaplan Executive Function System (D-KEFS) Color-Word Interference Test (CWIT)[127], peak performance was seen between

ages 16-19 years and stabilized through ages 30-39 years. Mild to moderate slowing was reported only at 80-89 years. The Word Reading condition of the CWIT peaked in the 20s and stabilized through the 40s. Little slowing was reported with aging.

Executive function was assessed using the Inhibition and Inhibition/Switching conditions of the CWIT[127]. On both of these conditions, the youngest and oldest age groups performed considerably slowest.

Nutrition status

Adequate nutrition early and throughout life plays a vital role in the development and maintenance of a healthy brain and cognitive status in humans. A number of nutrients have been associated with changes in cognitive status, although much research is still needed to clarify details. Work is needed, for example, to determine the critical time periods during which these nutrients are needed for brain and cognitive development. In addition, work is needed to confirm relationships between specific nutrients or combinations of nutrients with the development of specific regions of the brain and specific cognitive outcomes. The following nutrients may be associated with cognitive performance, and those on a Phe-restricted diet may be at increased risk of inadequate intake. Table 8 summarizes the expected risk of nutrient inadequacies for people with PKU for select nutrients associated with cognitive status.

Folate

Fortified grains and ready-to-eat cereals; meats; beans; and, leafy green vegetables are among the highest sources of dietary folate[128]. Folate is routinely added to medical foods containing micronutrients. Many studies related to folate and cognitive status have been conducted early or late in the life cycle. Adequate intake of folate prior

to conception promotes the proper development of the neural tube in the fetus[129]. In older adults, folate is positively associated with global cognitive function, psychomotor speed, information processing speed, and possibly memory[130-131], and is negatively associated with cognitive status during vitamin B12 deficiency[132].

Folate is a carrier of carbon in the process of methylation[133] and therefore should be considered alongside methionine, homocysteine, vitamin B12, and vitamin B6 status. Increased homocysteine, negatively associated with cognitive status, is seen with folate deficiency[133]. In addition, brain phospholipid metabolism may be impacted by folate deficiency (e.g., conversion of phosphatidylethanolamine to phosphatidylcholine)[134].

Iodine

Iodized salt, fish, seaweed, and dairy products are among the better sources of iodine[135]. Iodine is provided in micronutrient-containing medical foods; a supplemental source would be required if the medical food does not contain micronutrients. Iodine deficiency is the leading cause of preventable mental retardation[136]. Deficiency early in life is associated with poor visual–motor performance, motor skills, perceptual and neuromotor abilities, and development and intellectual quotients[137]. A major function of iodine is its role in the formation of the thyroid hormones triiodothyronine (T3) and thyroxine (T4); a low concentration of thyroid hormone is associated with learning and memory impairment[138].

Iron

Enriched grains, fortified ready-to-eat cereals, meats, and beans are among the foods highest in iron content[128]; because these foods are restricted or eliminated from the low Phe diet, iron is routinely added to micronutrient-containing medical foods.

Cognitive outcomes and iron status are positively related[139]. Iron status in children under 2 years is positively associated with cognitive outcomes later in life[133]. In addition, long-term iron supplementation in anemic children may improve cognitive outcomes[133]. Iron is important for myelination[140] and is a cofactor for tryptophan hydroxylase and tyrosine hydroxylase[133] needed for to synthesize the neurotransmitters serotonin and dopamine, respectively.

Protein

Meat, dairy, beans, and nuts contain the highest protein content per typical serving, although protein is also present in grains, vegetables, and fruits[128]. Because the low Phe diet relies on an overall restriction in intact protein, the main purpose of the Phe-free medical food is to provide the majority of essential amino acids. Brain size may be reduced with protein-energy malnutrition[141]. Head circumference, an indicator for brain size and protein-energy malnutrition[142], has been shown to be positively correlated with natural protein intake in infants with PKU[143]. In addition, several amino acids, components of proteins, are needed for neurotransmitter production or are neurotransmitters themselves.

Vitamin B12

Naturally occurring vitamin B12 is found exclusively in animal products such as meats, eggs, and dairy. Many ready-to-eat cereals are fortified with vitamin B12[128]. People adhering to the low Phe diet must obtain vitamin B12 either from micronutrient-containing medical food or supplement. Infants with Vitamin B12 deficiency show deterioration in development and slowed brain growth between ages 4-8 months. Improvements are seen with supplementation, but may not prevent long-term delays[144].

Deficient intake of vitamin B12 during the first 6 years of life and current low serum vitamin B12 is associated with decreased fluid intelligence in adolescents[145]. Other outcomes of deficiency include demylination[146], declines in sensory perception and cognitive ability, and psychiatric symptoms[147]. Importantly, symptoms of B12 deficiency may not correspond with biochemical markers of megaloblastic anemia particularly if folic acid status is adequate[147].

Vitamin B6

Fortified ready-to-eat cereals, meats, beans, nuts, potatoes, tomatoes, and bananas are among the highest food sources of vitamin B6[128]. Although measured amounts of cereals, potatoes, tomatoes, and bananas can be part of a low Phe diet, intake may still fall short of DRIs without provision from a medical food containing micronutrients or a multivitamin supplement.

Vitamin B6 is a cofactor in the synthetic pathways of the neurotransmitters serotonin, norepinephrine, dopamine, and gamma-aminobutyric acid[148]. Presentation of vitamin B6 deficiency includes convulsions in infants and irritability and confusion in adults[148]. A cross-sectional study of 812 adults (mean (SD) age 61.9 (12.6) years) revealed a positive association between plasma vitamin B6 (plasma pyridoxal 5'-phosphate) and performance in several cognitive domains (including overall cognitive, visual-spatial, working memory, abstract reasoning performance) before and after controlling for cardiovascular disease and its risk factors[149]. In contrast, a recent review revealed a lack of effect of vitamin B6 supplementation on cognitive performance in healthy older women (n=33, 75 mg vitamin B6, 5 weeks) and men (n=76, 20 mg vitamin

B6, 12 weeks) in two randomized controlled trials[150]. More evidence is needed to further the characterization of the relationship between vitamin B6 and cognitive performance.

Zinc

Zinc is found in highest concentrations in meats, beans, dairy, and fortified cereals[128], which must be eliminated or restricted from the low Phe diet due to their Phe content. Consumption of a medical food fortified with zinc or a dietary supplement containing zinc is necessary to achieve intakes consistent with the DRIs. Zinc deficiency can present in a number of ways, including impaired cognition in adults[151]. Animals deprived of zinc during brain development have shown impaired outcomes in domains including learning, memory, and attention[152]. Repletion of zinc plus other micronutrients may have positive cognitive and growth effects as shown in children with stunted growth (n=740; ages 6-9 years; 20 mg Zn plus micronutrients vs. 20 mg Zn vs. micronutrients)[153]. The mechanism for an association between zinc and cognition is under investigation. It is known that zinc is enriched in the synaptic vesicles of some glutamatergic neurons and is also associated with neuronal apoptosis[154]. Supplementation beyond the DRIs may not provide cognitive benefit. Maylor and colleagues observed that supplementation with 15 or 30 mg zinc per day for six months in healthy adults (n=387, ages 55-87 years) with sufficient baseline zinc intakes had a negligible effect on cognitive measures of visual and working memory, attention, and reaction time[155].

Table 8. The risk of nutrient deficiency for people with PKU for select nutrients associated with cognitive status

Nutrient	Risk for deficiency in PKU
Folate	Low protein diet with inadequate intake of: • fruits and vegetables • folate-containing medical food • other supplemental source
Iodine	Low protein diet with inadequate intake of: • fruits and vegetables grown in iodine rich soil • iodine-containing medical food • iodized salt • other supplemental source
Iron	Low protein diet with inadequate intake of: • iron-containing medical food • other supplemental source
Protein	Low protein diet with inadequate intake of: • medical food and phenylalanine allowance
Vitamin B12	Low protein diet with inadequate intake of: • vitamin B12-containing medical food • other supplemental source

Table 8 continued

Nutrient	Risk for deficiency in PKU
Vitamin B6	Low protein diet with inadequate intake of: • vitamin B6-containing medical food • other supplemental source
Zinc	Low protein diet with inadequate intake of: • Zinc-containing medical food • other supplemental source

Cognitive deficits in early treated PKU

As stated earlier, poor management of PKU, consisting of either under restriction or even overrestriction[156] of dietary Phe early in life can lead to severe, irreversible mental retardation. Mental retardation can be prevented and IQ within the normal range can be achieved with early and appropriate diet therapy. The Collaborative Study of Children Treated for Phenylketonuria found the primary predictors of IQ at age 6 years were maternal IQ, age of treatment initiation, and maintenance of plasma Phe concentrations within the recommended range[157]. Discontinuation of diet management after age five years may not have a large impact on IQ[158-159], but IQ may decline slightly unless treatment is continued until at least ages eight to twelve years[160-162]. Discontinuation may also result in increased psychopathology[158] and decreased information processing speed[163].

Even with early and continuous treatment, subtle deficits in cognitive performance are seen. A 2007 meta-analysis showed adolescents and adults who were

treated early and continuously for PKU had poorer outcomes in tasks of cognitive inhibition, cognitive processing speed, full scale intelligence quotient, attention, and motor control compared with controls without PKU. Cognitive processing speed showed the largest effect size (>0.9) and cognitive inhibition the second largest (>0.5)[164]. In addition, performance on tasks of working memory, strategic processing, and processing speed testing may deteriorate over time relative to controls without PKU[165-167] although the processing speed finding is not consistent[168].

Whether the cause of these subtle deficits in early, lifelong treated PKU is a result of abnormalities in neurochemistry or brain structure due to suboptimal current or past Phe control, current or past suboptimal nutrition due to the restrictive diet, or a combination of factors continues to be under investigation.

Neurochemistry

The "executive deficit hypothesis" asserts that declines in executive function in PKU are due to slight decreases in dopamine projecting to the dorsolateral prefrontal cortex of the brain. This would occur if plasma Phe concentrations are only moderately increased between 6-10 mg/dL (360-600 μmol/L)[169]. The findings of Diamond and colleagues support this hypothesis in a study of infants and children with PKU. They found that infants and children with moderately elevated plasma Phe concentrations, between 6 and 10 mg/dL, had poorer working memory and inhibitory control scores compared with controls and infants and children with PKU maintaining control below 6 mg/dL[169].

Brain structure

Another hypothesis states that white matter abnormalities (WMAs) in the form of dysmyelination, a breakdown in myelin sheath, contribute to changes in cognitive function, dopamine, and other neurotransmitters in PKU. Dysmyelination may occur due to increased concentrations of Phe or its metabolite, phenylpyruvic acid, inhibiting the activity of enzymes needed to produce cholesterol or fatty acids essential to myelin membranes[170-172]. WMAs may be reversible with improved plasma Phe control[173].

One study utilizing magnetic resonance imaging showed 14 patients with PKU displaying moderate, 12 displaying mild, six displaying no WMAs. All three groups displayed impaired cognitive outcomes, and participants with moderate WMAs showed the most deficits. Deficits seen in moderate WMAs were in the domains of IQ, divided attention, processing speed, verbal learning, visual learning, mental flexibility, and arithmetic. No clear pattern of progressive worsening was seen between the no WMA and mild WMA groups, although both groups displayed deficits (in the domains of IQ, processing speed, reading, and arithmetic). Notably, the moderate WMA group had a higher mean concurrent plasma Phe concentration (786±284 μmol/L) than the other two groups (400±132 μmol/L in the no WMA group and 435±196 μmol/L in the mild WMA group)[174].

Nutrition

Due to the restrictive nature of the low-Phe diet and the dependence on a supplemental synthetic formula (a Phe-free medical food), the possibility remains that nutrition could play a role in the subtle cognitive deficits seen in people treated early and lifelong for PKU. Because of the importance of DHA in the brain, the negligible intake of

preformed DHA in the low Phe diet, and the lower concentrations of plasma and RBC DHA in people with PKU, the question arises whether there is a relationship between DHA status and cognitive outcomes in PKU.

Supplementation with DHA in infants and children with PKU has been shown to improve plasma and RBC DHA content, measures of visually evoked potentials (VEPs), and motor skills. Agostoni and colleagues showed improved measures of pattern-reversal and flash visually evoked potential (VEP) in 10 children with PKU supplemented with LC-PUFAs (~9.3 mg DHA/kg body weight) for 12 months compared with 10 children with PKU supplemented with a placebo[8]. Beblo and colleagues also found improvements in two of four measures of VEP (n=36 with PKU supplemented with fish oil vs. n=12 controls without PKU) as well as improvements in motor skills in children with PKU supplemented with fish oil (15 mg DHA/kg body weight; n=24 with PKU supplemented with fish oil vs. n=11 controls without PKU) for three months compared with controls without PKU[9, 11]. Infants with LC-PUFA supplemented formula for 12 months improved pattern but not flash VEP as RBC DHA concentration increased[10].

These studies suggest there may be a cognitive benefit from supplementation of the low Phe diet with DHA in infants and children. Supplementation with DHA in adolescents and adults with PKU has not yet been studied.

Significance

Even with early and lifelong dietary treatment, adolescents and adults with PKU display minor cognitive deficits. The cause of these deficits is not confirmed but thought to be impacted by increased concentration of Phe or phenylketones in the brain, and resulting abnormalities in white matter and/or neurotransmitters in the brain. Because

decreased concentrations of DHA have been found in the blood lipids of patients with PKU, it has also been hypothesized that DHA may play a role in cognitive status in people treated early for PKU.

As part of a long term goal to optimize dietary treatment to enhance cognitive outcomes of people with PKU, the subsequent studies investigated the relationship between biomarkers of Phe control, DHA status, and measures of verbal ability, cognitive processing speed, and executive function in adolescent and adult females with PKU. The following research aims to offer clinicians and families evidence regarding the relationship between DHA supplementation and cognitive outcomes in adolescent and adult females with PKU.

Chapter 3. Preliminary Studies

Plasma Phe control consistently improves at the end of Metabolic Camp

Participants of the following studies were initially recruited from the Metabolic Camp for adolescent and adult women with inborn errors of metabolism held annually at Emory University. Participants travel from various parts of the United States to attend the Metabolic Camp, and about half of the campers attend for the first time. Singh and colleagues showed that the intervention of the annual Metabolic Camp has consistently shown clinically and statistically significant mean improvements in plasma Phe control at the end of camp as demonstrated in Table 9[175].

Table 9. Consistently improved plasma Phe control at the end of a Metabolic Camp for females with PKU

	Camp 1995 n=13 Mean (SD)	Camp 1996 n=21[a] Mean (SD)	Camp 1997 n=18[a] Mean (SD)	Camp 1998 n=22[a] Mean (SD)
Baseline plasma Phe, μmol/L	935 (401)	990 (349)	811 (398)	792 (350)
End-of-camp plasma Phe, μmol/L	487 (301)	564 (293)	534 (335)	453 (320)
P value	.001	<.0001	.0014	.0002

Definitions: Phe, phenylalanine; PKU, phenylketonuria.

[a] Includes participants with one or more previous exposures to camp experience.

Adapted from Table 3 in reference [175].

Consumers of medical foods without fat do not compensate for the missing fat

As described earlier, not all medical foods contain sources of fat. The following preliminary studies originated with the question of whether people with PKU consuming medical food without fat compensate for fat intake through other dietary sources. To investigate this first question, dietary intake prior to attending Metabolic Camp in 2004 or 2005 was assessed in 38 adolescent and adult females with PKU. As shown in Table 10, participants consuming medical food without fat (n=16) consumed significantly less total energy and energy from fat than participants consuming medical food with fat (n=22). Participants consuming medical food without fat consumed the same amount of fat from other foods as participants consuming medical food with fat. This finding indicated that participants consuming medical food without fat did not compensate by increasing fat intake through other foods[176-177].

Table 10. Energy intake in adolescent and adult females with PKU by medical food type

Intake[a]	Source	MF with Fat n=22 Mean (SD)	MF without Fat n=16 Mean (SD)	*P* value[b]
Age, y		18.7 (10)	23.2 (10.3)	.18
Energy, kcal/d	Total	1773 (501)	1458 (286)	.019
	Food	1088 (447)	1130 (281)	.73
	Medical Food	685 (362)	329 (166)	<.001
Fat, kcal/d	Total	502 (201)	300 (110)	<.001
	Food	338 (181)	292 (110)	.34
	Medical Food	163 (94)	8 (11)	<.001
Carbohydrate, kcal/d	Total	1050 (348)	936 (193)	.20
	Food	732 (327)	814 (214)	.36
	Medical Food	318 (229)	122 (107)	.001
Protein, kcal/d	Total	233 (73)	241 (67)	.74
	Food	44 (25)	44 (19)	.97
	Medical Food	189 (77)	197 (74)	.75

Abbreviations: MF, Medical Food; kcal, kilocalorie.

[a] Intakes analyzed using Amino Acid Analyzer v4.0 (Ross Products Division, Columbus, OH).

[b] Student's *t* test for independent samples.

Table using data from references [176-177].

Biomarkers of DHA status may not differ by type of medical food used

To investigate if the essential fatty acid status of adolescent and adult females with PKU taking medical foods without fat differed from those taking medical foods with fat, plasma and RBC total lipid fatty acid profiles were assessed in 15 eligible participants with PKU attending the Metabolic Camp in 2006. Even though estimated intakes of total fat and ALA were significantly different between medical food groups, the proportional content of plasma and RBC total lipid essential fatty acids and their longer chain derivatives were not different as seen in Table 11 (only select plasma biomarkers and diet intakes are shown). A larger sample size is needed to confirm these findings.

Table 11. Select biomarkers of plasma total lipid fatty acid status and dietary factors by medical food type in females of reproductive age with PKU

	MF without Fat n=7		MF with Fat n=8		*P* value[a]
	Median	(Range)	Median	(Range)	
Age, y	17	(13 – 41)	15	(12 - 49)	.54
BMI, kg/cm^2	29.8	(24.0 - 47.9)	22.5	(16.5 - 39.9)	.006
Plasma Phe, µmol/L	902	(245 - 1702)	640	(233 - 1099)	.19
Plasma Tyr, µmol/L	35	(22 - 54)	37	(23 - 46)	.87
Plasma TLFA, µg/mL	2885	(1755 – 4317)	2321	(1650 - 3011)	.40
Plasma LA, % TLFA	32.14	(19.97 - 41.34)	33.32	(31.04 - 38.89)	.54
Plasma AA, % TLFA	5.67	(3.93 - 8.70)	7.05	(4.30 - 9.45)	.40
Plasma ALA, % TLFA	0.62	(0.47 - 1.43)	0.71	(0.55 - 0.84)	.54
Plasma EPA, % TLFA	0.25	(0.11 - 0.40)	0.26	(0.13 - 0.45)	.61
Plasma DHA, % TLFA	0.93	(0.78 - 1.18)	0.86	(0.62 - 1.24)	.46
Dietary energy, kcal/d	1580	(873 - 1936)	1580	(856 - 2227)	.69
Dietary CHO, g/d	250	(109 - 364)	217	(122 - 364)	.96
Dietary total fat, g/d	32	(12 - 50)	57	(34 - 71)	.014
Dietary Phe, g/d	0.330	(0.060 - 0.730)	0.420	(0.220 - (1.050)	.69
Dietary protein, g/d	63	(40 - 87)	56	(22 - 66)	.34
Dietary tyrosine, g/d	5.54	(3.36 - 8.17)	4.58	(1.75 - 6.50)	.40
Dietary ALA, g/d	0.70	(0.04 - 1.48)	1.35	(0.66 - 1.76)	.014
Dietary EPA, g/d	0.00	(0.00 - 0.00)	0.00	(0.00 - 0.00)	1.0

Table 11 continued

	MF without Fat n=7		MF with Fat n=8		*P* value[a]
	Median	Range	Median	Range	
Dietary DHA, g/d	0.00	(0.00 - 0.00)	0.00	(0.00 - 0.00)	.69

Abbreviations: MF, Medical Food; BMI, body mass index; Phe, phenylalanine; Tyr, tyrosine; TLFA, total lipid fatty acid; LA, linoleic acid; AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; CHO, carbohydrate.

[a] Mann-Whitney U Test for independent samples.

[b] Dietary data analyzed using Food Processor SQL.

Table using data from references [178-179].

Biomarkers of EPA and DHA status are reduced in females with PKU

Although medical food type did not appear to relate to select fatty acid biomarkers, total lipid plasma and RBC proportions of EPA and DHA were significantly lower compared with adolescent and adult female laboratory controls without PKU as shown in Table 12 and Table 13.

Table 12. Plasma total lipid fatty acid status in adolescent and adult females with and without PKU

Marker	Units	PKU n=17 Mean (SD)	Controls n=23 Mean (SD)	*P* value[a]
Age	Years	21.06 (11.55)	33.02 (12.07)	.003
TLFA	μg/mL	2521 (669)	2569 (357)	.790
LA	% TLFA	32.58 (5.02)	28.91 (2.94)	.001
AA	% TLFA	6.41 (1.55)	6.22 (0.99)	.666
ALA	% TLFA	0.73 (0.24)	0.67 (0.25)	.410
EPA	% TLFA	0.26 (0.09)	0.73 (0.48)	<.001
DHA	% TLFA	0.89 (0.18)	3.01 (0.89)	<.001

Abbreviations: TLFA, total lipid fatty acid; LA, linoleic acid; AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

[a] Two-tailed independent samples *t* test.

Table using data from references [178-179].

Table 13. RBC total lipid fatty acid status in adolescent and adult females with and without PKU

Marker	Units	PKU n=17 Mean (SD)	Controls n=23 Mean (SD)	*P* value[a]
Age	Years	21.06 (11.55)	33.87 (12.06)	.002
TLFA	µg/mL	1941 (124)	1333 (197)	<.001
LA	% TLFA	10.76 (1.19)	9.69 (1.20)	.008
AA	% TLFA	14.48 (1.15)	14.05 (1.25)	.279
ALA	% TLFA	0.13 (0.04)	0.12 (0.05)	.751
EPA	% TLFA	0.22 (0.04)	0.47 (0.20)	<.001
DHA	% TLFA	2.55 (0.56)	3.88 (0.98)	<.001

Abbreviations: TLFA, total lipid fatty acid; LA, linoleic acid; AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid.

[a] Two-tailed independent samples *t* test.

Table using data from references [178-179].

As described in the Background, other studies have also shown decreased concentrations of DHA in blood fractions in individuals with PKU compared with controls without PKU. In an effort to understand the functional implications of decreased concentrations of DHA in adolescent and adult females with PKU, three studies are presented in this dissertation which focus on cognitive processing speed and executive functioning, cognitive domains in which individuals treated early for PKU have shown subtle deficits.

Study 1 (Chapter 5) reports the association between short-term improved Phe and change in performance on cognitive processing speed tasks in adolescent and adult females with PKU attending a week-long camp intervention. Study 2 (Chapter 6) examined the relationship between biomarkers of DHA status and measures of verbal ability, cognitive processing speed and executive function in adolescent and adult females with PKU controlling for plasma Phe in a cross-sectional study. Study 3 (Chapter 7) evaluated the impact of DHA supplementation on cognitive processing speed and executive function task performance in a randomized, placebo-controlled, double-blinded trial.

Chapter 4. Methods

The purpose of this chapter is to provide information on aspects of the methods which may be considered superfluous for inclusion in a manuscript submitted for publication to a scientific journal. These details focus on the outcome measures which include assessments of validity and reliability of the cognitive tests, laboratory methodologies used to assess biomarkers, and description of diet assessment methods. Descriptions of the demographic and medical information collected and aspects of Study 3 are also expounded upon. This chapter is not intended to describe all aspects of the methods employed in Studies 1, 2, and 3 since the reports (Chapter 5, Chapter 6, and Chapter 7, respectively) each have detailed methods sections.

Outcome measures

Main outcomes measured in Studies 1, 2, and 3 consisted of cognitive performance, biomarkers of Phe and DHA status, and diet intake. Demographic and medical information were also collected. Data collection procedures are detailed on page 208.

Cognitive Testing

Cognitive performance was assessed on tasks drawing upon verbal ability, cognitive processing speed, cognitive inhibition, and cognitive flexibility. Under the direction of a pediatric psychologist, the Peabody Picture Vocabulary Test (PPVT; Pearson, San Antonio, TX), Delis-Kaplan Executive Function System (D-KEFS; Pearson, San Antonio, TX) Color-Word Interference Test, and Woodcock-Johnson III (W-J III; Riverside Publishing, Rolling Meadows, IL) Tests of Cognitive Ability and Achievement

were administered to participants. The total battery of cognitive tests took approximately 45 minutes to complete.

Test administration and scoring

In Study 1 (Chapter 5), the PPVT and CWIT were administered individually to each participant by one of three licensed psychologists. For efficiency, in Study 1 the W-J III tests were administered in a group setting led by one licensed psychologist and four research assistants recorded participant finishing times. The W-J III tests could be completed either individually or in a group setting because participants recorded their test answers in test booklets. The group consisted of 30 participants at the beginning of camp and 31 at the end of camp. A diagram of the room in which group testing took place is on page 219.

The licensed psychologist who led the group testing is experienced in psychometric testing in clinical and research settings. Three research assistants held master's degrees in a health-related field, two of whom were pursuing a PhD in nutrition, and one research assistant was completing a bachelor's degree in biology.

As shown in Table 14, the pre-camp test measures served as the baseline measures for 20 and 13 of the participants enrolled and randomized into Studies 2 and 3 (Chapter 6 and Chapter 7), respectively. After Study 1, 21 additional participants were enrolled and 20 were randomized into Studies 2 and 3, respectively. These 21 additional participants were individually administered the tests by one trained tester (n=16) under the supervision of a licensed psychologist or by one of two licensed psychologists (n=5) at baseline. Follow up testing for the twenty-seven participants who completed Study 3

was individually administered by one trained tester (n=24) or one licensed psychologist (n=3).

Table 14. Administration of the W-J III test by environment and tester

	Study 1		Study 2	Study 3	
Environment	Pre-camp	End-of-camp		Baseline	Follow up
Group	n=20	n=20	n=20	n=13	
Individual			n=21	n=20	n=27
Individual tester					
Licensed Psychologist			n=5	n=4	n=3
Trained tester			n=16	n=16	n=24

The doctoral student investigator was the "trained tester" for these studies. The primary licensed psychologist/co-investigator trained and supervised the student investigator on the administration and scoring of the tests. The student investigator practiced administering the entire battery of tests to several volunteers and the supervising psychologist before administering the tests to research participants.

Due to the objective nature of the PPVT, CWIT, and W-J III tests, differences in test administrator and type of administration (i.e., group vs. individual) were not expected to affect test performance. To verify this expectation, performance on W-J III tests of all randomized participants in Study 3 (Chapter 7) was assessed. Thirteen participants completed the baseline W-J III tests in a group setting while twenty completed the tests individually. Mean scores on these tasks at baseline did not differ between the two

groups. Furthermore, a recent study showed a low frequency of errors of first year graduate students while administering or scoring the Decision Speed test of W-J III test[180].

To minimize scoring errors, each test was scored twice, with the second scoring occurring no sooner than 24-hours after the first scoring. Standard scores were calculated from raw scores using normative values included with the testing materials. 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[124, 126-127, 181]. Table 15 shows the standardization of each test to national demographics including the age groups involved in the each study. Table 16, Table 17, Table 18, and Table 19 show the reliability and validity estimates of the tests.

Verbal Ability

The PPVT-Third Edition, Form B was used to screen for intellectual functioning. The PPVT is (1) a verbally administered achievement test on standard English vocabulary, and (2) a screening test for verbal ability. Although the PPVT does not test overall intellectual capacity, it is highly correlated with measures of overall IQ as shown in Table 17[124]. The results from the PPVT were used to screen for inclusion in the study, to describe the study samples at baseline, and to provide a proxy measure for verbal intelligence.

The participant was asked to match a series of vocabulary terms with one of four pictures per term. The test is divided into 17 sets of 12 questions per set, and the sets are ordered in increasing difficulty. No time limit was given for the PPVT.

Participants scoring less than 70 (standard mean = 100, standard deviation = 15) on the PPVT were deemed to be severely impaired cognitively and were excluded from study due to potential difficulties in comprehending the instructions for the cognitive processing speed and executive function tasks.

Executive Functioning Skills

Inhibition and cognitive flexibility, two aspects of executive function, were evaluated using the Color-Word Interference Test (CWIT). CWIT evaluates automatic response inhibition and cognitive flexibility through four timed conditions (Color Naming, Word Reading, Inhibition, and Inhibition/Switching) and is based upon the original test developed by Stroop[182]. The Color Naming and Word Reading conditions assess fundamental skills needed to complete the Inhibition and Inhibition/Switching conditions. These initial two conditions allow for the differentiation between poor performance due to deficits in fundamental skills versus deficits in inhibition and/or cognitive flexibility. Performance on the CWIT has been shown to be sensitive to mean lifetime Phe concentrations in children and adolescents treated continuously from birth through at least 14 years old[183].

Participants were instructed to complete each condition as quickly as possible, and each condition had a maximum time limit. Described below are the four conditions which comprise the CWIT.

Color Naming

The Color Naming condition requires the participant to identify verbally a series of colors presented on a page.

Word Reading

The Word Reading condition requires the participant to read aloud a series of color names presented on a page.

Inhibition

The Inhibition condition requires the participant to indentify verbally the ink color a series of color names are printed in and not read the color names.

Inhibition/Switching

The Inhibition/Switching condition requires the participant to switch between two instructions. The participant is to indentify verbally the ink color a series of color names are printed in and not read the color names. If the color name is printed inside of a box, however, the participant is to read the color name and not name the ink color.

Cognitive Processing Speed

Processing speed was assessed using six simple, timed tests: Decision Speed, Pair Cancellation, Reading Fluency, and Math Fluency tests from the W-J III Tests of Cognitive Ability and Achievement; and the Color Naming and Word Reading conditions from the CWIT. Participants were instructed to complete each test as quickly as possible, and each test had a maximum time limit.

Decision Speed

The Decision Speed task is reliant on the ability to connect a concept with a name. Participants were instructed to circle two pictures in each row that are conceptually most similar.

Pair Cancellation

The Pair Cancellation task involves circling every instance of a specific pairing of pictures appearing on a single page.

Reading Fluency

The Reading Fluency task requires reading abilities. Participants were instructed to read a series of statements and circle if the statement is true or false.

Math Fluency

The Math Fluency task is related to math achievement and requires the participant to perform simple arithmetic calculations (addition, subtraction, and multiplication).

Color Naming

The Color Naming task requires the participant to identify verbally a series of colors presented on a page.

Word Reading

The Word Reading task requires the participant to read aloud a series of color names presented on a page.

Processing Speed Score

The six processing speed scores (Decision Speed, Pair Cancellation, Reading Fluency, Math Fluency, Color Naming, and Word Reading) were reduced to one processing speed factor using principal components analysis with a varimax rotation. For Study 2 (Chapter 6), the processing speed factor explained 66.6% of the total variance among the variables and had an eigenvalue of 3.99. For Study 3 (Chapter 7), 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 processing speed factors were standardized to a z-score.

Table 15. Standardization methods for the PPVT, CWIT, and W-J III cognitive tests

Test	Age range	Sample size	Standard
PPVT-III	2.5-90+ years	2725	Current Population Survey, March 1994, US Census Bureau[124]
CWIT	8-89 years	1750	Population Estimates, 2000, US Census Bureau[127]
W-J III	2-90+ years	8818	Population Projections, 2000, US Census Bureau[181]

Abbreviations: PPVT, Peabody Picture Vocabulary Test; CWIT, Color-Word Interference Test; W-J, Woodcock-Johnson.

Table 16. Reliability assessments for the PPVT, CWIT, and W-J III cognitive tests

Test	Subtest	Internal consistency	Stability[d]
PPVT-IIIB[124]		.95[a], .94[c]	.91 - .94[e]
CWIT[127]	Color Naming-Word Reading composite	.62 - .86[c]	
	Color Naming		.76
	Word Reading		.62
	Inhibition		.75
	Inhibition/Switching		.65
W-J III[181]	Decision Speed	.87[b]	.73 - .80
	Pair Cancellation	.81[b]	.69 - .84
	Reading Fluency	.90[b]	.80 - .94
	Math Fluency	.90[b]	.89 - .96

Abbreviations: PPVT, Peabody Picture Vocabulary Test; CWIT, Color-Word Interference Test; W-J, Woodcock-Johnson.

[a] Alpha reliability coefficients.

[b] Rasch analysis coefficients (median).

[c] Split-half reliability coefficients.

[d] Test-retest reliability coefficients.

[e] Range represents measures across age groups.

Table 17. Criterion validity for the PPVT

Comparison Test	Age (years)	Concurrent correlation with PPVT-IIIB
WISC-III (n=41)	7.9-14.3	Verbal IQ: .92
		Performance IQ: .84
		Full scale IQ: .90
OWLS (n=43)	8.1-12.8	Listening Comprehension: .77
		Oral Expression: .68
		Oral Composite: .77
KAIT (n=28)	13-17.7	Crystallized IQ: .91
		Fluid IQ: .85
		Composite IQ: .91
K-BIT (n=80)	18-71.1	Vocabulary: .80
		Matrices: .62
		Composite: .76

Abbreviations: PPVT, Peabody Picture Vocabulary Test; WISC-III, Wechsler Intelligence Scale for Children-Third Edition; IQ, Intelligence Quotient; OWLS, Oral and Written Language Scales; KAIT, Kaufman Adolescent and Adult Intelligence Test; K-BIT, Kaufman Brief Intelligence Test.

Data are from reference [124].

Table 18. Construct validity for the CWIT

Test/Condition	Construct validity	
	Inter-correlations[a]	Divergent[b]
CWIT		< .30
Color naming	.41 - .62	
Word reading	.41 - .62	
Inhibition	.45 - .63	
Inhibition/Switching	.41 - .63	

Abbreviations: CWIT, Color-Word Interference Test; CVLT-II: California Verbal Learning Test-Second Edition.

Data are from reference [127].

[a] Range represents measures across age groups.

[b] Compared with CVLT-II scores (ages 16-89 y).

Table 19. Construct validity of the W-J III tests using two factor analysis models

Model Name	Test	Correlation				
		Gs	Grw	Gq	NA	A3
CFA Broad Model	Decision Speed	.71				
	Pair Cancellation	.68				
	Reading Fluency	.43	.46			
	Math Fluency	.44		.47		
Broad/Narrow CHC	Decision Speed	.55			.22	
Factor Model	Pair Cancellation	.69				
	Reading Fluency	.31	.41		.22	
	Math Fluency	.43				.49

Abbreviations: Grw, Reading-Writing; Gs, Processing Speed; Gq, Mathematics; NA, Naming Facility; A3, Math Achievement.

Data are from references [126, 181].

Blood collection and analysis

Participants were instructed to begin fasting at least eight hours prior to the morning blood draw. Four to ten mL of venous blood was drawn into a sodium heparin tube (green top) for the assessment of plasma amino acid profile and plasma total lipid profile. Four to eight mL of venous blood was drawn into an ethylenediaminetetraacetic acid (EDTA) tube (lavender top) for the assessment of plasma and red blood cell total lipid fatty acid profiles. Seven to ten mL of blood was collected in a tube without anticoagulant (red top) or a serum separator tube for the assessment of serum vitamin B12. The maximum amount of blood drawn was limited by the amount stated in the informed consent document signed by the research participant. The procedures used for blood processing are listed on page 214. Excess plasma, serum, and red blood cells were stored frozen at -80°C for the purposes of repeating an analysis or the assessment of additional biomarkers contingent upon participant consent.

Blood amino acid profile

Amino acid analysis was 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[184].

For monthly monitoring or if a participant was unable to provide a venous sample, blood spot samples collected on filter paper were used to quantify whole blood whole blood Phe and tyrosine (Tyr) concentrations using liquid chromatography-tandem mass spectrometry (LC-MS/MS)[185]. Phe and tyrosine were extracted from the blood spots into

methanol containing internal standards (stable isotope labeled amino acids). Amino acid analysis was performed using a Micromass Quattro Micro tandem mass spectrometer with a Waters 2795 HPLC system. Amino acids were identified and quantified using NeoLynx software. Values of whole blood Phe analyzed by LC MS/MS are reportedly 19% lower than plasma Phe analyzed by ion-exchange chromatography[185]; therefore, a factor of 1.19 was multiplied to these values. Amino acid concentrations were reported as μmol/L. These values can be converted to mg/dL (mg%) by dividing by 60.54[186].

Plasma and RBC total lipid fatty acid profiles

Venous blood was drawn into ethylenediaminetetraacetic acid (EDTA) tubes. At least 2-3 mL of EDTA-preserved whole blood was shipped overnight at room temperature to the Peroxisomal Diseases Laboratory (PDL) at Kennedy Krieger Institute (Baltimore, MD) for processing and analysis. Excess plasma and RBCs were stored frozen at -80°C in case a repeat analysis was required. The PDL inspected each specimen for hemolysis. In the event of hemolysis, the PDL did not analyze the specimen. Instead, when available, frozen plasma collected at the same study visit was shipped overnight on dry ice to the PDL for analysis of plasma TLFA concentrations.

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 and colleagues[187] and is described in further detail in the paper authored by the scientists operating the PDL[188].

The plasma and RBCs were separated by centrifuging the sample at 3000 rpm for 10 minutes at 4° C. Plasma was aliquoted into a separate tube and frozen at -80°C until analysis. RBCs were washed twice in phosphate-buffered saline. Each time an equal

volume of saline was added to the RBCs, gently mixed, and centrifuged at 3000 rpm for 10 minutes at 4° C. The saline was discarded and the packed RBCs were transferred into a separate vial, flushed for 30 seconds under a nitrogen evaporator, and frozen at -80°C until analysis.

In order to prepare RBCs for analysis, two aliquots of 100 µL packed RBCs from each sample were each to a solvent (isopropanol:hexane, 2:3) rinsed tube containing 0.01 mg butylated hydroxytoluene (BHT). 50 µl MilliQ purified water was added to each tube, and to extract the fatty acids, 2:3 isopropanol:hexane was added. The tubes were placed on a rocker for 2 hours and vortexed every 15 minutes. Finally, each tube was centrifuged at 2500 rpm for 10 minutes and the supernatant was aliquoted into a tube containing internal standards. One of the tubes was destined for the measure of RBC plasmalogens using capillary gas liquid chromatography; because RBC plasmalogens are not the focus of this study, this method will not be discussed further. The second tube was destined for the quantitation of total lipid fatty acids using capillary gas chromatography-electron-capture negative-ion mass spectrometry (GS/MS) using the same methods used for analysis of plasma samples.

To prepare the tubes containing the internal standards, 2:1 $CHCl_3$:methanol plus BHT were added to new tubes and dried under nitrogen. C19:0 and C27:0 free fatty acid internal standards were added to the tubes and dried under nitrogen. A deuterium-labeled fatty acid mixture (D C16:0, D C18:0; D C10:0, D C12:0, D C14:0, D C20:0, D C22:0, D C24:0; D C15:0, D C26:0, D pristanate, D phytanate) mixed with 2:1 $CHCl_3$:methanol was next added to the tubes. The tubes were dried under nitrogen, and finally 100 µL of the sample (plasma or RBC) was added to the tubes containing internal standards.

The fatty acids (from plasma or RBC) were hydrolyzed from triglycerides and phospholipids first using acid hydrolysis by adding 0.6 N hydrochloric acid in acetonitrile and heating at 104°C for 45 minutes. After the sample cooled, base hydrolysis was conducted by adding 1.0 N methanolic sodium hydroxide and heating at 104°C for 45 minutes. After the sample cooled, 6 N HCl was added to reacidify the sample. Finally, the fatty acids were extracted upon addition of hexane. After centrifugation, the hexane layer was transferred to a new tube and evaporated under nitrogen.

Triethylamine and 10% pentafluorobenzyl bromide (PFB) in acetonitrile were mixed with the hydrolyzed fatty acids and kept at room temperature for at least 20 minutes in order to derivitize the fatty acids into PFB esters. The sample was washed using 0.1 N HCl and hexane and centrifuged. The hexane layer containing the PFB esters was transferred into a GC vial for injection on the GC/MS.

To analyze total lipid fatty acid content of the samples an Agilent 6890/5973 GC/MS operating in a negative chemical ionization mode using ammonia as a reagent was used. The analysis took 46.5-minutes per sample, and the type of column used to separate the PFB fatty acid esters was a highly polar AT-Silar-100 capillary column (30m x 0.25mm x 0.2 μm). Highly polar column enables the separation of carbon-hydrogen-based compounds with double and triple carbon-carbon bonds. The sample injection volume was 1 μl and injected with a 1:35 split ratio. Helium was used as the carrier gas.

Normal and abnormal controls were included in each sample run. In addition, 33 free fatty acids mixed in toluene at 9 different concentrations were analyzed in each sample run in order to calculate standard curves. Using the GS/MS analysis software, each fatty acid was matched to the deuterium-labeled internal standard with the closest

chain length, retention time, and concentration. Plasma and RBC fatty acid content in the following studies are presented as percent of total lipids; total lipid fatty acids are presented as μg/mL.

Calibration studies on the original method showed a mean coefficient of variance (CV) of 9.8% (n=9, r^2 >.923). Intra-assay CV ranged from 2.5 – 13.2% (n=17) and inter-assay CV ranged from 4.6 – 22.9% for C8 – C26 fatty acid-PFB esters from plasma. When added to control serum, 76 - 106% of fatty acids were recovered. Fatty acid concentrations were stable when refrigerated up to 7 days and frozen up to 3 months (-20°C or -70°C). Fatty acid concentrations were also stable after 5 freeze/thaw cycles[187].

Plasma and red blood cell DHA content are commonly used as biomarkers for brain DHA content because plasma and RBC long chain polyunsaturated fatty acid (LC-PUFA), specifically DHA, composition have been shown to change relative to neural membrane LC-PUFA composition in humans, neonatal pigs, and rats[26, 28-29]. Since DHA concentrations may adjust quicker in plasma than red blood cells[15], markers of the two were collected.

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 the participant by a registered dietitian at baseline and follow up in order to assess intakes including dietary energy; protein equivalent; total fat; carbohydrates; Phe; tyrosine; polyunsaturated, saturated, and monounsaturated fats; and LA, AA, ALA, EPA, and DHA. If a participant did not have a three-day diet record at baseline, a registered dietitian conducted a twenty-four hour dietary recall. While a single twenty-

four hour recall may not give a valid estimate of an individual's typical intake, it is not expected to bias the estimated mean intake of the group although the standard deviation may be overestimated[189]. Dietary intake data were analyzed using Nutrition Data System for Research (NDSR) software version 2009, developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN.

When a study visit was conducted at the baseline of the metabolic camp, the food records were also analyzed using at least one of three software programs by at least two different dietitians. The three software programs utilized were Amino Acid Analyzer v4.0 (Ross Products Division, Columbus, OH), The Food Processor SQL Nutrition and Fitness software[190], or NDSR software version 2008. For all three programs, ingredients were chosen based on similarity to actual foods reported by participant. For Food Processor SQL, completeness of nutrient data was an additional factor used in deciding which ingredients to choose. The final analyses were conducted using NDSR software version 2009 by one dietitian (SY) and results were compared with the previous analyses. Discrepancies between the different analyses were checked and resolved in order to minimize data entry errors.

Neurological status

Peripheral neuropathy is possible in both poorly controlled PKU as well as vitamin B12 deficiency. Under the supervision of a neurologist, peripheral sensory perception was screened for in Study 3 (Chapter 7) using the Rydel-Seiffer Graduated Tuning Fork and the Two-point Discriminator, two standardized sensory testing tools.

Questionnaires

The following questionnaires were completed by participants at study visits.

Health behavior survey

The health behavior questionnaire addressed behaviors related to cigarette smoking, body weight, and physical activity of the participants (see pages 253 and 254). The questionnaire was based upon the questions in three age-targeted health behavior questionnaires utilized in population-based surveillance by the Centers for Disease Control and Prevention[191-193].

Menstrual history

The menstrual history questionnaire was used to collect parity status and estimate estrogen status based on menstrual cycle (see page 260).

Medical Information Form

This form addressed medication and supplement usage as well as current and past management of PKU. The participant answered questions regarding prescribed and actual diet restriction and medical food intake (see page 247).

PKU Treatment History

The participant was asked further questions regarding treatment history of PKU. Questions include: method of PKU screening, diagnostic age, and early childhood control of blood Phe concentrations (see page 258).

Demographic Information Form

The demographic information requested included: race/ethnicity, marital status, household income and size, educational achievement, and parent/caregiver demographics (see page 249).

A poverty variable was used in order to report household income adjusted for household size. This variable was a two-level categorical variable based on the annual poverty thresholds from the US Census Bureau[194]. Household income on the Demographic Information Form is reported as income within a specific range and the actual income is not reported. If the poverty threshold for a participant fell within or above the range reported by the participant, the participant was categorized as being at or below the poverty threshold. The poverty threshold specific to the year that the participant completed the Demographic Information form was used.

Study 3 Methods

For Study 3, baseline 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)). At baseline, participants were randomized into one of two supplementation groups and instructed to take the supplements daily for 4.5 months. After 4.5 months following the start of supplementation, participants returned to the CIS for assessment. Methods of communication between the participants and study staff included telephone, electronic mail, postal mail, facsimile, and in person contact.

Off-site study visits

If necessary, the study coordinator/doctoral student investigator traveled to a location closer to the participant's home to conduct a study visit. When possible, all of the data were collected at the participant's local metabolic clinic. The procedures for an off-site study visit are detailed in Appendix: Methods. In three cases, cognitive testing

was conducted in a quiet room in a library. In one case, the blood draw and vital sign measures were collected at the participant's home.

Participant selection

Eligible participants were females with PKU, at least 12 years of age, and able to complete neuropsychological testing. Ineligible participants were those who were pregnant or currently taking supplemental n-3 LC-PUFAs.

Study visit evaluations

Participants received a history and physical exam at baseline at the CIS, and height, weight, and vital signs were assessed at each study visit. At the CIS, height was assessed using a digital stadiometer (Measurement Concepts and Quick Medical, North Bend, WA), weight was assessed using a digital scale (Stand-On Scale; Scaletronics, White Plains, NY), and vital signs were assessed using DINAMAP 2019205-001 monitors (GE Healthcare, Waukesha, WI). Baseline body mass index (BMI) was calculated using the standard formula BMI = (kg body weight) / (m^2 height).

Randomization

To reduce risk of bias and to promote similarity between the two groups with respect to known and unknown confounding factors and effect modifiers, several points in the study related to randomization were addressed (Table 20). First, participants were randomized into either the DHA supplement or placebo supplement group. Block randomization with a block size of four was used (See Appendix: Methods for further details). The CIS biostatistician developed the randomization assignments using a computer program and provided this list directly to the pharmacist at Emory University

Hospital Investigational Drug Services (IDS). The two supplements are described in the "Therapy" section on page 76.

Second, allocation of treatment was concealed from study investigators and participants through external storage and distribution by IDS.

Third, participants, data collectors, data analyzers, and investigators were masked (blinded) as to which group each participant belonged until the end of the data collection phase of the study. Masking was maintained by using supplements that are similar in appearance, weight, and smell.

Last, an intent-to-treat analysis was performed at the end of the study. To accomplish this, data were collected from all randomized participants regardless of participant compliance to the study protocol.

Table 20. Addressing bias

Issue	Potential bias	Action
Treatment allocation sequence generation	Selection bias	Computer-generated random number assignments
Allocation concealment	Selection bias	External storage and distribution of supplements
Blinding	Performance and detection bias	Mask participants, data collectors, data analyzers, and investigators to treatment allocation
Incomplete outcome data	Attrition bias	Perform intent to treat analysis

Where and when to call

Registration for this protocol was ongoing until sample size needs were met. The participants were directed to contact the principal investigator and doctoral student investigator with questions regarding the PKU & DHA Study by telephone, electronic mail, postal mail, facsimile, or in person.

Information to provide at entry

A short information session led by the student investigator was held at Metabolic Camps in 2007, 2008, and 2009 with time allotted for participant questions. Participants not attending Metabolic Camp were informed by the student investigator of the content discussed in the information session during the recruitment and/or baseline periods.

Participants received a study container before starting supplementation. The study container contained a study log book, supplies for monthly food records and filter paper, measuring cups and spoons, a ruler, a pen, and a supply of 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 study web-site was built for participants in the study to access study information and forms that were also given to the participants by mail, electronic mail, telephone, facsimile, or in person. This information included study staff contact information, directions to the study sites, and the PKU & DHA Study Information Sheet. Study forms include blank medication logs, illness logs, 3-day food records and instructions, filter paper blood test procedure instructions, and filter paper test requisition form.

Therapy

The number of capsules per day to provide each participant was based on baseline body weight. Based on previous dosing methods which resulted in increased plasma and erythrocyte DHA concentrations[8, 18], 10 (+5) mg DHA per 1 kg body weight per day or 1 capsule per 20 kg body weight per day were supplied by the supplement. Those randomized to receive DHA supplements received capsules containing DHA derived from the microalga *Schizochytrium spp* ("DHASCO-S"). Those randomized to receive placebo received capsules containing a mixture of soy and corn oils.

The capsules were provided by Martek Biosciences Corporation (Columbia, MD, USA). All capsules were provided in gelatin capsules containing approximately 500 mg of oil in the form of triglycerides and were all the same size, weight, color, and flavor. Ascorbyl palmitate and tocopherols are added to the DHASCO-S oil to prevent oxidation. The placebo oil is preserved with 60% of the ascorbyl palmitate and 100% of the tocopherols provided in the DHASCO-S capsules. The major fatty acids provided by each supplement are shown in Table 21[195]. Up to five months of product at a time was given to each participant by shipment or in-person.

Table 21. Select fatty acid content per capsule

Fatty acid name	Shorthand formula	DHASCO-S capsule[a,b]	Placebo capsule[a,c]
Palmitic acid	C16:0	114 mg	55 mg
Oleic acid	C18:1n-9	7 mg	113 mg
LA	C18:2n-6	3 mg	267 mg
ALA	C18:3n-3	<0.5 mg	32 mg
EPA	C20:5n-3	7 mg	<0.5 mg
DPAn-6	C22:5n-6	80 mg	<0.5 mg
DHA[d]	C22:6n-3	212 mg	<0.5 mg

Abbreviation: LA, linoleic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DPA, docosapentaenoic acid; DHA, docosahexaenoic acid.

[a] Absolute fatty acid contents estimated by dividing the average capsule fill weight by the average % fatty acid content.

[b] Average (range) capsule fill weight of the DHASCO-S capsule was 535.9 (506-560) mg.

[c] Average (range) capsule fill weight of the Placebo capsule was 528.2 (504-557) mg.

[d] Average (range) analyzed DHA content per capsule was 199.8 (190-210) mg DHA. Data shown are based on the Certificates of Analysis for the DHASCO-S and Corn/Soy Placebo lots used in the trial.

Expected dosage range

Based on participants with PKU (n=20) attending a previous Metabolic Camp who had a median age of 16.5 years (range: 12-50 years) and a median weight of 61.3 kg (range: 30.5-118.6 kg), the expected dosage range was as follows:

- Median number of capsules per day: 3 (range: 2-6)
- Median DHA per day: 11.2 mg/kg/day (range: 10.0-13.2 mg/kg/day)

Participant assessment

As seen in Table 22, follow up assessment after baseline was conducted at 4.5 months after supplementation start. Participants were asked to report any changes in supplement usage, illness, and medication usage between the study visits and at the end of the study. Participants were also asked to submit completed study log books, unused supplements, and all supplement bottles.

Table 22. Example study schedule

Date	Weekday	Visit	Day	Flexibility
7-Oct-07	Sunday	Baseline	1	
7-Oct-07	Sunday	(Start Supp)	1	+4 months
20-Feb-08	Wednesday	4.5 month	137	±2 weeks

Data collection

Data were collected at each visit as well as each month during supplementation. Assessment of the following was performed at both visits: plasma amino acid profile,

plasma and RBC total lipid fatty acid profiles, cognitive performance, 3-day diet intake, and questionnaires.

Monthly data collection

To monitor compliance to the study product and health status between study visits, participants were asked to submit a blood-spotted filter paper and 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 were reminded of each submission by telephone call or electronic mail.

Study log books

In addition to study instructions, the study log book contained study supplement, medication, and illness logs (see Appendix: Methods for excerpts from the study log book). The study supplement log was used to provide a secondary data source for calculating compliance (see calculation in the *Unused Supplement* section). The medication and illness logs were reviewed to identify any changes in health status, adverse events, and medication usage not otherwise told to the study staff.

Unused supplement

In order to assess compliance to supplementation, participants returned unused supplements at the end of the study. Compliance to supplementation was assessed by comparing the number of capsules taken with the number of capsules instructed to take and expressed as a percentage. Compliance was defined as taking 80% or more of supplements prescribed.

All reasonable and ethical attempts were made to collect follow up data on all randomized participants, regardless of adherence to allocated treatment.

Protection of Human Subjects

Because this study involved human participants, careful consideration was taken towards the development of methods sensitive to recruitment and consent procedures, risks and benefits, and data safety monitoring.

1. Risks to the Subjects

a. Human Subjects Involvement and Characteristics

This research involved female human participants attending the annual summer Metabolic Camp held at Emory University and those identified from metabolic clinics in the US. We anticipated a total of approximately 30-35 different participants with PKU to enroll in the camp each year. Their expected age range was 12 to 50 years. Because most of these participants had been diagnosed and treated early in life for PKU and subsequently followed in metabolic clinics, they were expected to be healthy.

Inclusion criteria included diagnosis with PKU, female, at least 12 years of age, and able to complete neuropsychological testing. Exclusion criteria included pregnancy or current supplementation with omega-3 fatty acids.

b. Sources of Materials

Research material obtained from the human participants included blood specimens, medical records, and data collected from verbal or written health-related tests, questionnaires, and logs.

Each participant was assigned an identification number upon enrollment into the study. Data collected during neuropsychological testing and blood draws was coded with

this number to ensure confidentiality. Only the investigators and data collectors had access to the identities of these participants.

c. Potential Risks

It was not anticipated that there would be any serious health risks in taking the DHA or placebo supplements as directed in the study. It was expected that there would be a risk of gastrointestinal distress if taken on an empty stomach or in large quantities. High intake (>3g) of omega-3 fatty acids (DHA is an omega-3 fatty acid) may increase the risk of bleeding. Omega-3 fatty acids may contribute to small decreases in blood pressure and small changes in blood glucose levels. Vitamin E needs may increase and blood low-density lipoprotein cholesterol concentrations may increase a small amount. The risks of drawing blood from a vein or finger stick are small and include rare infection, mild discomfort, and bruising to the site where the blood was drawn. There was always a risk, even though remote, of a loss of confidentiality. There may have been risks, discomforts or side effects that were not yet known.

2. Adequacy of Protection Against Risks

a. Recruitment and Informed Consent

Attendants of the 2007, 2008, and 2009 Annual Metabolic Camps and/or Metabolic Camp Annual Banquet at Emory University were identified as potential participants in the study. Clinicians such as genetic metabolic dietitians or physicians also identified participants during clinic visits and other interactions.

Identified participants were approached by mailed letter, electronic mailing, telephone call, or in person to introduce and recruit for the study. Recruitment was conducted first by the patient's clinician and followed up by the Principal Investigators if

the patient expressed interest and consented to contact regarding the study. In person recruitment was also conducted at information tables at conferences, banquets, or other gathering of patients with PKU or clinicians managing patients with PKU.

The following web-sites were also utilized for recruitment:

- http://www.clinicaltrials.gov (Identifier: NCT00892554)
- http://genetics.emory.edu/NUTRITION/pku_dha_study

Recruitment was planned to continue until at least 24 participants completed the study. Recruitment and enrollment was discontinued after thirty-three participants were randomized, twenty-seven participants completed the follow up visit, and twenty participants completed the study per protocol. The final participants were randomized in September 2009.

3. Consent Procedures

All participants gave consent to participate in research in accordance with the policies Emory University and federal regulations, including the Code of Federal Regulations, Title 45 (Public Welfare), Part 46 (Protection of Human Subjects). Prior to participation, each participant signed informed consent and Health Insurance Portability and Accountability Act forms. If a participant was between the ages of 12 and 16 years, the participant signed an assent form designed for children and the participant's legal authorized representative signed the consent form. If the participant was 17 years, both the participant and the participant's legal authorized representative signed the consent form.

a. Protection Against Risk

The study staff took every reasonable measure to prevent injury or harm to the subjects in this study. Because most participants took 1.2 gram or less of DHA (10 mg/kg body weight), participants were unlikely to experience the risks associated with omega-3 fatty acids described above. The following precautions, however, were taken. Participants were directed to take supplements with food, during a meal, or after a meal to reduce risk of gastrointestinal distress; blood pressure and plasma lipid profile were checked at study visits; and antioxidants were included as part of the DHA supplement. To minimize effects of adjustment to the supplement, participants were advised to increase supplement dosage gradually at the beginning of the trial (i.e., add 1 capsule per day per week). A trained professional drew blood and took proper safety precautions. The possible risks related to maintaining patient confidentiality were addressed by storing data in a locked file cabinet and maintaining data in a password-secured database.

The safety of the study was monitored through reports of adverse events from participants. Please see *5. Data and Safety Monitoring Plan* for further details.

4. Potential Benefits of the Proposed Research to the Subjects and Others

The tests and supplementation provided in this study were free of charge to participants. Participants received a small stipend for travel costs and inconvenience for being in the study or possible travel reimbursement as described in the consent form under the **Compensation/Cost** section. There is evidence that omega-3 fatty acids may improve blood pressure[196] and triglyceride levels[197] and provide cardiovascular disease prevention[198-199]. Other potential health benefits are either unrelated to this population or are being investigated.

The risks to the participants were minimal and very reasonable in relation to the anticipated benefits of the intervention.

a. Importance of the Knowledge to be Gained

Without early and appropriate preventative diet treatment, people with the rare autosomal recessive disorder PKU exhibit delays in development and mental retardation. Even with early and lifelong dietary treatment patients display minor neuropsychological deficits which intensify over time. The causal factor leading to neuropsychological deficits in PKU is thought to be increased concentration of Phe in the brain, particularly in early infancy and childhood; the biochemical mechanism, however, is still uncertain and may be multifactorial. Because decreased concentrations of DHA have been found in the blood lipids of patients with PKU, it has been hypothesized that DHA may play a role in neuropsychological status in PKU. Supplemental DHA has been shown to have a positive impact on visual evoked potentials and motor control in children with PKU, while its impact on other neuropsychological outcomes typically impacted in PKU have not yet been studied nor has supplementation been studied in adolescents and adults with PKU. As part of a long term goal to optimize neuropsychological outcomes of patients with PKU receiving diet treatment, this study was the first to investigate the influence of DHA supplementation on processing speed and executive function in patients with PKU. The results of this study aimed to offer a new line of evidence regarding the relationship between DHA supplementation and neuropsychological outcomes in adolescent and adult females with PKU.

5. Data Safety Monitoring Plan

Adherence and Monitoring Statement: The Data Safety Monitoring Plan (DSMP) outlined below adhered to the protocol approved by the CIS Research Advisory Committee and the Emory University School of Medicine IRB. An IRB-approved written informed consent was obtained from each participant at entry into the study; elements of informed consent included: (a) having the participant and/or parent/guardian/proxy review the study consent form; (b) having the investigator(s) or study staff meet with the participant and/or guardian/proxy to review the consent, confirm understanding, and answer any questions; and (c) once the investigator(s) or study staff are convinced that the protocol is understood and that there is agreement to participate, having the consent signed in the presence of a witness.

The Principal Investigator (PI) reviewed all data collection forms at least annually for completeness and accuracy of the data as well as protocol compliance. The PI reviewed this protocol on a continuing basis for participant safety and included the results of the review in annual progress reports submitted to the IRB and CIS Research Safety Advocate (RSA). As with all CIS protocols AEs and SAEs (below) were reviewed by the CIS Safety Advisory Subcommittee and the CIS RSA at scheduled monthly meetings.

Patient Monitoring was performed by the PI, the student investigator, the study physician/co-investigator and the ACTSI staff.

Patient safety data examination, monitoring procedures/oversight: All adverse events (AEs) were graded as to their attribution (unrelated to protocol, or possibly, probably, or definitely related to protocol). Any AE that was reported to either the PI or her designated research associates by a study participant or by medical staff caring for the

participant and which met the criteria was documented as such. This study was entered into the Emory CIS computerized database system to permit tracking of adverse events. This system was used by investigators to report "expected" AEs (predefined AEs which were monitored over the course of the trial–see below), "observed" AEs (AEs which occur but which may or may not have been anticipated), and all serious AEs (SAEs, see below); this system was used in this trial. SAEs were predefined as: any experience that suggests a significant hazard, such as events which: a) are fatal, b) are life threatening, c) result in permanent disability, d) require inpatient hospitalization, or e) involve cancer, a congenital anomaly, or drug overdose.

Any AEs were reported to the Emory CIS Research Safety Advocate (RSA), within 15 days of the event and any SAEs were to be reported to the RSA and the Emory IRB within 24-48 hours of the event. The standard Emory IRB reporting guidelines for AE and SAE reporting were also followed. The PI submitted an annual report to the IRB reporting any observed AEs or SAEs.

SAEs were also to be reported to the medical monitor at Martek Biosciences Corporation immediately by phone. The Serious Adverse Event Form was to be faxed to the medical monitor within five days. AEs were included in the final report to Martek following completion of the study. Martek defines an AE as:

> Any untoward medical occurrence in an investigational subject or recipient of a marketed or investigational product. The AE does not necessarily have to have a causal relationship with the treatment. An AE can be any unfavorable and unintended sign (including abnormal laboratory findings), symptom, or disease temporally associated with the use of the

product, whether or not considered to be related to the product (Nelson November 15, 2007).

Martek defines an SAE as:

> An adverse event that: results in death, is a life-threatening experience, requires in-patient hospitalization, results in a persistent or significant disability or incapacity, is a congenital anomaly or birth defect; or requires, based on reasonable medical judgment, a medical or surgical intervention to prevent one of the outcomes described above (Nelson November 15, 2007; CFR 312.32).

Potential ("expected") adverse events and plan for detecting problems and minimizing subject risk during this trial:

Expected adverse events were detailed in the Consent Form and included the following:

1. There may be mild discomfort and bruising to the site where the blood was drawn.
2. There may be a risk of gastrointestinal distress if taken on an empty stomach or if large quantities are taken.
3. High intake (>3g) of omega-3 fatty acids (DHA is in the family of omega-3 fatty acids) may increase the risk of bleeding.
4. Omega-3 fatty acids may contribute to small decreases in blood pressure and small changes in blood glucose levels.
5. Vitamin E needs may increase.
6. Low-density lipoprotein (bad cholesterol) levels may worsen a small amount.

There may be other risks that are currently unknown.

a. Procedures for minimizing risks:

1. Correct technique was used when drawing blood to minimize discomfort and bruising.
2. Participants were directed to take capsules with or following a meal.
3. The expected dosing range was 2-6 capsules per day, which provides 0.4-1.2 g DHA per day. Participants were told to take only their prescribed dose and not "make up" for missed doses.
4. Vital signs, including blood pressure, were monitored at the baseline and 4.5-month study visits.
5. Antioxidants were supplied with the supplement.
6. Blood was drawn to monitor total lipid profile at the baseline and 4.5-month study visits.

b. Plans for transmission of temporary or permanent suspension actions:

Any actions that mandated temporary or permanent suspension of study were to be transmitted to the CIS RSA, the Emory IRB, and, if appropriate, to Martek, the Food and Drug Association and the National Institutes of Health.

c. Plans for protecting subject confidentiality:

All information and materials was obtained for research purposes only and the data were kept in strict confidence. Confidentiality was assured by the use of participant codes rather than personal identifiers. The study database was secured, and information was only entered using participant identifier codes rather than personal identifiers. Electronic communication involved only coded, unidentifiable information. CIS adverse

event tracking utilized password-protected access, and all adverse event reports and annual summaries did not include participant-identifiable material.

d. Plans for assuring data accuracy and protocol human safety compliance:

The above detailed plans should assure data accuracy and human safety compliance for this research. These included computerized database management, and both CIS RSA and IRB oversight and communication. This plan, together with proposed monitoring by the RSA and the IRB, was determined to be sufficient without the addition of more faculty members to constitute a DSMB.

Inclusion of Women and Minorities

All females regardless of ethnicity or race were recruited for the study. Ethnic and racial variation among those who are diagnosed with PKU is limited as seen in Table 23 and Table 24. The investigators recruited from a national sample of patients attending a summer metabolic camp held at Emory University, a state-wide sample of patients attending the metabolic clinic at Emory University's Division of Medical Genetics, and other clinics around the United States. Due to the already small sample size of the study, there were not adequate sample sizes of racial and ethnic minorities with PKU to assess potential differences.

Only females, not males, were included in this study. One reason for this restriction is that the metabolic camp from which the study population was recruited is held for females only. If these pilot data warrant a larger clinical trial, males should be included in the larger clinical trial.

Table 23. The demographic distribution of PKU in the United States

	American Indian or Alaskan Native	Asian or Pacific Islander	Black, not of Hispanic Origin	Hispanic	White, not of Hispanic Origin	Other or Unknown	Total
Female	1%	1%	1%	12%	70%	15%	100%
Male	0%	1%	1%	10%	75%	13%	100%
Children	0.5%	1%	1%	11%	72.5%	14%	100%
Year 2002, National Cases (PKU)							

Table 24. The demographic distribution of PKU in the Emory Clinic Population

	American Indian or Alaskan Native	Asian or Pacific Islander	Black, not of Hispanic Origin	Hispanic	White, not of Hispanic Origin	Other or Unknown	Total
Female	1%	0%	2.5%	2.5%	94%	0%	100%
Male	0%	0%	1%	1%	97%	1%	100%
Children	1%	0%	3%	3%	92%	1%	100%
Year 1996-2002, Emory Children's Clinic							

Participation of persons under the age of 21

This study recruited persons 12 years of age and above. About half of the participants were expected to be between the ages of 12 and 21 years. Data collection occurred primarily in a clinic or hospital-based research environment at baseline and 4.5 months. Study staff experienced in working with children between the ages of 12 and 21 years of age collected the data and interacted with the participants.

Children under the age of 12 years were not be included in the study because both the educational material as well as the experience of being away from home requires a level of maturity for which children below 12 years of age, in the experience of this particular camp, may not be ready.

Biostatistical Design and Analysis

Data were analyzed and reported in accordance with the CONSORT (Consolidated Standards of Reporting Trials) statement[200].

Sample size calculations for Study 3

Several studies of people with PKU assess cognitive inhibition and processing speed as main outcome measures. This study, however, was the first to assess the effect of DHA supplementation on test performance of adolescents and adults with PKU. The target sample size was therefore informed by the following calculations.

Anticipated sample pool

Thirty to thirty-five participants typically attend the one-week Metabolic Camp intervention study.

Anticipated change in blood DHA concentrations

The sample size calculations were based upon 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 red blood cell total lipid DHA over the control group (Table 25). To achieve this level of improvement over a 4.5-month period at $\beta = .80$ and $\alpha = .05$, 9 participants and 14 participants would be needed in each group for improvement of plasma and erythrocyte total lipid DHA concentrations, respectively[201-202]. To allow for an expected participant loss to follow up of 25% over 4.5 months, a total of 35 participants were planned to be recruited.

Table 25. Plasma and RBC total lipid DHA status of children with PKU before and after supplementation

	Placebo		LC-PUFA[a]	
	Baseline Mean (SD)	Follow up Mean (SD)	Baseline Mean (SD)	Follow up Mean (SD)
Plasma DHA, % TLFA	1.2 (0.4)	1.1 (0.3)	1.1 (0.3)	2.3 (1.1)[b]
RBC DHA, % TLFA	1.1 (0.5)	1.5 (0.5)	1.3 (0.6)	2.8 (1.5)[b]

Abbreviations: RBC, red blood cell; DHA, docosahexaenoic acid; PKU, phenylketonuria; LC-PUFA, long chain polyunsaturated fatty acid; TLFA, total lipid fatty acids.

[a] Dosage: 10 mg DHA per kg body weight per day.

[b] Significantly different from baseline ($P < .05$).

Data from reference [201].

Anticipated change in neuropsychological outcomes

The same neuropsychological tests to be used in this study were used at the beginning and end of the metabolic camp intervention in 2007. Using the results of the camp study, in order to see a significant change ($\beta = .80$, $\alpha = .05$) in a total sample size of 24 participants, the minimum mean changes needed in this study are listed in Table 26.

Table 26. Minimum mean changes needed to see significant differences in neuropsychological outcomes for n=24

Test	Standard Scores Mean (SD)	Change in SS: Metabolic Camp 2007 Mean (SD)	Minimum mean changes in SS needed: PKU & DHA Study[a] Mean
Decision Speed[b]	100 (15)	22.30 (25.48)	18.28
Pair Cancellation[b]	100 (15)	13.70 (19.66)	14.10
Reading Fluency[b]	100 (15)	3.80 (24.30)	17.43
Math Fluency[b]	100 (15)	2.65 (26.04)	18.68
Word Reading[c]	10 (3)	0.16 (1.21)	2.71
Color Naming[c]	10 (3)	0.00 (2.26)	2.93
Inhibition[c]	10 (3)	1.58 (1.46)	3.07
Switching[c]	10 (3)	2.21 (2.10)	3.19

Abbreviations: SS, standard score; SD, standard deviation.

[a] Assumptions: n=12 per group, $\beta \geq .80$, $\alpha \leq .05$.

[b] n=20 females with PKU, ages 12-47 years.

[c] n=19 females with PKU, ages 12-47 years.

Chapter 5. Short term changes in plasma phenylalanine and cognitive processing speed in females of reproductive age with phenylketonuria attending a metabolic camp

Short term changes in plasma phenylalanine and cognitive processing speed in females of reproductive age with phenylketonuria attending a metabolic camp

Sarah H. L. Yi,[1] MS, RD, Julie A. Kable,[2] PhD, Rani H. Singh,[3] PhD, RD

[1]Nutrition & Health Sciences Program of the Graduate Division of Biological & Biomedical Sciences, Emory University, [2]Department of Pediatrics, Emory University School of Medicine, [3]Department of Human Genetics, Emory University School of Medicine

All correspondence concerning this article should be addressed to Rani H. Singh, PhD, RD, Department of Human Genetics, Emory University, 2165 N. Decatur Rd, Decatur, GA 30033, USA. E-mail: rsingh@emory.edu

Funding: This work was supported by the Clinical and Translational Science Award program, National Institutes of Health, National Center for Research Resources (PHS Grant UL1 RR025008), the General Clinical Research Center program, National Institutes of Health, National Center for Research Resources (PHS Grant M01 RR0039), and private donations. The camp was supported by camper tuition payments, tuition scholarships from the Georgia Department of Human Resources, and donations from private donors.

Conflict of Interest: None declared.

Acknowledgements: We thank the participants and their families, clinicians, research assistants, camp staff, and clinical research staff for their involvement and support.

Abstract

Objective: The objective of this study was to assess the impact of a 1-week camp intervention on cognitive processing speed in females with phenylketonuria (PKU).

Methods: Plasma phenylalanine (Phe), processing speed measures, and diet intake of 20 females with PKU (ages 12-47 years) were assessed at baseline and end-of-camp. Processing speed was assessed in group format by Woodcock-Johnson III timed tests of Decision Speed and Pair Cancellation.

Results: As expected, mean plasma Phe concentrations and processing speed scores significantly improved ($p < .001$) and Pair Cancellation scores negatively correlated with change in plasma Phe concentrations after controlling for verbal ability ($R = -.54$, $p = .008$). Teenage campers had significantly higher change scores versus the comparison sample on the Pair Cancellation task ($p = .003$, $n = 14$); adult campers exhibited higher change scores at the level of a trend ($p = .106$, $n = 6$) versus the comparison sample.

Conclusions: Pair cancellation may be more sensitive to improved plasma Phe due to the task's reliance on attention and concentration. Short-term improved plasma Phe achieved in a camp setting appears to be associated with improved processing speed reliant on attention.

Keywords: phenylketonuria; phenylalanine; cognitive processing speed; camp intervention

Introduction

Phenylketonuria (PKU; OMIM 261600) is an autosomal recessive disorder that, if not treated soon after birth, can lead to severe developmental delays, eczema, seizures, and behavioral disorders[35]. PKU is generally caused by a mutation in the gene encoding for phenylalanine (Phe) hydroxylase (PAH; EC 1.14.16.1) resulting in little or no PAH activity. Consequently, Phe metabolism is impaired and Phe in the plasma, brain, and other tissues increases above normal concentrations which can ultimately lead to neurological and psychological dysfunction[38, 203]. Successful prevention of the harmful effects of PKU requires dietary Phe restriction and adequate nutrient provision through an amino acid-rich, Phe-free medical food initiated within the first few weeks of life[36, 204].

When maintained for a minimum of the first eight to twelve years of life, the diet treatment for PKU allows for the attainment of intelligence comparable to other family members without PKU and controls[160-162]. Thereafter, when the diet is discontinued or poorly managed, psychological[158, 205-206], neurological[207], and cognitive[163, 208] declines commonly occur. In addition, for women, noncompliance during pregnancy places the fetus at risk for mental retardation, congenital heart defect, and other harmful outcomes[209]. Because of these negative outcomes, lifelong treatment is generally recommended[34, 210].

Even with early, lifelong treatment, subtle deficits in performance are seen on cognitive tasks in adolescents and adults with PKU, particularly in the domains of executive function (inhibition), attention, and processing speed[164, 211-212]. Processing speed is postulated to be a fundamental component of cognition[125], and is significantly related to concurrent and lifelong control of blood Phe concentrations[165]. Feldmann and

colleagues found lower scores on processing speed tasks in teens and young adults with PKU compared with teens and young adults with diabetes, and these lower scores were strongly correlated with concurrent and lifelong serum Phe concentrations[165].

Based on their recent meta-analysis, Albrecht and coworkers proposed there would be no difference in choice reaction time, an index of processing speed, between people with and without PKU when plasma Phe is well-controlled, which was defined as no higher than 320 μmol/L (5.3 mg/dL) for ages 7-13 years and 570 μmol/L (9.4 mg/dL) for ages 13-18 years[213]; inadequate data were available to evaluate this theory for adults. Furthermore, brief interventions have shown the reversibility of the effects of blood Phe concentrations on performance of processing speed tasks including sustained attention[3-4], reaction time[1, 4-5], and response latency[2] in individuals of varied treatment histories.

In this study, we assessed the impact of a 1-week Metabolic Camp intervention on measures of cognitive processing speed. The Metabolic Camp is held annually at Emory University (Atlanta, GA) for females of reproductive age with PKU and other inborn errors of metabolism. The camp's mission is to improve dietary adherence; prevent maternal PKU through educational, nutritional, and social support strategies; and evaluate these strategies through ongoing research. The camp intervention has been effective in reducing average plasma Phe concentrations of participants within the brief period[175]. We predicted that: (1) both performance on processing speed tasks and control of plasma Phe concentrations would improve at the end of the brief intervention, (2) improved performance would exceed a practice effect, and (3) changes in plasma Phe concentrations and processing speed performance would be related.

Methods

Participants/Intervention

A convenience sample of teen and adult women with PKU attending the aforementioned camp were recruited for this study. To advertise, brochures were mailed to previous campers and distributed by clinicians to patients throughout the US. Eligible participants were diagnosed with PKU and willing and able to participate in camp and research activities. To ensure test comprehension, those performing below two standard deviations on a standardized verbal ability task were excluded. Tuition scholarships and travel assistance were available for those with financial need.

Participants 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). Participants between ages 12 and 16 years signed an assent form designed for children and the participant's legal authorized representative signed an informed consent form. Participants aged 17 years and the participant's legal authorized representative signed the consent form.

The test-retest reliability control group data were obtained from the W-J III technical manual[181] to compare with changes observed in the camp participants. The purpose of this comparison was to evaluate if changes exceeded a practice effect. The test-retest study was conducted as part of the psychometric testing of the W-J III speeded tests. The test-retest participants consisted of healthy males and females aged 14-17 years (n=52) and 26-79 years (n=54), respectively. The time elapsed from baseline to follow up for the test-retest study was 1 day. As the results of the test-retest study were presented as

W-scores by age groups in the technical manual, the comparison between changes in the camp study was also presented this way.

Measures

The camp has been conducted as an ongoing clinical research protocol, thus a strong research infrastructure was already in place for this study. Baseline cognitive data were collected on the first and last afternoons and fasting blood was drawn on the first and last mornings of camp. Individual testing of verbal ability was performed at the hospital-based research center or in a private room in a camp house. Group testing of processing speed was performed in a camp house in which rows of tables and chairs were arranged. Each cognitive test was standardized to national demographics and demonstrates adequate reliability and validity[124, 127, 181].

Verbal Ability

The Peabody Picture Vocabulary Test—Third Edition, Form B (PPVT-IIIB) is (1) a verbally administered achievement test on standard English vocabulary, and (2) a screening test for verbal ability. Although the PPVT does not test overall intellectual capacity, it is highly correlated with measures of verbal and overall IQ[124]; therefore, the score was also utilized to index cognitive ability. The test was individually administered once to each participant during the camp week by a licensed psychologist or a clinical psychology post-doctoral fellow.

Cognitive Processing Speed

Processing speed was assessed using two simple, timed tests: the Decision Speed and Pair Cancellation tests from the Woodcock-Johnson III Tests of Cognitive Ability and Achievement (W-J III; Riverside Publishing, Rolling Meadows, IL). Participants in

the camp study were administered the W-J III tests in a group setting led by a licensed psychologist and research assistants recorded participant finishing times. Participants were instructed to complete each test as quickly as possible.

Decision Speed

The Decision Speed task is reliant on the ability to connect a concept with a name. Participants were instructed to circle two pictures in each row that were conceptually most similar.

Pair Cancellation

The Pair Cancellation task involves circling every instance of a specific pairing of pictures appearing on a single page.

Blood amino acid profile

Fasting venous blood was collected into sodium heparin tubes to assess plasma amino acid profiles. Amino acid analyses were performed by Emory Genetics Laboratory's Biochemical Genetics Laboratory. 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 [184].

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) [185]. Phe and Tyr were extracted from the blood spots into methanol containing internal standards (stable isotope labeled amino acids). Amino acid analysis was performed using a Micromass Quattro Micro tandem mass spectrometer with a Waters 2795 HPLC system. Amino acids were identified and quantified using NeoLynx software. Values of

whole blood Phe analyzed by LC MS/MS are about 19% lower than plasma Phe analyzed by ion-exchange chromatography[185]; therefore, a factor of 1.19 was used to determine these values. The same type of analysis was used for each participant at baseline and end-of-camp blood draws. Amino acid concentrations are reported as μmol/L.

Nutrient analysis

Three-day food records were collected and reviewed by a registered dietitian at baseline and end-of-camp in order to assess intakes of energy, intact protein, protein from medical food, phenylalanine, and tyrosine. If a participant did not have a three-day food record at baseline, a registered dietitian conducted a twenty-four hour dietary recall. 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.

Percent intake of estimated energy requirement (EER) was calculated using age-group-based equations. The equation used for girls aged 18 years or younger, assuming a low physical activity level, was EER = 135.3 − (30.8 × age [y]) + 1.16 × (10.0 × weight [kg] + 934 × height [m]) + 25 kcal; the equation used for women , assuming a low physical activity level, was EER = 354 – 6.91 × age (y) + 1.12 × (9.36 × wt [kg] + 726 × ht [m])[39].

Data analysis

Baseline and end-of-camp outcomes were compared using repeated measures analysis of variance (ANOVA). Comparisons between the camp participants and the test-retest controls were conducted using independent samples *t* test. Mean change and standard deviation of the change in performance of the test-retest controls were calculated

using the method for "imputing standard deviations for changes from baseline" described in the Cochrane Handbook (http://www.cochrane-handbook.org: section 16.1.3.2). Relationships between variables were assessed using Pearson product-moment correlation coefficient. To control for cognitive ability, PPVT standard score was added as a covariate. Multiple linear regression was used to assess the proportion of variance in task performance explained by the variance in plasma Phe concentrations and PPVT standard scores. To ensure the response to intervention was similar by age group, the analyses were also conducted according to age group (<18 years and ≥18 years). A two-tailed *p*-value ≤ .05 was considered statistically significant; *p*-values between > .05 and ≤ .10 were considered trends. Statistical analyses were performed using SPSS Statistics 17.0 (SPSS Inc., Chicago, IL).

Results

Of the 31 camp attendees diagnosed with an inborn error of metabolism, 27 had PKU. Three of the 27 did not consent to research, two did not pass the verbal ability screening test, one did not participate in baseline testing, and one discontinued due to homesickness. Twenty participants were included in this analysis.

Participants ranged in age from 12 to 47 years, with 14 under 18 years ("teens") (Table 27). While all participants under 18 years reported having health insurance, two of the six adults reported having no health insurance. The majority of the participants resided in the southern region of the US.

The teens were treated early and continuously for PKU, but only three of the six adults were detected through newborn screening with treatment starting as a newborn. The other three adult participants were diagnosed prior to implementation of newborn screening. Of these latter three, two started treatment within the first four months of life and one started treatment at 6 years. All six adults discontinued treatment at some point between the ages of 5 and 16 years and resumed treatment as adults. One early treated adult resumed treatment at the start of the camp after several years off diet.

Reported intakes at baseline revealed a mean intake of 138 ± 97% of prescribed Phe. Seventy percent reported consuming 100% of prescribed medical food. One participant was not consuming medical food at baseline. Of those consuming medical food, 84% of total protein was derived from medical food. Mean energy intake at baseline was 74 ± 22% of estimated energy requirement. Further intake data are shown in Table 28.

Distribution of baseline plasma Phe for the two age groups was similar. One participant had a plasma Phe concentration under 360 μmol/L, eight participants had plasma Phe concentrations between 360 and 600 μmol/L, and the remaining eleven had plasma Phe concentrations over 600 μmol/L (overall range: 353-1357 μmol/L). Mean plasma Phe significantly improved with the camp intervention ($p < .001$; Table 28, Figure 4), and fourteen of the twenty participants completed the camp with plasma Phe concentration below 600 μmol/L (five below 360 μmol/L). Phe concentrations of two participants increased at end-of-camp, however, one remained below 600 μmol/L.

Although participants under 18 years performed on average 8.5 points higher on the PPVT than those ≥18 years (105.3 ± 13.1 vs. 96.8 ± 14.2), the two age groups responded similarly to the intervention. Participants improved an average standard score of 21.6 ± 14.3 on the Decision Speed task and 12.9 ± 8.8 on the Pair Cancellation task ($p < .001$; Table 28, Figure 4). The mean improvements exceeded those of the test-retest controls, but were only statistically significant on the Pair Cancellation task in the teens ($p = .006$, Figure 5).

Mean changes in Decision Speed score were not related to changes in plasma Phe ($r = .082$, $p = .731$); the changes remained unrelated after controlling for verbal ability ($R = .091$, $p = .721$). Mean changes in Pair Cancellation score and plasma Phe were negatively related ($r = -.333$, $p = .151$); this correlation was significant after controlling for verbal ability ($R = -.604$, $p = .008$). Verbal ability and change in plasma Phe accounted for 49% (27.6%, 21.4% respectively; $p = .003$) of the variation in the Pair Cancellation change score.

Discussion

As expected, mean plasma Phe and Processing Speed scores significantly improved following the one-week camp-based intervention. Improvements in processing speed scores were greater than those achieved by the test-retest controls, indicating that the improvements were above a learning or practice effect. Changes in Decision Speed did not correspond with plasma Phe changes. Changes in Pair Cancellation corresponded, as expected, with changes in plasma Phe after controlling for verbal ability.

The Pair Cancellation task may be more sensitive to improved plasma Phe control than the Decision Speed task due to the former task's reliance on sustained attention. The Decision Speed task involves semantic processing which may not be as affected by concurrent plasma Phe. Other interventions have shown a positive impact of plasma Phe reduction on attentional domains[1, 3-4]; the relationship between semantic processing and change in plasma Phe has not previously been reported. A larger sample size, preferably with a control group, would be necessary to confirm the differing relationships of the two cognitive tests with plasma Phe.

Improved cognitive outcomes have also been seen in other interventions designed to decrease plasma Phe concentrations[1, 3, 5]. Huijbregts and colleagues demonstrated improved performance with modest decreases (mean: -186 μmol/L) in plasma Phe of young adolescents on tasks utilizing memory, sustained attention, and speed of processing[3]. Likewise, participants with modest increases in plasma Phe (mean: +260 μmol/L) showed less improvement or declines in performance.

The limitations of this study must be acknowledged. A control group with PKU assessed over the same time period as the camp participants would have been ideal for

the purposes of confirming the improved processing speed performance was not simply a learning effect. In addition, the test-retest controls were administered the follow up test only 24 hours after the initial test while the camp participants were administered the follow up test 96 hours after the initial test. It is possible that the differences in changes in performance of camp groups would have been even larger compared with the test-retest controls if the time elapsed had been the same (i.e., 96 hours) in both groups.

The small sample size may have limited the ability to detect subtle relationships between changes in plasma Phe and performance on processing speed tasks. In addition, the treatment histories of the participants were heterogeneous. While participants <18 years of age were treated early and continuously, actual lifelong compliance may have been variable. Treatment history varies even more in those ≥18 years of age due to differences in newborn screening practices and treatment recommendations. This heterogeneity may have resulted in variability in treatment outcomes as a result of differences in initial cognitive integrity. Even so, when data were analyzed by age group, the groups responded in a similar direction to the intervention.

These findings extend to females of reproductive age with PKU. Since neither children nor adolescent and adult males with PKU were included, caution should be taken when attempting to generalize results. In addition, these results may not extend to those who have normal concentrations of brain Phe despite increased plasma Phe[214].

The long term consequences of the camp intervention on cognitive outcomes were not evaluated in this study. It was previously shown that plasma Phe control returned to baseline levels by 4 months after the camp intervention[175]. Thus, the improvements in plasma Phe, and in turn cognitive processing speed, seen in this study are also likely to be

temporary, lasting as long as participants maintain adherence to their diet Phe and medical food prescriptions.

Adherence to a restrictive diet is difficult to maintain, particularly in adolescence and adulthood. Although difficult, it is essential that adolescent and adult women with PKU maintain plasma Phe concentrations within treatment range to prevent the detrimental effects of maternal PKU on a developing fetus in the event of a planned or unplanned pregnancy. Identifying factors to help motivate women to maintain plasma Phe concentrations within treatment range continues to be a struggle for clinicians in the US and internationally. Offering intensive interventions several times during the year which utilize a structure similar to a camp may be one way to help women of reproductive age with PKU maintain dietary adherence for longer periods.

Additionally, communicating to women the cognitive benefits of improved plasma Phe control over a short time period, as observed during Metabolic Camp, may help improve perceived benefits of diet adherence in women with PKU and in turn improve motivation to adhere to the diet. At the least, this knowledge may promote maintenance of plasma Phe control during important events such as taking an exam in school or giving a presentation at work. In the aforementioned study, knowledge about PKU and the diet treatment significantly improved at the end of camp and persisted over the 12-month follow up period, even though plasma Phe control was only temporary. Perceived benefits of adhering to the diet, however, did not change from baseline at any time point including at the end of camp[175]. Further research should investigate if communicating the short-term cognitive benefits would improve perceived benefits and thereby prolong plasma Phe control.

Conclusion

Short-term improved plasma Phe was associated with concurrent improved processing speed on a task requiring attention in females of reproductive age attending a metabolic camp. This finding confirms what has been seen in inpatient and outpatient-based interventions. Adherence to a restrictive diet is difficult to maintain, particularly in adolescence and adulthood. Communication of the potential cognitive benefits of short-term improved plasma Phe control may be helpful in motivating compliance. Offering frequent, intensive interventions to women of reproductive age with PKU may be one strategy to prolong improved plasma Phe control.

Table 27. Baseline characteristics of female camp participants with phenylketonuria stratified by age

Variable		<18 years n=14	≥18 years n=6
Age, mean (SD), y		14.9 (2.0)	38.1 (6.5)
Verbal ability, mean (SD), SS		105.3 (13.1)	96.8 (14.2)
Phe prescription, mean (SD), mg/d		318 (98)	376 (153)
Plasma Phe, mean (SD), µmol/L[a]		699.0 (293.8)	768.5 (337.5)
Plasma Tyr, mean (SD), µmol/L[b]		35.5 (15.3)	38.3 (8.2)
Overweight, No. (%)[c]		4 (29)	5 (83)
Parental marital status, No. (%)[d]	Married	11 (79)	
Participant marital status, No. (%)[e]	Married		4 (67)
Mother education, No. (%)	High school degree	14 (100)	
Father education, No. (%)	High school degree	10/13 (77)	
Participant education, No. (%)	High school degree		6 (100)
Employment, No. (%)	Employed		3 (50)
Poverty, No. (%)[f]		2 (18)	0 (0)
Race/ethnicity, No. (%)[g]	White/Caucasian	12 (86)	6 (100)
Insured, No. (%)		14 (100)	4 (66)
Residence, No. (%)[h]	South	14 (100)	5 (83)

Table 27 continued

Abbreviations: PKU, phenylketonuria; SS, standard score; Phe, phenylalanine; Tyr, tyrosine.

[a] Treatment range: 120-360 μmol/L.

[b] Normal range (2-18 years): 24-115 μmol/L; normal range (>18 years): 41-78 μmol/L.

[c] ≤19 years: at risk for overweight + overweight defined as >85th percentile, >19 years: overweight defined as BMI >25.0.

[d] Data shown for participants <18 years old.

[e] Data shown for participants ≥18 years old.

[f] At or below poverty threshold based on annual poverty thresholds calculated by the US Census Bureau (<18 years, n=11).

[g] Participants self-identified race/ethnicity as white/Caucasian or black/African American.

[h] US Census region.

Table 28. Baseline and end-of-camp measures of cognitive processing speed and metabolic control in females of reproductive age with PKU

Field	Task	Baseline Mean (SD)	End-of-camp Mean (SD)	Intervention Effect[a]
Cognitive	Decision Speed, SS	96.1 (15.8)	117.6 (20.3)	$F(1, 19) = 45.3$, $p < .001$
Processing Speed	Pair Cancellation, SS	98.0 (13.4)	110.9 (14.6)	$F(1, 19) = 43.5$, $p < .001$
	Decision Speed Correct[b]	34.5 (6.0)	37.5 (4.2)	$F(1, 19) = 21.2$, $p < .001$
	Pair Cancellation Correct[c]	62.0 (7.7)	64.6 (6.5)	$F(1, 19) = 12.9$, $p < .002$
Biomarkers	Plasma Phe, µmol/L	720 (300)	472 (201)	$F(1, 19) = 15.1$, $p < .001$
	Plasma Tyr, µmol/L	36.4 (13.4)	35.4 (12.9)	$F(1, 19) = 0.1$, $p = .752$
Nutrient intake[d,e]	Phe, mg/d	477 (466)	385 (125)	$F(1, 18) = 1.0$, $p = .321$
	Tyr, mg/d	5015 (1317)	5585 (1288)	$F(1, 18) = 4.2$, $p = .055$
	Energy, kcal/d	1551 (394)	1974 (394)	$F(1, 18) = 18.5$, $p < .001$
	Intact Protein, g/d	10.9 (9.7)	8.8 (2.6)	$F(1, 18) = 1.3$, $p = .262$
	MF Protein, g pro eq/d	46.5 (16.8)	52.7 (14.8)	$F(1, 18) = 4.7$, $p = .044$

Table 28 continued

Abbreviations: PKU, phenylketonuria; SS, standard score; Phe, phenylalanine; Tyr, tyrosine; MF, medical food.

[a] Repeated Measures ANOVA.

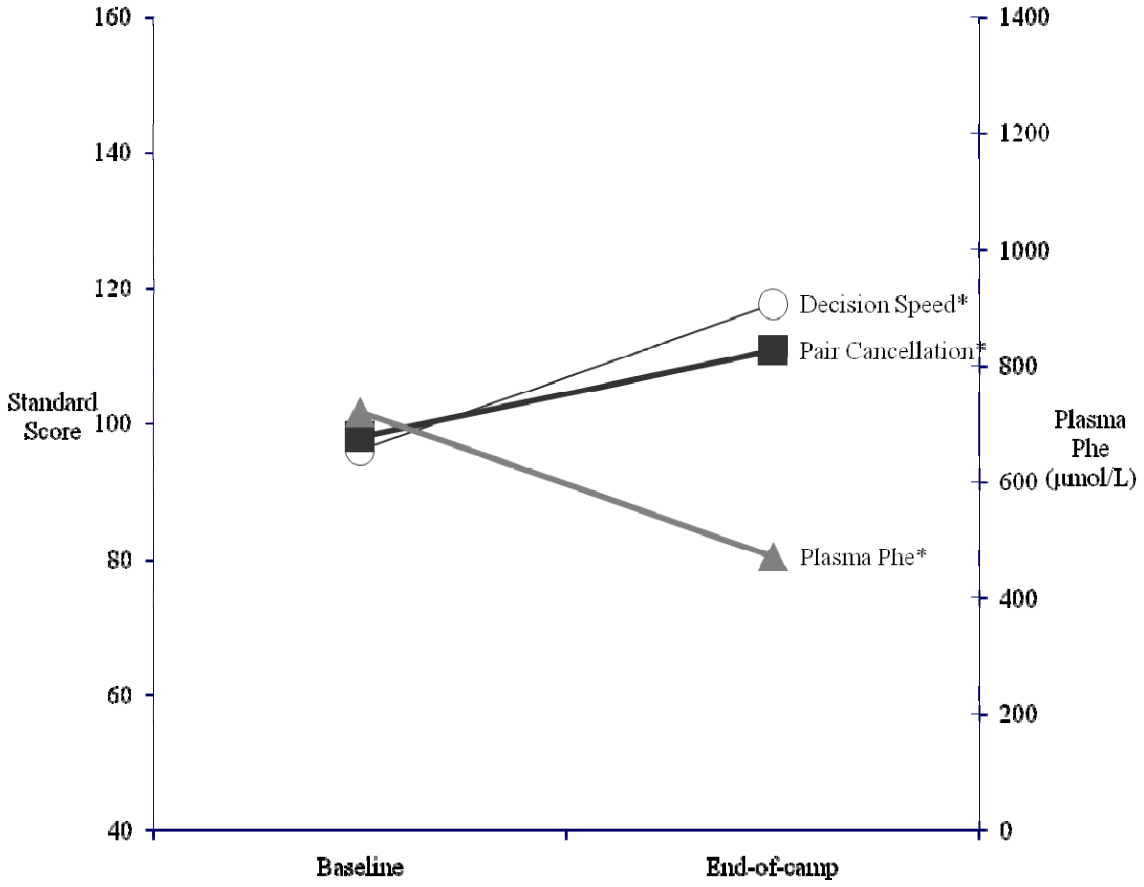
[b] Decision Speed possible correct: 40.

[c] Pair Cancellation possible correct: 69.

[d] Dietary intake data were analyzed using Nutrient Data System for Research software version 2009, developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN.

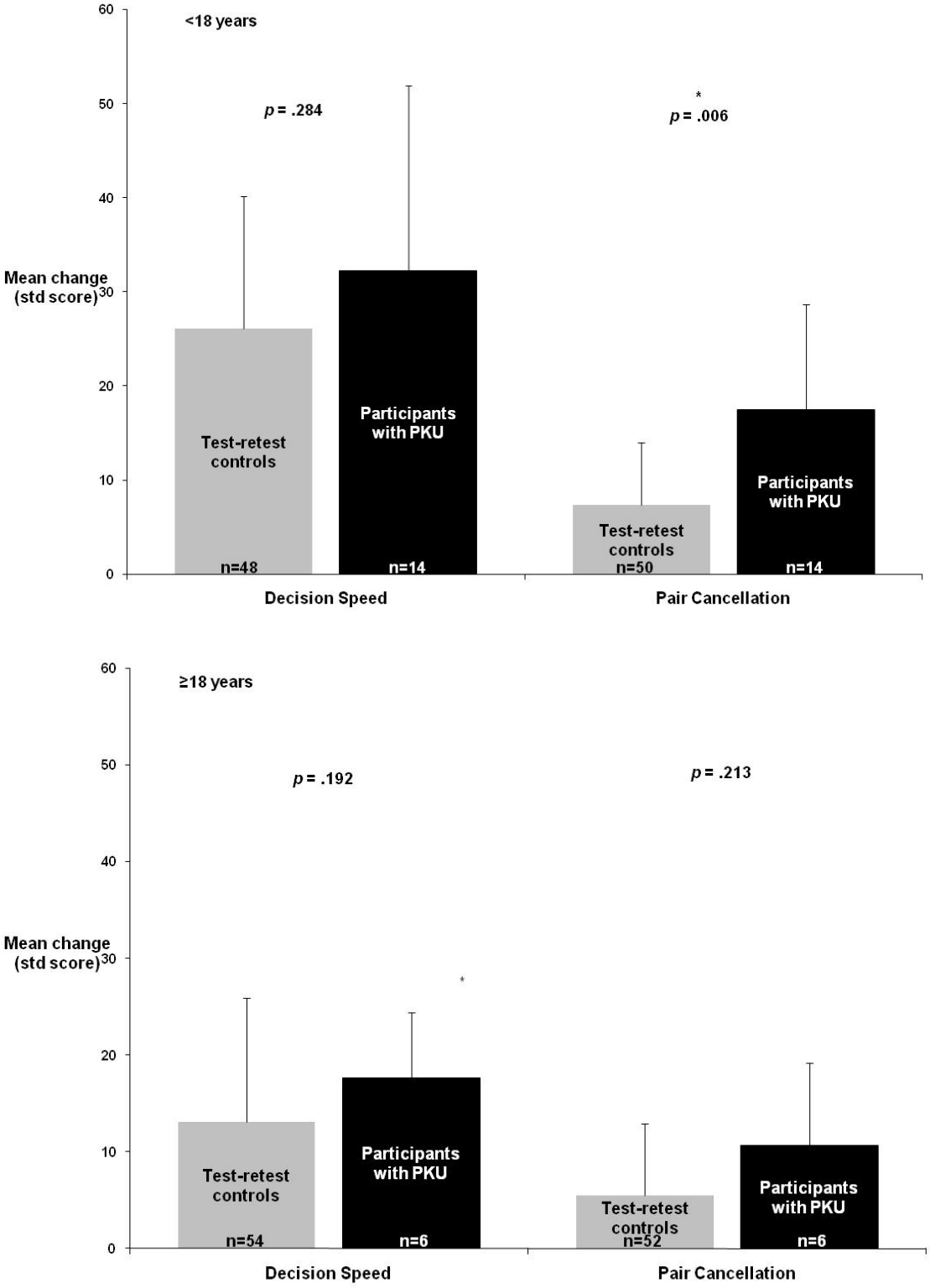
[e] n=19.

Figure 4. Baseline and end-of-camp cognitive processing speed task performance and biomarkers of metabolic control in females of reproductive age with PKU



n = 20; **p* < .001

Figure 5. Changes in performance on cognitive processing speed tasks (Mean (SD)) by age group: test-retest controls vs. females with PKU attending a camp intervention



Chapter 6. Verbal performance is associated with red blood cell docosahexaenoic acid accounting for plasma phenylalanine in females of reproductive age with phenylketonuria

Title: Verbal performance is associated with red blood cell docosahexaenoic acid accounting for plasma phenylalanine in females of reproductive age with phenylketonuria

Authors: S.H.L. Yi[1], J.A. Kable[2], M.L. Evatt[3], R.H. Singh[4].

Institutional affiliations: [1]Emory University, Nutrition & Health Sciences Program of the Graduate Division of Biological & Biomedical Sciences, [2]Emory University, School of Medicine, Department of Pediatrics, [3]Department of Veterans Affairs Medical Center, Atlanta, GA and Emory University School of Medicine, Department of Neurology, [4]Emory University School of Medicine, Department of Human Genetics

Corresponding author:

Rani H. Singh

2165 N. Decatur Road

Decatur, GA 30033

Tel: 404-778-8519

Fax: 404-778-8562

rsingh@emory.edu

Requests for reprints: Rani H. Singh, 2165 N. Decatur Road, Decatur, GA 30033

Summary word count: 248

Text word count: 3263

Figures: 0

Tables: 8

Summary

Diet therapy for phenylketonuria (PKU) requires restricted phenylalanine (Phe) intake with the majority of protein and other nutrients derived from synthetic medical food. The fatty acid docosahexaenoic acid (DHA) is important in brain development and function; low blood concentrations of DHA, however, have been reported in people treated for PKU. While the implications of low blood DHA are unclear, subtle cognitive deficits have been reported in those treated early and continuously for PKU. This study investigated the relationship between DHA status and cognitive performance in 41 females 12 years and older with PKU. Participants were attending the baseline visit of a research-based camp or a supplementation trial. The domains of verbal ability, processing speed, and executive function were assessed using standardized tests. Proportions of DHA in plasma and red blood cell (RBC) total lipids were assessed using gas chromatography/mass spectrometry. Percent plasma and RBC total lipid DHA were significantly lower in the participants compared with laboratory controls ($P < .001$), and participants consumed no appreciable DHA according to diet records. Plasma and RBC DHA both negatively correlated with plasma Phe ($P < .02$), and performance on the verbal ability task positively correlated with RBC DHA controlling for plasma Phe ($R = .32$, $P = .03$). The relationship between DHA and domains related to verbal ability, such as learning and memory, should be confirmed in a controlled trial. Domains of processing speed and executive function may require a larger sample size in order to clarify any association with DHA.

Synopsis: A positive association is seen between verbal performance and red blood cell docosahexaenoic acid status accounting for plasma phenylalanine control in females of reproductive age with phenylketonuria.

Abbreviated title (running head): Docosahexaenoic acid and cognitive performance in phenylketonuria

References to electronic databases: Phenylketonuria (PKU; OMIM 261600), phenylalanine hydroxylase (PAH; EC 1.14.16.1), docosahexaenoic acid (DHA; PubChem ID 445580)

List of abbreviations

AA	20:4(n-6)	arachidonic acid
ALA	18:3(n-3)	alpha-linolenic acid
DHA	22:6(n-3)	docosahexaenoic acid
DPAn-6	22:5(n-6)	docosapentaenoic acid
EPA	20:5(n-3)	eicosapentaenoic acid
LA	18:2(n-6)	linoleic acid
LC-PUFA		long-chain polyunsaturated fatty acid
MF		medical food
mPKU		maternal PKU
n-3 PUFA		a polyunsaturated fatty acid containing the first instance of unsaturation (i.e., double bond) at the third carbon from the methyl end of the fatty acid
PAH		phenylalanine hydroxylase
Phe		phenylalanine
PKU		phenylketonuria
PUFA		polyunsaturated fatty acid
RBC		red blood cell
Tyr		tyrosine

Each author contributed to the planning, conduct, and reporting of the work described in this article.

Rani H. Singh serves as guarantor for the article, accepts full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish.

All authors confirm that they have no competing interests for declaration.

The authors confirm independence from the funding sources, and the content of the article has not been influenced by funding sources.

Source(s) of support: This research was supported in part by grants from United to Support Metabolic Disorders (USMD-PKU) in America; and PHS Grant UL1 RR025008 from the Clinical and Translational Science Award program and PHS Grant M01 RR0039 from the General Clinical Research Center program, National Institutes of Health, National Center for Research Resources. This material is also the result of work supported with resources at the Atlanta VA Medical Center.

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.

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; EC 1.14.16.1). When diagnosed and treated soon after birth, associated developmental delays and behavioral disturbances can be prevented[35-36]; currently, lifelong diet treatment is recommended[34] to prevent cognitive, neurological, and psychiatric declines[158, 163, 205-208].

Despite successful prevention of mental retardation, 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[165, 211-212, 215]. Debate remains over the existence, nature, etiology, and clinical significance of these deficits, but two recent meta-analyses of studies including children, adolescents, and adults treated early for PKU confirm a significant effect of PKU compared with controls across the aforementioned and other domains[164, 216].

Individuals treated early in life for PKU on average show lower plasma and red blood cell (RBC) content of the n-3 fatty acid docosahexaenoic acid (DHA) compared with controls without PKU[6-7, 217-218]. DHA can be synthesized endogenously from the n-3 polyunsaturated fatty acid (PUFA) alpha-linolenic acid (ALA), which is found in some vegetable oils, however this process is generally inefficient in humans[57]. The enzymes which catalyze the desaturation and elongation of ALA to DHA also catalyze the conversion of LA to its longer chain derivative arachidonic acid (AA). Presence of LA in the U.S. food supply is higher than ALA[219], and increased intake of LA relative to ALA

is thought to competitively inhibit the conversion of ALA to longer-chain n-3 fatty acids including eicosapentaenoic acid (EPA) and DHA[59-61].

Because DHA appears to have multiple effects on the central nervous system, the mechanism(s) by which DHA may affect cognitive performance continue to be clarified. DHA is the primary n-3 PUFA in the brain, and comprises 10-13.5% of total lipids of the cerebral cortex[27, 220]. DHA is found primarily in membrane phospholipids and appears to affect a variety of structures and processes in the body, including cell membrane structure and neurochemistry. Effects on the cell membrane include increasing membrane disorder[221], enhancing lateral compressibility of the membrane[222], and allowing for vesicle formation and fusion[223]. DHA is a precursor to bioactive molecules neuroprotectin-D1 and resolvins, and appears to regulate neuronal apoptosis[224]. DHA supplementation may also increase brain concentrations of nitric oxide synthetase, dopamine, serotonin, and brain-derived neurotrophic factor[104, 225].

It has been proposed that inadequate docosahexaenoic acid (DHA; PubChem ID 445580) concentrations in neural lipids may be related to the minor cognitive deficits in people treated early for PKU[119, 218, 226]. The question relevant to adolescents and adults with PKU is whether blood concentrations of DHA are related to cognitive performance. In infants and children with PKU, small but significant improvements have been shown in visual evoked potentials and motor skills with improved percent blood DHA[8-11]. Studies have not yet investigated the relationship between biomarkers of DHA status and cognitive status in adolescents and adults with PKU. The present study evaluated the hypothesis that improved DHA status is associated with improved cognitive status. Specifically, we expected individuals with adequate plasma Phe control and improved

DHA concentrations to have the highest scores on cognitive tests and individuals with poor plasma Phe control and decreased DHA concentrations to have the lowest scores. In order to investigate this hypothesis, we compared the performance of females of reproductive age with PKU stratified into in four categories of plasma Phe control and DHA status on tasks of verbal ability, processing speed, and executive function in a cross-sectional study.

Participants and Methods

A convenience sample of participants attending the baseline visit of a research-based metabolic camp or a trial of supplemental DHA were included in this cross-sectional study. All participants were females ages 12 years and above, diagnosed with PKU, and not consuming supplemental DHA. 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).

Procedures

Plasma amino acid profile, plasma and RBC total lipid fatty acid profiles, cognitive status, and three-day diet intakes were assessed as described below.

Blood amino acid profile

Amino acid analyses were performed by Emory Genetics Laboratory's Biochemical Genetics Laboratory. 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[184]. Amino acid concentrations are reported as μmol/L. These values can be converted to mg/dL (mg%) by dividing by 60.54[186].

Plasma and RBC total lipid fatty acid profiles

Plasma and RBC total lipid fatty acid profiles were assessed by the Peroxisomal Diseases Laboratory at Kennedy Krieger Institute. Venous blood was drawn into tubes containing ethylenediaminetetraacetic acid (EDTA), and the whole blood was 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 and colleagues[187]. The content of plasma and RBC DHA are presented as percentage of total lipids; total lipid fatty acid concentrations are presented as μg/mL.

Diet assessment

Participants were given instructions and materials for the documentation of food intake and portion size estimation. Three-day food records were collected and reviewed with participants by a registered dietitian. Twenty-four hour recalls were conducted if food records were not available. 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. Nutrients assessed included energy, protein, medical food protein, fat, carbohydrates, Phe, Tyr, linoleic acid (LA), arachidonic acid (AA), alpha-linolenic acid (ALA), eicosapentaenoic acid (EPA), and DHA.

Cognitive assessments

Performance was assessed on tasks drawing upon the domains of verbal ability, executive function, and processing speed. Standard scores were calculated from raw scores using normative values included with the testing materials. The standard scores are age-adjusted 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[124, 126-127, 181].

Verbal Ability

The Peabody Picture Vocabulary Test—Third Edition, Form B (PPVT-IIIB) is (1) a verbally administered achievement test on standard English vocabulary, and (2) a screening test for verbal ability. Although the PPVT does not test overall intellectual capacity, it is highly correlated with measures of overall IQ[124]. 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 were used as a screening tool, to describe the sample, and as a proxy for verbal intelligence.

Participants scoring less than 70 (standard mean = 100, standard deviation = 15) on the PPVT were deemed to be severely impaired cognitively and were excluded from study. The results of the PPVT tests for these participants were not included in the analysis because complete cognitive data (i.e., performance on cognitive processing speed and executive function tasks) were not available.

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) and were 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[182]. Performance on the CWIT has been shown to be sensitive to

mean lifetime blood Phe concentrations in children and adolescents treated continuously from soon after birth through at least 14 years old[183]. Color Naming and Word Reading are fundamental skills needed to complete the Inhibition and Inhibition/Switching tasks and were also evaluated to differentiate poor performance due to deficits in fundamental skills versus inhibition and/or cognitive flexibility.

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 in the camp study took the W-J III tests in a group setting led by a licensed psychologist and research assistants recorded participant finishing times. Participants in the trial took the W-J III tests individually by a trained tester under the supervision of a licensed psychologist. Participants were instructed to complete each test as quickly as possible.

The Decision Speed task is reliant on the ability to connect a concept with a name. Participants were instructed to circle two pictures in each row that were conceptually most similar. The Pair Cancellation task involves circling every instance of a specific pairing of pictures appearing on a single page. The Reading Fluency task requires reading abilities. Participants were instructed to read a series of statements and record if the statement was true or false. The Math Fluency task is related to math achievement and requires the participant to perform simple arithmetic calculations (addition, subtraction, and multiplication). The Color Naming task requires the participant to identify verbally a

series of colors presented on a page. The Word Reading task requires participants to read aloud a series of color names presented on a page.

The six processing speed scores were reduced to one processing speed factor using principal components analysis with a varimax rotation. The processing speed factor explained 66.6% of the total variance among the variables and had an eigenvalue of 3.99. For analysis, the processing speed factor was standardized to a z-score.

Statistical analysis

Participant characteristics, biomarkers, nutrient intake, and cognitive performance were presented as mean ± standard deviation (SD) for continuous variables or as number and percent for categorical variables. Pearson product-moment correlation coefficient was used to perform simple correlation analyses. Participants were categorized into two groups based on plasma Phe concentrations; low Phe was defined as having a plasma Phe concentration <600 μmol/L and high Phe as having a plasma Phe concentration ≥600 μmol/L. Categories of RBC DHA status were constructed using the sample median RBC DHA value of each age group (12-17 and ≥18 years); the high DHA category was RBC greater than or equal to the sample median for age, and low DHA category less than the sample median for age. Intergroup comparisons for continuous variables were made using two-way ANOVA. The plasma Phe and RBC DHA categorical variables were then collapsed into one, four-level categorical variable, and multiple comparison testing was conducted using Tukey's post-hoc test. Intergroup comparisons for categorical variables were made using Fisher's Exact Test. Multiple linear regression was used to assess the proportion of variance in cognitive performance attributed to the variation in plasma or RBC DHA controlling for plasma Phe concentrations. A P value ≤.05 was considered

statistically significant, and *P* values between >.05 and ≤.10 were considered trends informative for future research. Statistical analyses were performed using SPSS Statistics 17.0 (SPSS Inc.; Chicago, IL).

Results

Of the 47 individuals with PKU attending the baseline visit, three declined participation, two had impaired scores on the screening test (PPVT SS<70), and one was not able to provide venous blood at the blood draw. A final sample of 41 participants was included in this analysis. As shown in Table 29, the four participant groups were similar in most demographic characteristics. Average Phe prescription was lower in Group 3 (high Phe/high DHA) compared with the other groups. A higher percentage of participants in Group 4 (high Phe/low DHA) reported lower maternal education. In addition, higher percentages of participants in Groups 3 (high Phe/high DHA) and 4 (high Phe/low DHA) were at or below the poverty threshold compared with the other two groups.

On average, participants displayed concentrations of plasma Phe exceeding treatment recommendations (Table 30), and plasma and RBC DHA contents significantly below laboratory controls (Table 31, Table 32). Concentrations of plasma Phe were inversely correlated with plasma DHA (r = -.42, P = .003, n = 41) and RBC DHA (r = -.33, P = .02, n = 41).

Table 33 shows no differences in estimated intake of DHA or Phe between the four groups of participants. Absolute intake of LA and ALA significantly differed by group, but the dietary LA:ALA ratios were similar (range: 7.7±1.6 – 8.4±1.7; $F_{2, 36}$ = 0.2, P = .84). Participants with poor plasma Phe concentrations (Groups 2 and 4; Phe ≥ 600 μmol/L) reportedly consumed significantly less energy during the three days prior to the blood draw compared with participants with better plasma Phe (Phe < 600 μmol/L; $F_{1,37}$ = 5.8, P = .02).

Plasma Phe concentration was inversely correlated with measures of verbal ability (r = -.45, P = .002, n = 41), cognitive processing speed (r = -.37, P = .009, n = 41), cognitive inhibition (r = -.32, P = .02, n = 41), and cognitive flexibility (r = -.35, P = .02, n = 38).

RBC DHA content was positively related to performance on the PPVT before and after adjusting for plasma Phe (r = .42, P = .003, n = 41; R = .32, P = .02, n = 41, respectively). RBC DHA explained an additional 9.3% of the variance in PPVT performance after controlling for plasma Phe ($\Delta F_{1, 38} = 4.8$, P = .03). An initial trend in the relationship between plasma DHA and PPVT (r = .20, P = 0.10, n=41) was diminished after controlling for plasma Phe (R = .02, P = 0.46, n=41). Plasma and RBC DHA were not found to be related to measures of cognitive processing speed, inhibition, and flexibility.

As shown in Table 34, PPVT scores differed significantly by group status. Specifically, Group 1 (low Phe/high DHA) performed significantly better on the PPVT than Group 4 (high Phe/low DHA; Tukey's post-hoc test: mean difference: 15.89, std error: 4.30, P = .004; 95% confidence interval: 4.32-27.46). Controlling for maternal education did not change this relationship (Table 52). Differences in performance speed and accuracy on the processing speed and executive function tasks were not seen between the four groups (Table 34, Table 35, Table 36).

Discussion and Conclusions

In this study, combined high RBC DHA content and low plasma Phe concentrations was associated with the highest mean performance on a verbal ability task in adolescent and adult women with PKU. Relationships between biomarkers of DHA status and performance on tasks of processing speed and executive function, however, were not apparent.

Due to the cross-sectional nature of this study, the results that participants with the best Phe and DHA status had the highest mean PPVT scores and those with the poorest Phe and DHA status had the lowest mean PPVT scores suggest a number of possible interpretations. If consistent with the stated hypothesis, plasma Phe control <600 μmol/L and improved DHA status may allow for enhanced performance on specific cognitive tasks, such as verbal ability.

Verbal ability has been shown to be related to DHA status in other studies. Similar to the present study, performance on the PPVT and whole blood DHA concentrations ($r^2 = .14$, $P = .018$) were positively related in healthy four year old children supplemented with 400 mg DHA for four months[91]. They found no differences, however in change in performance on tests of verbal ability between the DHA- and placebo-supplemented groups[91]. Furthermore, Birch and colleagues found four year olds who were fed formula supplemented with DHA (0.36% fatty acids) and AA (0.72% fatty acids) as infants had verbal IQ similar to breastfed infants[86]. Four year olds fed formula without preformed LC-PUFAs as infants, however, had significantly lower verbal IQ than those who were breastfed.

Not all studies have shown statistically significant differences, however this is likely due to inadequate statistical power[47-48, 227]. The lower concentrations of blood DHA seen in people with PKU are comparable with those seen in other populations consuming diets lacking pre-formed long-chain polyunsaturated fatty acids such as a vegan diet[120] or the low protein diet to treat maple syrup urine disease[228] and are thus suspected to be an artifact of the restrictive diet.

Interestingly, in this study those with low Phe/low DHA and high Phe/high DHA, similarly displayed average performance on the PPVT. This suggests a protective effect of DHA in the domain of verbal ability in the event of poor plasma Phe control. It also suggests that while important, plasma Phe control may not be the sole diet-related predictor of cognitive status in PKU.

These results could be interpreted alternatively that participants with higher performance on the PPVT (low Phe/high DHA) are more compliant with their diet therapy and thus have both better control of plasma Phe and a higher quality diet. Also, people with average PPVT scores (Groups 2 and 3: low Phe/low DHA and high Phe/high DHA Groups) may have diet-related health behaviors that are more beneficial than those with lower scores (Group 4: high Phe/low DHA).

The lack of association between measures of processing speed and executive function and biomarkers of DHA suggests that the mechanism by which DHA is related to PPVT performance may be distinct. In the aforementioned study, Ryan and Nelson also found no differences in change in performance on tests of sustained attention, inhibition, and processing speed between the DHA- and placebo-supplemented groups[91]. de Groot and colleagues similarly 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[97-98]. In a smaller study, Llorente and colleagues also found no significant differences in cross-sectional measures of processing speed and executive function in 27 lactating women supplemented with 200 mg DHA or placebo; however, small improvements in performance were seen in the supplemented group, but the lack of significance may have been due to inadequate statistical power[99].

In this study, lower reported mean energy intakes were seen in the two groups with plasma Phe >600 μmol/L. This finding was consistent with results of a separate analysis of the camp intervention in which we showed that change in energy intake was inversely correlated with change in plasma Phe concentration (Yi et al 2010, unpublished manuscript). A common explanation for this relationship is that in individuals with PKU inadequate energy and/or protein intake may lead to catabolism of skeletal muscle and subsequent release of Phe into the circulation[38]; additional studies are still needed to confirm this interpretation.

Because of the small sample size in the current study, extra care was taken in the interpretation of the results. Scores standardized to age were used for all cognitive outcomes. Also, to reduce the chance of a significant result due to random error, multiple variables assessing the same domain were aggregated for analysis whenever possible. In addition, because this study was conducted only with females, these results are not necessarily extendable to males.

Conclusions

This is the first study assessing the relationship between cognitive performance and DHA status in adolescents and adults with PKU. This study found a positive

relationship between RBC DHA and performance on a verbal ability task, but did not find support for a relationship between performance on tasks of processing speed and executive function. A controlled trial should be conducted to confirm the relationship between DHA status and verbal ability; this trial should also assess performance on tasks utilizing memory and learning.

Acknowledgements

We thank the participants and their families, clinicians, research assistants, camp staff, and ACTSI staff for their involvement and support. We also thank Ngoc-Anh Le, PhD; Usha Ramakrishnan, PhD; Kevin M. Sullivan, PhD; and Thomas R. Ziegler, MD for encouraging this analysis. This research was supported in part by grants from United to Support Metabolic Disorders (USMD-PKU) in America; private donations; and, PHS Grant UL1 RR025008 from the Clinical and Translational Science Award program and PHS Grant M01 RR0039 from the General Clinical Research Center program, National Institutes of Health, National Center for Research Resources. This material is also the result of work supported with resources at the Atlanta VA Medical Center; Dr. Evatt receives salary support from the Atlanta VA Medical Center.

Tables

Table 29. Characteristics of females of reproductive age with PKU stratified by plasma Phe and RBC DHA status

		Low Phe/ High DHA[a] Group 1, n=12	Low Phe/ Low DHA Group 2, n=7	High Phe/ High DHA Group 3, n=8	High Phe/ Low DHA Group 4, n=14
Age, mean (SD), y		24.2 (10.7)	23.0 (9.1)	21.9 (8.1)	25.2 (12.2)
Phe prescription, mean (SD), mg/d[b]		384 (132)	434 (104)	265 (69)	350 (127)
Medical food prescription, mean (SD), g pro eq/d[c]		54.1 (15.1)	59.4 (11.8)	49.4 (8.6)	56.9 (7.7)
Poverty, No. (%)[d]		1 (11)	1 (17)	3 (43)	4 (36.4)
Overweight, No. (%)[e]		4 (33)	4 (57)	4 (50)	8 (57)
Maternal education, No. (%)	HS degree or less	2 (17)	3 (43)	4 (50)	9 (64)
Race/ethnicity, No. (%)[f]	white/Caucasian	11 (92)	7 (100)	7 (88)	12 (86)
Insured, No. (%)		12 (100)	6 (86)	6/7 (86)	13 (93)
Residence, No. (%)[g]	South	10 (83)	5 (71)	6 (75)	14 (100)

Table 29 continued

Abbreviations: PKU, phenylketonuria; Phe, phenylalanine; DHA, docosahexaenoic acid; pro eq, protein equivalent; HS, high school.

[a] Low Phe: <600 μmol/L; High DHA: RBC DHA median by age group, <18 years: ≥ 2.23%; ≥18 years: ≥ 2.40%.

[b] Phe prescription: Group 3, n=7.

[c] Medical food prescription: Group 3, n=1 taking large neutral amino acids not included in mean; Group 4, n=13.

[d] At or below poverty threshold based on annual poverty thresholds calculated by the US Census Bureau (Group 1, n=9; Group 2, n=6; Group 3, n=7; Group 4, n=11).

[e] <20 years: at risk for overweight + overweight defined as BMI ≥85th percentile, ≥20 years: overweight defined as BMI ≥25.0.

[f] Participants self-identified race/ethnicity as white/Caucasian, black/African American, or Native American.

[g] US Census regions; other participants from Northeast, West, or Midwest.

Table 30. Biomarkers of metabolic control in females of reproductive age with PKU stratified by age group

	<18 years		≥18 years		Treatment Range[a]
	PKU	Reference	PKU	Reference	
	n=20		n=21		
	Mean (SD)	Range	Mean (SD)	Range	
Plasma Phe, µmol/L	843.1 (463.9)	26-91	707.8 (464.8)	49-76	120-360
Plasma Tyr, µmol/L	42.1 (20.1)	24-115	48.5 (24.5)	41-78	
Phe : Tyr Ratio	23.9 (14.0)		16.3 (11.3)		

Abbreviations: PKU, phenylketonuria; Phe, phenylalanine; Tyr, tyrosine.

[a] NIH consensus statement (2000) treatment range goals: <12 years, preconception, and pregnancy: 120-360 µmol/L; adolescents: 120-600; adults: 120-900[34].

Table 31. Select biomarkers of plasma total lipid fatty acid status in females of reproductive age with PKU compared with female laboratory controls without PKU

	<18 years		≥18 years		Statistics	
	PKU	Lab controls	PKU	Lab controls		
	n=20	n=5	n=21	n=17		
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	*F* test[a]	*P* value
Age, y	15.1 (1.8)	15.7 (0.97)	32.3 (7.4)	38.7 (8.1)	$F_{1, 61} = 10.9$	.002
Plasma LA, % TLFA	30.37 (4.24)	27.97 (4.49)	29.78 (3.71)	29.33 (2.45)	$F_{1, 61} = 1.2$	.28
Plasma AA, % TLFA	5.96 (1.44)	5.86 (1.09)	6.86 (1.40)	6.38 (0.970)	$F_{1, 61} = 0.2$	.65
Plasma ALA, % TLFA	0.841(0.201)	0.612 (0.085)	0.839 (0.340)	0.691 (0.289)	$F_{1, 61} = 5.5$	.02
Plasma EPA, % TLFA	0.271(0.087)	1.027 (0.436)	0.365 (0.200)	0.618 (0.459)	$F_{1, 61} = 23.1$	<.001
Plasma DHA, % TLFA	0.932 (0.299)	3.177 (1.129)	1.137 (0.326)	2.889 (0.817)	$F_{1, 61} = 157.8$	<.001
Plasma AA/DHA ratio	6.67 (1.47)	2.16 (1.13)	6.45 (2.10)	2.36 (0.68)	$F_{1, 58} = 110.6$	<.001
Plasma TLFA, µg/mL	2476 (1100)	2609 (322)	2588 (786)	2547 (382)	$F_{1, 58} = 0.0$	.90

Table 31 continued

Abbreviations: PKU, phenylketonuria; DHA, docosahexaenoic acid; LA, linoleic acid; AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; TLFA, total lipid fatty acids.

[a] 1-way ANOVA: all PKU (n=41) vs. all lab controls (n=23).

Table 32. Select biomarkers of RBC total lipid fatty acid status in females of reproductive age with PKU compared with female laboratory controls without PKU

	<18 years		≥18 years		Statistics	
	PKU n=20	Lab controls n=5	PKU n=21	Lab controls n=20		
	Mean (SD)	Mean (SD)	Mean (SD)	Mean (SD)	*F* test[a]	*P* value
Age, y	15.1 (1.8)	13 (2.1)	32.3 (7.4)	36.8 (9.9)	$F_{1, 64} = 7.9$	.007
RBC LA, % TLFA	9.85 (1.15)	9.57 (1.09)	9.73 (1.28)	9.63 (1.22)	$F_{1, 64} = 0.3$	.58
RBC AA, % TLFA	12.45 (1.17)	13.64 (0.49)	12.71 (1.75)	14.08 (1.34)	$F_{1, 64} = 15.9$	<.001
RBC ALA, % TLFA	0.130 (0.025)	0.079 (0.021)	0.126 (0.036)	0.125 (0.048)	$F_{1, 64} = 1.4$	.23
RBC EPA, % TLFA	0.196 (0.030)	0.332 (0.121)	0.282 (0.133)	0.494 (0.205)	$F_{1, 64} = 34.6$	<.001
RBC DHA, % TLFA	2.12 (0.50)	3.66 (0.45)	2.69 (0.82)	3.92 (1.04)	$F_{1, 64} = 48.8$	<.001
RBC AA/DHA ratio	6.27 (1.99)	3.77 (0.48)	5.13 (1.57)	3.88 (1.21)	$F_{1, 64} = 20.1$	<.001
RBC TLFA, µg/mL	1329 (165)	1423 (145)	1453 (257)	1328 (204)	$F_{1, 64} = 0.7$	.41

Table 32 continued

Abbreviations: PKU, phenylketonuria; DHA, docosahexaenoic acid; LA, linoleic acid; AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; TLFA, total lipid fatty acids.

[a] 1-way ANOVA: all PKU (n=41) vs. all lab controls (n=25).

Table 33. Estimates of diet intake in females of reproductive age with PKU stratified by plasma Phe and RBC DHA status

	Low Phe/ High DHA[a] Group 1, n=11 Mean (SD)	Low Phe/ Low DHA Group 2, n=7 Mean (SD)	High Phe/ High DHA Group 3, n=7 Mean (SD)	High Phe/ Low DHA Group 4, n=14 Mean (SD)	Statistics *F* test[b]	*P* value
Energy, kcal/d[c]	1799 (362)	1793 (631)	1461 (373)	1491 (357)	$F_{3, 35} = 1.8$	.16
Protein, g pro eq/d	60.4 (10.3)	66.5 (24.2)	57.6 (12.2)	52.9 (10.8)	$F_{3, 35} = 1.6$	.22
MF Protein, g pro eq/d	51.9 (14.0)	53.7 (17.9)	48.2 (7.3)	40.3 (18.3)	$F_{3, 36} = 1.7$	.19
Fat, g/d	61 (24)	52 (12)	38 (16)	46 (16)	$F_{3, 35} = 2.6$	.07
Carbohydrates, g/d	266 (72)	275 (116)	234 (63)	224 (65)	$F_{3, 35} = 1.0$	.41
Phe, g/d	.526 (.328)	.581 (.535)	.373 (.273)	.564 (.563)	$F_{3, 35} = 0.3$	.81
Tyr, g/d	5.535 (.910)	5.648 (1.834)	5.350 (1.109)	4.515 (1.515)	$F_{3, 35} = 1.7$	.20
LA, g/d	18.3 (6.9)	14.5 (4.2)	8.9 (2.6)	13.2 (6.3)	$F_{3, 35} = 4.0$	.01
AA, g/d	.002 (.002)	.014 (.023)	.006 (.008)	.008 (.014)	$F_{3, 35} = 1.3$	.30
ALA, g/d	2.49 (1.23)	1.95 (.67)	1.12 (.30)	1.57 (.62)	$F_{3, 35} = 4.6$	.008

Table 33 continued

	Low Phe/ High DHA[a] Group 1, n=11 Mean (SD)	Low Phe/ Low DHA Group 2, n=7 Mean (SD)	High Phe/ High DHA Group 3, n=7 Mean (SD)	High Phe/ Low DHA Group 4, n=14 Mean (SD)	Statistics *F* test[b]	*P* value
EPA, g/d	.000 (.001)	.002 (.003)	.000 (.001)	.001 (.001)	$F_{3, 35} = 1.2$	.31
DHA, g/d	.001 (.001)	.003 (.005)	.002 (.003)	.001 (.002)	$F_{3, 35} = 1.0$	.39

Abbreviations: PKU, phenylketonuria; Phe, phenylalanine; DHA, docosahexaenoic acid; pro eq, protein equivalent; MF, medical food; Tyr, tyrosine; LA, linoleic acid; AA, arachidonic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid.

[a] Low Phe: <600 µmol/L; High DHA: RBC DHA median by age group, <18 years: ≥ 2.23%; ≥18 years: ≥ 2.40%.

[b] Two-way ANOVA; statistics include interaction factor.

[c] Dietary intake data were analyzed using Nutrient Data System for Research software version 2009, developed by the Nutrition Coordinating Center (NCC), University of Minnesota, Minneapolis, MN.

Table 34. Cognitive performance in females of reproductive age with PKU stratified by plasma Phe and RBC DHA status

Domain	Low Phe/ High DHA[a] Group 1, n=12 Mean (SD)	Low Phe/ Low DHA Group 2, n=7 Mean (SD)	High Phe/ High DHA Group 3, n=8 Mean (SD)	High Phe/ Low DHA Group 4, n=14 Mean (SD)	Statistics	
					F test[b]	*P* value
Verbal ability, SS[c]	108.8 (12.4)	100.9 (10.9)	100.4 (11.7)	92.9 (9.0)	$F_{2, 38} = 7.0$	.003
Processing speed, factor score	102.8 (7.3)	101.4 (15.8)	101.6 (12.2)	97.4 (18.3)	$F_{2, 38} = 0.5$	.61
Cognitive inhibition, SS	11.3 (2.6)	9.4 (3.7)	9.4 (4.7)	9.4 (3.8)	$F_{2, 38} = 0.8$	.48
Cognitive flexibility, SS[d]	9.9 (2.7)	10.2 (4.6)	10.0 (3.7)	9.2 (4.5)	$F_{2, 35} = 0.1$	.90

Abbreviations: PKU, phenylketonuria; Phe, phenylalanine; DHA, docosahexaenoic acid; SS, standard score.

[a] Low Phe: <600 μmol/L; High DHA: RBC DHA median by age group, <18 years: ≥ 2.23%; ≥18 years: ≥ 2.40%.

[b] Two-way ANOVA.

[c] PPVT standard scores of Group 1 significantly differed compared with scores of Group 4 (mean difference = 15.9, *P* = .004; 95% confidence interval: 4.3, 27.5).

[d] Cognitive flexibility: Group 2, n=6; Group 3, n=7; Group 4, n=13.

Table 35. Number and percent of participants making 2 or more errors on processing speed and executive function tasks of the Color-Word Interference Test by females of reproductive age with PKU stratified by plasma Phe and RBC DHA status

Task[a]	Low Phe/ High DHA[b] Group 1, n=12 No. (%)	Low Phe/ Low DHA Group 2, n=7 No. (%)	High Phe/ High DHA Group 3, n=8 No. (%)	High Phe/ Low DHA Group 4, n=14 No. (%)	Statistics Exact test[c]	*P* value
Color Naming, SS[d]	0 (0)	1 (14)	2 (25)	4 (29)	4.4	.20
Word Reading, SS[d]	1 (8)	0 (0)	1 (13)	2 (14)	1.2	.91
Inhibition, SS[e]	4 (33)	3 (43)	4 (50)	10 (71)	4.0	.28
Inhibition/Switching, SS[e,f]	7 (58)	5 (71)	4 (50)	9 (69)	1.2	.82

Abbreviations: PKU, phenylketonuria; Phe, phenylalanine; DHA, docosahexaenoic acid; SS, standard score.

[a] 80 items per task.

[b] Low Phe: <600 μmol/L; High DHA: RBC DHA median by age group, <18 years: ≥ 2.23%; ≥18 years: ≥ 2.40%.

[c] Fisher's Exact Test.

[d] Cognitive processing speed.

[e] Executive function.

[f] Cognitive flexibility: Group 4, n=13.

Table 36. Average number of correct answers on W-J III processing speed tasks by females of reproductive age with PKU stratified by plasma Phe and RBC DHA status

	Low Phe High DHA[a] Group 1, n=12 Mean (SD)	Low Phe Low DHA Group 2, n=7 Mean (SD)	High Phe High DHA Group 3, n=8 Mean (SD)	High Phe Low DHA Group 4, n=14 Mean (SD)	Statistics *F* test[b]	*P* value
W-J Decision Speed[c]	35.0 (4.3)	34.1 (6.7)	35.6 (3.2)	33.9 (5.9)	$F_{2, 38} = 0.3$	.72
W-J Pair Cancellation[d]	64.8 (2.8)	62.1 (7.9)	62.4 (6.4)	58.1 (9.2)	$F_{2, 38} = 3.1$	.06
W-J Reading Fluency[e]	78.3 (17.3)	73.4 (19.3)	70.6 (19.4)	65.1 (18.5)	$F_{2, 38} = 1.7$	.19
W-J Math Fluency[f]	112.8 (27.1)	107.7 (41.4)	111.0 (26.0)	90.2 (32.6)	$F_{2, 38} = 1.8$	.18
≥1 error	No. (%)	No. (%)	No. (%)	No. (%)	Exact test[g]	*P* value
W-J Reading Fluency	4 (33)	1 (14)	4 (50)	5 (36)	2.1	.63

Table 36 continued

Abbreviations: W-J, Woodcock-Johnson; PKU, phenylketonuria; Phe, phenylalanine; DHA, docosahexaenoic acid.

[a] Low Phe: <600 μmol/L; High DHA: RBC DHA median by age group, <18 years: ≥2.23%; ≥18 years: ≥2.40%.

[b] Two-way ANOVA.

[c] Possible number correct: 0-40.

[d] Possible number correct: 0-69.

[e] Possible number correct: 0-98.

[f] Possible number correct: 0-160.

[g] Fisher's Exact Test.

Chapter 7. 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

Title: 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

Authors: S.H.L. Yi[1], J.A. Kable[2], M.L. Evatt[3], R.H. Singh[4].

Institutional affiliations: [1]Emory University, Nutrition & Health Sciences Program of the Graduate Division of Biological & Biomedical Sciences, [2]Emory University, School of Medicine, Department of Pediatrics, [3]Department of Veterans Affairs Medical Center, Atlanta, GA and Emory University School of Medicine, Department of Neurology, [4]Emory University School of Medicine, Department of Human Genetics

Corresponding author:

Rani H. Singh

2165 N. Decatur Road

Decatur, GA 30033

Tel: 404-778-8519

Fax: 404-778-8562

rsingh@emory.edu

Requests for reprints: Rani H. Singh, 2165 N. Decatur Road, Decatur, GA 30033

Summary word count: 328

Text word count: 4727

Tables: 8

Figures: 1

Abstract

The functional implications of low blood docosahexaenoic acid (DHA) in adolescents and adults with phenylketonuria (PKU) are not known. The objective of this study was to investigate the effect of supplemental DHA on cognitive processing speed and executive function, two domains in which individuals treated early for PKU exhibit subtle deficits. In a parallel, randomized, placebo-controlled, double-blind clinical trial, 33 females with PKU ages 12-47 years were randomly assigned to receive either a DHA (10 mg/kg/day) or placebo supplement for 4.5 months. Main outcomes included performance on tasks involving cognitive processing speed, inhibition, and flexibility. Changes in plasma Phe, diet intake, and biomarkers of DHA status; compliance; and adverse events were also assessed. Seventeen participants were randomized to the DHA Group and 16 to the Placebo Group. The intention to treat analysis included 27 participants (DHA Group: n=12; Placebo Group: n=15). The per protocol analysis included 20 participants (DHA Group: n=9; Placebo Group: n=11). At follow up, plasma and RBC biomarkers of DHA were significantly higher in the DHA Group. Assessed performance on cognitive processing speed, inhibition, and flexibility tasks was not different between the two groups at follow up nor was plasma Phe control. The proportion of participants reporting adverse events deemed related to treatment was similar between the two groups. No serious adverse events were reported. In this small pilot study, supplementation with DHA at a dose of 10 mg/kg/day for 4.5 months effectively increased biomarkers of DHA status, and did not show an increase in treatment-related adverse events compared with controls taking placebo. In conclusion, alternative cognitive domains, such as verbal ability, memory, and learning, may be more

responsive to DHA supplementation and should be used in future trials investigating the cognitive effects of DHA. While the implications of low blood DHA in adolescents and adults with PKU still remain to be answered, this study confirms that supplementation with pre-formed DHA appears to be safe and effective in increasing biomarkers of DHA. www.clinicaltrials.gov; Identifier: NCT00892554

Keywords: docosahexaenoic acid; phenylketonuria; phenylalanine; cognitive tests; protein-restricted diet; clinical trial

List of abbreviations

AA	20:4(n-6)	arachidonic acid
ALA	18:3(n-3)	alpha-linolenic acid
DHA	22:6(n-3)	docosahexaenoic acid
DPAn-6	22:5(n-6)	docosapentaenoic acid
EPA	20:5(n-3)	eicosapentaenoic acid
LA	18:2(n-6)	linoleic acid
LC-PUFA		long-chain polyunsaturated fatty acid
MF		medical food
mPKU		maternal PKU
PAH		phenylalanine hydroxylase
Phe		phenylalanine
PKU		phenylketonuria
PUFA		polyunsaturated fatty acid
Tyr		tyrosine

Each author contributed to the planning, conduct, and reporting of the work described in this article.

Rani H. Singh serves as guarantor for the article, accepts full responsibility for the work and/or the conduct of the study, had access to the data, and controlled the decision to publish.

All authors confirm that they have no competing interests for declaration.

The authors confirm independence from the funding sources, and the content of the article has not been influenced by funding sources.

Source(s) of support: This research was supported in part by grants from United to Support Metabolic Disorders (USMD-PKU) in America; and PHS Grant UL1 RR025008 from the Clinical and Translational Science Award program and PHS Grant M01 RR0039 from the General Clinical Research Center program, National Institutes of Health, National Center for Research Resources. Study supplements were provided by Martek Biosciences Corporation. This material is also the result of work supported with resources at the Atlanta VA Medical Center.

Roles of the funding sources:

- United to Support Metabolic Disorders (USMD-PKU) in America had no involvement in the study design; collection, analysis, and interpretation of the data; writing of the report; and the decision to submit the report for publication.
- The Clinical and Translational Science Award and General Clinical Research Center program was involved in the critique of the study design, data collection, and analysis of the data; but, not involved in the original study design, interpretation of the data, writing the report, and the decision to submit the report for publication.
- Martek Biosciences Corporation reviewed this report prior to publication and minor feedback was given. Martek had no involvement in the study design; collection, analysis, and interpretation of the data; writing the report; and the decision to submit the report for publication.
- The Atlanta VA Medical Center had no involvement in the study design; collection, analysis, and interpretation of the data; writing the report; and the decision to submit the report for publication.

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.

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[35-36]. The traditional treatment for PKU consists of a diet restricted in the amino acid phenylalanine (Phe) with the majority of dietary protein equivalents provided by a Phe-free, amino acid-based medical food. Achievement of a diet low in Phe relies on choosing foods 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). The goal of treatment is to control plasma Phe concentrations while maintaining adequate nutrient intake. Currently, lifelong diet treatment is recommended[34] to prevent cognitive, neurological, and psychiatric declines[158, 163, 205-208].

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[165, 211-212, 215]. Debate remains over the existence, nature, etiology, and clinical significance of these deficits, but two recent meta-analyses of studies including children, adolescents, and adults treated early for PKU confirm a significant effect of PKU compared with controls across the aforementioned and other domains[164, 216].

DHA is a major fatty acid in the brain[27, 220] and its presence in the cell membrane affects multiple membrane properties including increased membrane disorder [221], enhanced lateral membrane compressibility[222], and formation and fusion of synaptic

vesicles [223]. 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 [69-72, 229]. Animal studies continue to reveal positive effects of DHA adequacy and supplementation on behavior and cognitive performance [21]; however, human studies have not reached a consensus on the effect of DHA status on cognitive performance in infants, children, adults, or older adults [230].

DHA is a precursor to the bioactive molecules neuroprotectin D1 and resolvins[224, 231]; and, increased brain concentrations of nitric oxide synthetase, dopamine and serotonin, brain-derived neurotrophic factor have been shown in DHA-supplemented animals[104, 225]. DHA appears to regulate neuronal apoptosis[224, 232] and n-3 fatty acids may regulate neurogenesis[233-234] 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.

Individuals treated early in life for PKU show on average lower plasma and RBC percentage of DHA compared with controls without PKU [6-7, 217-218]. Accordingly, it has been proposed that inadequate DHA concentrations in neural lipids may be related to cognitive deficits in people treated early for PKU[119, 218, 226].

Supplementation with preformed DHA in children with PKU significantly improved plasma and red blood cell (RBC) DHA content in children with PKU using doses of 10-15 mg/kg/day DHA for 3-12 months [8-9, 11, 18]. It is hypothesized that high intake of linoleic acid (LA) relative to alpha-linolenic acid (ALA) inhibits endogenous synthesis of DHA from ALA due to competition since arachidonic acid (AA) synthesis from LA necessitates the same desaturase and elongase enzymes[59-61]. Improving the

dietary linoleic:alpha-linoleic acid (LA:ALA) ratio, however, has shown either a small change [62] or no change in blood DHA concentrations [18] in children with PKU. This latter finding is consistent with isotope tracer studies[57] and ALA supplementation trials[63-65] which demonstrate the inefficiency of conversion from ALA to DHA in humans.

In infants and children with PKU, small but significant improvements have been shown in visual function and motor skills with improved blood DHA from supplementation for a period as short as 3 months [8-11]. Studies have not yet 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 performance on tests of cognitive processing speed, inhibition, and flexibility would be higher compared with those supplemented with placebo after 4.5 months of supplementation with 10 mg/kg DHA (www.clinicaltrials.gov; Identifier: NCT00892554).

2. Materials and Methods

2.1 Study participants

Eligible participants were females with PKU and aged at least 12 years. They were not eligible if pregnant, currently taking supplemental DHA, or scored less than 2 standard deviations below average on a standardized verbal ability task.

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)). If necessary, the primary data collector for the study traveled to a location closer to the participant to complete data collection.

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 web-site was created for participants in the study as an additional way to access study information (see Appendix: Methods for examples).

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 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 [8, 18]. 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[9, 11]. DHA was provided in microalgae oil ("DHASCO-S") capsules. Each DHASCO-S capsule contained approximately 200 mg DHA and is described in detail elsewhere [195]. 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 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 diet intake at follow up and compliance 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 [184].

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[185]; therefore, the blood Phe concentrations obtained by LC MS/MS were multiplied by a factor of 1.19. Amino acid concentrations are reported as μmol/L. These values can be converted to mg/dL (commonly referred to as "mg%") by dividing by 60.54[186].

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 and colleagues[187]. Plasma and RBC DHA content are presented as percent of total lipids; total lipid fatty acids are presented as μg/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, protein, medical food protein, fat, carbohydrates, Phe, and Tyr; the n-6 PUFAs LA and AA; the n-3 PUFAs ALA, EPA, and DHA were estimated.

Baseline 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. 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 [124, 126-127, 181].

2.4.4.1 Verbal Ability

The Peabody Picture Vocabulary Test—Third Edition, Form B (PPVT-IIIB) 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[235]. 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[183]. 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 versus inhibition and/or cognitive flexibility.

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.

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 and research assistants recorded participant finishing times. 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 twenty completed individually. Mean scores on these tasks did not differ between those who completed the tasks in the group setting versus individually.

2.4.4.3.1 Decision Speed

The Decision Speed task relies on the ability to connect a concept with a name. 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.

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.

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).

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.

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.

2.4.4.3.7 Processing Speed Score

The six processing speed scores were reduced 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 $\beta = .80$ and $\alpha = .05$, 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 [201-202]. 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 (Yi et al 2010, unpublished manuscript), in order to see a significant change ($\beta = .80$, $\alpha = .05$) 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[200]. A two-tailed P value $\leq .05$ 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 energy, macronutrient, amino acid, and fatty acid intakes; 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[236].

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

Biomarkers of DHA status were compared to evaluate the effectiveness of DHA supplementation.

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.

Changes in plasma Phe concentration and diet intake were assessed in order to evaluate potential confounding effects.

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 & protocol deviations

Figure 6 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 37, 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.

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 (Table 39, Table 40).

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 (plasma DHA, intention to treat (n=24): 3.05 (0.93)% vs. 1.06 (0.88)%, $P < .001$; per protocol (n=19): 3.33 (0.70)% vs. 0.99 (0.65)%, $P < .001$; RBC DHA, intention to treat (n=24): 5.49 (1.72)% vs. 2.44 (1.57)%, $P < .001$; per protocol (n=19): 5.98 (1.35)% vs. 2.47 (1.26)%, $P < .001$). The DHA Group and Placebo Group had similar proportions of compliance to allocated treatment (Table 42). Mean plasma Phe at follow up did not differ between groups after controlling for baseline concentrations (intention to treat (n=27): 861 (608) µmol/L vs. 895 (540) µmol/L, $P = .837$; per protocol (n=20): 770 (492) µmol/L vs. 856 (443) µmol/L, $P = .575$).

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 43). The categories of reported adverse events deemed to be related to

treatment also did not differ by treatment group (Table 44). No serious adverse event was reported.

4. Discussion

This 4.5 month pilot study failed to find an effect of DHA supplementation on measures of cognitive processing speed, inhibition, and flexibility in females of reproductive age with PKU. 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.

One possible reason for the lack of cognitive effects seen in this study is that the domains tested, namely 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 [91], lactating women [99], and older adults [102, 237]. 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[97-98].

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 four months of supplementation compared with those receiving placebo capsules (n=10)[237]. 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. In a cross-sectional study performed at baseline in the present study, verbal ability was significantly correlated with

RBC DHA content after accounting for plasma Phe concentrations (Yi et al 2010, unpublished manuscript). These findings suggest that measures of verbal fluency, memory, and learning may be affected by DHA to a greater extent than cognitive processing speed, inhibition, and flexibility.

Alternatively, known and unknown methodological issues in this study could be contributing to the lack of effect. In the trial of lactating women, for example, the sample size was only 27 and the dosage was 200 mg DHA during the first four months after delivery. Small improved performance was seen on the Color Naming (cognitive processing speed) and Interference (cognitive inhibition) conditions of the Color Word Interference Test in the supplemented group; however, the differences were not statistically significant[99]. A larger sample size and higher dose of supplemental DHA may have improved the power to detect an effect of DHA on measures of cognitive processing speed and inhibition.

The dosage of DHA used in this and other recent studies assessing cognitive processing speed and/or executive function range between approximately 200 mg and 1000 mg per day. While this dosage may produce changes in other domains, it may be inadequate for affecting measures of cognitive processing speed and executive function.

Correspondingly, there may be a threshold for neural cell membrane DHA composition after 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[238]. Building upon this proposal, McNamara 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[239]. 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 achieved in this study.

The small sample size in the present study may have limited the power to assess 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 (see Table 57). Recruitment was conducted through multiple avenues, and participants from other states were included. Because the incidence of PKU is relatively uncommon[34], 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 results.

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

5. Conclusions

This is the first report of a trial conducted with adolescents and adults with PKU assessing the impact of supplemental DHA on measures of cognitive processing speed and executive function, two domains commonly affected in people with PKU even with

early and lifelong treatment. This study suggests a larger sample size is required to investigate potential associations between biomarkers of DHA status and the domains of cognitive processing speed, inhibition, and flexibility. 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[240-241], 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[82, 84].

Acknowledgements

We thank the participants and their families, clinicians, camp staff, EUH Investigational Drug Service, and ACTSI staff for their involvement and support. We also thank Ngoc Anh Le, PhD; Usha Ramakrishnan, PhD; Kevin M. Sullivan, PhD; and Thomas R. Ziegler, MD for helpful feedback and suggestions. This research was supported in part by grants from United to Support Metabolic Disorders (USMD-PKU) in America; and PHS Grant UL1 RR025008 from the Clinical and Translational Science Award program and PHS Grant M01 RR0039 from the General Clinical Research Center program, National Institutes of Health, National Center for Research Resources. Study supplements were provided by Martek Biosciences Corporation. This material is also the result of work supported with resources at the Atlanta VA Medical Center; Dr. Evatt receives salary support from the Atlanta VA Medical Center.

Tables

Table 37. 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), y		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.

[a] Based on annual poverty thresholds calculated by the US Census Bureau (DHA Group, n=15; Placebo Group, n=14).

[b] Participants self-identified race/ethnicity as white/Caucasian, black/African American, or Native American.

[c] US Census regions; other participants from Northeast, West, or Midwest.

Table 38. Baseline health characteristics and cognitive performance of all of females with PKU randomized to receive DHA or Placebo supplement

Baseline characteristics	DHA Group n=17	Placebo Group n=16
Phe prescription, mean (SD), mg/d	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/d[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/d[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/d [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/d [b]	4955 (1535)	5435 (1356)
Dietary folate, mean (SD), µg/d [b]	732 (217)	830 (341)
Dietary iron, mean (SD), mg/d [b]	24.4 (9.6)	27.7 (8.9)
Dietary zinc, mean (SD), mg/d [b]	19.6 (8.9)	23.3 (9.4)
Dietary vitamin B12, mean (SD), µg/d [b]	5.6 (2.3)	7.7 (3.6)
Dietary vitamin B6, mean (SD), mg/d [b]	3.0 (1.3)	3.2 (1.4)
BMI, mean (SD), kg/m^2	25.8 (6.6)	30.4 (9.4)

Table 38 continued

Baseline characteristics	DHA Group n=17	Placebo Group n=16
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 & RBC TLFA, n=15.

[c] Smoker defined as smoking cigarettes at least once per week.

Table 39. 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 n=12 Mean (SD)[c]	Placebo Group n=15 Mean (SD)	ANCOVA[a,b] Difference	95% CI	*P* value
Cognitive processing speed, factor score	98.8 (5.3)	101.0 (5.4)	-2.23	-6.5, 2.0	.29
Cognitive inhibition, SS	11.3 (1.5)	11.4 (1.5)	-0.05	-1.3, 1.2	.93
Cognitive flexibility, SS[d]	11.1 (1.4)	10.8 (1.4)	0.28	-0.9, 1.5	.63

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

[a] ANCOVA model: follow up score = $\beta 0 + \beta 1$(baseline score) + $\beta 2$(treatment group) + error.

[b] Statistics are presented for $\beta 2$(treatment group).

[c] Mean (SD) adjusted for baseline score.

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

Table 40. The effect of DHA supplementation on mean follow up cognitive test scores controlling for baseline score in females with PKU: per protocol analysis

	DHA Group n=9 Mean (SD)[c]	Placebo Group n=11 Mean (SD)	ANCOVA[a,b] Difference	95% CI	*P* value
Cognitive processing speed, factor score	101.7 (5.1)	101.6 (5.1)	0.14	-4.7, 5.0	.95
Cognitive inhibition, SS	11.7 (1.5)	11.4 (1.5)	0.25	-1.2, 1.7	.72
Cognitive flexibility, SS[d]	11.6 (1.3)	11.1 (1.3)	0.49	-0.9, 1.9	.45

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

[a] ANCOVA model: follow up score = $\beta 0 + \beta 1$(baseline score) + $\beta 2$(treatment group) + error.

[b] Statistics are presented for $\beta 2$(treatment group).

[c] Mean and standard deviation adjusted for baseline score.

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

Table 41. Estimates of participant compliance to treatment allocation in females with PKU: intention-to-treat analysis

	DHA Group n=11 No. (%)	Placebo Group n=14 No. (%)	Fisher's Exact Test *P* value
Self-reported capsules taken[a]	8 (67)	10 (67)	1.0
Plasma DHA[b]	9 (82)	13 (100)	.20
RBC DHA[c]	10 (91)	13 (100)	.46

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

[c] Placebo Group: Plasma DHA, RBC DHA, n=13.

[d] Expected change in RBC DHA: ≥1.3% in DHA Group and <1.3% in Placebo Group.

Table 42. Estimates of participant compliance to treatment allocation in females with PKU: per protocol analysis

	DHA Group n=9 No. (%)	Placebo Group n=11 No. (%)	Fisher's Exact Test *P* value
Self-reported capsules taken[a]	8 (89)	10 (91)	1.0
Plasma DHA[b]	8 (89)	10 (100)	.47
RBC DHA[c]	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 change in plasma DHA: ≥1.2% in DHA Group and <1.2% in Placebo Group.

[c] Placebo Group: Plasma DHA, RBC DHA, n=10.

[d] Expected change in RBC DHA: ≥1.3% in DHA Group and <1.3% in Placebo Group.

Table 43. Summary of reported adverse events by intention-to-treat group in females with PKU

Adverse Event[a]	DHA Group n=17 No. (%)	Placebo Group n=15[b] No. (%)	Fisher's Exact Test *P* value
1 or more adverse events	14 (82)	15 (100)	.23
Angioedema	0 (0)	1 (7)	.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)	.09
Other[c]	13 (76)	14 (93)	.34

Abbreviation: 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 44. Adverse events judged possibly, probably, or definitely associated with supplement in females with PKU

Adverse Event[a,b]	DHA Group n=17 No. (%)	Placebo Group n=15[b] No. (%)	Fisher's Exact Test *P* value
Angioedema	0 (0)	1 (7)	.47
Diarrhea	2 (12)	2 (13)	1.0
Fishy eructation	1 (6)	0 (0)	1.0
Nausea	1 (6)	3 (20)	.32
Vomiting	0 (0)	1 (7)	.47

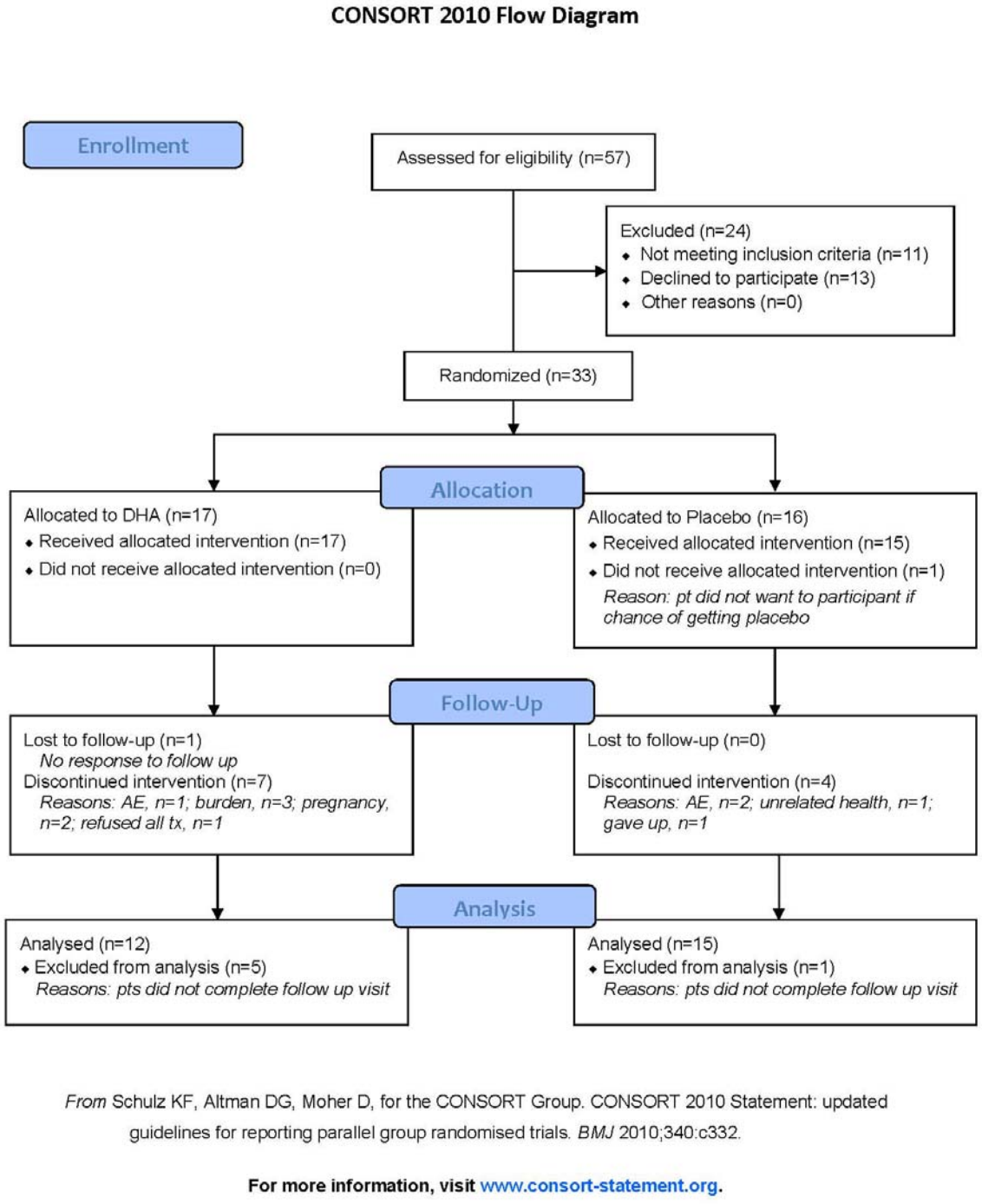
Abbreviation: PKU, phenylketonuria; DHA, docosahexaenoic acid.

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

[b] Association determined by investigators.

Captions to illustrations

Figure 6. 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



From reference [242].

Chapter 8. Conclusions

These studies demonstrated that multiple diet-related factors are related to blood Phe control, and that blood Phe control may not be the sole diet-related predictor of cognitive performance.

Study one demonstrated improved plasma Phe and cognitive processing speed in females of reproductive age at the end of a metabolic camp. Second, improved performance appeared to exceed a practice effect in both measures when compared with the test-retest controls; however, a larger sample size is needed to reach statistical significance. Third, changes in Pair Cancellation scores corresponded with changes in plasma Phe after controlling for verbal ability.

The results of study 2 confirmed that individuals with PKU have lower proportions of DHA in plasma and RBC total lipids compared with controls without PKU. Second, dietary intake of pre-formed DHA was close to nil for individuals following a Phe-restricted diet. Third, dietary LA/ALA ratio was unrelated to RBC DHA level. Fourth, the cognitive effects of DHA may be domain-specific, with specific effects related to verbal ability but not executive function or processing speed.

Study 3 did not show a difference between the DHA and placebo groups in performance on tasks of cognitive processing speed, inhibition, or flexibility. Either the study was underpowered to detect an effect of DHA supplementation or these domains are not specifically affected by DHA. Statistically and clinically significant improved plasma and red blood cell concentrations of DHA were seen in those supplemented with DHA, which confirms previous investigations. Importantly, there was no difference in

reported adverse events between the two groups, which is also consistent with other studies.

Clinical implications

The findings of Study 1 confirm the positive relationship between improved plasma Phe and processing speed, however, these improvements are likely to be temporary, lasting as long as participants maintain adherence to their diet Phe and medical food prescriptions. Adherence to a restrictive diet is difficult to maintain, particularly in adolescence and adulthood. Although difficult, it is essential that adolescent and adult women with PKU maintain plasma Phe concentrations within treatment range to prevent the detrimental effects of maternal PKU on a developing fetus in the event of a planned or unplanned pregnancy.

Communicating to women the cognitive benefits of improved Phe control over a short time period, as observed during Metabolic Camp, may help improve perceived benefits of diet adherence in women with PKU and in turn improve motivation to adhere to the diet. Alternatively, more frequent interventions may be warranted in this population.

Study 2 suggests that improved DHA status in addition to adequate Phe control may benefit aspects of cognitive performance, specifically verbal ability. While the findings of this study are observational and deserve further investigation, the findings suggest diet adequacy and quality beyond Phe control may be beneficial.

Study 3 affirms that more research is needed before pre-formed DHA supplements should be recommended for the specific purpose of improving or preserving cognition. However, this does not preclude the recommendation to supplement with

preformed DHA for the purposes of improving biomarkers of DHA status and achieving dietary recommendations. The current DRI for alpha-linolenic acid allows up to 10% of the intake from n-3 LC-PUFAs[39] (e.g., 0.06%-0.12% total energy or 133-267 mg EPA + DHA in a 2000 kcalorie diet). Several national and international organizations[240-241] recommend consumption of two servings of fish per week or 500 mg EPA + DHA per day for the primary prevention of cardiovascular disease. In addition, some experts recommend a minimum maternal intake of 2-300 mg pre-formed DHA per day during pregnancy and lactation[82, 84]. Alternatively, the American Dietetic Association suggests that vegetarians decrease the n-6:n-3 ratio by increasing intake of omega-3 rich oils to improve ALA to DHA conversion; with the caveat that pregnant and lactating vegetarian women may still benefit from pre-formed DHA[243].

Suggestions for future research

Communication of the findings of Study 1 to patients, that blood Phe concentrations and processing speed can be improved in a short period, may help motivate adherence to diet therapy. Future research should investigate if sharing these and other related research results with campers as part of the camp intervention will improve long-term compliance.

If possible, future studies investigating the short-term changes in cognitive performance associated with a metabolic camp should include a control group. The test-retest reliability sample used in Study 1 was useful estimating the magnitude of improved performance on the processing speed tasks without an intervention. A more ideal comparison, though, would have been a test-retest comparison group with PKU with a similar elapsed time to the time elapsed in the camp study (i.e., 4 days instead of 1 day).

At the very least, the control group could have the plasma Phe concentrations and cognitive performance measured according to the same schedule. Incorporating a social component, even if not a camp, would be helpful in reducing the potentially confounding influence of social interactions and support on cognitive performance.

The finding that although performance on the Decision Speed and Pair Cancellation tasks both significantly improved at the end of camp, only Pair Cancellation changes were related to changes in plasma Phe concentration was interesting. Realizing the different properties of the two tasks helped to explain the differing results: that a speeded task dependent on attention and concentration would be responsive to changes in plasma Phe, but a speeded task dependent on semantic processing skills would not. This finding raises the question of whether it is processing speed that is affected by plasma Phe control or other qualities present in many speeded tasks (i.e., attention and concentration) are affected by plasma Phe control.

Study 2 had a number of strengths that should be retained or built upon in future studies. For this population, there was a relatively large sample size and the age groups were balanced between teens (12-17 years) and adults (18-47 years). Several cognitive domains affected in early treated PKU were assessed using standardized cognitive tests. And, nutrient intake was assessed alongside biomarkers and cognitive performance.

The limitations to Study 2 reveal suggestions for future research. As a small observational study, confounding factors, such as maternal education, are difficult to control for when evaluating the relationship between cognitive performance, DHA status, and Phe status. In addition, as a cross-sectional study, it is impossible to know the directionality of relationship; do people with better plasma Phe control and DHA status

because they have higher cognitive performance on a verbal ability task or do the improved Phe and DHA improve cognitive performance? A randomized-controlled trial would help to balance potential confounding factors between the participant groups and will allow investigators to investigate directionality of the relationship. Specifically, a randomized controlled trial should be conducted to confirm the relationship between DHA status and verbal ability observed in the cross-sectional study. The trial should utilize tasks designed to assess verbal ability, learning, and memory since these domains have shown the strongest link with DHA status in animal and human studies.

The major strength of Study 3 was that it was a randomized, placebo-controlled, double-blinded trial. This allowed us to investigate the effect of supplementation of DHA on cognitive performance while balancing potential confounding factors between the DHA and Placebo supplemented groups. Of the 33 participants, 13 withdrew from the study.

While a certain percentage of study participants is expected to withdraw from any study, and their right to do so should be respected, it is essential for investigators to ensure that study participation places minimal burden to the participant. Several participants who withdrew from the study cited the burden of taking capsules every day as their primary reason for withdrawing from the study. If the trial involves participants consuming medical food, investigators should consider adding DHA to the medical food instead of capsules to ease participant burden and potentially improve participant retention. Clearly what constitutes a burden is subjective; several participants did not think that taking the supplements was a burden when asked.

Recruitment of an adequate sample size was challenging, and participants from other states and clinics who enrolled in the study were critical in getting close to the goal sample size. Future studies should consider a multi-center approach to allow for larger sample sizes.

Another limitation of all of these studies is that they are not generalizable to males. While it is a high priority to ensure that females of reproductive age with PKU are following their diet prescriptions and practicing health behaviors to allow for optimal fetal outcomes in the event of a pregnancy, males treated early for PKU in this age group also show cognitive deficits. Ideally, a future trial should include both males and females in order to provide more generalizable information to the PKU community.

In these studies, a wide age range was included (i.e., 12 to 47 years). Narrowing the age range of participants included in the study and/or having adequate sample sizes to allow for stratification of analysis by age is also important since there may be specific differences in response to any intervention by age group. Adolescents, for example, may be accumulating DHA in the brain at a higher rate than adults. If DHA supplementation does affect certain cognitive domains, there may be differences in magnitude of response to supplementation by age group.

Finally, we plan to expand upon the work of the preliminary studies. Specifically, we plan to investigate with a larger sample size whether there is no difference in essential fatty acid status between consumers of medical foods with fat vs. without fat. In this analysis we will aim to confirm the finding that consumers of medical foods without fat do not compensate for the missing fat.

Chapter 9. Appendix: Background

Table 45. Summary of reports of blood DHA status in people with PKU

Author	Year	n	Location	Age (y)	Phe (µmol/L)	DHA: units, blood fraction	Result	Controls	*P*
LaVoie[227]	2009	20	US (OR)	0.75-7		µmol/L RBC TLFAs	58.0±3.7[a]	68.6±4.2	.070
Fiori[244]	2006	43	Milan, Italy	14±2	on diet	% Plasma TL EPA+DHA	0.6±0.3[b]	1.6±0.5	<.01
		16		13±1.9	off diet	% Plasma TL EPA+DHA	0.8±0.2		
Moseley[6]	2002	27	US (CA)	10-50	831.2±366.1	% Plasma TLFAs	1.14±0.38[b]	1.33±0.48	<.001
						% RBC TLFAs	2.91±0.92	3.46±1.05	.013
Acosta[48]	2001	13	US locations	3.0±0.4	Phenex group	% Plasma TLFAs	1.65±0.11[a]	2.10±0.17	ns
						% RBC TLFAs	0.77±0.06	0.96±0.10	ns
		6		5.0±1.0	Phenylfree group	% Plasma TLFAs	1.80±0.14	1.97±0.29	ns
						% RBC TLFAs	0.85±0.13	0.97±0.22	ns
		7		5.8±1.3	XPhe Max group	% Plasma TLFAs	1.62±0.14	2.36±0.28	ns
						% RBC TLFAs	0.72±0.07	1.23±0.24	<.05
van Gool[7]	2000	9	Netherlands	0.5-25	200-500	% Plasma PLs	1.41±0.11[a]	2.74±0.17	.0002
						% RBC PLs	1.26±0.01	2.53±0.13	.0001
Pöge[47]	1998	8	Germany	1-6	272 (½-yr med)	% Plasma PLs	1.72 (0.92-2.42)[c]	2.15 (0.87-4.19)	ns
						% Plasma CE	0.25 (0.14-0.41)	0.54 (0.16-0.68)	<.05
						% RBC PC	1.02 (0.43-1.14)	1.25 (0.70-1.48)	<.05

Table 45 continued

Author	Year	n	Location	Age (y)	Phe (µmol/L)	DHA: units, blood fraction	Result	Controls	*P*
						% RBC PE	4.21 (1.76-5.31)	5.85 (4.80-6.68)	<.01
		9		11-16	714 (½-yr med)	% Plasma PLs	2.13 (0.79-3.97)	2.38 (1.59-3.11)	ns
						% Plasma CE	0.34 (0.12-0.74)	0.51 (0.29-0.62)	ns
						% RBC PC	1.22 (0.41-2.24)	1.80 (1.16-2.02)	ns
						% RBC PE	5.35 (3.73-8.03)	6.28 (5.89-7.98)	ns
Cockburn[218]	1996	16	Scotland	10-16	on treatment	% RBC PLs	3.08+0.87[b]	4.92±0.82	<.0001
						% RBC PE	4.46±1.59	6.03±1.47	.013
Agostoni[18]	1995	10	Milan, Italy	5-10: BLFO	324±75 (1y ave)	% Plasma TLFAs	0.65±0.10[b]	1.78±0.52	<.05
		11		5-10: BLBCO	284±139 (1y ave)	% Plasma TLFAs	0.66±0.12	1.78±0.52	<.05
Galli[217]	1991	15	Milan, Italy	3-12	380±76	% Plasma TLFAs	0.51±0.04[a]	1.01±0.08	<.01
						% Plasma PLs	0.50±0.02	1.21±0.29	<.02
						% RBC TLFAs	0.99±0.14	1.77±0.49	ns

Table 45 continued

Abbreviations: Phe, phenylalanine; DHA, docosahexaenoic acid; OR, Oregon; RBC, red blood cell; TLFA, total lipid fatty acids; TL, total lipid; EPA, eicosapentaenoic acid; CA, California; XPhe Max, XPhe Maxamaid or XPhe Maxamum; PL, phospholipid; CE, cholesterol ester; PC, phosphatidylcholine; PE, phosphatidylethanolamine; BLFO, baseline fish oil group; BLBCO, baseline blackcurrant seed oil group.

[a] Mean ± SEM.

[b] Mean ± SD.

[c] Median (min-max).

Table 46. Fat source(s) and select nutrients in medical foods containing fat available in June 2007

Medical Food[a]	Phenylfree 2	Periflex Advance	XPhe Maxamum Drink Box	Phenylfree 2 HP	Phenex-2	PhenylAde Amino Acid Bar	Phenylade	Add-Ins	Phlexy-10 Bar
Company	Mead Johnson	Nutricia	Nutricia	Mead Johnson	Abbott Nutrition	Applied Nutrition	Applied Nutrition	Nutricia	Nutricia
Canola oil		✓	✓						
Soybean oil	✓		✓	✓	✓				✓H
Coconut oil		✓FR			✓	✓[b]	✓PH		
Safflower oil		✓HO			✓HO				
Sunflower oil			✓HO						
Palm oil								✓H	✓H
Peanut oil									✓
Cocoa butter						✓			
Flaxseed oil									
Fish oil									
Other[c]		✓	✓		✓	✓	✓		✓
Energy (kcal)	280	165	160	146	205	405	240	129	268

Table 46 continued

Medical Food[a]	Phenylfree 2	Periflex Advance	XPhe Maxamum Drink Box	Phenylfree 2 HP	Phenex-2	PhenylAde Amino Acid Bar	Phenylade	Add-Ins	Phlexy-10 Bar
Company	Mead Johnson	Nutricia	Nutricia	Mead Johnson	Abbott Nutrition	Applied Nutrition	Applied Nutrition	Nutricia	Nutricia
Protein eq (g)	15	15	15	15	15	15	15	15	15
Fat (g)	5.9	5.4	5	2.4	7	26-27	7.8	7.6	6.7
LA (g)	3.178	0.879	0.710	1.205	1.100	0.375	0.228	0.355	1.152
ALA (g)	0.420	0.240	0.210	0.161	0.113	0.105	0.036	0.035	0.018
EPA (g)									
DHA (g)									

Abbreviations: LA, linoleic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; Protein eq, protein equivalent; H, hydrogenated; FR, fractionated; PH, partially hydrogenated; HO, high oleic.

[a] Medical foods containing >1g fat/15 g protein eq for older children and adults with PKU in the US.

[b] In 2 of 3 flavors.

[c] Other: other sources of fatty acids including additives (e.g., mono and diglycerides and emulsifiers).

Table 47. Fat source(s) and select nutrients in medical foods containing fat in new products available as of January 2010

Medical Food[a]	PhenylAde Essential	PKUCoolers
Company	Applied Nutrition	Vitaflo USA
Canola oil	✓	
Soybean oil	✓	
Coconut oil	✓	
Safflower oil	✓	
Sunflower oil		
Palm oil		
Peanut oil		
Cocoa butter		
Flaxseed oil	✓	
Fish oil		✓
Other[b]		✓
Energy (kcal)	234	92
Protein eq (g)	15	15
Fat (g)	7.5	0.5
LA (g)	1.215	0.005
ALA (g)	0.315	0.003
EPA (g)		0.023
DHA (g)		0.100

Abbreviations: LA, linoleic acid; ALA, alpha-linolenic acid; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; Protein eq, protein equivalent.

[a] Medical foods containing >1g fat/15 g protein eq for older children and adults with PKU in the US.

[b] Other: other sources of fatty acids including additives (e.g., mono and diglycerides and emulsifiers).

Chapter 10. Appendix: Methods

PKU & DHA Study

GCRC Day-To-Day Protocol

Outpatient Procedure

GCRC Study #: 7014 / **Title** The impact of docosahexaenoic acid status and phenylalanine control on neuropsychological status in females with phenylketonuria

Principal Investigator Rani H. Singh, PhD, RD, LD; PIC #14097

Co-Investigator/Coordinator Sarah Yi, MS, RD, LD; phone: 404-778-1286

Co-Investigator/Physician Marian L. Evatt, MD, MSCR; pager: 404-778-3801 ID#15496

Co-Investigator/Psychologist Julie A. Kable, PhD

Participant Name _____ **Study Visit: ___Baseline**

Medical Record # _____ (please check) **___Follow up**

Diagnosis Phenylketonuria

Admission Date _____

Appointment Time _____

Day: _____, **Date:** _____, **___:___ AM/PM – ___:___ AM/PM**

_____ 1. NPO after midnight except water.

_____ 2. Notify **Sarah Yi at 404-778-1286** of patient's arrival.

_____ 3. On-call: 1. _____ (***PIC #*** _____); 2. _____ (***PIC #*** _____).

_____ 4. Informed consent will be signed upon admission unless signed previously.

_____ 5. Admit to GCRC.

_____ 6. Obtain Vital signs: blood pressure _ _ _ / _ _ _ mmHg, heart rate _ _ _ bpm, temperature _ _ _ . _°F, respiratory rate __ breaths per min, pain __ (0-10, low-high).

_____ 7. Obtain Weight _ _ _ . _ _ (kg) Height _ _ _ . _ _ (cm) **(GCRC Nursing Staff)**.

_____ 8. Complete 1-page questionnaire before blood draw **(Coordinator/Nursing)**.

_____ 9. Blood Draw (draw in the following order):

9a. Drip from syringe 5 spots of blood on filter paper and allow to dry **(Nursing/ Phlebotomist)**.

9b. Draw 10 mL venous blood (fasting) into a red top tube **(Nursing/Phlebotomist)**. Protect specimen from light. Send to **Core Lab** for processing.

9c. Draw 10 mL venous blood (fasting) into a sodium heparin green top tube **(Nursing/Phlebotomist)**. Send to **Core Lab** for processing.

9d. Draw 8 mL venous blood (fasting) into an EDTA lavender top tube **(Nursing/Phlebotomist)**. Samples are to be kept at room temperature and given to **Coordinator**.

_____ 10. Low phenylalanine snack to be provided following blood draw **(Bionutrition)**.

_____ 11. H & P to be done by _____ (***PIC #*** _____). *Optional after Visit 1.*

_____ Brief neurological testing, if not conducted by physician **(Trained tester)**.

_____ 13. Neuropsychological testing to be done in a quiet room or cubicle with accordion partition after snack **(Psychologist/trained tester)**.

_____ 14. Collect and review for accuracy 3-day food records **(Dietitian/Bionutrition)**.

_____ 15. Complete additional questionnaires **(Coordinator).**

_____ 16. Review study plan and study materials with participant **(Coordinator)**.

_____ 17. The patient may be discharged after data collection has been completed **(Nursing)**.

Physician's Signature: _____ PIC #: _____ Date: _____

Variance and Actions:	Nurse Signature	Initials
_____	_____	_____
_____	_____	_____

Inpatient Procedure

PKU & DHA Study / GCRC Day-To-Day Protocol / Inpatient Procedure / Page 1 of 2

GCRC Study #: 7014 / **Title:** The impact of docosahexaenoic acid status and phenylalanine control on neuropsychological status in females with phenylketonuria

Participant Name _____ **Admission Date** _____ **Study Visit: ___1 ___2**

Medical Record # _____ **Appointment Time** _____ (please check)

Diagnosis Phenylketonuria_____

Day 0: _____, **Date:** _____, **___:___ PM – ___:___ PM**

_____ 1. Notify **Sarah Yi at 404-778-1286** of patient's arrival (leave voicemail).

_____ 2. Informed consent will be signed upon admission unless signed previously.

_____ 3. Admit to GCRC.

_____ 4. Obtain Vital signs: blood pressure __ __ __ / __ __ __ mmHg, heart rate __ __ __ bpm, temperature __ __ __ . __ °F, respiratory rate _____ breaths per minute, pain ____ (0-10, low-high).

_____ 5. Obtain Weight __ __ __ . __ __ (kg) Height __ __ __ . __ __ (cm) **(GCRC Nursing Staff)**.

_____ 6. H & P to be done by _____ (***PIC #_____***) (Day 0 or Day 1).

_____ 7. Brief neurological testing, if not conducted by physician **(Trained tester).**

_____ 8. Neuropsychological Testing to be done in a quiet room after snack **(Psychologist or trained tester)**.

_____ 9. Collect and review for accuracy 3-day food records **(Dietitian or GCRC Bionutrition)**.

_____ 10. Complete additional questionnaires **(Coordinator).**

_____ 11. Review study materials with participant **(Coordinator)**.

_____ 12. Low-phenylalanine dinner to be provided at 6 PM. Water provided at meals and as needed.

_____ 13. Low phenylalanine snacks to be provided at 3 PM and 9 PM.

_____ 14. On-call: 1. Dr. Singh PIC #14097, 2. Dr. Evatt pager 404-778-3801 ID#15496.

_____ 15. NPO after midnight except water.

Physician's Signature: _____ PIC #: _____ Date: _____

Variance and Actions:	Nurse Signature	Initials
_____	_____	_____
_____	_____	_____
_____	_____	_____

PKU & DHA Study / GCRC Day-To-Day Protocol / Inpatient Procedure: Inpatient Procedure / Page 2 of 2

GCRC Study #: 7014 / **Title:** The impact of docosahexaenoic acid status and phenylalanine control on neuropsychological status in females with phenylketonuria

Participant Name _____ **Admission Date** _____ **Study Visit: ___1 ___2**

Medical Record # _____ **Appointment Time** _____ (please check)

Diagnosis Phenylketonuria_____

Day 1: _____, **Date:** _____, **___:___ AM – ___:___ AM**

_____ 1. NPO after midnight except water.

_____ 2. Obtain Vital signs: blood pressure __ __ __ / __ __ __ mmHg, heart rate __ __ __ bpm, temperature __ __ __ . __ °F, respiratory rate _____ breaths per minute, pain ____ (0-10, low-high).

_____ 3. Obtain Weight __ __ __ . __ __ (kg) Height __ __ __ . __ __ (cm) **(GCRC Nursing Staff)**.

_____ 4. Complete 1-page questionnaire before blood draw **(Coordinator/GCRC Nursing Staff)**.

_____ 5. Blood Draw (draw in the following order) **(GCRC Nursing Staff/Phlebotomist)**:

5a. Drip from syringe 5 spots of blood on filter paper and allow to dry.

5b. Draw 10 mL venous blood (fasting) into a red top tube. Wrap in foil to protect from light.

5c. Draw 10 mL venous blood (fasting) into a sodium heparin green top tube.

5d. Draw 10 mL venous blood (fasting) into an EDTA lavender top tube.

_____ 6. Send all blood draw tubes to **GCRC Core Lab** for processing.

_____ 7. Low phenylalanine breakfast to be provided following blood draw **(GCRC Bionutrition Staff)**.

_____ 8. Complete any remaining tasks from Day 0.

_____ 9. The patient may be discharged after data collection has been completed.

Physician's Signature: _____ PIC #: _____ Date: _____

Variance and Actions:	Nurse Signature	Initials
_____	_____	_____
_____	_____	_____
_____	_____	_____

PKU & DHA Study, GCRC #7014

GCRC Core Lab

Blood processing instructions

Study title: The impact of docosahexaenoic acid status and phenylalanine control on neuropsychological status in females with phenylketonuria

Principal Investigator: Rani H. Singh, PhD, RD, LD; PIC #14097

Investigator/Coordinator: Sarah Yi, MS, RD, LD; phone: 404-778-1286

Bar code label #1	Bar code label #2

Red top tube (10 mL)

- ☐ Protect specimen from light by wrapping in foil.
- ☐ Allow blood to clot for 30 minutes from time of collection, upright at room temperature.
- ☐ Spin sample to separate serum (3000 rpm x 10 minutes at 4^{o}C).
- ☐ Aliquot serum into 4 cryovials each containing 1-2 mL of serum.
- ☐ Store cryovials frozen at -80°C in the GCRC Core Lab.

Green top tube (sodium heparin) (10 mL)

- ☐ Spin sample to separate plasma (3000 rpm x 10 minutes at 4^{o}C).
- ☐ Aliquot 0.5-1 mL plasma into a vial for lipid profile.

Blood processing instructions, continued

- Store vial frozen in specimen box at GCRC Core Lab for Cardiovascular Specialties Lab (Dr. Anh Le).

☐ Aliquot 1 mL plasma into a vial for amino acid profile.

- Store vial in the refrigerator at GCRC Core Lab.
- Study coordinator will pick up on the same day.

☐ Aliquot remaining plasma into 2 cryovials each containing 1-2 mL plasma

- Store vials frozen in specimen box at -80°C in the GCRC Core Lab.

Lavender top tube (EDTA) (10 mL)

☐ Pour 2-3 mL whole blood into storage tube with strong cap.

- Store tube at room temperature.
- Study coordinator will pick up on the same day.

☐ Spin the remaining sample to separate plasma (3000 rpm x 10 minutes at 4^{o}C).

- Aliquot plasma into 2 cryovial(s) each containing 1-2 mL plasma.
- Cryovial(s) should be stored frozen at -80°C in the GCRC Core Lab.

PKU & DHA Study

Off Site Procedure

Vital Signs

Participant Name _____

Collection Date _____

Collection Location _____

Please initial completed tasks:

_____ 1. Obtain vital signs Time: **___:___ AM/PM**

1a. Blood pressure __ __ __ / __ __ __ mm Hg

1b. Heart rate __ __ __ beats per minute

1c. Temperature __ __ __ . __ °F

1d. Respiratory rate _____ breaths per minute

1e. Pain ____ (0-10, low-high)

_____ 2. Obtain height and weight: Time: **___:___ AM/PM**

2a. Weight __ __ __ . __ __ (kg) *(if different from above)*

2b. Height __ __ __ . __ __ (cm)

Notes:	Signature	Initials

PKU & DHA Study

Off Site Procedure

Blood Draw

Participant Name _____

Collection Date _____

Collection Location _____

Please initial completed tasks:

_____ 1. NPO after midnight except water.

_____ 2. Complete 1-page questionnaire before blood draw.

_____ 3. Blood Draw (draw in the following order): Time: **___:___ AM/PM**

3a. Drip from syringe 5 spots of blood on filter paper and allow to dry.

3b. Draw 10 mL venous blood (fasting) into SST tube. Protect specimen from light by wrapping in foil.

3c. Draw 2x4 or 1x10 mL whole venous blood (fasting) into a sodium heparin (green top) tube. Samples are to be kept at room temperature.

3d. Draw 2x4 mL venous blood (fasting) into an EDTA (lavender top) tube. Samples are to be kept at room temperature.

PKU & DHA Study

Off Site Procedure

Blood Processing & Shipment

Participant Name _____

Collection Date _____

Collection Location _____

SST tube (tiger top) (10 mL)

- ☐ Protect specimen from light by wrapping in **foil**.
- ☐ Allow blood to **clot** for 30 minutes from time of collection, upright at room temperature.
- ☐ **Spin** sample to separate serum (3000 rpm x 15 minutes at 4^{o}C).
- ☐ **Refrigerate** sample until ready to package
- ☐ Pack tube(s) into FedEx box with ice packs.
- ☐ Send overnight chilled (not frozen) with ice packs to Emory University
    - Must be delivered within 24 hours of collection

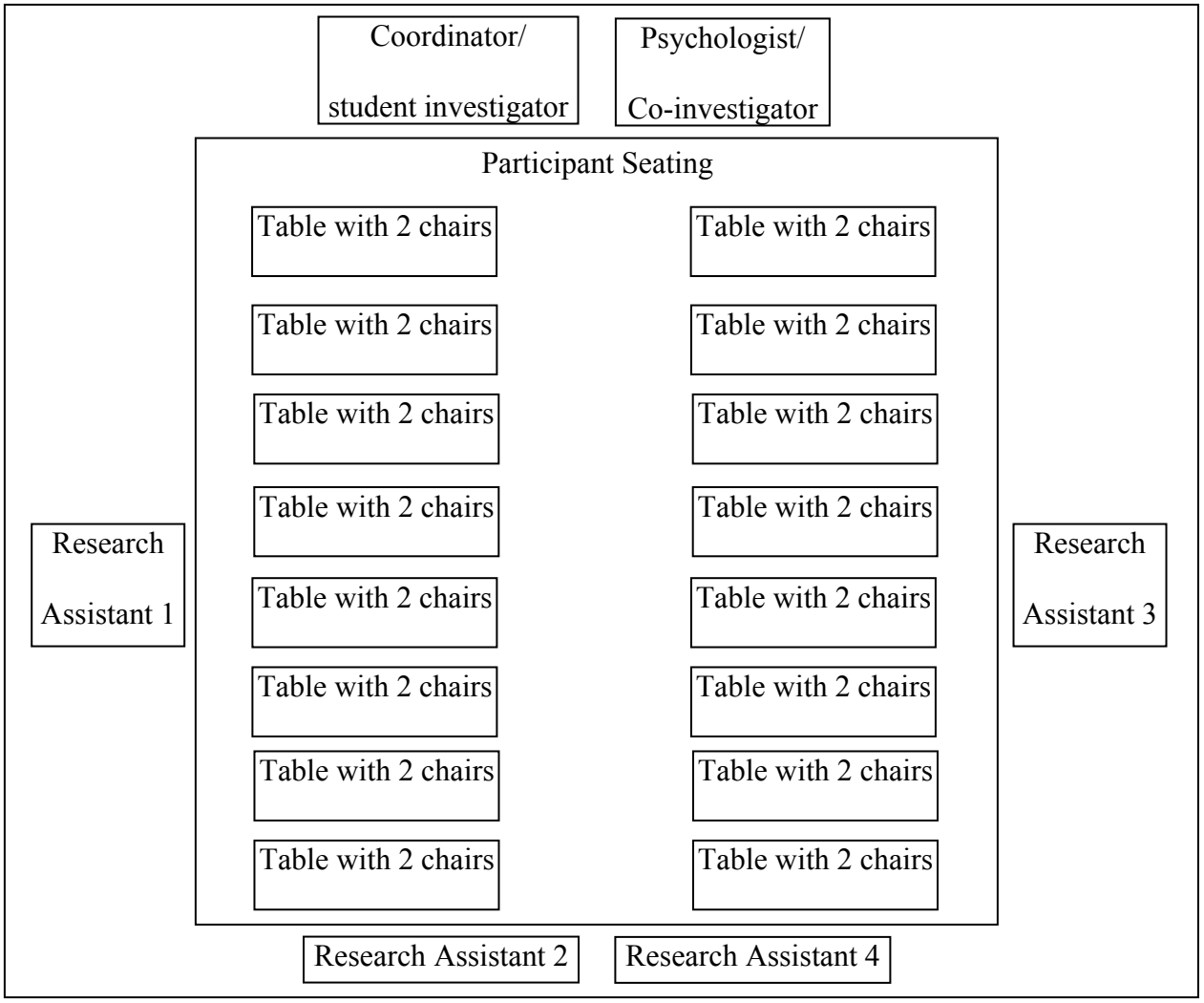
Sodium heparin tube (green top) (10 mL)

- ☐ Send overnight at **room temperature** to Emory University
    - Must be delivered within 24 hours of collection

EDTA tube (lavender top) (2 x 4 mL)

- ☐ Send tube 1 overnight at **room temperature** to Kennedy Krieger Institute
- ☐ Send tube 2 overnight at **room temperature** to Emory University
    - Must be delivered within 24 hours of collection

Group Testing (W-J III): Room Map



Participants were seated by last name in alphabetical order (A→ Z) starting in the upper left corner

Group Testing (W-J III): Research Assistant Data Collection Sheet

Research Assistant Name

Research Assistant Number _____

Stopwatch Number _____

- *Start timing at 0:00*
- *Participants who complete the testing early (before 3:00) will raise their hand*
- *Record the time and name of any participants finishing early*
    - *RA1 and RA2 will focus on the participants in the front half of the room*
    - *RA3 and RA4 will focus on the participants in the back half of the room*

Test Number (16, 20, 2, 6)	**Time (min:sec)**	**Participant Name (First name, Last initial)**	**Participant ID**

Scoring Datasheet for W-J III Tests

PKU & DHA Study

Participant ID: _____

Test Date: _____

Definitions:	
Correct:	correct answer
Commission Error:	wrong answer
Omission Error:	missing answer

Decision Speed – COG 16

Total Correct _____

Time _____

Standard Score_____

Pair Cancellation – COG 20

Total Correct _____

Total Commission Errors _____

Time _____

Standard Score _____

Reading Fluency – ACH 2 – Version A / B

Total Correct _____

Total Errors _____

Time _____

Standard Score_____

Math Fluency – ACH 6 – Version A / B

Total Correct _____

Total Errors _____

Time _____

Standard Score_____

Website for Study 3

Singh Research Group

Emory PKU & DHA Study

EMORY UNIVERSITY SCHOOL OF MEDICINE

- Home
- Contact Information
- Directions to Emory
- Study Forms
- Filter Paper Test
- Filter Paper Test Example
- Study Staff
- Study Funding
- Emory Links

If you may be interested in participating, please read below for more information:

Invitation Letter

Study Flyer

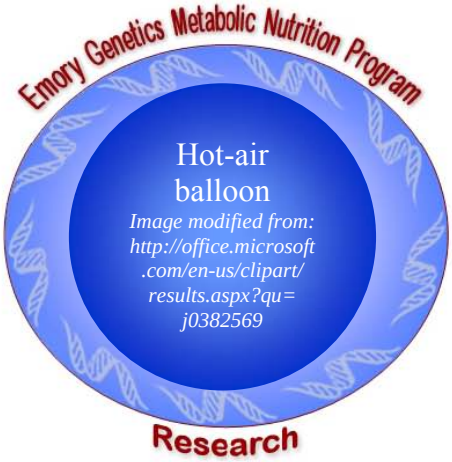
Contact information:

- Telephone: 404-778-1286
- e-mail: sarah.yi@emory.edu
- Toll-free: dial 1-800-366-1502, press 0, ask for extension 8-1286

Last updated March 18, 2009

Website designed by Sarah Yi using PersonalWebKit template

Logbook for Study 3



PKU & DHA Study

Log Book

for

___ ___ ___

from

Month ___ to Month ___

EMORY
UNIVERSITY
SCHOOL OF
MEDICINE

PKU & DHA Study

Participant ID ___ ___ ___

Participant Initials ___ ___ ___

Prescribed dose ___ capsules per day

Next visit date _____

This log book should be used between the dates of:

_____ and _____

(Study Month ___ and Study Month ___)

Date log book sent/given _____

EMORY UNIVERSITY SCHOOL OF MEDICINE

Department of Human Genetics
Division of Medical Genetics
www.genetics.emory.edu

Dear PKU & DHA Study Participant,

Thank you for choosing to take part in the PKU & DHA study! We are eager to start the study with you!

Study Purpose
For this study, we are asking you to take either a DHA or placebo supplement for 4.5 months to learn whether the DHA supplement will have a beneficial impact on your blood levels of DHA and your brain function.

Study Visit Schedule
Because we want this study to be a convenient part of your life, we will have only two study visits. These visits be at the beginning (0 months) and the end (4.5 months) of the study. The visits will take place either at the Emory University Hospital's General Clinical Research Center, the Emory Genetics Clinic, or at a clinic closer to your home. We will be contacting you to schedule these visits, but you can also feel free to contact Sarah, the main study contact, to schedule your study visits at the phone number or e-mail below.

Monthly Communication
Every month we will be checking in with you. We will ask you to:
1. **Update us** on any changes in medications and if you have gotten sick.
2. **Send us** a filter paper and 3-day diet record.

DHA & PKU Study Log Book
This Log Book has your "Study Supplement Instructions" and other important documents for you to use during this study. The pages you need to complete are on yellow paper.

Mailing the Supplements
If we need to give you more supplements during the study we will send you the supply of study supplements. We will mail these to you via Federal Express to your home address. Please contact Sarah with any specific delivery needs. Alternatively, you can pick up your supplements at the Emory Genetics Clinic. Please save all unused supplements and any empty bottles to return at the end of the study.

DHA & PKU Study Container
We are sending you a DHA & PKU Study Container to store your supplements, study Log Book, and other items. Please keep **all** of your study materials **in this container**!

We look forward to working with you on this exciting project. Please call or e-mail us with any questions or concerns you may have.

Sincerely,

Rani H. Singh, PhD, RD, LD and Sarah Yi, MS, RD, LD
Principal Investigator Doctoral Student Investigator

EMORY UNIVERSITY SCHOOL OF MEDICINE

Table of Contents

EMORY UNIVERSITY SCHOOL OF MEDICINE

Contact Information

To enroll in the study, schedule a study visit, and ask other study-related questions, please contact:

Sarah Yi, MS, RD
Study Coordinator/Co-Investigator
404-778-1286
sarah.yi@emory.edu

Mary Jane Kennedy, RN
Backup Study Coordinator
404-778-8522
mjkennedy@genetics.emory.edu

For more information concerning the research and research-related risks or injuries, you may call:

Rani H. Singh, PhD, RD, LD
Principal Investigator
404-778-8566
rsingh@genetics.emory.edu

To call any of the above phone numbers toll-free, dial 1-800-366-1502, press 0, and ask to be connected to one of the extensions below:

- Sarah Yi ext. 81286
- Mary Jane Kennedy ext. 88522
- Dr. Rani H. Singh ext. 88566

Other Contact Information:

Fax	404-778-8562
Address	Emory Genetics Metabolic Nutrition Program 2165 North Decatur Road Decatur, GA 30033-5307
Study Website	http://www.genetics.emory.edu/NUTRITION/pku_dha_study

If you have any questions regarding your rights as a study participant, you may call:

Colleen K. Dilorio, PhD
Interim Chair
Emory University Institutional Review Board
404-712-0720

EMORY UNIVERSITY SCHOOL OF MEDICINE

Study Details

STUDY OVERVIEW

The purpose of the PKU & DHA Study is to learn whether DHA supplementation improves brain function in people with PKU. You have been randomly assigned to take DHA or placebo (mixture of corn and soy oils) capsules for 4.5 months. Listed below are important details of the study:

STUDY VISITS

Schedule of study visits

The PKU & DHA Study has 2 visits:

1. beginning of the study (baseline visit)
2. 4.5 month visit (end of the study)

Your next visit is visit _____. If you have not scheduled this visit or do not know when you are scheduled, please contact us at 404-778-1286.

How to prepare for study visits

1. Complete a 3-day diet record before your study visit
2. Bring this log book and all supplement bottles
3. Fast starting at midnight the morning of the study visit

Study visit location

The study visits will usually be conducted at the General Clinical Research Center (GCRC) at Emory University. You have the option of spending the night at the GCRC or arriving at the GCRC in the morning for the study visit.

What to expect at study visits

- Fasting blood draw
- Neuropsych testing
- 3-day diet record collection and review
- Height and weight
- Vital signs
- History & physical exam

MONTHLY MAILINGS

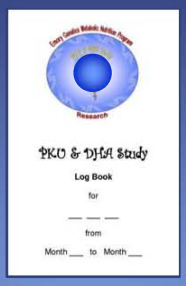
Every month we will ask you to mail the below items.

Month ___	**Month ___**	**Month ___**	**Month ___**
Mail by _____	Mail by _____	Mail by _____	Mail by _____
☐ Filter paper card	☐ Filter paper card	☐ Filter paper card	☐ Filter paper card
☐ 3-day diet record	☐ 3-day diet record	☐ 3-day diet record	☐ 3-day diet record
☐ Test requisition form	☐ Test requisition form	☐ Test requisition form	☐ Test requisition form

MONTHLY TELEPHONE COMMUNICATION

Every month we will contact you by telephone to check on how you are doing. During these phone calls we will go over the **PKU & DHA Study Log Book** and ask if you have any questions.

STUDY CONTAINER CONTENTS



Log Book
- Contact information
- Supplement instructions
- Medication log
- Illness log
- Supplement calendar

Supplements
- 5-month supply

Diet Records
- 3-day diet records
- Ruler
- Measuring cups and spoons

Filter Paper Cards
- Filter paper blood test procedure
- Filter paper cards
- Test requisition forms
- Self-addressed envelopes

EMORY UNIVERSITY SCHOOL OF MEDICINE

Study Supplement Instructions

Initials: ___ ___ ___

Dose

Take ____ capsules every day.

- Start date: _____
- Time you plan to take supplement daily: _____ AM / PM

Directions

- Take your study supplement with a meal or snack.
- Drink a glass of water or other beverage with your study supplement.
- Take your study supplement at the same time every day.
- Please do not share your study supplement with anyone.
- If you forget to take your dose, take it as soon as you remember. If it is almost time for the next dose, skip the missed dose and take your next dose at the normal time. Take only one dose per day.
- Record any missed doses on the Supplement Calendar (pages 8-11)
  - If you miss a daily dose, write zero (0) on the day you missed
  - If you take only part of your daily dose, write the number of capsules you actually took on that day
- Store the study supplements in the plastic study container. Avoid exposure to heat, direct sunlight, and moisture.
- If you have any questions, please call or e-mail Sarah or Dr. Singh:

Sarah	Dr. Singh
Phone: 404-778-1286	Phone: 404-778-8566
e-mail: sarah.yi@emory.edu	e-mail: rsingh@genetics.emory.edu

- **Please save all unused capsules in the bottles. We will send you the shipping materials to send the bottles to:**

Emory Genetics Metabolic Nutrition Program, Attn: Sarah Yi
2165 North Decatur Road; Decatur, GA 30033-5307

EMORY
UNIVERSITY
SCHOOL OF
MEDICINE

Supplement Calendar

Directions

- If you miss a daily dose, write zero (0) on the day you missed
- If you take only part of your daily dose, write the number of capsules you actually took on that day
- If you take your entire daily dose, leave the day blank

Month _____ Year _____

Sunday	Monday	Tuesday	Wednesday	Thursday	Friday	Saturday

EMORY UNIVERSITY SCHOOL OF MEDICINE

Emory PKU & DHA Study

Medication Log

Your Initials: ___ ___ ___
First Middle Last

Medication Name	Dose	How many doses per day?	Start Date *Month/Day/Year*	Stop Date *Month/Day/Year*	Reason for taking medication
Example: Regular Strength Tylenol	*2 tablets*	*1*	*Friday, 12/28/2007*	*Friday, 12/28/2007*	*Headache*

Please call or e-mail Sarah with questions and updates at 404-778-1286 or sarah.yi@emory.edu

EMORY UNIVERSITY SCHOOL OF MEDICINE

Emory PKU & DHA Study

Illness Log

Your Initials: ___ ___ ___
First Middle Last

Illness	Start Date *Month/Day/Year*	Stop Date *Month/Day/Year*	Did you list medication on Medication Log?	Life-threatening?*	Comments
Headache	*Friday, 12/28/2007*	*Friday, 12/28/2007*	☑Yes ☐No ☐N/A	☐Yes ☑No	*Stayed up late - didn't get enough sleep last night*
			☐Yes ☐No ☐N/A	☐Yes ☐No	
			☐Yes ☐No ☐N/A	☐Yes ☐No	
			☐Yes ☐No ☐N/A	☐Yes ☐No	
			☐Yes ☐No ☐N/A	☐Yes ☐No	
			☐Yes ☐No ☐N/A	☐Yes ☐No	
			☐Yes ☐No ☐N/A	☐Yes ☐No	
			☐Yes ☐No ☐N/A	☐Yes ☐No	
			☐Yes ☐No ☐N/A	☐Yes ☐No	
			☐Yes ☐No ☐N/A	☐Yes ☐No	
			☐Yes ☐No ☐N/A	☐Yes ☐No	
			☐Yes ☐No ☐N/A	☐Yes ☐No	
			☐Yes ☐No ☐N/A	☐Yes ☐No	
			☐Yes ☐No ☐N/A	☐Yes ☐No	
			☐Yes ☐No ☐N/A	☐Yes ☐No	

*Examples of **Life-threatening illness**: serious illness, requiring admission to hospital overnight, or disabling permanently

In case of serious or life-threatening illness, seek emergency treatment. Afterwards, please inform Sarah (404-778-1286) or Dr. Singh (404-778-8566)

EMORY UNIVERSITY SCHOOL OF MEDICINE

Filter Paper Blood Test Procedure

Important: *Blood tests are analyzed every Thursday and results are reported on Fridays. Please mail the filter paper by Monday so that it arrives by Wednesday. All tests that arrive after Wednesday afternoon will not be reported until the following Friday.*

Blood spots should be taken at least 1.5-2 hours after a meal

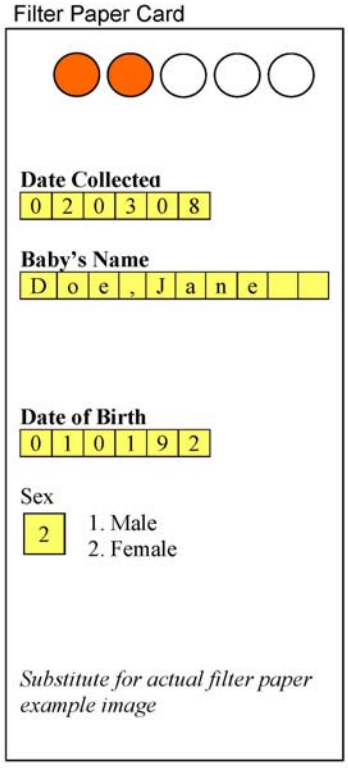
1. Fill out filter paper card with patient's name, date of birth, collection date.
2. Fill out parts of pages 1 and 3 of the Emory Genetics Lab Test Requisition Form where highlighted.
3. Do not touch the filter paper before or after applying blood spot.
4. Wash hands with soap and warm water and dry thoroughly.
5. Clean fingertip to be pricked with 70% alcohol pad and air dry. **Tip**: *You can rub the fingertip to increase the blood flow before pricking.*
6. Prick side of fingertip near fingernail with a sterile lancet. Lancets can be found in any drug store. Discard the lancet after use.
7. Allow drop of blood to form. Wipe first drop of blood away with gauze or cotton ball. Allow second drop of blood to form.
8. Apply large drop of blood to filter paper. One drop will fill a circle from front to back. Do not layer additional drops of blood, and apply blood to only one side of the filter paper.
9. Fill **TWO** circles on card.
10. Re-stick finger if blood does not flow freely. Follow steps 5 to 8.
11. Allow to dry for four hours on a flat, clean, nonabsorbent surface that is away from heat and sunlight.
12. Please send the filter paper, a copy of insurance card, and test requisition form completed in the provided envelope to: Emory Genetics Metabolic Nutrition Program; Attn: Sarah Yi; 2165 North Decatur Road; Decatur, GA 30033-5307

- The cost of this test is $50.00 which is billed to your insurance.
- If your insurance does not cover this test or if you do not have insurance, please contact Sarah Yi at 404-778-1286. To make a toll-free call, dial 1-800-366-1502, press 0, and ask to be connected to extension 81286.

EMORY UNIVERSITY SCHOOL OF MEDICINE

Filter Paper Blood Test Procedure

Filter Paper Card



Date Collected

0	2	0	3	0	8

Baby's Name

D	o	e	,	J	a	n	e		

Date of Birth

0	1	0	1	9	2

Sex

2

1. Male
2. Female

Substitute for actual filter paper example image

Test Requisition Form: Page 1

EMORY GENETICS LABORATORY TEST REQUISITION FORM - Page 1 of 3

2165 N. Decatur Rd., Atlanta, GA. 30033 • (404)778-8500 or 1-800-366-1502 • FAX (404)778-8559 • www.geneticslab.emory.edu

PATIENT INFORMATION

Last Name: Doe First: Jane MI: A

Parent Name (if pt is a minor)/Spouse:

DOB: 01 / 01 / 1992 Gender: Male (Female) Unknown

Address: 1 DNA Road

City: Decatur State: GA Zip: 30033

Preferred Phone# (404) 555 - 0100

Is this #: (Home) Work Cell Other:

Other # where pt. can be reached ( )

SS# - - Pt. ID/Med Rec#:

Ethnicity of Pt. (circle all that apply): African-American Asian Caucasian/NW European Eastern Indian Hispanic Jewish-Ashkenazi Jewish-Sephardic Mediterranean Native American Native Hawaiian/Other Pacific Islander Other:

PHYSICIAN ORDERING TEST:

SPECIMEN INFORMATION

Date Collected: 02 / 03 / 08 Time: 8 : 30 (AM)/PM

SAMPLE TYPE

Serum(S) Urine(U) Blood(B) Plasma(P) POC

Fibroblast Cult(FC) Amniotic Fl(AF) ✓ Blood Spot Card(BS) CSF

Skin(SK) DNA Chorionic Villi(CV) Muscle(M) Buccal Cells(BC)

Other:

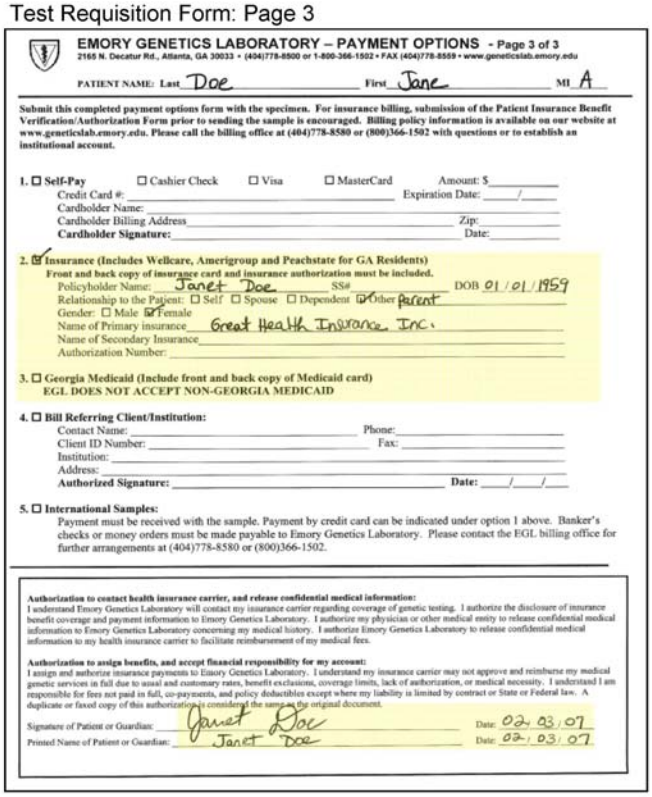
FOR SPECIMEN REQUIREMENTS: WWW.GENETICSLAB.EMORY.EDU

REASONS FOR REFERRAL:

PREVIOUS LAB RESULTS:

What time did you last eat or drink something (besides water)? : AM/PM

Test Requisition Form: Page 3



EMORY GENETICS LABORATORY – PAYMENT OPTIONS - Page 3 of 3

2165 N. Decatur Rd., Atlanta, GA 30033 • (404)778-8500 or 1-800-366-1502 • FAX (404)778-8559 • www.geneticslab.emory.edu

PATIENT NAME: Last Doe First Jane MI A

Submit this completed payment options form with the specimen. For insurance billing, submission of the Patient Insurance Benefit Verification/Authorization Form prior to sending the sample is encouraged. Billing policy information is available on our website at www.geneticslab.emory.edu. Please call the billing office at (404)778-8580 or (800)366-1502 with questions or to establish an institutional account.

1. ☐ **Self-Pay** ☐ Cashier Check ☐ Visa ☐ MasterCard Amount: $
Credit Card #: Expiration Date: /
Cardholder Name:
Cardholder Billing Address Zip:
Cardholder Signature: Date:

2. ☑ **Insurance (Includes Wellcare, Amerigroup and Peachstate for GA Residents)**
Front and back copy of insurance card and insurance authorization must be included.
Policyholder Name: Janet Doe SS# DOB 01 / 01 / 1959
Relationship to the Patient: ☐ Self ☐ Spouse ☐ Dependent ☑ Other Parent
Gender: ☐ Male ☑ Female
Name of Primary insurance Great Health Insurance, Inc.
Name of Secondary Insurance
Authorization Number:

3. ☐ **Georgia Medicaid (Include front and back copy of Medicaid card)**
EGL DOES NOT ACCEPT NON-GEORGIA MEDICAID

4. ☐ **Bill Referring Client/Institution:**
Contact Name: Phone:
Client ID Number: Fax:
Institution:
Address:
Authorized Signature: **Date:** / /

5. ☐ **International Samples:**
Payment must be received with the sample. Payment by credit card can be indicated under option 1 above. Banker's checks or money orders must be made payable to Emory Genetics Laboratory. Please contact the EGL billing office for further arrangements at (404)778-8580 or (800)366-1502.

Authorization to contact health insurance carrier, and release confidential medical information:
I understand Emory Genetics Laboratory will contact my insurance carrier regarding coverage of genetic testing. I authorize the disclosure of insurance benefit coverage and payment information to Emory Genetics Laboratory. I authorize my physician or other medical entity to release confidential medical information to Emory Genetics Laboratory concerning my medical history. I authorize Emory Genetics Laboratory to release confidential medical information to my health insurance carrier to facilitate reimbursement of my medical fees.

Authorization to assign benefits, and accept financial responsibility for my account:
I assign and authorize insurance payments to Emory Genetics Laboratory. I understand my insurance carrier may not approve and reimburse my medical genetic services in full due to usual and customary rates, benefit exclusions, coverage limits, lack of authorization, or medical necessity. I understand I am responsible for fees not paid in full, co-payments, and policy deductibles except where my liability is limited by contract or State or Federal law. A duplicate or faxed copy of this authorization is considered the same as the original document.

Signature of Patient or Guardian: Janet Doe Date: 02 / 03 / 07

Printed Name of Patient or Guardian: Janet Doe Date: 02 / 03 / 07

PKU & DHA Study

404-778-1286 ~ http://www.genetics.emory.edu/NUTRITION/pku_dha_study

EMORY UNIVERSITY SCHOOL OF MEDICINE

PKU & DHA Study

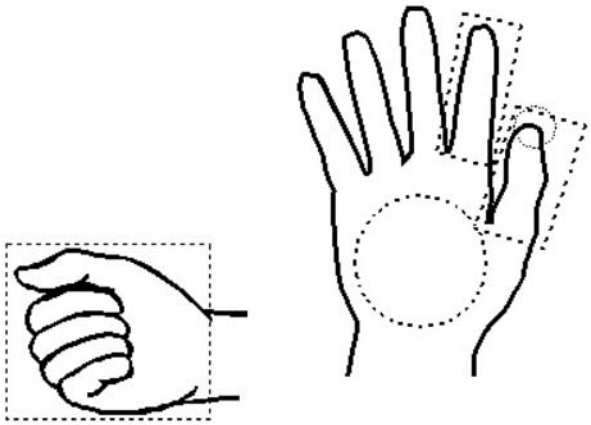
Three-Day Food Record Instructions

- We will ask you to keep a three-day food record:
  - ◇ Three days before each study visit, and
  - ◇ Three days before taking a blood sample for each filter paper card
- **Please record <u>everything</u> you actually eat and drink** for three days in a row
- Weigh and/or measure your food before you eat it
  - ◇ Subtract any amount that you did not eat and record your actual intake on the food record
- To estimate portion sizes, use the instructions in this packet, measuring spoons and cups, a ruler, and a scale
- Record what you eat and drink as soon as you can to reduce the chance of forgetting
- Write only one food or drink item per line
- Estimate the amount of phenylalanine (Phe) in milligrams or exchanges for the serving of food or drink that you actually ate (15 mg of Phe equals 1 Phe exchange)
- **Double-check your food record.** Did you remember to write down:
  - ☐ All meals, snacks, nibbling, and beverages including cocktails?
  - ☐ Recipes?
  - ☐ Ingredients used in mixed dishes, sandwiches, etc.?
  - ☐ Seasonings, spices, or condiments added to foods?
  - ☐ Whether weights are for cooked or raw portions?
  - ☐ How the food was prepared (uncooked vs. cooked)
    - ◇ Specify how the food was cooked: baked, boiled, broiled, fried, grilled, steamed, toasted
  - ☐ If the food is a specialty low protein item?
  - ☐ Whether there was ice in any beverages?

If you have any questions about your food record,
please call Sarah at 404-778-1286 or Dr. Singh at 404-778-8566

EMORY
UNIVERSITY
SCHOOL OF
MEDICINE

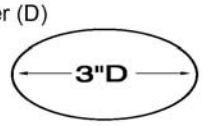
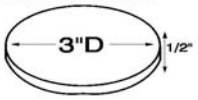
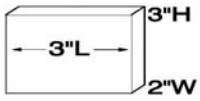
A Handy Way to Estimate Portion Size



Part of Hand	Portion Size	Examples of Foods
• Whole Fist	1 cup	2 servings of vegetables, 1 piece of fruit
• Palm of Hand	1 oz	1 serving of snack chips or pretzels
• Finger Length	2.5 inches	Diameter of 1 fruit serving or 1 tennis ball
• Whole Thumb	2 Tbsp, 20 mL, 1 fl oz, 1 oz, 28 g	1 serving of low protein peanut butter or cheese
• Thumb Tip	1 tsp, 5 mL	1 serving of margarine

EMORY UNIVERSITY SCHOOL OF MEDICINE

Ways to Describe Portion Size

Description	Examples
Measuring cups (C), teaspoons (t or tsp), tablespoons (T or Tbsp)	Vegetables, canned or frozen fruits, pasta, casseroles, all liquids such as water, beverages, soups, sauces, salad dressings, sorbet, or smoothies.
Fluid Ounces (fl oz)	All liquids such as water, beverages, soups, sauces, salad dressings, sorbet, or smoothies.
Weight in grams (g) or ounces (oz)	Any solid food such as low protein cheese, frozen entrees, or dry medical food powder (formula).
Fraction of the whole	1/8 of 9" pie or 1/4 of 6" cantaloupe.
Diameter (D) 	Any sphere, such as a 3" diameter apple, roll, or tomato.
Diameter and Thickness 	Any cylinder or disk, such as a pancake, cracker, cookie, or low protein burger (eg: a 3" diameter, 1/2" thick low protein burger patty).
Length and Height and Width 	Any rectangle or square such as a 3" long, 3" high, 2" wide piece of chocolate cake or low protein bread.
Length and Height and Width of Arc 	Any wedge, such as a slice of low protein pizza or pie.

★SAMPLE★

Participant Initials: ___ ___ ___

Day of Week: _____

Date: _____ / __ __ / __ __ __ __
Month Day Year

Food Record: DAY _____, Page 1

	Medical Food (Phe-free formula)		Other Ingredients			Volume Mixed	Volume Consumed
	Product Name	Amount	Other Ingredients	Brand	Amount		
1	*Phenex-2*	*100 g*	*Strawberry Syrup*	*Hershey's*	*1 T*	*24 fl oz*	*19 fl oz*
			Water				
2	*Amino Acid Blend*	*26 g*	*Lemonade Mix*	*Country Time*	*3/8 cap (51 g)*	*24 fl oz*	*50%*
			Water				
3							

# of servings per day: _____

Please circle: Did you take the SAME, MORE, or LESS medical food (Phe-free formula) than usual?

Medications		
Medication Name	Brand Name	Total amount taken
Advil 200 mg Ibuprofen per tablet	*Wyeth*	*1 Tablet*

Vitamins, Minerals, or Other Supplements		
Supplement Name	Brand Name	Total Amount taken
Calcium 500 mg tablets with Vitamin D	*CVS*	*1 Tablet*

Please continue on the reverse side if you need more space.

★SAMPLE★

Participant Initials: ___ ___ ___

Date: _____ / __ __ / __ __ __ __
Month Day Year

Food Record: DAY _____, Page 3

Time/ Meal*	Food Item • Be specific. Only one item per line! • Write "low-pro" if a specialty low protein item	Amount Specify oz, tsp, etc	Cooking method	Type of fat used when cooking	Where eaten? H (home) R (restaurant) O (other)	Brand/ Restaurant	Phe (mg)	Exchanges 1 exchange = 15 mg Phe

* Meal: B=Breakfast; L=Lunch; D=Dinner; SN=Snack

Total (page 2 + page 3): 379 **mg** 28.5 **Ex**

Please circle: Did you eat the SAME, MORE, or LESS food than usual?

Please describe anything that affected your diet while keeping today's food record.

Forgot formula at home this morning. Drank as much as I could when I got home today, but got too full to drink all of it.

Please continue on the reverse side if you need more space.

★SAMPLE★
Recipes

Recipe 1: *Low-protein spaghetti* *2 oz* *Dry Low-Pro Spaghetti (Loprofin)* *4 cups* *Water* *1 Tbsp* *Butter (Cabot salted butter)* *1 tsp* *Salt* *Boiled for 10 minutes.*	Recipe 2:
Recipe 3:	Recipe 4:

EMORY UNIVERSITY SCHOOL OF MEDICINE

Directions to the GCRC

Address

W. Dean Warren General Clinical Research Center (GCRC)
Emory University Hospital, Suite GG-23
1364 Clifton Road, NE
Atlanta, GA 30322

Parking

Parking for the Emory University Hospital is available in front of the Emergency Department and across Clifton road in the visitor parking tower. We will provide you with a voucher to pay for your parking.

Directions

From the Northeast (I-85):
Take I-85 South to Clairmont Road (Exit #91). Turn left on Clairmont Road and follow the signs towards Decatur. Continue on Clairmont Road for 3.8 miles to North Decatur Road. Turn right on North Decatur Road. Proceed one mile to Clifton Road. Turn Right on Clifton Road. The hospital and ambulance entrance are located 0.2 miles on the left. Clinic Buildings A and B, as well as Visitor Parking, are located 0.2 miles on the right.

From the North (GA 400):
Take GA 400 South to Sidney Marcus Boulevard exit. Turn left on Sidney Marcus Boulevard. Turn left on Buford Highway. Turn right on Lenox Road. Get on I-85 North. Take I-85 North to Clairmont Road (Exit #91). Turn right on Clairmont Road and follow the signs towards Decatur. Continue on Clairmont Road for 3.8 miles to North Decatur Road. Turn right on North Decatur Road. Proceed one mile to Clifton Road. Turn right on Clifton Road. The hospital and ambulance entrance are located 0.2 miles on the left. Clinic Buildings A and B, as well as Visitor Parking, are located 0.2 miles on the right.

-or-

Take GA 400 South to I-285. Take I-285 East for approximately 5.6 miles. Take I-85 South to Clairmont Road (Exit #91). Turn left on Clairmont Road and follow the signs towards Decatur. Continue on Clairmont Road for 3.8 miles to North Decatur Road. Turn right on North Decatur Road. Proceed one mile to Clifton Road. Turn right on Clifton Road. The hospital and ambulance entrance are located 0.2 miles on the left. Clinic Buildings A and B, as well as Visitor Parking, are located 0.2 miles on the right.

From the South (I-75):
Take I-75 North to Freedom Parkway/Carter Center/International Boulevard (Exit #248-C). Bear right off the exit and continue straight for approximately 2.1 miles following the signs to the Carter Center and Moreland Avenue. Turn left on Moreland Avenue. Continue north on Moreland Avenue (Moreland Avenue becomes Briarcliff Road) for approximately 1.7 miles. Turn right on North Decatur Road and travel approximately 1.1 miles, going through Emory Village. Turn left on Clifton Road. The hospital and ambulance entrance are located 0.2 miles on the left. Clinic Buildings A and B, as well as Visitor Parking, are located 0.2 miles on the right.

EMORY UNIVERSITY SCHOOL OF MEDICINE

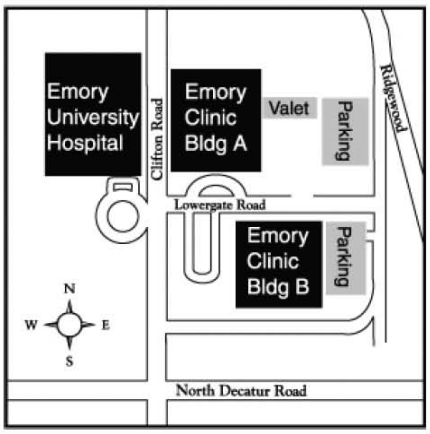
Directions to the GCRC, Continued

From the East (US 78):
Take 285 to Stone Mountain Freeway/US-78, follow signs towards Decatur. Proceed on Scott Blvd towards North Decatur Road. Turn Right on North Decatur Road. Proceed approximately two miles on North Decatur Road. Turn right on Clifton Road. The hospital and ambulance entrance are located 0.2 miles on the left. Clinic Buildings A and B, as well as Visitor Parking, are located 0.2 miles on the right.

From the East (I-20):
Take I-20 West to Moreland Avenue (Exit 60). Turn right (north) on Moreland Avenue. Continue on Moreland Avenue (Moreland Avenue becomes Briarcliff Road) for approximately 3.4 miles. Turn right on North Decatur Road and travel approximately 1.1 miles, going through Emory Village. Turn left on Clifton Road. The hospital and ambulance entrance are located 0.2 miles on the left. Clinic Buildings A and B, as well as Visitor Parking, are located 0.2 miles on the right.

From the Northwest (I-75):
Take I-75S to I-85N. Take I-85 to Clairmont Road (Exit #91). Turn right and follow the signs towards Decatur. Stay on Clairmont Road for 3.8 miles to North Decatur Road. Turn right on North Decatur Road. Proceed one mile to Clifton Road. Turn right on Clifton Road. The hospital and ambulance entrance are located 0.2 miles on the left. Clinic Buildings A and B, as well as Visitor Parking, are located 0.2 miles on the right.

From the West (I-20):
Take I-20 East to Moreland Avenue (Exit 60). Turn right (north) on Moreland Avenue. Continue on Moreland Avenue (Moreland Avenue becomes Briarcliff Road) for approximately 3.4 miles. Turn right on North Decatur Road and travel approximately 1.1 miles, going through Emory Village. Turn left on Clifton Road. The hospital and ambulance entrance are located 0.2 miles on the left. Clinic Buildings A and B, as well as Visitor Parking, are located 0.2 miles on the right.



EMORY UNIVERSITY SCHOOL OF MEDICINE

Directions to Emory Genetics

Address

Emory Genetics Clinic Building
2165 North Decatur Rd.
Decatur, GA 30033

Parking

Free parking is available in front of the building.

Directions

From Interstate 20 East
Take exit 60-B, the Moreland Avenue North exit. Turn right on Moreland and follow it approximately 3 miles. Moreland will change to Briarcliff Road once it crosses Ponce de Leon Avenue. Continue straight on Briarcliff approximately 2 miles to North Decatur Road. Turn right and follow North Decatur one mile to the Oxford Road intersection. Stay on North Decatur for 2 miles until you reach the intersection of North Decatur and Clairmont Rd. **See below** ***

From Interstate 20 West
Take exit 60-B, the Moreland Avenue North exit. Turn right on Moreland and follow it approximately 3 miles. Moreland will change to Briarcliff Road once it crosses Ponce de Leon Avenue. Continue straight on Briarcliff approximately 2 miles to North Decatur Road. Turn right and follow North Decatur one mile to the Oxford Road intersection. Stay on North Decatur for 2 miles until you reach the intersection of North Decatur and Clairmont Rd. ***

From Interstate 75 North or South
Take exit 248-C, the Freedom Parkway exit. Cross Boulevard; continue on Freedom Parkway; veer left at split; continue until it ends at Ponce de Leon Avenue; then turn right. Off Ponce, turn left on Briarcliff Road. Go approximately 2 miles to North Decatur Road. Turn right and follow North Decatur Road one mile to the Oxford Road intersection. Stay on North Decatur for 2 miles until you reach the intersection of North Decatur and Clairmont Rd. ***

From Interstate 85 North and Hartsfield Atlanta International Airport
The airport is located in the southwest section of the city, approximately 25 minutes from the Emory University campus. Visitors driving from the airport should take I-85 North. To Emory from Interstate 85 North, take exit 248-C, the Freedom Parkway exit. Cross Boulevard; continue on Freedom Parkway; veer left at split; continue until it ends at Ponce de Leon Avenue; then turn right. Off Ponce, turn left on Briarcliff Road. Go approximately 2 miles to North Decatur Road. Turn right and follow North Decatur Road one mile to the Oxford Road intersection. Stay on North Decatur for 2 miles until you reach the intersection of North Decatur and Clairmont Rd. ***

***Continue straight on North Decatur through the intersection. We are located just past the Publix on the right.

From Interstate 85 South
Take exit 91, the Clairmont Road exit. Turn left (east) on Clairmont and follow it approximately 3 miles to North Decatur Road. Turn left on North Decatur. We are just past the Publix on the right.

EMORY UNIVERSITY SCHOOL OF MEDICINE

Notes

EMORY UNIVERSITY SCHOOL OF MEDICINE

Notes

Questionnaires

Medical Information Form

ID # _ _ _

MEDICAL INFORMATION FORM

Please complete this form and return it in the enclosed envelope.

PATIENT INFORMATION

Formula Prescription
For example: Phenylfree 2 180 grams+
Kool-Aid 2 Tbsp+water to make 20 fl oz _____

Diet Prescription
For example: 20 exchanges, 300 mg Phe,
or 6 grams of protein per day _____

Current Medications
Include dose and how often _____

Current Supplements
vitamins, minerals, herbal _____

List any operations or serious
injuries and date(s) _____

Activities to be encouraged or
limited by physician's advice _____

Any current or previous psychological counseling? ☐ Yes ☐ No Date: _____

Any current or previous psychiatric care? ☐ Yes ☐ No Date: _____

Any previous psychiatric hospitalization? ☐ Yes ☐ No Date: _____

Have you menstruated? ☐ Yes ☐ No

Is your menstrual history normal? ☐ Yes ☐ No

Do you have children? ☐ Yes ☐ No If yes, how many? _____

Ages of child/children: _____

ID # __ __ __

Page 2: MEDICAL INFORMATION FORM

DIET RESTRICTION *(for example: low Phe or low Leu diet)*	**FORMULA** *(for example: Phe-free or Leu free formula)*
I first started my diet restriction when I was: ☐ A baby ☐ _____ years old ☐ I have never restricted my diet	**I first started taking my formula when I was:** ☐ A baby ☐ _____ years old ☐ I have never taken formula
I stopped my diet restriction between ages: ☐ _____ and _____ years old ☐ I have never stopped my diet restriction	**I stopped taking my formula between ages:** ☐ _____ and _____ years old ☐ I have never stopped taking my formula
I have kept the diet restriction since I was: ☐ A baby ☐ _____ years old ☐ I am not currently on a diet restriction	**I have taken formula since I was:** ☐ A baby ☐ _____ years old ☐ I am not currently taking formula
Even though I currently keep a diet restriction: ☐ Some days I eat high protein foods like cheese, beans, eggs, meat/chicken/fish, nuts ☐ Some days I eat too much potatoes/French fries, bread, pasta, or _____ ☐ I don't currently keep a diet restriction	**Even though I currently take formula:** ☐ Some days don't finish all of my formula ☐ Some days I don't drink any of my formula ☐ I don't currently take formula

During the year, how often do you visit a doctor's office or clinic for:

- Routine check-ups? _____
- Diet evaluations and blood levels (not including check-ups or other illnesses)? _____
- Illnesses (not including check-ups or diet evals/blood levels)? _____

Which method(s) do you use to monitor your blood levels (Phe, Leu, other) and how often?

For example: weekly, monthly, every other month

☐ Filter paper blood spots — How often? _____

☐ Blood drawn in Clinic or Doctor's office — How often? _____

My levels (for example, blood Phe or blood Leu) are:

☐ Usually within the recommended range

☐ Usually above the recommended range

☐ Sometimes within recommended range and sometimes above recommended range

☐ Other: _____

Have you ever participated in any **other** educational program for PKU, MSUD, or other inborn errors of metabolism (not including Metabolic Camp at Emory)?

☐ Yes ☐ No If yes, when and where? _____

Do you have health insurance? ☐ Yes ☐ No

Demographic Information Form

ID # __ __ __

DEMOGRAPHIC INFORMATION FORM

Please complete this form and return it in the enclosed envelope.

The following answers will be used for research purposes only and will be kept strictly confidential.

YOUR DEMOGRAPHICS:

What racial or ethnic group do you belong to?

- ☐ Caucasian
- ☐ African American
- ☐ Hispanic/Latino
- ☐ Asian
- ☐ Other: _____
- ☐ Mixed race: _____

What is your marital status?

- ☐ Single (never married)
- ☐ Married
- ☐ Widowed
- ☐ Separated
- ☐ Divorced
- ☐ Other: _____

YOUR EMPLOYMENT:

Are you employed? ☐ Yes ☐ No

If yes, what type of work do you do? _____

YOUR CURRENT HOUSEHOLD:

Excluding yourself, how many adults (age 18 and older) currently live with you? _____

- Who do you live with (check all that apply)?
  - ☐ Biological mother
  - ☐ Biological father
  - ☐ Adopted mother
  - ☐ Adopted father
  - ☐ Grandmother
  - ☐ Grandfather
  - ☐ Aunt
  - ☐ Uncle
  - ☐ Husband
  - ☐ Roommate
  - ☐ Foster caretaker
  - ☐ Other _____

Excluding yourself, how many children under the age of 18 currently live with you? _____

- Number of siblings in household (include step-siblings): _____

Who is the primary caretaker of you (check **all** that apply)?

- ☐ Self
- ☐ Biological parents
- ☐ Biological mother
- ☐ Biological father
- ☐ Adopted mother
- ☐ Adopted father
- ☐ Grandmother
- ☐ Grandfather
- ☐ Aunt
- ☐ Uncle
- ☐ Foster caretaker
- ☐ Other _____

What is the combined household income currently? (Check appropriate average)

- ☐ $5,000 or less
- ☐ $5,001 – 10,000
- ☐ $10,001 –20,000
- ☐ $20,001 – 30,000
- ☐ $30,001 –40,000
- ☐ $40,001 – 50,000
- ☐ $50,001 –60,000
- ☐ $60,001 – 70,000
- ☐ $70,001 – 100,000
- ☐ More than $100,000

ID # __ __ __

Page 2: DEMOGRAPHIC INFORMATION FORM

- *If you do not (or did not) live with your biological parents for part or all of your childhood (birth through 18 years), please <u>also</u> describe the caretaker(s) who you lived with (for example, your adopted parents).*
- *If you are an adult, please answer based on your childhood (between birth and age 18 years).*

YOUR BIOLOGICAL PARENTS:
How long have you lived (or did you live) with your biological parents? _____ years

What is the marital status of your parents?
- ☐ Married to each other
- ☐ Separated
- ☐ Divorced
- ☐ Widowed
- ☐ Married to other people
- ☐ Single and never married
- ☐ Living with partner
- ☐ Unknown
- ☐ Other

<u>Your biological mother</u>
Mother's age: _____ years

Mother's occupation (give title if possible)
(or occupation before retirement/lay off)
_____
How many hours per week are spent:
working: ____ with camper: ____

Mother's racial/ethnic group:
- ☐ Caucasian
- ☐ African American
- ☐ Hispanic/Latino
- ☐ Asian
- ☐ Other: _____
- ☐ Mixed race: _____

<u>Your biological father</u>
Father's age: _____ years

Father's occupation (give title if possible)
(or occupation before retirement/lay off)
_____
How many hours per week are spent:
working: ____ with camper: ____

Father's racial/ethnic group:
- ☐ Caucasian
- ☐ African American
- ☐ Hispanic/Latino
- ☐ Asian
- ☐ Other: _____
- ☐ Mixed race: _____

YOUR GUARDIANS/CARETAKERS:
How long have you lived (or did you live) with your primary caretakers? _____ years

What is the marital status of your caretakers?
- ☐ Married to each other
- ☐ Separated
- ☐ Divorced
- ☐ Widowed
- ☐ Married to other people
- ☐ Single and never married
- ☐ Living with partner
- ☐ Unknown
- ☐ Other

<u>Caretaker 1</u>
Caretaker 1's age: _____ years
Caregiver 1's sex: ☐ FEMALE ☐ MALE
Caretaker 1's occupation (give title if possible)
(or occupation before retirement/lay off)
_____
How many hours per week are spent:
working: ____ with camper: ____

Caretaker 1's racial/ethnic group:
- ☐ Caucasian
- ☐ African American
- ☐ Hispanic/Latino
- ☐ Asian
- ☐ Other: _____
- ☐ Mixed race: _____

<u>Caretaker 2</u>
Caretaker 2's age: _____ years
Caregiver 1's sex: ☐ FEMALE ☐ MALE
Caretaker 2's occupation (give title if possible)
(or occupation before retirement/lay off)
_____
How many hours per week are spent:
working: ____ with camper: ____

Caretaker 2's racial/ethnic group:
- ☐ Caucasian
- ☐ African American
- ☐ Hispanic/Latino
- ☐ Asian
- ☐ Other: _____
- ☐ Mixed race: _____

ID # __ __ __

Page 3: DEMOGRAPHIC INFORMATION FORM

EDUCATION:

What is the highest grade of school completed by you, your biological parents, your caregiver(s), and your spouse (if applicable)?

You	Biological Mother	Biological Father	Caretaker 1	Caretaker 2	Spouse	
☐	☐	☐	☐	☐	☐	No school completed
☐	☐	☐	☐	☐	☐	Nursery school/preschool to 4th grade
☐	☐	☐	☐	☐	☐	5th or 6th grade
☐	☐	☐	☐	☐	☐	7th or 8th grade
☐	☐	☐	☐	☐	☐	9th grade
☐	☐	☐	☐	☐	☐	10th grade
☐	☐	☐	☐	☐	☐	11th grade
☐	☐	☐	☐	☐	☐	12th grade, no diploma
☐	☐	☐	☐	☐	☐	High school graduate (diploma or equivalent, such as GED)
☐	☐	☐	☐	☐	☐	Technical training (for example: mechanic or plumber)
☐	☐	☐	☐	☐	☐	Some college credit, but less than 1 year
☐	☐	☐	☐	☐	☐	1 or more years of college, no degree
☐	☐	☐	☐	☐	☐	Associate's degree (for example: AA, AS)
☐	☐	☐	☐	☐	☐	Bachelor's degree (for example: BA, AB, BS)
☐	☐	☐	☐	☐	☐	Some graduate school, no degree
☐	☐	☐	☐	☐	☐	Master's degree (for example: MA, MS)
☐	☐	☐	☐	☐	☐	Professional degree (for example: MD, DDS, DVM, JD, PsyD)
☐	☐	☐	☐	☐	☐	Doctorate degree (for example: PhD, EdD)
☐	☐	☐	☐	☐	☐	Other: _____
☐	☐	☐	☐	☐	☐	Unknown

Do you/did you require extra help in school, such as special education classes or an individualized education program (IEP)? ☐ Yes ☐ No If yes, describe: _____

Other Medical Information Form

OTHER MEDICAL INFORMATION FORM

Please complete this form and return it in the enclosed envelope.

NAME (FIRST, MIDDLE, LAST): _____

SEX: ☐ FEMALE ☐ MALE BIRTH DATE: _____ SOCIAL SEC #: ____-____-_____

ADDRESS: _____

_____

HOME PHONE: ( ) _____ OKAY TO LEAVE MESSAGE? ☐ Yes ☐ No

_____ PHONE: ( ) _____ OKAY TO LEAVE MESSAGE? ☐ Yes ☐ No

ELECTRONIC MAIL: _____ DIAGNOSIS: _____

Name of Dietitian: _____ Dietitian Phone #: ( ) _____

Name of Metabolic Clinic: _____

Name of Physician: _____ Physician Phone #: ( ) _____

EMERGENCY CONTACT INFORMATION:

NAME: _____ PHONE #: ( ) _____

RELATIONSHIP TO CAMPER: _____

Parent/Guardian Information if < 18 years old

MOTHER'S NAME: _____ ADDRESS: _____

PHONE # (HOME): _____ PHONE # (WORK/CELL): _____

FATHER'S NAME: _____ ADDRESS: _____

PHONE # (HOME): _____ PHONE # (WORK/CELL): _____

INSURANCE INFORMATION:

Primary Insurance

Name of primary insurance: _____

Policyholder's name: _____ SS#: ____-____-_____

Policyholder's relationship to the camper: ☐ Self ☐ Spouse ☐ Parent ☐ Other

Policyholder's gender: ☐ Male ☐ Female Policyholder's birth date: _____

Secondary Insurance

Name of primary insurance: _____

Policyholder's name: _____ SS#: ____-____-_____

Policyholder's relationship to the camper: ☐ Self ☐ Spouse ☐ Parent ☐ Other

Policyholder's gender: ☐ Male ☐ Female Policyholder's birth date: _____

Please include a copy of both sides of your insurance card.

Pre-Blood Draw Questionnaire

Date: _____
ID #: _____
FML: _____

PKU & DHA Study

Before we draw your blood, please answer these questions. Please answer honestly. Your answers help make sure the blood test results are accurate. All information will be kept confidential.

1. Have you eaten any food, formula/medical food, juice, soda, or taken vitamins (not including water) since midnight last night?

Yes No If yes, what did you eat and when? _____

2. What time did you last eat or drink something (besides water)? ____:____ AM/PM

3. Does anyone in your current household smoke cigarettes?

Yes No

4. How often do you usually smoke cigarettes?

A I do not smoke
B At least one time per day
C At least one time per week
D At least one time per month
E Less than one time per month
F Less than one time per year

If you circled B, C, D, E, or F, please answer the following questions about the past 3 days:

5. During the past 3 days, which days did you smoke cigarettes?

□ I did not smoke during the past 3 days
□ Today:
□ Yesterday:
□ The day before yesterday:

6. During the past 3 days, on the days you smoked, how many cigarettes did you smoke per day?

A I did not smoke cigarettes during the past 3 days
B Less than 1 cigarette per day
C 1 cigarette per day
D 2 to 5 cigarettes per day
E 6 to 10 cigarettes per day
F 11 to 20 cigarettes per day
G More than 20 cigarettes per day

Thank you!

Weight Control and Physical Activity Questionnaire

Dear Participant,

This survey is about health behavior. Your honest answers will help us make sure females with metabolic disorders get the best care possible.

The answers you give will be kept private. Answer the questions based on what you really do.

Completing the survey is voluntary. If you are not comfortable answering a question, just leave it blank.

Make sure to read every question. When you are finished, follow the instructions of the person giving you the survey.

Thank you for your help!

The next 7 questions ask about body weight.

Date: _____
ID #: _____
DOB: _____

Please circle the best answer

1. How do **you** describe your weight?
   a. Very underweight
   b. Slightly underweight
   c. About the right weight
   d. Slightly overweight
   e. Very overweight

2. Which of the following are you trying to do about your weight?
   a. **Lose** weight
   b. **Gain** weight
   c. **Stay** the same weight
   d. I am **not trying to do anything** about my weight

Please check all the apply

During the past 30 days:

3. In order to **lose or keep from gaining weight**, did you:
   □ I am not trying to lose or keep from gaining weight
   □ Exercise
   □ Eat less food, fewer calories or foods low in fat
   □ Drink less formula/medical food than your prescription
   □ Go without eating for 24 hours or more (also called fasting)
   □ Take any diet pills, powders, or liquids without a doctor's advice
   □ Vomit or take laxatives

Please circle the best answer

During the past 30 days:

4. In order to **gain weight**, did you drink more formula than your prescription?
   a. I am not trying to gain weight
   b. Yes
   c. No

5. In the past 12 months, has a doctor, nurse, or dietitian given you advice about your weight?
   a. Yes, I was given advice to lose weight
   b. Yes, I was given advice to gain weight
   c. Yes, I was given advice to maintain weight
   d. No, I was not given advice about my weight

6. Compared with one year ago (last _____), have you:
   a. Gained weight
   b. Lost weight
   c. Stayed the same weight

7. Compared with one year ago (last _____), have you:
   a. Grown taller
   b. Lost height
   c. Stayed the same height

The next 4 questions ask about physical activity.

Date: _____
ID #: _____

Please circle the best answer

1. In the past week (_____ - _____), on how many days were you:

a. exercising or physically active for **at least 20 minutes that made you sweat and breathe hard**?
Examples: basketball, soccer, running, swimming laps, fast bicycling, fast dancing, or similar aerobic activities

a. 0 days
b. 1 day
c. 2 days
d. 3 days
e. 4 days
f. 5 days
g. 6 days
h. 7 days

b. physically active for **at least 30 minutes** that did **not** make you sweat or breathe hard?
Examples: fast walking, slow bicycling, skating, pushing a lawn mower, or mopping floors

a. 0 days
b. 1 day
c. 2 days
d. 3 days
e. 4 days
f. 5 days
g. 6 days
h. 7 days

c. lightly active for **at least 30 minutes?**
Examples: slow walking, light house chores, or stretching

a. 0 days
b. 1 day
c. 2 days
d. 3 days
e. 4 days
f. 5 days
g. 6 days
h. 7 days

2. Compared with the past year, was your activity level in the past week the same, lower, or higher than usual?

a. The same
b. Lower
c. Higher

3. On an average school or work day, how many hours do you watch TV per day?

a. I do not watch TV on an average school/work day
b. Less than 1 hour per day
c. 1 hour per day
d. 2 hours per day
e. 3 hours per day
f. 4 hours per day
g. 5 or more hours per day

4. On an average weekend or vacation day, how many hours do you watch TV per day?

a. I do not watch TV on an weekend/vacation day
b. Less than 1 hour per day
c. 1 hour per day
d. 2 hours per day
e. 3 hours per day
f. 4 hours per day
g. 5 or more hours per day

Thank you for completing this survey!

References

Physical Activity
Ainsworth BE, Haskell WL, Whitt MC, Irwin ML, Swartz AM, Strath SJ, O'Brien WL, Bassett DR Jr, Schmitz KH, Emplaincourt PO, Jacobs DR Jr, Leon AS. Compendium of physical activities: an update of activity codes and MET intensities. Med Sci Sports Exerc. 2000 Sep;32(9 Suppl):S498-504.

Pate RR, Pratt M, Blair SN, Haskell WL, Macera CA, Bouchard C, Buchner D, Ettinger W, Heath GW, King AC, et al. Physical activity and public health. A recommendation from the Centers for Disease Control and Prevention and the American College of Sports Medicine. JAMA. 1995 Feb 1;273(5):402-7. Review.

YRBSS
Grunbaum JA, Kann L, Kinchen S, Ross J, Hawkins J, Lowry R, Harris WA, McManus T, Chyen D, Collins J. Youth risk behavior surveillance--United States, 2003. MMWR Surveill Summ. 2004 May 21;53(2):1-96. Erratum in: MMWR Morb Mortal Wkly Rep. 2004 Jun 25;53(24):536. MMWR Morb Mortal Wkly Rep. 2005 Jun 24;54(24):608.
http://www.cdc.gov/mmwr/preview/mmwrhtml/ss5302a1.htm; accessed May 12, 2006.

2005 CDC YRBSS High School Questionnaire

Treatment History Questionnaire

Phenylketonuria Transition Project **Clinical Treatment Summary**
Date of summary:
ID #:
Age:

I. Health Status:
General health
Specific concerns

II. PKU Treatment History:	
Age at Dx:	Newborn peak phe level:
Identified by Newborn Screening?	Which state?
Blood phe levels:	
Mean blood phe level 1st year:	Range:
Mean blood phe level age 1-5 years:	Range:
Mean blood phe level age 6-10 years:	Range:
Mean blood phe level age 11-15 years:	Range:
Mean blood phe level age 16-present:	Range:
Mean blood phe level in past year:	Range:

III. Intellectual Functioning:

General assessment

Psychometric Evaluations

Date	Age	Test	Score	Comments

School History:

Grade completed:	Academic goals:
Resources required:	

Cristine M. Trahms Program for Phenylketonuria
University of Washington - CHDD - Box 357920, Seattle, WA 98195

(206) 598-1800, Toll Free in Washington State 877-685-3015
http://depts.washington.edu/pku

IV. Current PKU Treatment:

Phenylalanine prescription:
Medical food prescription:
Medications:
Comments:

V. Support System/Level of Independence:

Where living?		
Completed high school?		IEP in place?
Working?	What job?	Hours/week:
Marital status:		
Insurance coverage:		

VI. Level of Independence:
Rank as **1=Independent,** able to complete task; **2=Parent support** required for activity or task; **3=Dependent** on parents and others for activity or task

Task	Rating	Comments
Provides regular blood levels:	1 2 3	
Makes/keeps appointments:	1 2 3	
Understands basics of disorder:	1 2 3	
Understands basics of treatment:	1 2 3	
Success with self-management:	1 2 3	
Prepares meals:	1 2 3	
Monitors own medications:	1 2 3	
Does food shopping:	1 2 3	
Driver's license:	Yes/No	
Checking account:	1 2 3	

VII. General Assessment of Transition Readiness:

Completed Adolescent Autonomy Checklist?
Can describe plans for future?
Has met with Transition Team to develop Timeline?
Has met with Genetic Counselor to discuss adult PKU and reproductive issues?
Has met with Nutritionist to discuss adult PKU dietary management?

Cristine M. Trahms Program for Phenylketonuria
University of Washington - CHDD - Box 357920, Seattle, WA 98195

(206) 598-1800, Toll Free in Washington State 877-685-3015
http://depts.washington.edu/pku

Menstrual History Questionnaire

PKU & DHA Study

Date: _____
ID #: _____
FML: _____

Menstrual history

1. Have you ever had your period? Yes No
2. When did you first start getting your period?

   Age: _____ years
   Date: _____

3. Do you still get your period (i.e., pre/peri/post menopausal)? Yes No
4. What was the first day of your last period?

   Date: _____

5. How often do you get your period?
   a. Every _____ days/weeks/months/years
   b. Is your period regular? Yes No
   c. Have you ever gone for a time without getting your period (besides pregnancy)? Yes No
   d. If Yes, how long? _____ days/weeks/months/years
6. Are your periods painful? Yes No
   a. Do you ever take medication to reduce the pain? Yes No
   b. If yes, which medication(s)?
7. Have you ever been pregnant? Yes No
   a. # pregnancies: _____
   b. Last 3-trimester pregnancy: _____

Notes:

Randomization plan

Block randomization (using one block size):

1. Total number of participants to randomize: 52

2. Number of participants per block: 4

3. Total number of blocks: 13

4. Starting ID number: 101

5. Treatment groups: DHA or Placebo

- Randomization plan designed by student investigator
- Allocation sequence generated by CIS biostatistician
- Sequence given by the CIS biostatistician directly to the pharmacist at Investigational Drug Services for implementation

Chapter 11. Appendix: Results

Study 1

Table 48. Relationships between changes in cognitive processing speed, markers of metabolic control, and dietary intake in females with PKU attending a metabolic camp intervention

	△ Plasma Phe[a]		△Plasma Tyr[a]		△Energy intake[b]		△MF Protein intake[a]		△Phe intake[b]	
	r[c]	*p*[d]	*r*	*p*	*r*	*p*	*r*	*p*	*r*	*p*
△ Decision Speed	.082	.731	-.321	.167	.315	.189	-.091	.703	.292	.225
△ Pair Cancellation	-.333	.151	-.160	.501	.379	.109	.037	.878	-.003	.990
△ Plasma Phe					-.432	.065	-.232	.324	-.102	.677
△Plasma Tyr	-.035	.883			-.237	.329	-.107	.653	-.076	.757

Abbreviations: PKU, phenylketonuria; △, change; Phe, phenylalanine; Tyr, tyrosine; MF, medical food.

[a] $n = 20$.

[b] $n = 19$.

[c] Pearson's correlation coefficient.

[d] Two-tailed *p* value.

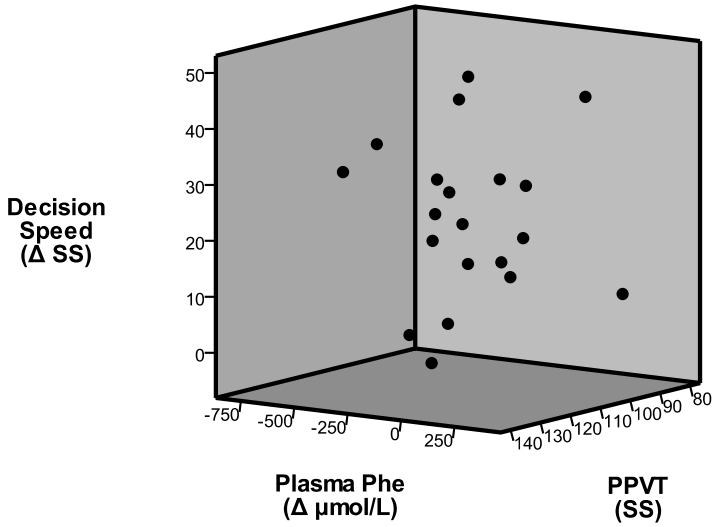
Table 49. Baseline and end-of-camp plasma Phe concentrations by change in intakes of energy and protein from medical food in females with PKU attending a metabolic camp intervention

	Increased intake			Decreased intake			Statistic
	n	Baseline	End-of-camp	n	Baseline	End-of-camp	
		Increased energy intake			Increased energy intake		
Plasma Phe, mean (SD), μmol/L	16	768[a] (311)	452 (199)	3	573 (164)	631 (186)	$F(1, 17) = 5.2$, $p = .036$
		Increased medical food protein intake			Increased medical food protein intake		
Plasma Phe, mean (SD), μmol/L	8	924 (320)	526 (209)	12	584 (200)	436 (196)	$F(1, 18) = 4.4$, $p = .051$

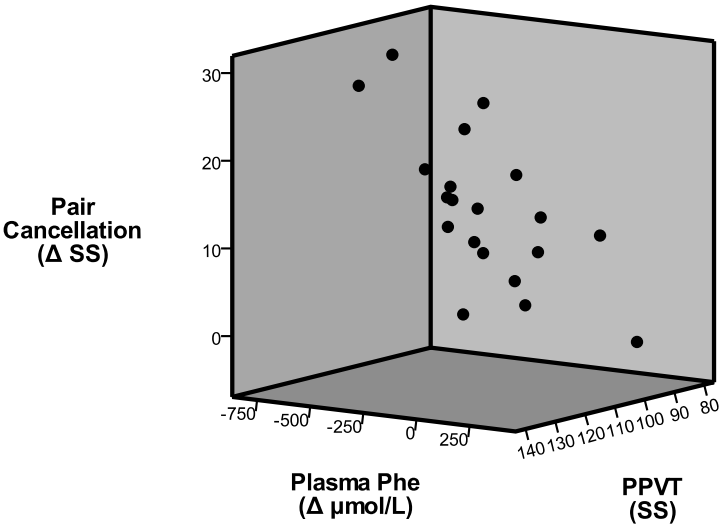
Abbreviations: Phe, phenylalanine; PKU, phenylketonuria.

[a]Plasma Phe (μmol/L).

Figure 7. The relationship between changes in plasma Phe concentration and performance on cognitive processing speed tasks



$n = 20$, $R = .091$, $p = .721$



$n = 20$, $R = -.604$, $p = .008$

Study 2

Table 50. Categories of plasma Phe and RBC DHA as predictors of verbal ability using linear regression

Model Summary[c]

Model	R	R Square	Adjusted R Square	Std. Error of the Estimate	Change Statistics				
					R Square Change	F Change	df1	df2	Sig. F Change
1	.420[a]	.177	.156	11.309	.177	8.377	1	39	.006
2	.519[b]	.270	.231	10.791	.093	4.833	1	38	.034

a. Predictors: (Constant), Phecat

b. Predictors: (Constant), Phecat, pctRBC_DHA_BLcat

c. Dependent Variable: PPVTSS

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B		Collinearity Statistics	
		B	Std. Error	Beta			Lower Bound	Upper Bound	Tolerance	VIF
1	(Constant)	105.842	2.594		40.795	.000	100.594	111.090		
	Phecat	-10.251	3.542	-.420	-2.894	.006	-17.415	-3.087	1.000	1.000
2	(Constant)	100.984	3.318		30.431	.000	94.266	107.702		
	Phecat	-8.190	3.507	-.336	-2.335	.025	-15.290	-1.090	.929	1.077
	pctRBC_DHA_BLcat	7.692	3.499	.316	2.198	.034	.609	14.775	.929	1.077

a. Dependent Variable: PPVTSS

Table 51. Categories of plasma Phe and RBC DHA as predictors of verbal ability using ANCOVA

Tests of Between-Subjects Effects

Dependent Variable:PPVTSS

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	1634.190[a]	2	817.095	7.017	.003	.270	14.034	.907
Intercept	413810.743	1	413810.743	3553.606	.000	.989	3553.606	1.000
Phecat	635.009	1	635.009	5.453	.025	.125	5.453	.624
pctRBC_DHA_BLcat	562.815	1	562.815	4.833	.034	.113	4.833	.573
Error	4425.029	38	116.448					
Total	418864.000	41						
Corrected Total	6059.220	40						

a. R Squared = .270 (Adjusted R Squared = .231)

b. Computed using alpha = .05

Parameter Estimates

Dependent Variable:PPVTSS

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval		Partial Eta Squared	Noncent. Parameter	Observed Power[a]
					Lower Bound	Upper Bound			
Intercept	100.486	3.202	31.385	.000	94.004	106.967	.963	31.385	1.000
[Phecat=.00]	8.190	3.507	2.335	.025	1.090	15.290	.125	2.335	.624
[Phecat=1.00]	0[b]	.	.	.	.	.	.	.	.
[pctRBC_DHA_BLcat=.00]	-7.692	3.499	-2.198	.034	-14.775	-.609	.113	2.198	.573
[pctRBC_DHA_BLcat=1.00]	0[b]	.	.	.	.	.	.	.	.

a. Computed using alpha = .05

b. This parameter is set to zero because it is redundant.

Coefficients[a]

Model		Unstandardized Coefficients		Standardized Coefficients	t	Sig.	95.0% Confidence Interval for B		Collinearity Statistics	
		B	Std. Error	Beta			Lower Bound	Upper Bound	Tolerance	VIF
1	(Constant)	100.984	3.318		30.431	.000	94.266	107.702		
	Phecat	-8.190	3.507	-.336	-2.335	.025	-15.290	-1.090	.929	1.077
	pctRBC_DHA_BLcat	7.692	3.499	.316	2.198	.034	.609	14.775	.929	1.077
2	(Constant)	92.506	6.690		13.827	.000	78.950	106.061		
	Phecat	-6.745	3.597	-.277	-1.875	.069	-14.034	.543	.858	1.166
	pctRBC_DHA_BLcat	6.650	3.523	.273	1.888	.067	-.488	13.787	.890	1.124
	mated2cat	5.260	3.621	.215	1.453	.155	-2.077	12.597	.855	1.170

a. Dependent Variable: PPVTSS

Table 52. Categories of plasma Phe and RBC DHA as predictors of verbal ability controlling for maternal education (categorical) using ANCOVA

Tests of Between-Subjects Effects

Dependent Variable: PPVTSS (verbal ability)

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	1872.970[a]	3	624.323	5.518	.003	.309	16.554	.915
Intercept	400435.779	1	400435.779	3539.236	.000	.990	3539.236	1.000
Plasma Phe cat	397.812	1	397.812	3.516	.069	.087	3.516	.447
RBC DHA cat	403.135	1	403.135	3.563	.067	.088	3.563	.452
Maternal education cat	238.780	1	238.780	2.110	.155	.054	2.110	.293
Error	4186.250	37	113.142					
Total	418864.000	41						
Corrected Total	6059.220	40						

a. R Squared = .309 (Adjusted R Squared = .253)

b. Computed using alpha = .05

Note: maternal education category may attenuate the relationship between plasma Phe, RBC DHA, and performance on a verbal ability task. A larger sample size would be needed to investigate these relationships with adequate statistical power.

Parameter Estimates

Dependent Variable:PPVTSS

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval		Partial Eta Squared	Noncent. Parameter	Observed Power[a]
					Lower Bound	Upper Bound			
Intercept	102.931	3.577	28.779	.000	95.684	110.178	.957	28.779	1.000
[Phecat=.00]	6.745	3.597	1.875	.069	-.543	14.034	.087	1.875	.447
[Phecat=1.00]	0[b]	.	.	.	.	.	.	.	.
[pctRBC_DHA_BLcat=.00]	-6.650	3.523	-1.888	.067	-13.787	.488	.088	1.888	.452
[pctRBC_DHA_BLcat=1.00]	0[b]	.	.	.	.	.	.	.	.
[mated2cat=1.00]	-5.260	3.621	-1.453	.155	-12.597	2.077	.054	1.453	.293
[mated2cat=2.00]	0[b]	.	.	.	.	.	.	.	.

a. Computed using alpha = .05

b. This parameter is set to zero because it is redundant.

Univariate Analysis of Variance

Descriptive Statistics

Dependent Variable:PPVTSS

Phe_DHA_cat	mated2cat	Mean	Std. Deviation	N
1.00	1.00	101.00	7.071	2
	2.00	110.30	12.876	10
	Total	108.75	12.381	12
2.00	1.00	99.00	14.000	3
	2.00	102.25	10.079	4
	Total	100.86	10.915	7
3.00	1.00	93.00	9.764	4
	2.00	107.75	9.032	4
	Total	100.38	11.747	8
4.00	1.00	93.44	6.948	9
	2.00	91.80	12.872	5
	Total	92.86	9.020	14
Total	1.00	95.11	8.649	18
	2.00	104.43	13.331	23
	Total	100.34	12.308	41

Tests of Between-Subjects Effects

Dependent Variable:PPVTSS

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	1873.120[a]	4	468.280	4.027	.008	.309	16.109	.870
Intercept	374291.264	1	374291.264	3218.864	.000	.989	3218.864	1.000
Phe_DHA_cat	995.330	3	331.777	2.853	.051	.192	8.560	.634
mated2cat	238.597	1	238.597	2.052	.161	.054	2.052	.286
Error	4186.100	36	116.281					
Total	418864.000	41						
Corrected Total	6059.220	40						

a. R Squared = .309 (Adjusted R Squared = .232)

b. Computed using alpha = .05

Parameter Estimates

Dependent Variable:PPVTSS

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval		Partial Eta Squared	Noncent. Parameter	Observed Power[a]
					Lower Bound	Upper Bound			
Intercept	96.244	3.728	25.818	.000	88.684	103.805	.949	25.818	1.000
[Phe_DHA_cat=1.00]	13.384	4.589	2.916	.006	4.076	22.692	.191	2.916	.810
[Phe_DHA_cat=2.00]	6.871	5.054	1.360	.182	-3.378	17.120	.049	1.360	.263
[Phe_DHA_cat=3.00]	6.765	4.808	1.407	.168	-2.986	16.516	.052	1.407	.278
[Phe_DHA_cat=4.00]	0[b]	.	.	.	.	.	.	.	.
[mated2cat=1.00]	-5.269	3.678	-1.432	.161	-12.728	2.191	.054	1.432	.286
[mated2cat=2.00]	0[b]	.	.	.	.	.	.	.	.

a. Computed using alpha = .05

b. This parameter is set to zero because it is redundant.

Descriptive Statistics

Dependent Variable:PPVTSS

Phe_DHA_cat	mated2cat	Mean	Std. Deviation	N
1.00	1.00	101.00	7.071	2
	2.00	110.30	12.876	10
	Total	108.75	12.381	12
4.00	1.00	93.44	6.948	9
	2.00	91.80	12.872	5
	Total	92.86	9.020	14
Total	1.00	94.82	7.278	11
	2.00	104.13	15.343	15
	Total	100.19	13.230	26

Tests of Between-Subjects Effects

Dependent Variable:PPVTSS

Source	Type III Sum of Squares	df	Mean Square	F	Sig.	Partial Eta Squared	Noncent. Parameter	Observed Power[b]
Corrected Model	1653.449[a]	2	826.725	6.984	.004	.378	13.968	.889
Intercept	249605.997	1	249605.997	2108.632	.000	.989	2108.632	1.000
Phe_DHA_cat	1102.781	1	1102.781	9.316	.006	.288	9.316	.832
mated2cat	21.375	1	21.375	.181	.675	.008	.181	.069
Error	2722.589	23	118.373					
Total	265377.000	26						
Corrected Total	4376.038	25						

a. R Squared = .378 (Adjusted R Squared = .324)

b. Computed using alpha = .05

Parameter Estimates

Dependent Variable:PPVTSS

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval		Partial Eta Squared	Noncent. Parameter	Observed Power[a]
					Lower Bound	Upper Bound			
Intercept	94.202	4.299	21.915	.000	85.310	103.095	.954	21.915	1.000
[Phe_DHA_cat=1.00]	14.896	4.880	3.052	.006	4.800	24.992	.288	3.052	.832
[Phe_DHA_cat=4.00]	0[b]	.	.	.	.	.	.	.	.
[mated2cat=1.00]	-2.093	4.925	-.425	.675	-12.280	8.095	.008	.425	.069
[mated2cat=2.00]	0[b]	.	.	.	.	.	.	.	.

a. Computed using alpha = .05

b. This parameter is set to zero because it is redundant.

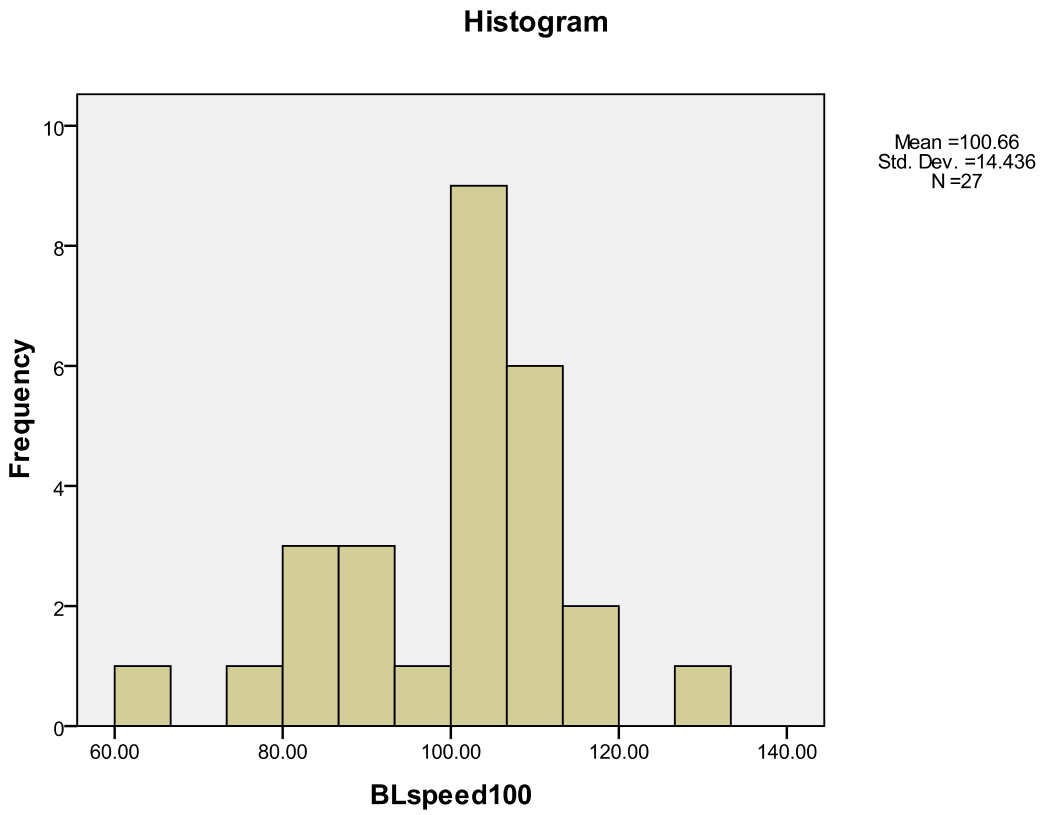
Study 3

Distribution of key outcome variables:

Baseline processing speed factor score

n=27

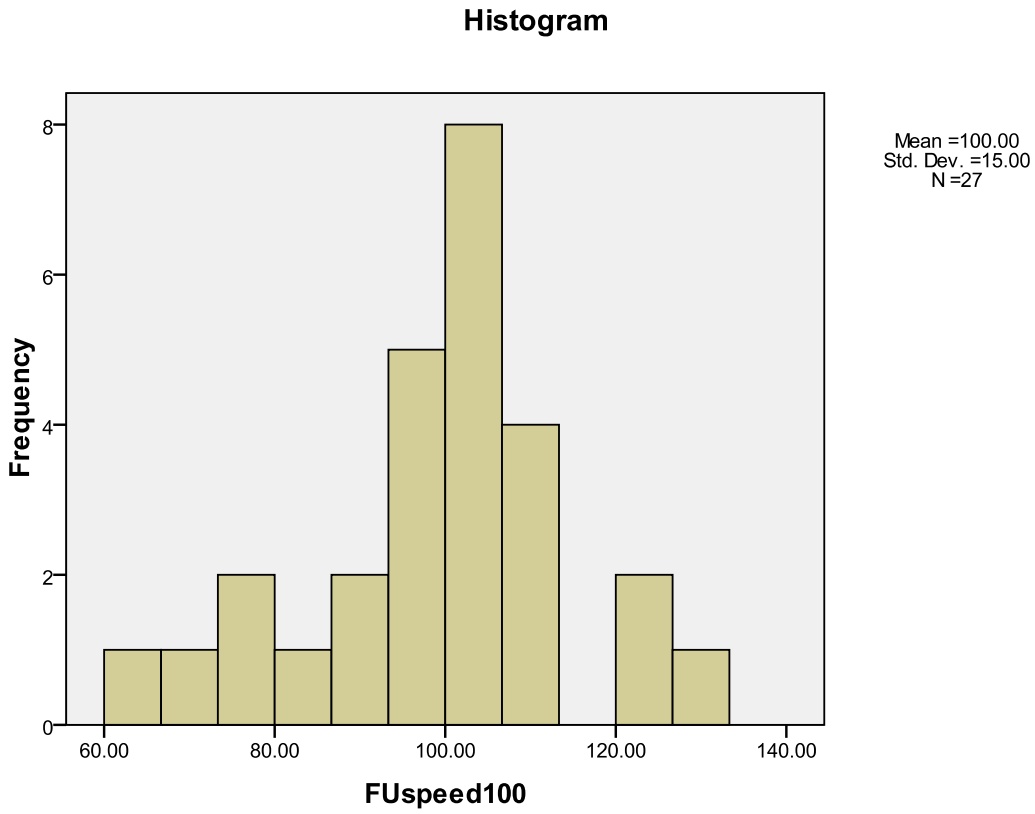
Shapiro-Wilk test: *P* = .282



Follow up processing speed factor score

n=27

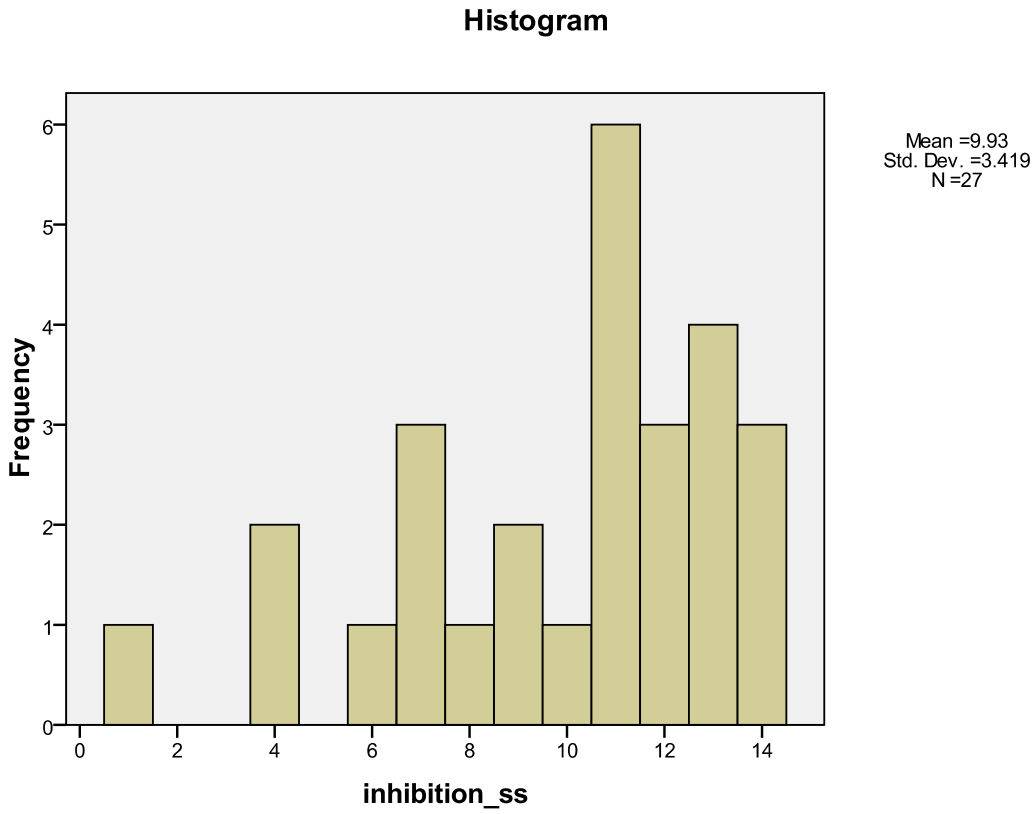
Shapiro-Wilk test: *P* = .326



Baseline cognitive inhibition score

n=27

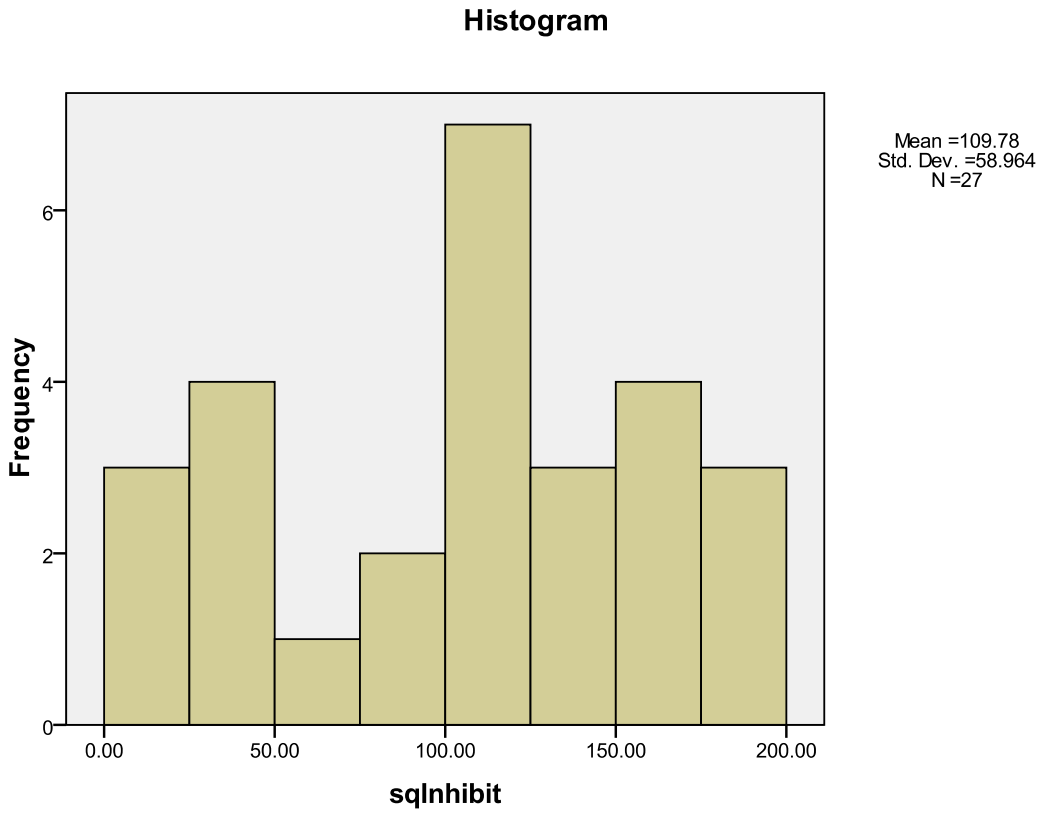
Shapiro-Wilk test: $P = .019$



Baseline cognitive inhibition score: squared

n=27

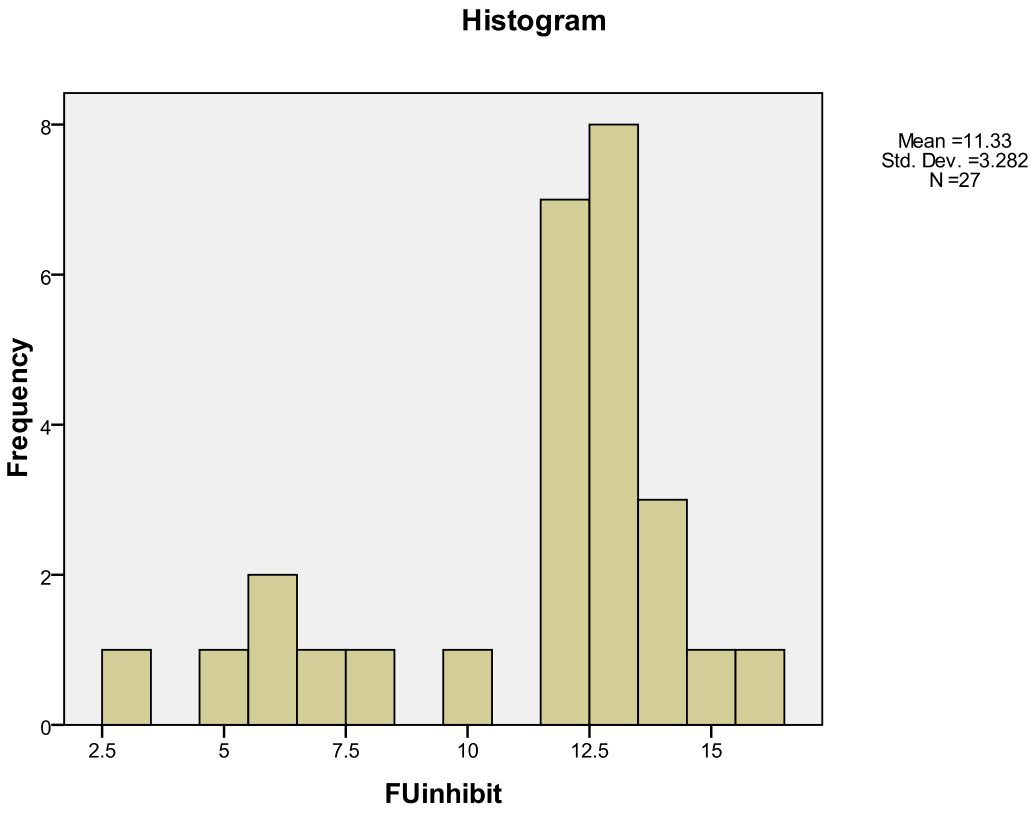
Shapiro-Wilk test: $P = .136$



Follow up cognitive inhibition score

n=27

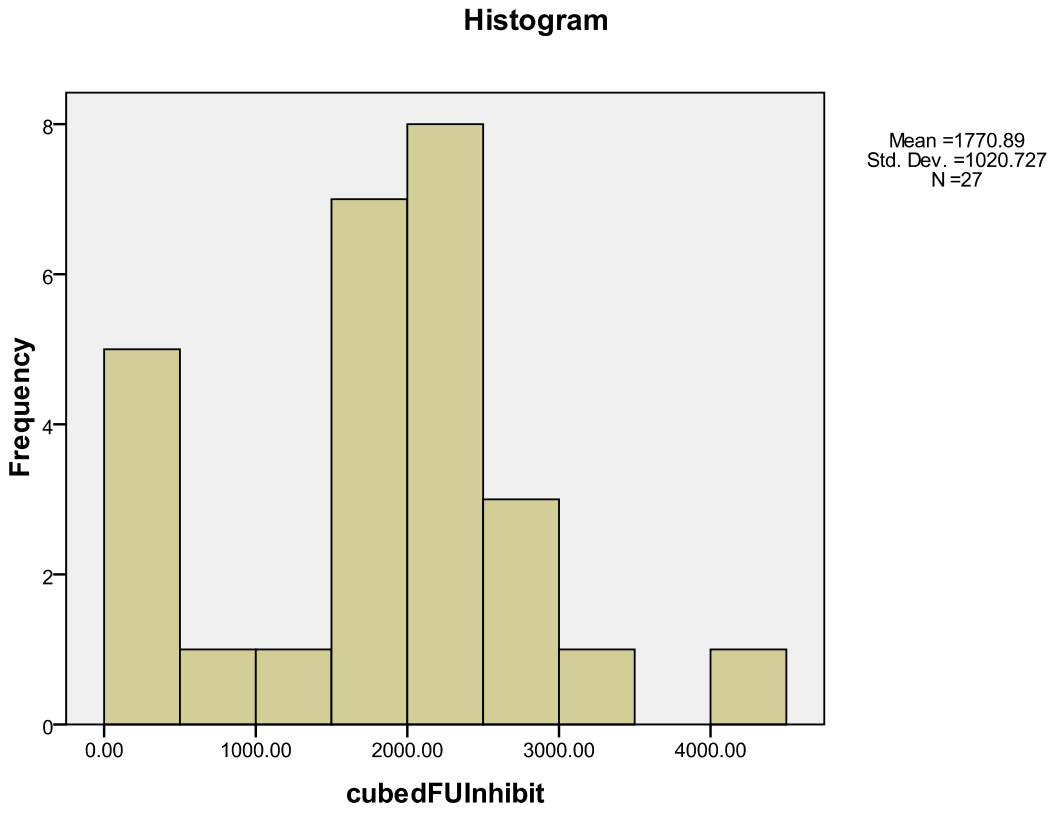
Shapiro-Wilk test: $P = .001$



Follow up cognitive inhibition score: cubed

n=27

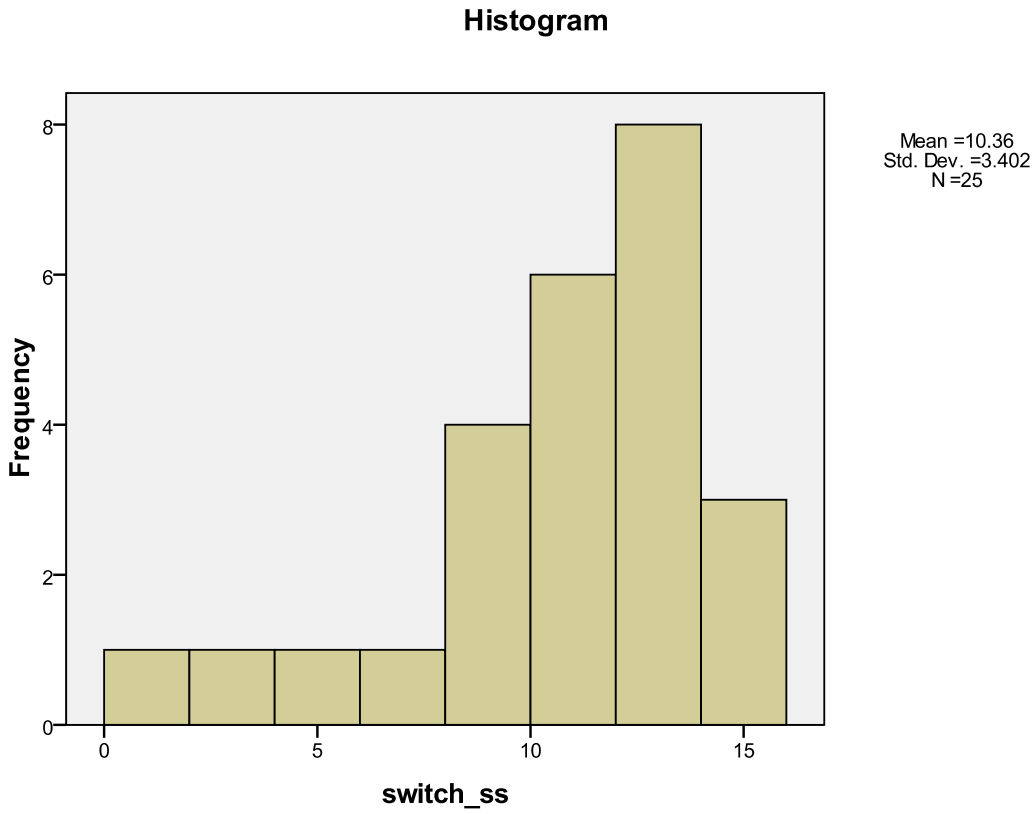
Shapiro-Wilk test: P = .041



Baseline cognitive flexibility score

n=25

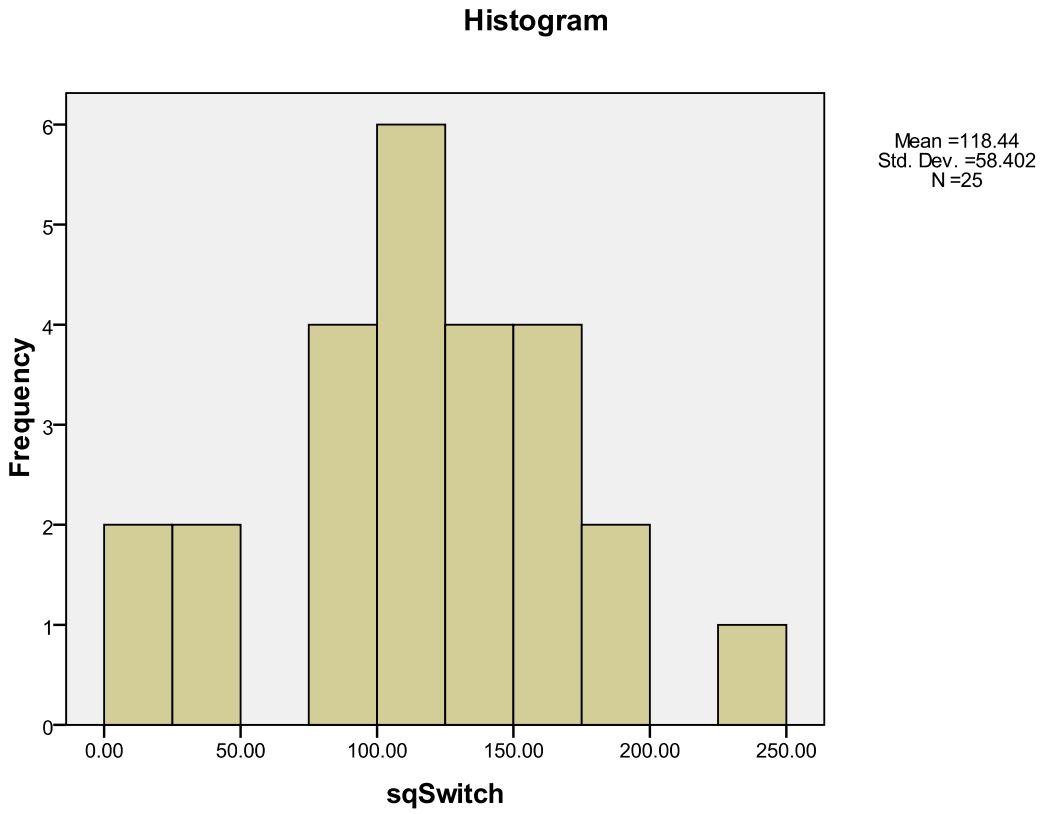
Shapiro-Wilk test: $P = .009$



Baseline cognitive flexibility score: squared

n=25

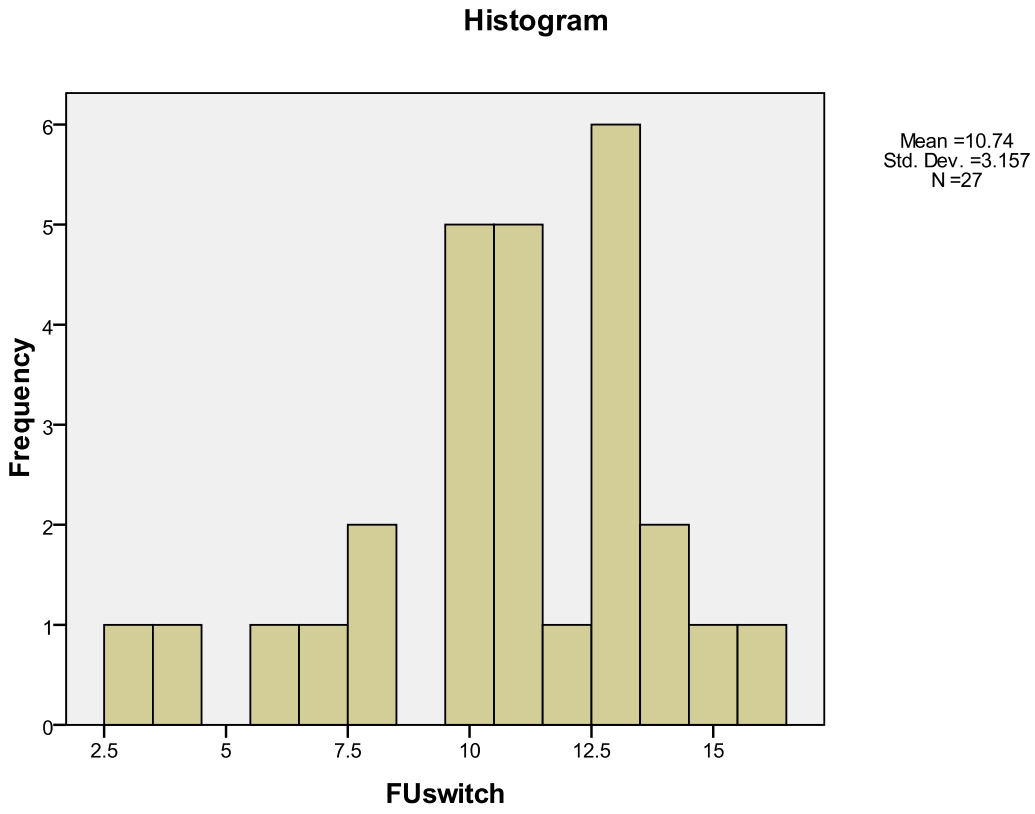
Shapiro-Wilk test: $P = .547$



Follow up cognitive flexibility score

n=25

Shapiro-Wilk test: $P = .107$



Intent to treat analyses using transformed variables

n=27

Parameter Estimates

Dependent Variable:cubedFUInhibit

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval		Partial Eta Squared	Noncent. Parameter	Observed Power[a]
					Lower	Upper			
Intercept	127.460	228.676	.557	.582	-344.504	599.425	.013	.557	.083
sqInhibit	15.086	1.744	8.648	.000	11.485	18.686	.757	8.648	1.000
[TxGrp_num=1]	-28.468	203.129	-.140	.890	-447.705	390.770	.001	.140	.052
[TxGrp_num=2]	0[b]	.	.	.	.	.	.	.	.

a. Computed using alpha = .05

b. This parameter is set to zero because it is redundant.

Parameter Estimates

Dependent Variable:FUswitch

Parameter	B	Std. Error	t	Sig.	95% Confidence Interval		Partial Eta Squared	Noncent. Parameter	Observed Power[a]
					Lower	Upper			
Intercept	5.459	.635	8.594	.000	4.142	6.776	.771	8.594	1.000
sqSwitch	.045	.005	9.429	.000	.035	.055	.802	9.429	1.000
[TxGrp_num=1]	.434	.557	.779	.444	-.721	1.590	.027	.779	.116
[TxGrp_num=2]	0[b]	.	.	.	.	.	.	.	.

a. Computed using alpha = .05

b. This parameter is set to zero because it is redundant.

Per protocol analyses using transformed variables

n=20

Parameter Estimates

Dependent Variable:cubedFUInhibit

Parameter	B	Std. Error	t	Sig.	95% CI		Partial Eta Squared	Noncent. Parameter	Observed Power[a]
					Lower	Upper			
Intercept	215.125	282.950	.760	.457	-381.848	812.098	.033	.760	.111
sqInhibit	14.231	2.049	6.946	.000	9.909	18.554	.739	6.946	1.000
[TxGrp_num=1]	47.641	257.877	.185	.856	-496.432	591.714	.002	.185	.053
[TxGrp_num=2]	0[b]	.	.	.	.	.	.	.	.

a. Computed using alpha = .05

b. This parameter is set to zero because it is redundant.

Parameter Estimates

Dependent Variable:FUswitch

Parameter	B	Std. Error	t	Sig.	95% CI		Partial Eta Squared	Noncent. Parameter	Observed Power[a]
					Lower	Upper			
Intercept	5.541	.834	6.643	.000	3.763	7.319	.746	6.643	1.000
sqSwitch	.045	.006	6.934	.000	.031	.058	.762	6.934	1.000
[TxGrp_num=1]	.552	.741	.744	.468	-1.028	2.131	.036	.744	.107
[TxGrp_num=2]	0[b]	.	.	.	.	.	.	.	.

a. Computed using alpha = .05

b. This parameter is set to zero because it is redundant.

Transformed vs. untransformed variable usage in ANCOVA for Study 3

ANCOVA is commonly used in the analysis of randomized controlled trials that have baseline measures assessed prior to randomization, and these same measures are repeated at follow up. Based on simulation studies, Vickers concluded ANCOVA may be preferred even when data are moderately skewed[245]. The preceding tables demonstrate the ANCOVA results using variables transformed to better approximate the normal distribution. The results do not differ from those using the untransformed variables; therefore, the untransformed variables were kept for the final report.

.

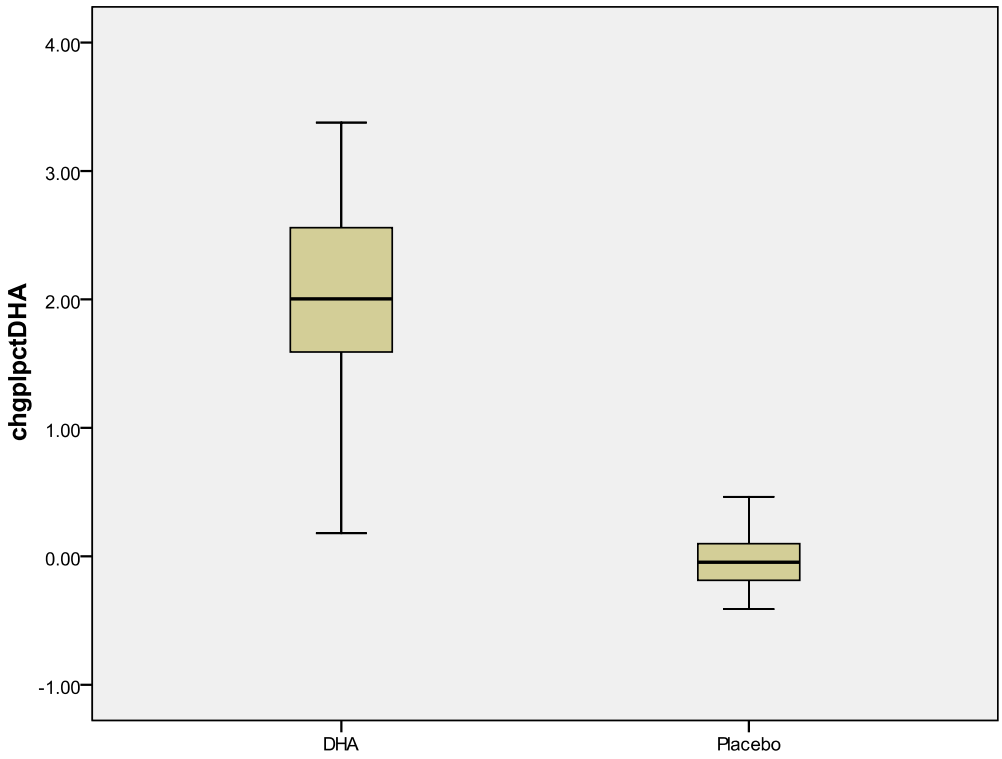
Table 53. Summary of baseline and follow up plasma and RBC total lipid % DHA for each participant categorized by treatment allocation (N=33)

DHA Group	Participant	% plasma DHA		% RBC DHA	
		Baseline	Follow up	Baseline	Follow up
Per protocol	1	1.89	3.52	4.10	6.73
	2	1.43	3.44	4.09	5.94
	3	1.32	3.95	2.68	7.04
	4	1.04	3.73	2.06	7.14
	5	1.03	2.11	2.37	3.88
	6	1.00	2.77	1.92	5.61
	7	.97	3.46	2.40	4.48
	8	.93	4.31	2.43	6.67
	9	.89	2.94	2.89	6.97
Intention to treat	10	1.54	3.10	3.24	5.33
	11	.75	.	1.91	.
	12	.75	.93	2.42	2.06
Withdrawn	13	1.16	.	3.05	.
	14	.95	.	1.58	.
	15	.95	.	1.61	.
	16	.94	.	2.25	.
	17	.61	.	1.21	.

Table 53 continued

Placebo Group	Participant	% plasma DHA		% RBC DHA	
		Baseline	Follow up	Baseline	Follow up
Per protocol	1	1.86	1.45	3.26	3.19
	2	1.52	1.27	3.39	3.04
	3	1.36	1.46	3.41	2.71
	4	1.15	.83	2.17	2.04
	5	.98	.79	3.82	3.08
	6	.96	.81	1.82	1.82
	7	.94	1.24	2.57	3.58
	8	.69	.54	1.65	1.67
	9	.66	.67	2.04	1.38
	10	.58	.53	1.13	1.49
	11	.	1.04	.	1.87
Intention to treat	12	1.25	1.34	2.52	2.68
	13	.82	.	2.36	.
	14	.70	1.14	1.46	1.74
	15	.46	.92	1.83	1.87
Withdrawn	16	.70	.	1.70	.

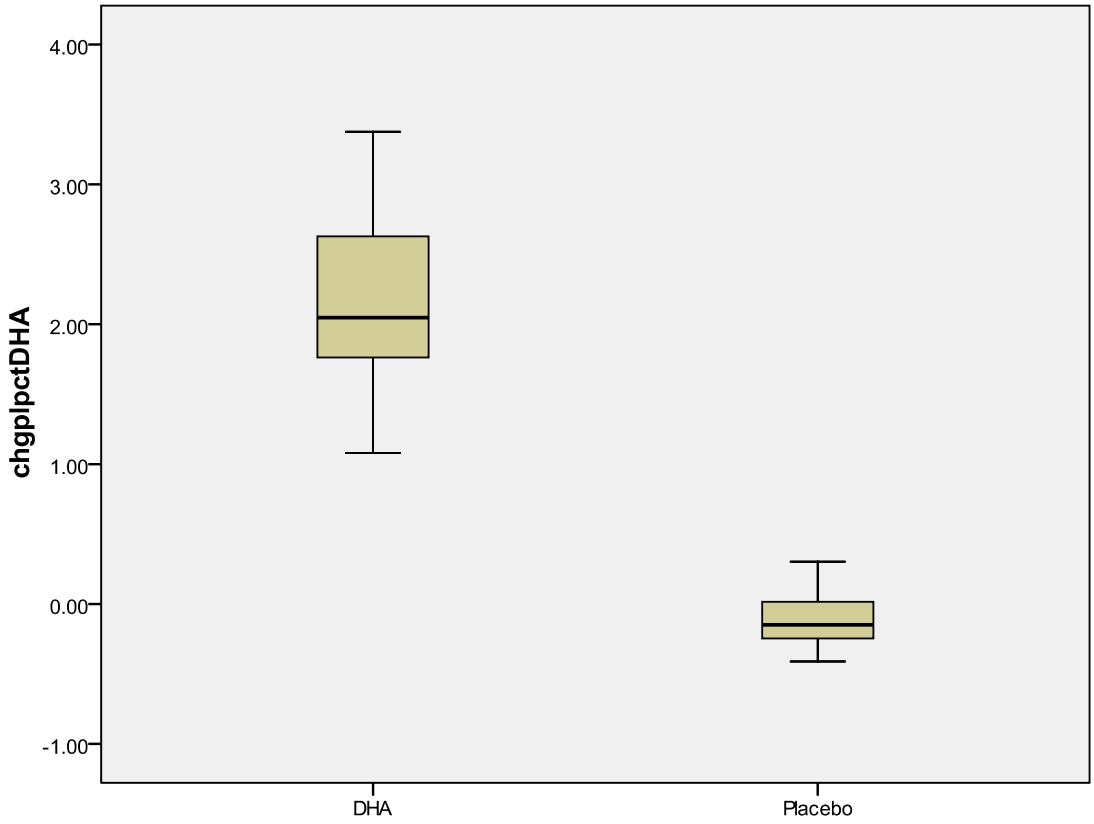
Figure 8. Change in plasma total lipid % DHA by treatment group: intention to treat



	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
DHA	11	1.9487	.86756	.26158	1.3659	2.5316	.18	3.38
Placebo	13	-.0071	.27875	.07731	-.1756	.1613	-.41	.46
Total	24	.8893	1.16567	.23794	.3971	1.3815	-.41	3.38

ANOVA	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	22.793	1	22.793	59.280	.000
Within Groups	8.459	22	.384		
Total	31.252	23			

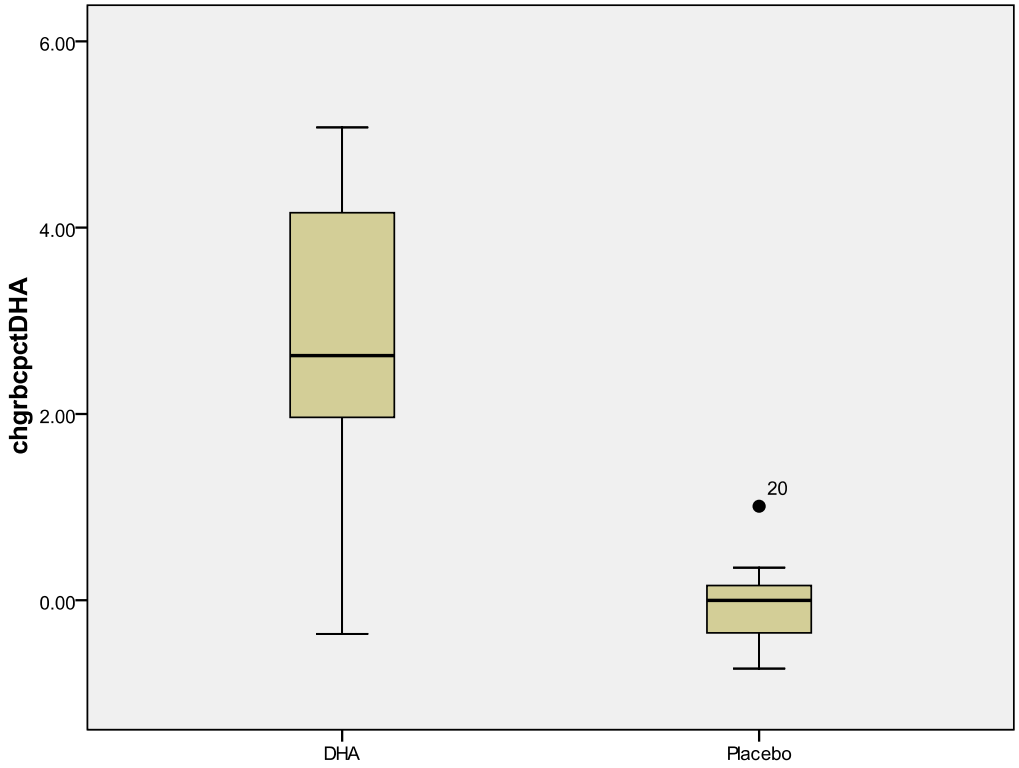
Figure 9. Change in plasma total lipid % DHA by treatment group: per protocol



	N	Mean	Std. Deviation	Std. Error	95% CI		Minimum	Maximum
					Lower Bound	Upper Bound		
DHA	9	2.1892	.68194	.22731	1.6650	2.7134	1.08	3.38
Placebo	10	-.1090	.20987	.06637	-.2591	.0411	-.41	.30
Total	19	.9796	1.27227	.29188	.3664	1.5928	-.41	3.38

ANOVA	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	25.019	1	25.019	103.318	.000
Within Groups	4.117	17	.242		
Total	29.136	18			

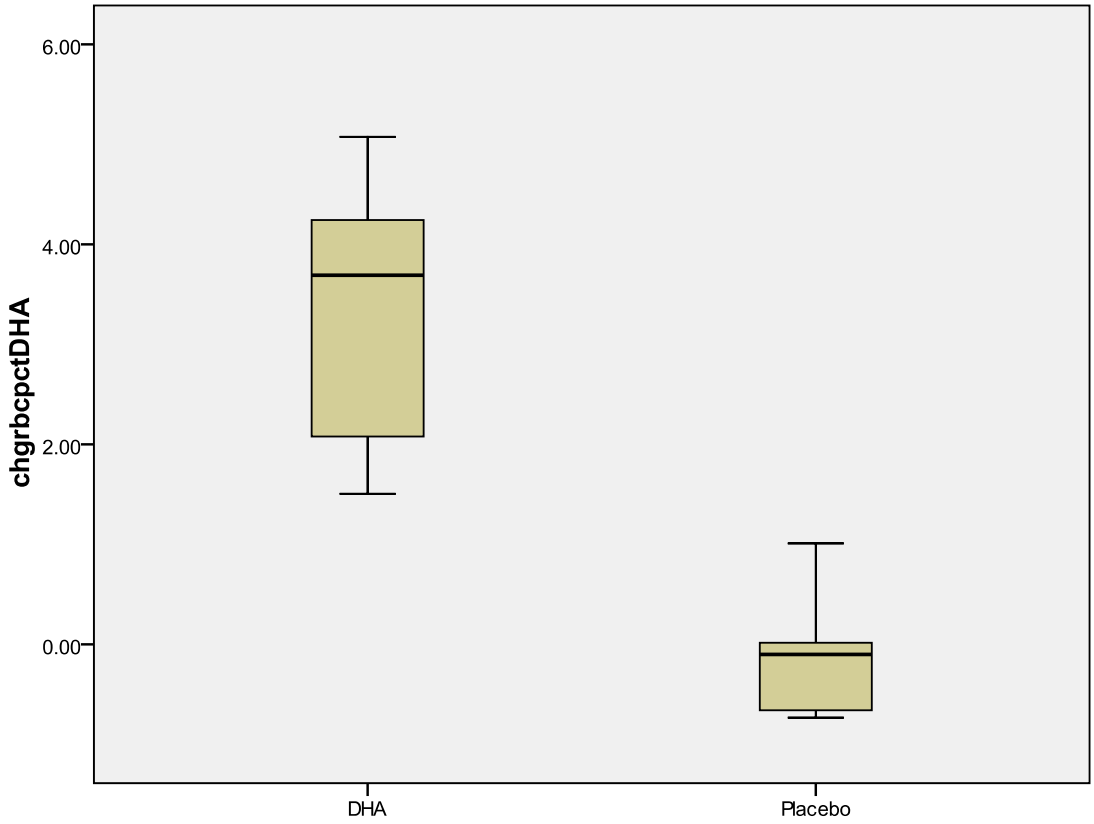
Figure 10. Change in RBC total lipid % DHA by treatment group: intention to treat



	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval for Mean		Minimum	Maximum
					Lower Bound	Upper Bound		
DHA	11	2.8403	1.60446	.48376	1.7625	3.9182	-.36	5.08
Placebo	13	-.0604	.48307	.13398	-.3523	.2315	-.73	1.01
Total	24	1.2691	1.84955	.37754	.4881	2.0501	-.73	5.08

ANOVA	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	50.136	1	50.136	38.643	.000
Within Groups	28.543	22	1.297		
Total	78.679	23			

Figure 11. Change in RBC total lipid % DHA by treatment group: per protocol



	N	Mean	Std. Deviation	Std. Error	95% Confidence Interval		Minimum	Maximum
					Lower Bound	Upper Bound		
DHA	9	3.2788	1.28513	.42838	2.2910	4.2667	1.50	5.08
Placebo	10	-.1269	.53554	.16935	-.5100	.2562	-.73	1.01
Total	19	1.4863	1.98238	.45479	.5308	2.4418	-.73	5.08

ANOVA	Sum of Squares	df	Mean Square	F	Sig.
Between Groups	54.943	1	54.943	59.140	.000
Within Groups	15.794	17	.929		
Total	70.737	18			

Table 54. Relationship between changes in measures of cognitive and DHA status in females with PKU

	Plasma DHA[a]			RBC DHA[a]		
	n	*r*[b]	*P*[c]	n	*r*	*P*
Processing Speed	24	.01	.96	24	-.02	.93
Inhibition	24	-.11	.61	24	.04	.84
Switching	22	-.01	.95	22	-.02	.95

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

[a] % DHA in total lipid.

[b] Pearson's correlation.

[c] Two-tailed *P* value.

Table 55. Relationship between changes in measures of cognitive and DHA status in females with PKU, controlling for plasma Phe

	Plasma DHA[a]			RBC DHA[a]		
	n	$R^{b,c}$	P^{d}	n	$R^{b,c}$	P^{d}
Processing Speed	24	-.02	.94	24	-.02	.92
Inhibition	24	-.11	.62	24	.04	.85
Switching	22	.01	.96	22	.05	.82

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

[a] % DHA in total lipid.

[b] Pearson's correlation.

[c] Controlling for change in plasma Phe.

[d] Two-tailed *P* value.

Table 56. Relationship between changes in measures of cognitive and DHA status in females with PKU, controlling for verbal ability

	Plasma DHA[a]			RBC DHA[a]		
	n	$R^{b,c}$	P^{d}	n	$R^{b,c}$	P^{d}
Processing Speed	24	-.01	.97	24	-.02	.93
Inhibition	24	-.10	.66	24	.02	.92
Switching	22	.01	.96	22	-.02	.95

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

[a] % DHA in total lipid.

[b] Pearson's correlation.

[c] Controlling for verbal ability assessed by performance on the Peabody Picture Vocabulary Test.

[d] Two-tailed *P* value.

Table 57. Post-hoc sample size calculation: estimated sample size needed to detect a significant difference between DHA and Placebo supplemented groups in measures of cognitive processing speed, inhibition, and flexibility at follow up utilizing ANCOVA

	Data Source	Cognitive Processing Speed	Cognitive Inhibition	Cognitive Flexibility
Difference	Per protocol analysis	0.14	0.25	0.49
$SD_{Y(a,b)}$	Standardized SD	15	3	3
$r^2_{xy(a,b)}$	Per protocol analysis	.88	.81	.84
Significance (alpha)		.05	.05	.05
Power (beta)		.80	.80	.80
Tailedness		2-tailed	2-tailed	2-tailed
Sample size per group[a]		853	426	95

Abbreviations: DHA, docosahexaenoic acid; ANCOVA, analysis of variance; SD, standard deviation.

[a] Estimated using the power analysis calculator in the web version of the Handbook of Biological Statistics (http://udel.edu/~mcdonald/statancova.html; accessed January 28, 2010)[246].

Chapter 12. Literature Cited

1. Pietz J, Schmidt E, Matthis P, Kobialka B, Kutscha A, de Sonneville L. EEGs in phenylketonuria. I: Follow-up to adulthood; II: Short-term diet-related changes in EEGs and cognitive function. *Dev Med Child Neurol.* Jan 1993;35(1):54-64.

2. Anderson VE, Siegel FS. Behavioral and biochemical correlates of diet change in phenylketonuria. *Pediatr Res.* Jan 1976;10(1):10-17.

3. Huijbregts SC, de Sonneville LM, Licht R, van Spronsen FJ, Sergeant JA. Short-term dietary interventions in children and adolescents with treated phenylketonuria: effects on neuropsychological outcome of a well-controlled population. *J Inherit Metab Dis.* Oct 2002;25(6):419-430.

4. Schmidt E, Rupp A, Burgard P, Pietz J, Weglage J, de Sonneville L. Sustained attention in adult phenylketonuria: the influence of the concurrent phenylalanine-blood-level. *J Clin Exp Neuropsychol.* Oct 1994;16(5):681-688.

5. Krause W, Halminski M, McDonald L, et al. Biochemical and neuropsychological effects of elevated plasma phenylalanine in patients with treated phenylketonuria. A model for the study of phenylalanine and brain function in man. *J Clin Invest.* Jan 1985;75(1):40-48.

6. Moseley K, Koch R, Moser AB. Lipid status and long-chain polyunsaturated fatty acid concentrations in adults and adolescents with phenylketonuria on phenylalanine-restricted diet. *J Inherit Metab Dis.* Feb 2002;25(1):56-64.

7. van Gool CJ, van Houwelingen AC, Hornstra G. The essential fatty acid status in phenylketonuria patients under treatment. *J Nutr Biochem.* Nov 2000;11(11-12):543-547.

8. Agostoni C, Massetto N, Biasucci G, et al. Effects of long-chain polyunsaturated fatty acid supplementation on fatty acid status and visual function in treated children with hyperphenylalaninemia. *J Pediatr.* Oct 2000;137(4):504-509.

9. Beblo S, Reinhardt H, Muntau AC, Mueller-Felber W, Roscher AA, Koletzko B. Fish oil supplementation improves visual evoked potentials in children with phenylketonuria. *Neurology.* Oct 23 2001;57(8):1488-1491.

10. Agostoni C, Harvie A, McCulloch DL, et al. A randomized trial of long-chain polyunsaturated fatty acid supplementation in infants with phenylketonuria. *Dev Med Child Neurol.* Mar 2006;48(3):207-212.

11. Beblo S, Reinhardt H, Demmelmair H, Muntau AC, Koletzko B. Effect of fish oil supplementation on fatty acid status, coordination, and fine motor skills in children with phenylketonuria. *J Pediatr.* May 2007;150(5):479-484.

12. Sun Q, Ma J, Campos H, Hankinson SE, Hu FB. Comparison between plasma and erythrocyte fatty acid content as biomarkers of fatty acid intake in US women. *Am J Clin Nutr.* Jul 2007;86(1):74-81.

13. Tjonneland A, Overvad K, Thorling E, Ewertz M. Adipose tissue fatty acids as biomarkers of dietary exposure in Danish men and women. *Am J Clin Nutr.* May 1993;57(5):629-633.

14. Harris WS, Pottala JV, Sands SA, Jones PG. Comparison of the effects of fish and fish-oil capsules on the n 3 fatty acid content of blood cells and plasma phospholipids. *Am J Clin Nutr.* Dec 2007;86(6):1621-1625.

15. Cao J, Schwichtenberg KA, Hanson NQ, Tsai MY. Incorporation and clearance of omega-3 fatty acids in erythrocyte membranes and plasma phospholipids. *Clin Chem.* Dec 2006;52(12):2265-2272.

16. Katan MB, Deslypere JP, van Birgelen AP, Penders M, Zegwaard M. Kinetics of the incorporation of dietary fatty acids into serum cholesteryl esters, erythrocyte membranes, and adipose tissue: an 18-month controlled study. *J Lipid Res.* Oct 1997;38(10):2012-2022.

17. Nelson GJ, Schmidt PS, Bartolini GL, Kelley DS, Kyle D. The effect of dietary docosahexaenoic acid on platelet function, platelet fatty acid composition, and blood coagulation in humans. *Lipids.* Nov 1997;32(11):1129-1136.

18. Agostoni C, Riva E, Biasucci G, et al. The effects of n-3 and n-6 polyunsaturated fatty acids on plasma lipids and fatty acids of treated phenylketonuric children. *Prostaglandins Leukot Essent Fatty Acids.* Dec 1995;53(6):401-404.

19. Innis SM, de La Presa Owens S. Dietary fatty acid composition in pregnancy alters neurite membrane fatty acids and dopamine in newborn rat brain. *J Nutr.* Jan 2001;131(1):118-122.

20. de la Presa Owens S, Innis SM. Docosahexaenoic and arachidonic acid prevent a decrease in dopaminergic and serotoninergic neurotransmitters in frontal cortex caused by a linoleic and alpha-linolenic acid deficient diet in formula-fed piglets. *J Nutr.* Nov 1999;129(11):2088-2093.

21. Chung WL, Chen JJ, Su HM. Fish oil supplementation of control and (n-3) fatty acid-deficient male rats enhances reference and working memory performance and increases brain regional docosahexaenoic acid levels. *J Nutr.* Jun 2008;138(6):1165-1171.

22. Levant B, Ozias MK, Carlson SE. Diet (n-3) polyunsaturated fatty acid content and parity interact to alter maternal rat brain phospholipid fatty acid composition. *J Nutr.* Aug 2006;136(8):2236-2242.

23. Levant B, Ozias MK, Jones KA, Carlson SE. Differential effects of modulation of docosahexaenoic acid content during development in specific regions of rat brain. *Lipids.* May 2006;41(5):407-414.

24. DeMar JC, Jr., Ma K, Bell JM, Rapoport SI. Half-lives of docosahexaenoic acid in rat brain phospholipids are prolonged by 15 weeks of nutritional deprivation of n-3 polyunsaturated fatty acids. *J Neurochem.* Dec 2004;91(5):1125-1137.

25. Connor WE, Neuringer M, Lin DS. Dietary effects on brain fatty acid composition: the reversibility of n-3 fatty acid deficiency and turnover of docosahexaenoic acid in the brain, erythrocytes, and plasma of rhesus monkeys. *J Lipid Res.* Feb 1990;31(2):237-247.

26. Huang MC, Brenna JT, Chao AC, Tschanz C, Diersen-Schade DA, Hung HC. Differential Tissue Dose Responses of (n-3) and (n-6) PUFA in Neonatal Piglets Fed Docosahexaenoate and Arachidonoate. *J Nutr.* Sep 2007;137(9):2049-2055.

27. Carver JD, Benford VJ, Han B, Cantor AB. The relationship between age and the fatty acid composition of cerebral cortex and erythrocytes in human subjects. *Brain Res Bull.* Sep 15 2001;56(2):79-85.

28. Ward GR, Huang YS, Bobik E, et al. Long-chain polyunsaturated fatty acid levels in formulae influence deposition of docosahexaenoic acid and arachidonic acid in brain and red blood cells of artificially reared neonatal rats. *J Nutr.* Dec 1998;128(12):2473-2487.

29. Carlson SE, Carver JD, House SG. High fat diets varying in ratios of polyunsaturated to saturated fatty acid and linoleic to linolenic acid: a comparison of rat neural and red cell membrane phospholipids. *J Nutr.* May 1986;116(5):718-725.

30. Whalley LJ, Fox HC, Wahle KW, Starr JM, Deary IJ. Cognitive aging, childhood intelligence, and the use of food supplements: possible involvement of n-3 fatty acids. *Am J Clin Nutr.* Dec 2004;80(6):1650-1657.

31. Heude B, Ducimetiere P, Berr C. Cognitive decline and fatty acid composition of erythrocyte membranes--The EVA Study. *Am J Clin Nutr.* Apr 2003;77(4):803-808.

32. Kotani S, Sakaguchi E, Warashina S, et al. Dietary supplementation of arachidonic and docosahexaenoic acids improves cognitive dysfunction. *Neurosci Res.* Oct 2006;56(2):159-164.

33. Kaye CI, Accurso F, La Franchi S, et al. Newborn screening fact sheets. *Pediatrics.* Sep 2006;118(3):e934-963.

34. National Institutes of Health Consensus Development Panel. National Institutes of Health Consensus Development Conference Statement: phenylketonuria: screening and management, October 16-18, 2000. *Pediatrics.* Oct 2001;108(4):972-982.

35. Paine RS. The variability in manifestations of untreated patients with phenylketonuria (phenylpyruvic aciduria). *Pediatrics.* Aug 1957;20(2):290-302.

36. Bickel H, Gerrard J, Hickmans EM. Influence of phenylalanine intake on phenylketonuria. *Lancet.* Oct 17 1953;265(6790):812-813.

37. Curtius HC, Vollmin JA, Baerlocher K. The use of deuterated phenylalanine for the elucidation of the phenylalanine-tyrosine metabolism. *Clin Chim Acta.* Mar 1972;37:277-285.

38. Scriver CR, Kaufman S. Hyperphenylalaninemia: phenylalanine hydroxylase deficiency. In: Scriver CR, Beaudet AL, Sly WS, Valle D, eds. *The Metabolic & Molecular Bases of Inherited Disease*. 8 ed. New York: McGraw-Hill; 2001:1667-1724.

39. Institute of Medicine. *Dietary Reference Intakes for Energy, Carbohydrate, Fiber, Fat, Fatty Acids, Cholesterol, Protein, and Amino Acids (Macronutrients).* Washington, D.C.: National Academies Press;2002.

40. Guldberg P, Rey F, Zschocke J, et al. A European multicenter study of phenylalanine hydroxylase deficiency: classification of 105 mutations and a general system for genotype-based prediction of metabolic phenotype. *Am J Hum Genet.* Jul 1998;63(1):71-79.

41. Cristine M. Trahms Program for Phenylketonuria. PKU Food Bull's Eye. http://depts.washington.edu/pku/PDFs2/PKUFoodTarget.pdf. Accessed February 11, 2010.

42. Schulz B, Bremer HJ. Nutrient intake and food consumption of adolescents and young adults with phenylketonuria. *Acta Paediatr.* Jul 1995;84(7):743-748.

43. Hanley WB, Feigenbaum A, Clarke JT, Schoonheyt W, Austin V. Vitamin B12 deficiency in adolescents and young adults with phenylketonuria. *Lancet.* Oct 16 1993;342(8877):997.

44. Gropper SS, Acosta PB, Clarke-Sheehan N, Wenz E, Cheng M, Koch R. Trace element status of children with PKU and normal children. *J Am Diet Assoc.* Apr 1988;88(4):459-465.

45. MacDonald A. Diet and compliance in phenylketonuria. *Eur J Pediatr.* Oct 2000;159 Suppl 2:S136-141.

46. Lyman FL, Lyman JK. Dietary management of phenylketonuria with lofenalac. *Arch Pediatr.* May 1960;77:212-220.

47. Poge AP, Baumann K, Muller E, Leichsenring M, Schmidt H, Bremer HJ. Long-chain polyunsaturated fatty acids in plasma and erythrocyte membrane lipids of children with phenylketonuria after controlled linoleic acid intake. *J Inherit Metab Dis.* Jun 1998;21(4):373-381.

48. Acosta PB, Yannicelli S, Singh R, et al. Intake and blood levels of fatty acids in treated patients with phenylketonuria. *J Pediatr Gastroenterol Nutr.* Sep 2001;33(3):253-259.

49. Greve LC, Wheeler MD, Green-Burgeson DK, Zorn EM. Breast-feeding in the management of the newborn with phenylketonuria: a practical approach to dietary therapy. *J Am Diet Assoc.* Mar 1994;94(3):305-309.

50. New infant formula additives approved by FDA. *AAP News.* 2002;20(5):209-210.

51. Neuringer M, Connor WE, Lin DS, Barstad L, Luck S. Biochemical and functional effects of prenatal and postnatal omega 3 fatty acid deficiency on retina

and brain in rhesus monkeys. *Proc Natl Acad Sci U S A.* Jun 1986;83(11):4021-4025.

52. Kim HY. Novel metabolism of docosahexaenoic acid in neural cells. *J Biol Chem.* Jun 29 2007;282(26):18661-18665.

53. McNamara RK, Carlson SE. Role of omega-3 fatty acids in brain development and function: potential implications for the pathogenesis and prevention of psychopathology. *Prostaglandins Leukot Essent Fatty Acids.* Oct-Nov 2006;75(4-5):329-349.

54. Brenna JT, Diau GY. The influence of dietary docosahexaenoic acid and arachidonic acid on central nervous system polyunsaturated fatty acid composition. *Prostaglandins Leukot Essent Fatty Acids.* Nov-Dec 2007;77(5-6):247-250.

55. Umhau JC, Zhou W, Carson RE, et al. Imaging incorporation of circulating docosahexaenoic acid into the human brain using positron emission tomography. *J Lipid Res.* Jul 2009;50(7):1259-1268.

56. Brenna JT. Efficiency of conversion of alpha-linolenic acid to long chain n-3 fatty acids in man. *Curr Opin Clin Nutr Metab Care.* Mar 2002;5(2):127-132.

57. Pawlosky RJ, Hibbeln JR, Novotny JA, Salem N, Jr. Physiological compartmental analysis of alpha-linolenic acid metabolism in adult humans. *J Lipid Res.* Aug 2001;42(8):1257-1265.

58. Innis SM. Essential fatty acids in growth and development. *Prog Lipid Res.* 1991;30(1):39-103.

59. Arbuckle LD, MacKinnon MJ, Innis SM. Formula 18:2(n-6) and 18:3(n-3) content and ratio influence long-chain polyunsaturated fatty acids in the developing piglet liver and central nervous system. *J Nutr.* Feb 1994;124(2):289-298.

60. Liou YA, King DJ, Zibrik D, Innis SM. Decreasing linoleic acid with constant alpha-linolenic acid in dietary fats increases (n-3) eicosapentaenoic acid in plasma phospholipids in healthy men. *J Nutr.* Apr 2007;137(4):945-952.

61. Blank C, Neumann MA, Makrides M, Gibson RA. Optimizing DHA levels in piglets by lowering the linoleic acid to alpha-linolenic acid ratio. *J Lipid Res.* Sep 2002;43(9):1537-1543.

62. Cleary MA, Feillet F, White FJ, et al. Randomised controlled trial of essential fatty acid supplementation in phenylketonuria. *Eur J Clin Nutr.* Jul 2006;60(7):915-920.

63. Francois CA, Connor SL, Bolewicz LC, Connor WE. Supplementing lactating women with flaxseed oil does not increase docosahexaenoic acid in their milk. *Am J Clin Nutr.* Jan 2003;77(1):226-233.

64. Harper CR, Edwards MJ, DeFilipis AP, Jacobson TA. Flaxseed oil increases the plasma concentrations of cardioprotective (n-3) fatty acids in humans. *J Nutr.* Jan 2006;136(1):83-87.

65. de Groot RH, Hornstra G, van Houwelingen AC, Roumen F. Effect of alpha-linolenic acid supplementation during pregnancy on maternal and neonatal polyunsaturated fatty acid status and pregnancy outcome. *Am J Clin Nutr.* Feb 2004;79(2):251-260.

66. Brenna JT, Salem N, Jr., Sinclair AJ, Cunnane SC. alpha-Linolenic acid supplementation and conversion to n-3 long-chain polyunsaturated fatty acids in humans. *Prostaglandins Leukot Essent Fatty Acids.* Feb-Mar 2009;80(2-3):85-91.

67. Brown AJ, Pang E, Roberts DC. Persistent changes in the fatty acid composition of erythrocyte membranes after moderate intake of n-3 polyunsaturated fatty acids: study design implications. *Am J Clin Nutr.* Oct 1991;54(4):668-673.

68. Innis SM. Plasma and red blood cell fatty acid values as indexes of essential fatty acids in the developing organs of infants fed with milk or formulas. *J Pediatr.* Apr 1992;120(4 Pt 2):S78-86.

69. Lamptey MS, Walker BL. A possible essential role for dietary linolenic acid in the development of the young rat. *J Nutr.* Jan 1976;106(1):86-93.

70. Yamamoto N, Saitoh M, Moriuchi A, Nomura M, Okuyama H. Effect of dietary alpha-linolenate/linoleate balance on brain lipid compositions and learning ability of rats. *J Lipid Res.* Feb 1987;28(2):144-151.

71. Yamamoto N, Hashimoto A, Takemoto Y, et al. Effect of the dietary alpha-linolenate/linoleate balance on lipid compositions and learning ability of rats. II. Discrimination process, extinction process, and glycolipid compositions. *J Lipid Res.* Aug 1988;29(8):1013-1021.

72. Suzuki H, Park SJ, Tamura M, Ando S. Effect of the long-term feeding of dietary lipids on the learning ability, fatty acid composition of brain stem phospholipids and synaptic membrane fluidity in adult mice: a comparison of sardine oil diet with palm oil diet. *Mech Ageing Dev.* Mar 16 1998;101(1-2):119-128.

73. Martinez M. Tissue levels of polyunsaturated fatty acids during early human development. *J Pediatr.* Apr 1992;120(4 Pt 2):S129-138.

74. Hanebutt FL, Demmelmair H, Schiessl B, Larque E, Koletzko B. Long-chain polyunsaturated fatty acid (LC-PUFA) transfer across the placenta. *Clin Nutr.* Oct 2008;27(5):685-693.

75. Dunstan JA, Simmer K, Dixon G, Prescott SL. Cognitive assessment of children at age 2(1/2) years after maternal fish oil supplementation in pregnancy: a randomised controlled trial. *Arch Dis Child Fetal Neonatal Ed.* Jan 2008;93(1):F45-50.

76. Agostoni C, Marangoni F, Stival G, et al. Whole blood fatty acid composition differs in term versus mildly preterm infants: small versus matched appropriate for gestational age. *Pediatr Res.* Sep 2008;64(3):298-302.

77. Diau GY, Hsieh AT, Sarkadi-Nagy EA, Wijendran V, Nathanielsz PW, Brenna JT. The influence of long chain polyunsaturate supplementation on docosahexaenoic acid and arachidonic acid in baboon neonate central nervous system. *BMC Med.* 2005;3:11.

78. Tanaka K, Kon N, Ohkawa N, Yoshikawa N, Shimizu T. Does breastfeeding in the neonatal period influence the cognitive function of very-low-birth-weight infants at 5 years of age? *Brain Dev.* Apr 2009;31(4):288-293.

79. Henriksen C, Haugholt K, Lindgren M, et al. Improved cognitive development among preterm infants attributable to early supplementation of human milk with docosahexaenoic acid and arachidonic acid. *Pediatrics.* Jun 2008;121(6):1137-1145.

80. Smithers LG, Collins CT, Simmonds LA, Gibson RA, McPhee A, Makrides M. Feeding preterm infants milk with a higher dose of docosahexaenoic acid than that used in current practice does not influence language or behavior in early childhood: a follow-up study of a randomized controlled trial. *Am J Clin Nutr.* Mar 2010;91(3):628-634.

81. Makrides M, Neumann MA, Byard RW, Simmer K, Gibson RA. Fatty acid composition of brain, retina, and erythrocytes in breast- and formula-fed infants. *Am J Clin Nutr.* Aug 1994;60(2):189-194.

82. Koletzko B, Lien E, Agostoni C, et al. The roles of long-chain polyunsaturated fatty acids in pregnancy, lactation and infancy: review of current knowledge and consensus recommendations. *J Perinat Med.* 2008;36(1):5-14.

83. Kris-Etherton PM, Innis S, American Dietetic Association, Dietitians of Canada. Position of the American Dietetic Association and Dietitians of Canada: dietary fatty acids. *J Am Diet Assoc.* Sep 2007;107(9):1599-1611.

84. Simopoulos AP, Leaf A, Salem N, Jr. Workshop on the Essentiality of and Recommended Dietary Intakes for Omega-6 and Omega-3 Fatty Acids. *J Am Coll Nutr.* Oct 1999;18(5):487-489.

85. McCann JC, Ames BN. Is docosahexaenoic acid, an n-3 long-chain polyunsaturated fatty acid, required for development of normal brain function? An overview of evidence from cognitive and behavioral tests in humans and animals. *Am J Clin Nutr.* Aug 2005;82(2):281-295.

86. Birch EE, Garfield S, Castaneda Y, Hughbanks-Wheaton D, Uauy R, Hoffman D. Visual acuity and cognitive outcomes at 4 years of age in a double-blind,

randomized trial of long-chain polyunsaturated fatty acid-supplemented infant formula. *Early Hum Dev.* May 2007;83(5):279-284.

87. Auestad N, Scott DT, Janowsky JS, et al. Visual, cognitive, and language assessments at 39 months: a follow-up study of children fed formulas containing long-chain polyunsaturated fatty acids to 1 year of age. *Pediatrics.* Sep 2003;112(3 Pt 1):e177-183.

88. Morley R. Nutrition and cognitive development. *Nutrition.* Oct 1998;14(10):752-754.

89. Cheatham CL, Colombo J, Carlson SE. N-3 fatty acids and cognitive and visual acuity development: methodologic and conceptual considerations. *Am J Clin Nutr.* Jun 2006;83(6 Suppl):1458S-1466S.

90. Zhang J, Hebert JR, Muldoon MF. Dietary fat intake is associated with psychosocial and cognitive functioning of school-aged children in the United States. *J Nutr.* Aug 2005;135(8):1967-1973.

91. Ryan AS, Nelson EB. Assessing the effect of docosahexaenoic acid on cognitive functions in healthy, preschool children: a randomized, placebo-controlled, double-blind study. *Clin Pediatr (Phila).* May 2008;47(4):355-362.

92. Hamazaki K, Syafruddin D, Tunru IS, et al. The effects of docosahexaenoic acid-rich fish oil on behavior, school attendance rate and malaria infection in school children - a double-blind, randomized, placebo-controlled trial in Lampung, Indonesia. *Asia Pac J Clin Nutr.* 2008;17(2):258-263.

93. Martinez M. Developmental profiles of polyunsaturated fatty acids in the brain of normal infants and patients with peroxisomal diseases: severe deficiency of

docosahexaenoic acid in Zellweger's and pseudo-Zellweger's syndromes. *World Rev Nutr Diet.* 1991;66:87-102.

94. Martinez M, Vazquez E, Garcia-Silva MT, et al. Therapeutic effects of docosahexaenoic acid ethyl ester in patients with generalized peroxisomal disorders. *Am J Clin Nutr.* Jan 2000;71(1 Suppl):376S-385S.

95. Al MD, van Houwelingen AC, Kester AD, Hasaart TH, de Jong AE, Hornstra G. Maternal essential fatty acid patterns during normal pregnancy and their relationship to the neonatal essential fatty acid status. *Br J Nutr.* Jul 1995;74(1):55-68.

96. Otto SJ, van Houwelingen AC, Badart-Smook A, Hornstra G. Comparison of the peripartum and postpartum phospholipid polyunsaturated fatty acid profiles of lactating and nonlactating women. *Am J Clin Nutr.* Jun 2001;73(6):1074-1079.

97. de Groot RH, Adam J, Jolles J, Hornstra. Alpha-linolenic acid supplementation during human pregnancy does not effect cognitive functioning. *Prostaglandins Leukot Essent Fatty Acids.* Jan 2004;70(1):41-47.

98. de Groot RH, Hornstra G, Jolles J. Exploratory study into the relation between plasma phospholipid fatty acid status and cognitive performance. *Prostaglandins Leukot Essent Fatty Acids.* Mar 2007;76(3):165-172.

99. Llorente AM, Jensen CL, Voigt RG, Fraley JK, Berretta MC, Heird WC. Effect of maternal docosahexaenoic acid supplementation on postpartum depression and information processing. *Am J Obstet Gynecol.* May 2003;188(5):1348-1353.

100. O'Connor DL. Long chain polyunsaturated fatty acids and phenylketonuria (symposium). Paper presented at: Genetic Metabolic Dietitians International Conference: Bridging Science and Clinical Practice; April 25, 2008; Atlanta, GA.

101. Manzato E, Roselli della Rovere G, Zambon S, et al. Cognitive functions are not affected by dietary fatty acids in elderly subjects in the Pro.V.A. study population. *Aging Clin Exp Res.* Feb 2003;15(1):83-86.

102. van de Rest O, Geleijnse JM, Kok FJ, et al. Effect of fish oil on cognitive performance in older subjects: a randomized, controlled trial. *Neurology.* Aug 5 2008;71(6):430-438.

103. Wisdom NM, Callahan JL, Hawkins KA. The effects of apolipoprotein E on non-impaired cognitive functioning: A meta-analysis. *Neurobiol Aging.* Mar 12 2009.

104. Jiang L, Shi Y, Long Y, Yang Z. The influence of orally administered docosahexaenoic acid on monoamine neurotransmitter, nerve growth factor, and brain-derived neurotrophic factor in aged mice. *Pharmaceutical Biology (Formerly International Journal of Pharmacognosy).* 2009;47:584-591.

105. Morrow JD, Frei B, Longmire AW, et al. Increase in circulating products of lipid peroxidation (F2-isoprostanes) in smokers. Smoking as a cause of oxidative damage. *N Engl J Med.* May 4 1995;332(18):1198-1203.

106. Reilly M, Delanty N, Lawson JA, FitzGerald GA. Modulation of oxidant stress in vivo in chronic cigarette smokers. *Circulation.* Jul 1 1996;94(1):19-25.

107. Simon JA, Fong J, Bernert JT, Jr., Browner WS. Relation of smoking and alcohol consumption to serum fatty acids. *Am J Epidemiol.* Aug 15 1996;144(4):325-334.

108. Hibbeln JR, Makino KK, Martin CE, Dickerson F, Boronow J, Fenton WS. Smoking, gender, and dietary influences on erythrocyte essential fatty acid composition among patients with schizophrenia or schizoaffective disorder. *Biol Psychiatry.* Mar 1 2003;53(5):431-441.

109. Agostoni C, Riva E, Giovannini M, et al. Maternal smoking habits are associated with differences in infants' long-chain polyunsaturated fatty acids in whole blood: a case-control study. *Arch Dis Child.* May 2008;93(5):414-418.

110. Pawlosky RJ, Hibbeln JR, Salem N, Jr. Compartmental analyses of plasma n-3 essential fatty acids among male and female smokers and nonsmokers. *J Lipid Res.* Apr 2007;48(4):935-943.

111. Amitai N, Markou A. Chronic nicotine improves cognitive performance in a test of attention but does not attenuate cognitive disruption induced by repeated phencyclidine administration. *Psychopharmacology (Berl).* Jan 2009;202(1-3):275-286.

112. Levant B, Ozias MK, Carlson SE. Diet (n-3) polyunsaturated fatty acid content and parity affect liver and erythrocyte phospholipid fatty acid composition in female rats. *J Nutr.* Nov 2007;137(11):2425-2430.

113. Bonham MP, Duffy EM, Wallace JM, et al. Habitual fish consumption does not prevent a decrease in LCPUFA status in pregnant women (the Seychelles Child Development Nutrition Study). *Prostaglandins Leukot Essent Fatty Acids.* Jun 2008;78(6):343-350.

114. Henry JD, Rendell PG. A review of the impact of pregnancy on memory function. *J Clin Exp Neuropsychol.* Nov 2007;29(8):793-803.

115. Burdge G. Alpha-linolenic acid metabolism in men and women: nutritional and biological implications. *Curr Opin Clin Nutr Metab Care.* Mar 2004;7(2):137-144.

116. Giltay EJ, Gooren LJ, Toorians AW, Katan MB, Zock PL. Docosahexaenoic acid concentrations are higher in women than in men because of estrogenic effects. *Am J Clin Nutr.* Nov 2004;80(5):1167-1174.

117. Farage MA, Osborn TW, MacLean AB. Cognitive, sensory, and emotional changes associated with the menstrual cycle: a review. *Arch Gynecol Obstet.* Oct 2008;278(4):299-307.

118. Cohen BE, Garg SK, Ali S, Harris WS, Whooley MA. Red blood cell docosahexaenoic acid and eicosapentaenoic acid concentrations are positively associated with socioeconomic status in patients with established coronary artery disease: data from the Heart and Soul Study. *J Nutr.* Jun 2008;138(6):1135-1140.

119. Infante JP, Huszagh VA. Impaired arachidonic (20:4n-6) and docosahexaenoic (22:6n-3) acid synthesis by phenylalanine metabolites as etiological factors in the neuropathology of phenylketonuria. *Mol Genet Metab.* Mar 2001;72(3):185-198.

120. Rosell MS, Lloyd-Wright Z, Appleby PN, Sanders TA, Allen NE, Key TJ. Long-chain n-3 polyunsaturated fatty acids in plasma in British meat-eating, vegetarian, and vegan men. *Am J Clin Nutr.* Aug 2005;82(2):327-334.

121. Geppert J, Kraft V, Demmelmair H, Koletzko B. Docosahexaenoic acid supplementation in vegetarians effectively increases omega-3 index: a randomized trial. *Lipids.* Aug 2005;40(8):807-814.

122. Agren JJ, Tormala ML, Nenonen MT, Hanninen OO. Fatty acid composition of erythrocyte, platelet, and serum lipids in strict vegans. *Lipids.* Apr 1995;30(4):365-369.

123. Sanders TA, Ellis FR, Dickerson JW. Studies of vegans: the fatty acid composition of plasma choline phosphoglycerides, erythrocytes, adipose tissue, and breast milk, and some indicators of susceptibility to ischemic heart disease in vegans and omnivore controls. *Am J Clin Nutr.* May 1978;31(5):805-813.

124. Dunn LM, Dunn LM. *Examiner's Manual for the Peabody Picture Vocabulary Test--Third Edition (PPVT-III)*. Circle Pines, MN: American Guidance Service; 1997.

125. Colombo J. *Infant cognition : predicting later intellectual functioning*. Newbury Park, Calif.: Sage Publications; 1993.

126. Schrank FA, McGrew KS, Woodcock RW. *Technical Abstract (Assessment Service Bulletin No. 2).* Itasca: Riverside Publishing;2001.

127. Delis DC, Kaplan E, Kramer JH. *Delis-Kaplan Executive Function System: Technical Manual*. San Antonio, TX: The Psychological Corporation; 2001.

128. USDA National Nutrient Database for Standard Reference, Release 22. U.S. Department of Agriculture, Agricultural Research Service; 2009. http://www.ars.usda.gov/ba/bhnrc/ndl. Accessed March 9, 2010.

129. Prevention of neural tube defects: results of the Medical Research Council Vitamin Study. MRC Vitamin Study Research Group. *Lancet.* Jul 20 1991;338(8760):131-137.

130. de Lau LM, Refsum H, Smith AD, Johnston C, Breteler MM. Plasma folate concentration and cognitive performance: Rotterdam Scan Study. *Am J Clin Nutr.* Sep 2007;86(3):728-734.

131. Durga J, van Boxtel MP, Schouten EG, et al. Effect of 3-year folic acid supplementation on cognitive function in older adults in the FACIT trial: a randomised, double blind, controlled trial. *Lancet.* Jan 20 2007;369(9557):208-216.

132. Morris MS, Jacques PF, Rosenberg IH, Selhub J. Folate and vitamin B-12 status in relation to anemia, macrocytosis, and cognitive impairment in older Americans in the age of folic acid fortification. *Am J Clin Nutr.* Jan 2007;85(1):193-200.

133. Bryan J, Osendarp S, Hughes D, Calvaresi E, Baghurst K, van Klinken JW. Nutrients for cognitive development in school-aged children. *Nutr Rev.* Aug 2004;62(8):295-306.

134. Troen AM, Chao WH, Crivello NA, et al. Cognitive impairment in folate-deficient rats corresponds to depleted brain phosphatidylcholine and is prevented by dietary methionine without lowering plasma homocysteine. *J Nutr.* Dec 2008;138(12):2502-2509.

135. Pennington JAT. *Bowes & Church's Food Values of Portions Commonly Used*. 17th ed. Baltimore: Lippincott Williams & Wilkins; 1998.

136. de Benoist B, Andersson M, Egli I, Takkouche B, Allen H, eds. *Iodine status worldwide*. Geneva: World Health Organization; 2004.

137. Delange F. The role of iodine in brain development. *Proc Nutr Soc.* Feb 2000;59(1):75-79.

138. Rivas M, Naranjo JR. Thyroid hormones, learning and memory. *Genes Brain Behav.* Jun 2007;6 Suppl 1:40-44.

139. Khedr E, Hamed SA, Elbeih E, El-Shereef H, Ahmad Y, Ahmed S. Iron states and cognitive abilities in young adults: neuropsychological and neurophysiological assessment. *Eur Arch Psychiatry Clin Neurosci.* Dec 2008;258(8):489-496.

140. Todorich B, Pasquini JM, Garcia CI, Paez PM, Connor JR. Oligodendrocytes and myelination: the role of iron. *Glia.* Apr 1 2009;57(5):467-478.

141. Georgieff MK. Nutrition and the developing brain: nutrient priorities and measurement. *Am J Clin Nutr.* Feb 2007;85(2):614S-620S.

142. Gibson RS. *Principles of Nutritional Assessment*. 2nd ed. New York: Oxford University Press; 2005.

143. Hoeksma M, Van Rijn M, Verkerk PH, et al. The intake of total protein, natural protein and protein substitute and growth of height and head circumference in Dutch infants with phenylketonuria. *J Inherit Metab Dis.* 2005;28(6):845-854.

144. Graham SM, Arvela OM, Wise GA. Long-term neurologic consequences of nutritional vitamin B12 deficiency in infants. *J Pediatr.* Nov 1992;121(5 Pt 1):710-714.

145. Louwman MW, van Dusseldorp M, van de Vijver FJ, et al. Signs of impaired cognitive function in adolescents with marginal cobalamin status. *Am J Clin Nutr.* Sep 2000;72(3):762-769.

146. Groff JL, Gropper SS. *Advanced Nutrition and Human Metabolism*. 3rd ed. Belmont: Wadsworth/Thomson Learning; 2000.

147. Savage DG, Lindenbaum J. Neurological complications of acquired cobalamin deficiency: clinical aspects. *Baillieres Clin Haematol.* Sep 1995;8(3):657-678.

148. Leklem JE. Vitamin B_6. In: Shils ME, Olson JA, Shike M, Ross AC, eds. *Modern Nutrition in Health and Disease*. 9th ed. Baltimore: Lippincott Williams & Wilkins; 1999:413-421.

149. Elias MF, Robbins MA, Budge MM, et al. Homocysteine, folate, and vitamins B6 and B12 blood levels in relation to cognitive performance: the Maine-Syracuse study. *Psychosom Med.* Jul-Aug 2006;68(4):547-554.

150. Malouf R, Grimley Evans J. The effect of vitamin B6 on cognition. *Cochrane Database Syst Rev.* 2003(4):CD004393.

151. Dibley MJ. Zinc. In: Bowman BA, Russell RM, eds. *Present Knowledge in Nutrition*. 8th ed. Washington, DC: International Life Sciences Institute; 2001:329-343.

152. Bhatnagar S, Taneja S. Zinc and cognitive development. *Br J Nutr.* May 2001;85 Suppl 2:S139-145.

153. Sandstead HH, Penland JG, Alcock NW, et al. Effects of repletion with zinc and other micronutrients on neuropsychologic performance and growth of Chinese children. *Am J Clin Nutr.* Aug 1998;68(2 Suppl):470S-475S.

154. Cuajungco MP, Lees GJ. Zinc metabolism in the brain: relevance to human neurodegenerative disorders. *Neurobiol Dis.* 1997;4(3-4):137-169.

155. Maylor EA, Simpson EE, Secker DL, et al. Effects of zinc supplementation on cognitive function in healthy middle-aged and older adults: the ZENITH study. *Br J Nutr.* Oct 2006;96(4):752-760.

156. Hanley WB, Linsao L, Davidson W, Moes CA. Malnutrition with early treatment of phenylketonuria. *Pediatr Res.* Jul 1970;4(4):318-327.

157. Williamson ML, Koch R, Azen C, Chang C. Correlates of intelligence test results in treated phenylketonuric children. *Pediatrics.* Aug 1981;68(2):161-167.

158. Fisch RO, Chang PN, Weisberg S, Guldberg P, Guttler F, Tsai MY. Phenylketonuric patients decades after diet. *J Inherit Metab Dis.* 1995;18(3):347-353.

159. Koff E, Kammerer B, Boyle P, Pueschel SM. Intelligence and phenylketonuria: effects of diet termination. *J Pediatr.* Apr 1979;94(4):534-537.

160. Azen CG, Koch R, Friedman EG, et al. Intellectual development in 12-year-old children treated for phenylketonuria. *Am J Dis Child.* Jan 1991;145(1):35-39.

161. Holtzman NA, Kronmal RA, van Doorninck W, Azen C, Koch R. Effect of age at loss of dietary control on intellectual performance and behavior of children with phenylketonuria. *N Engl J Med.* Mar 6 1986;314(10):593-598.

162. Burgard P, Rey F, Rupp A, Abadie V, Rey J. Neuropsychologic functions of early treated patients with phenylketonuria, on and off diet: results of a cross-national and cross-sectional study. *Pediatr Res.* Mar 1997;41(3):368-374.

163. Moyle JJ, Fox AM, Bynevelt M, Arthur M, Burnett JR. A neuropsychological profile of off-diet adults with phenylketonuria. *J Clin Exp Neuropsychol.* May 2007;29(4):436-441.

164. Moyle JJ, Fox AM, Arthur M, Bynevelt M, Burnett JR. Meta-Analysis of Neuropsychological Symptoms of Adolescents and Adults with PKU. *Neuropsychol Rev.* Jun 2007;17(2):91-101.

165. Feldmann R, Denecke J, Grenzebach M, Weglage J. Frontal lobe-dependent functions in treated phenylketonuria: blood phenylalanine concentrations and long-term deficits in adolescents and young adults. *J Inherit Metab Dis.* 2005;28(4):445-455.

166. White DA, Nortz MJ, Mandernach T, Huntington K, Steiner RD. Age-related working memory impairments in children with prefrontal dysfunction associated with phenylketonuria. *J Int Neuropsychol Soc.* Jan 2002;8(1):1-11.

167. White DA, Nortz MJ, Mandernach T, Huntington K, Steiner RD. Deficits in memory strategy use related to prefrontal dysfunction during early development: evidence from children with phenylketonuria. *Neuropsychology.* Apr 2001;15(2):221-229.

168. Araujo GC, Christ SE, Steiner RD, et al. Response monitoring in children with phenylketonuria. *Neuropsychology.* Jan 2009;23(1):130-134.

169. Diamond A, Prevor MB, Callender G, Druin DP. Prefrontal cortex cognitive deficits in children treated early and continuously for PKU. *Monogr Soc Res Child Dev.* 1997;62(4):i-v, 1-208.

170. Joseph B, Dyer CA. Relationship between myelin production and dopamine synthesis in the PKU mouse brain. *J Neurochem.* Aug 2003;86(3):615-626.

171. Shefer S, Tint GS, Jean-Guillaume D, et al. Is there a relationship between 3-hydroxy-3-methylglutaryl coenzyme a reductase activity and forebrain pathology in the PKU mouse? *J Neurosci Res.* Sep 1 2000;61(5):549-563.

172. Land JM, Clark JB. Effect of phenylpyruvate on enzymes involved in fatty acid synthesis in rat brain. *Biochem J.* Jun 1973;134(2):545-555.

173. Walter JH, White F, Wraith JE, Jenkins JP, Wilson BP. Complete reversal of moderate/severe brain MRI abnormalities in a patient with classical phenylketonuria. *J Inherit Metab Dis.* Jul 1997;20(3):367-369.

174. Anderson PJ, Wood SJ, Francis DE, et al. Neuropsychological functioning in children with early-treated phenylketonuria: impact of white matter abnormalities. *Dev Med Child Neurol.* Apr 2004;46(4):230-238.

175. Singh RH, Kable JA, Guerrero NV, Sullivan KM, Elsas LJ, 2nd. Impact of a camp experience on phenylalanine levels, knowledge, attitudes, and health beliefs relevant to nutrition management of phenylketonuria in adolescent girls. *J Am Diet Assoc.* Jul 2000;100(7):797-803.

176. Leatham Yi SH, Kennedy MJ, Singh RH. Nutrient compensation in adolescent and adult females with phenylketonuria (poster). *Genetic Metabolic Dietitians International*. Atlanta, GA, 2006.

177. Leatham Yi SH, Kennedy MJ, Singh RH. Nutrient adequacy & compensation in females with phenylketonuria (talk). *SERGG Annual Meeting*. Atlanta, GA, 2006.

178. Yi SHL, Moser AB, Singh RH. Relationship between medical food type consumption and plasma polyunsaturated fatty acid status of females of childbearing age with phenylketonuria (poster). *Experimental Biology 2007*. Washington, D.C., 2007.

179. Yi SHL, Moser AB, Singh RH. The assessment of omega-3 fatty acid status using 3-day diet records of childbearing age with phenylketonuria (poster). *SERGG Annual Meeting*. New Orleans, LA, 2007.

180. Ramos E, Alfonso VC, Schermerhorn SM. Graduate students' administration and scoring errors on the Woodcock-Johnson III Tests of Cognitive Abilities. *Psychology in the Schools.* 2009;46(7):650-657.

181. McGrew KS, Woodcock RW. *Woodcock-Johnson III Technical Manual*. Itasca, IL: The Riverside Publishing Company; 2001.

182. Stroop JR. Studies of interference in serial verbal reactions. *J Exp Psychol.* 1935;18(6):643-662.

183. VanZutphen KH, Packman W, Sporri L, et al. Executive functioning in children and adolescents with phenylketonuria. *Clin Genet.* Jul 2007;72(1):13-18.

184. Slocum RH, Cummings A. Amino acid analysis of physiological samples. In: Hommos F, ed. *Techniques in Diagnostic Human Biochemical Genetics*. New York: Wiley Liss; 1991.

185. Gregory CO, Yu C, Singh RH. Blood phenylalanine monitoring for dietary compliance among patients with phenylketonuria: comparison of methods. *Genet Med.* Nov 2007;9(11):761-765.

186. NIST Chemistry WebBook. 2008. http://webbook.nist.gov. Accessed January 16, 2010.

187. Lagerstedt SA, Hinrichs DR, Batt SM, Magera MJ, Rinaldo P, McConnell JP. Quantitative determination of plasma c8-c26 total fatty acids for the biochemical diagnosis of nutritional and metabolic disorders. *Mol Genet Metab.* May 2001;73(1):38-45.

188. Steinberg S, Jones R, Tiffany C, Moser A. Investigational methods for peroxisomal disorders. *Curr Protoc Hum Genet.* Jul 2008;Chapter 17:Unit 17 16.

189. Willett W. *Nutritional Epidemiology*. 2nd ed. New York: Oxford University Press; 1998.

190. ESHA_Research. The Food Processor SQL. http://www.esha.com/foodprosql. Accessed December 24, 2007.

191. CDC. Behavioral Risk Factor Surveillance System Survey Questionnaire. Atlanta, Georgia: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention; 2005.

192. CDC. Youth Risk Behavior Surveillance System Survey Questionnaire. Atlanta, Georgia: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention; 2005.

193. CDC. Youth Risk Behavior Surveillance System Survey Questionnaire: Middle School Questionnaire. Atlanta, Georgia: U.S. Department of Health and Human Services, Centers for Disease Control and Prevention; 2005.

194. U.S. Census Bureau, Housing and Household Economic Statistics Division. Poverty Thresholds. http://www.census.gov/hhes/www/poverty/threshld.html. Accessed March 11, 2010.

195. Arterburn LM, Oken HA, Hoffman JP, et al. Bioequivalence of Docosahexaenoic Acid from Different Algal Oils in Capsules and in a DHA-Fortified Food. *Lipids.* Nov 2007;42(11):1011-1024.

196. Ueshima H, Stamler J, Elliott P, et al. Food omega-3 fatty acid intake of individuals (total, linolenic acid, long-chain) and their blood pressure: INTERMAP study. *Hypertension.* Aug 2007;50(2):313-319.

197. Jacobson TA, Miller M, Schaefer EJ. Hypertriglyceridemia and cardiovascular risk reduction. *Clin Ther.* May 2007;29(5):763-777.

198. Harper CR, Jacobson TA. Usefulness of omega-3 fatty acids and the prevention of coronary heart disease. *Am J Cardiol.* Dec 1 2005;96(11):1521-1529.

199. He K, Song Y, Daviglus ML, et al. Accumulated evidence on fish consumption and coronary heart disease mortality: a meta-analysis of cohort studies. *Circulation.* Jun 8 2004;109(22):2705-2711.

200. Moher D, Schulz KF, Altman DG. The CONSORT statement: revised recommendations for improving the quality of reports of parallel-group randomised trials. *Lancet.* Apr 14 2001;357(9263):1191-1194.

201. Agostoni C, Verduci E, Massetto N, et al. Long term effects of long chain polyunsaturated fats in hyperphenylalaninemic children. *Arch Dis Child.* Jul 2003;88(7):582-583.

202. Lenth RV. Java Applets for Power and Sample Size [Computer software]. 2006; http://www.stat.uiowa.edu/~rlenth/Power. Accessed March 20, 2007.

203. Huttenlocher PR. The neuropathology of phenylketonuria: human and animal studies. *Eur J Pediatr.* Oct 2000;159 Suppl 2:S102-106.

204. Pietz J, Dunckelmann R, Rupp A, et al. Neurological outcome in adult patients with early-treated phenylketonuria. *Eur J Pediatr.* Oct 1998;157(10):824-830.

205. Waisbren SE, Levy HL. Agoraphobia in phenylketonuria. *J Inherit Metab Dis.* 1991;14(5):755-764.

206. Waisbren SE, Zaff J. Personality disorder in young women with treated phenylketonuria. *J Inherit Metab Dis.* 1994;17(5):584-592.

207. Cerone R, Schiaffino MC, Di Stefano S, Veneselli E. Phenylketonuria: diet for life or not? *Acta Paediatr.* Jun 1999;88(6):664-666.

208. Channon S, Goodman G, Zlotowitz S, Mockler C, Lee PJ. Effects of dietary management of phenylketonuria on long-term cognitive outcome. *Arch Dis Child.* Mar 2007;92(3):213-218.

209. Levy HL, Ghavami M. Maternal phenylketonuria: a metabolic teratogen. *Teratology.* Mar 1996;53(3):176-184.

210. Recommendations on the dietary management of phenylketonuria. Report of Medical Research Council Working Party on Phenylketonuria. *Arch Dis Child.* Mar 1993;68(3):426-427.

211. Channon S, Mockler C, Lee P. Executive functioning and speed of processing in phenylketonuria. *Neuropsychology.* Sep 2005;19(5):679-686.

212. Waisbren SE, Brown MJ, de Sonneville LM, Levy HL. Review of neuropsychological functioning in treated phenylketonuria: an information processing approach. *Acta Paediatr Suppl.* Dec 1994;407:98-103.

213. Albrecht J, Garbade SF, Burgard P. Neuropsychological speed tests and blood phenylalanine levels in patients with phenylketonuria: A meta-analysis. *Neurosci Biobehav Rev.* Mar 2009;33(3):414-421.

214. Moller HE, Weglage J, Wiedermann D, Ullrich K. Blood-brain barrier phenylalanine transport and individual vulnerability in phenylketonuria. *J Cereb Blood Flow Metab.* Nov 1998;18(11):1184-1191.

215. Brumm VL, Azen C, Moats RA, et al. Neuropsychological outcome of subjects participating in the PKU adult collaborative study: a preliminary review. *J Inherit Metab Dis.* 2004;27(5):549-566.

216. DeRoche K, Welsh M. Twenty-five years of research on neurocognitive outcomes in early-treated phenylketonuria: intelligence and executive function. *Dev Neuropsychol.* 2008;33(4):474-504.

217. Galli C, Agostoni C, Mosconi C, Riva E, Salari PC, Giovannini M. Reduced plasma C-20 and C-22 polyunsaturated fatty acids in children with phenylketonuria during dietary intervention. *J Pediatr.* Oct 1991;119(4):562-567.

218. Cockburn F, Clark BJ, Caine EA, et al. Fatty acids in the stability of neuronal membrane: relevance to PKU. *Int Pediatr.* 1996;11(1):56-60.

219. Kris-Etherton PM, Taylor DS, Yu-Poth S, et al. Polyunsaturated fatty acids in the food chain in the United States. *Am J Clin Nutr.* Jan 2000;71(1 Suppl):179S-188S.

220. Spector AA. Plasma free fatty acid and lipoproteins as sources of polyunsaturated fatty acid for the brain. *J Mol Neurosci.* Apr-Jun 2001;16(2-3):159-165; discussion 215-121.

221. Wassall SR, Stillwell W. Docosahexaenoic acid domains: the ultimate non-raft membrane domain. *Chem Phys Lipids.* May 2008;153(1):57-63.

222. Holte LL, Peter SA, Sinnwell TM, Gawrisch K. 2H nuclear magnetic resonance order parameter profiles suggest a change of molecular shape for phosphatidylcholines containing a polyunsaturated acyl chain. *Biophys J.* Jun 1995;68(6):2396-2403.

223. Jenski LJ, Stillwell W. Role of Docosahexaenoic Acid in Determining Membrane Structure and Function. In: Mostofsky D, Yehuda S, Salem N, Jr., eds. *Fatty Acids: Physiological and Behavioral Functions*. Totowa: Human Press Inc.; 1999:41-62.

224. Salem N, Jr., Litman B, Kim HY, Gawrisch K. Mechanisms of action of docosahexaenoic acid in the nervous system. *Lipids.* Sep 2001;36(9):945-959.

225. Engstrom K, Saldeen AS, Yang B, Mehta JL, Saldeen T. Effect of fish oils containing different amounts of EPA, DHA, and antioxidants on plasma and brain fatty acids and brain nitric oxide synthase activity in rats. *Ups J Med Sci.* 2009;114(4):206-213.

226. Koletzko B, Beblo S, Demmelmair H, Muller-Felber W, Hanebutt FL. Does dietary DHA improve neural function in children? Observations in phenylketonuria. *Prostaglandins Leukot Essent Fatty Acids.* Aug-Sep 2009;81(2-3):159-164.

227. LaVoie SM, Harding CO, Gillingham MB. Normal Fatty Acid Concentrations in Young Children With Phenylketonuria. *Top Clin Nutr.* October/December 2009 2009;24(4):333-340.

228. Mazer LM, Yi SH, Singh RH. Docosahexaenoic acid status in females of reproductive age with maple syrup urine disease. *J Inherit Metab Dis.* Mar 9 2010;Mar 9 [Epub ahead of print].

229. Reisbick S, Neuringer M, Gohl E, Wald R, Anderson GJ. Visual attention in infant monkeys: effects of dietary fatty acids and age. *Dev Psychol.* May 1997;33(3):387-395.

230. Riediger ND, Othman RA, Suh M, Moghadasian MH. A systemic review of the roles of n-3 fatty acids in health and disease. *J Am Diet Assoc.* Apr 2009;109(4):668-679.

231. Bazan NG. Cellular and molecular events mediated by docosahexaenoic acid-derived neuroprotectin D1 signaling in photoreceptor cell survival and brain protection. *Prostaglandins Leukot Essent Fatty Acids.* Aug-Sep 2009;81(2-3):205-211.

232. Akbar M, Calderon F, Wen Z, Kim HY. Docosahexaenoic acid: a positive modulator of Akt signaling in neuronal survival. *Proc Natl Acad Sci U S A.* Aug 2 2005;102(31):10858-10863.

233. Beltz BS, Tlusty MF, Benton JL, Sandeman DC. Omega-3 fatty acids upregulate adult neurogenesis. *Neurosci Lett.* Mar 26 2007;415(2):154-158.

234. Venna VR, Deplanque D, Allet C, Belarbi K, Hamdane M, Bordet R. PUFA induce antidepressant-like effects in parallel to structural and molecular changes in the hippocampus. *Psychoneuroendocrinology.* Feb 2009;34(2):199-211.

235. Stroop JR. *Studies of interference in serial verbal reactions* [PhD]. Nashville, TN, George Peabody College for Teachers; 1935.

236. Altman DG, Schulz KF, Moher D, et al. The revised CONSORT statement for reporting randomized trials: explanation and elaboration. *Ann Intern Med.* Apr 17 2001;134(8):663-694.

237. Johnson EJ, McDonald K, Caldarella SM, Chung HY, Troen AM, Snodderly DM. Cognitive findings of an exploratory trial of docosahexaenoic acid and lutein supplementation in older women. *Nutr Neurosci.* Apr 2008;11(2):75-83.

238. Harris WS, Von Schacky C. The Omega-3 Index: a new risk factor for death from coronary heart disease? *Prev Med.* Jul 2004;39(1):212-220.

239. McNamara RK. Evaluation of docosahexaenoic acid deficiency as a preventable risk factor for recurrent affective disorders: current status, future directions, and dietary recommendations. *Prostaglandins Leukot Essent Fatty Acids.* Aug-Sep 2009;81(2-3):223-231.

240. Lichtenstein AH, Appel LJ, Brands M, et al. Diet and lifestyle recommendations revision 2006: a scientific statement from the American Heart Association Nutrition Committee. *Circulation.* Jul 4 2006;114(1):82-96.

241. Cunnane S, Drevon C, Harris WS, Sinclair A, Spector A. *Recommendations for intake of polyunsaturated fatty acids in healthy adults*: International Society for the Study of Fatty Acids and Lipids; June 2004.

242. Schulz KF, Altman DG, Moher D. CONSORT 2010 statement: updated guidelines for reporting parallel group randomised trials. *BMJ.* 2010;340:c332.

243. Craig WJ, Mangels AR. Position of the American Dietetic Association: vegetarian diets. *J Am Diet Assoc.* Jul 2009;109(7):1266-1282.

244. Fiori L, Casero D, Minghetti D, et al. Plasma long-chain polyunsaturated fatty acids in PKU patients on diet-therapy and free-diet group in adolescence age. *J Inherit Metab Dis.* Aug 2006;29(Suppl 1):31.

245. Vickers AJ. Parametric versus non-parametric statistics in the analysis of randomized trials with non-normally distributed data. *BMC Med Res Methodol.* 2005;5:35.

246. McDonald JH. Handbook of Biological Statistics. September 14, 2009 ed. Baltimore: Sparky House Publishing; 2009: http://udel.edu/~mcdonald/statancova.html. Accessed January 28, 2010.