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A cross-sectional study of docosahexaenoic acid status and cognitive outcomes in females of reproductive age with phenylketonuria

Sarah H. L. Yi,

Nutrition & Health Sciences Program of the Graduate Division of Biological & Biomedical Sciences, Emory University, Atlanta, GA, USA

Julie A. Kable,

Department of Pediatrics, Emory University School of Medicine, Atlanta, GA, USA

Marian L. Evatt, and

Department of Veterans Affairs Medical Center, Atlanta, GA, USA

Rani H. Singh

Nutrition & Health Sciences Program of the Graduate Division of Biological & Biomedical Sciences, Emory University, Atlanta, GA, USA

Department of Human Genetics, Emory University School of Medicine, Atlanta, GA, USA

2165 N. Decatur Road, Decatur, GA 30033, USA

Rani H. Singh: rsingh@emory.edu

Abstract

Diet therapy for phenylketonuria (PKU) requires restricted phenylalanine (Phe) intake, with the majority of protein and other nutrients coming from synthetic medical food. The fatty acid docosahexaenoic acid (DHA) is important in brain development and function; however, there are reports of low blood DHA concentrations in people treated for PKU. Although the implications of this low blood DHA are unclear, subtle cognitive deficits have been reported in those treated early and continuously for PKU. For this study, we 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. We assessed the domains of verbal ability, processing speed, and executive function using standardized tests, and the proportions of DHA in plasma and red blood cell (RBC) total lipids 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

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Correspondence to: Rani H. Singh, rsingh@emory.edu.

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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 to clarify any association with DHA.

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, the developmental delays and behavioral disturbances associated with it can be prevented (Bickel et al. 1953; Paine 1957); currently, lifelong diet treatment is recommended (National Institutes of Health Consensus Development Panel 2001) to prevent cognitive, neurological, and psychiatric declines (Cerone et al. 1999; Channon et al. 2007; Fisch et al. 1995; Moyle et al. 2007a, b; Waisbren and Levy 1991; Waisbren and Zaff 1994).

Despite successful prevention of mental retardation, adolescents and adults treated early for PKU reportedly still display minor cognitive deficits in domains that include processing speed, executive function (inhibition), attention, and overall IQ (Brumm et al. 2004; Channon et al. 2005; Feldmann et al. 2005; Waisbren et al. 1994). Debate continues 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 across the aforementioned domains, as well as others (DeRoche and Welsh 2008; Moyle et al. 2007a, b).

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; PubChem ID 445580) compared with controls without PKU (Cockburn et al. 1996; Galli et al. 1991; Moseley et al. 2002; van Gool et al. 2000). 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 (Pawlosky et al. 2001). The enzymes that catalyze the desaturation and elongation of ALA to DHA also catalyze the conversion of linoleic acid (LA) to its longer-chain derivative arachidonic acid (AA). The presence of LA in the US food supply is higher than ALA (Kris-Etherton et al. 2000), and a higher 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 (Arbuckle et al. 1994; Blank et al. 2002; Liou et al. 2007).

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 (Carver et al. 2001; Spector 2001). 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 (Wassall and Stillwell 2008), enhancing lateral compressibility of the membrane (Holte et al. 1995), and allowing for vesicle formation and fusion (Jenski and Stillwell 1999). DHA is a precursor to bioactive molecules neuroprotectin D1 and resolvins and appears to regulate neuronal apoptosis (Bazan 2009; Salem et al. 2001). DHA supplementation may also increase brain concentrations of nitric oxide synthetase, dopamine, serotonin, and brain-derived neurotrophic factor (Engstrom et al. 2009; Jiang et al. 2009).

Inadequate DHA concentrations in neural lipids may be related to the minor cognitive deficits in people treated early for PKU (Cockburn et al. 1996; Infante and Huszagh 2001; Koletzko et al. 2009). 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 are seen in visual evoked potentials and motor skills with improved blood DHA content (Agostoni et al. 2000, 2006; Beblo et al. 2001, 2007). Studies have yet to investigate 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 blood DHA content to have the highest scores on cognitive tests, and individuals with poor plasma Phe control and decreased blood DHA content to have the lowest scores. To investigate this hypothesis, we compared the performance of females of reproductive age with PKU stratified into 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 who had been diagnosed with PKU and were not consuming supplemental DHA. The Emory University Institutional Review Board approved this study. Participants, and a parent or guardian if the participant was under 18 years old, 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 3-day diet intakes were assessed as described below. 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.

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 (Slocum and Cummings 1991). Amino acid concentrations are reported as $\mu\text{mol/L}$. These values can be converted to mg/dL ($\text{mg}\%$) by dividing by 60.54 (National Institute of Standards and Technology; NIST Chemistry WebBook 2008).

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 (Baltimore, MD, USA). 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 (Lagerstedt et al. 2001). The content of plasma and RBC DHA are presented as percentage of total lipids; total lipid fatty acid concentrations are presented as $\mu\text{g/mL}$.

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, USA. Nutrients assessed included energy, protein, medical food protein, fat, carbohydrates, Phe, Tyr, LA, AA, ALA, EPA, and DHA.

Cognitive assessments—We assessed performance on tasks that draw 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 that have been standardized to national demographics. Evaluations demonstrate adequate reliability and validity of the tests (Delis et al. 2001; Dunn and Dunn 1997; McGrew and Woodcock 2001; Schrank et al. 2001).

The verbal ability and executive function tests were individually administered, and attention and focus of the examinee was maintained by the examiner as part of study procedures. The verbal ability task generally takes about 10–15 min to complete; however, examinees are given an unlimited time period. The two executive function tests each have a time limit of 180 s. The processing speed tests are very brief, and are unlikely to be impacted by poor attentional regulatory skills. Two of the processing speed tests each have a time limit of 90 s; the other four tests each have a time limit of 180 s.

Verbal ability

The Peabody Picture Vocabulary Test—Third Edition, Form B (PPVT-III B) is (1) a verbally administered achievement test on standard English vocabulary, and (2) a screening test for

verbal ability. Although the PPVT does not test overall intellectual capacity, it is highly correlated with measures of overall IQ (Dunn and Dunn 1997). 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 as a result of concern with their ability to understand the task demands of the other tests. 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 unavailable.

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 (1935). 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 (VanZutphen et al. 2007). 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, USA), 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 with a trained tester under the supervision of a licensed psychologist. Participants were instructed to complete each test as quickly as possible. Twenty-one participants completed the baseline W-J III in a group setting while 20 completed individually. Mean scores on these tasks did not differ between those who completed the tasks in the group setting versus individually.

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. 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. We used the Pearson product-moment correlation coefficient 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 the 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, Chicago, IL, USA).

Results

Of the 47 individuals with PKU attending the baseline visit, 3 declined participation, 2 had impaired scores on the screening test (PPVT SS<70), and 1 was unable to provide venous blood at the blood draw, for a final sample of 41 participants in this analysis. As shown in Table 1, the four participant groups were similar in most demographic characteristics. The 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.

Average plasma Phe concentrations exceeded treatment recommendations in the 20 adolescents. Average plasma Phe concentrations were within treatment recommendations for adults but exceeded preconception recommendations (Table 1S of supplementary

material). As shown in Table 2, average plasma and RBC DHA contents were significantly lower than adolescent and adult female laboratory controls without PKU. Concentrations of plasma Phe were inversely correlated with plasma DHA ($r=-.42$, $P=.003$, $n=41$) and RBC DHA ($r=-.33$, $P=.02$, $n=41$; additional data in Tables 2S and 3S of supplementary material).

Table 3 reveals 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 $\mu\text{mol/L}$) reportedly consumed significantly less energy during the 3 days prior to the blood draw compared with participants with better plasma Phe (Phe <600 $\mu\text{mol/L}$; $F_{1,37}=5.8$, $P=.02$).

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 ($F_{1,38}=4.8$, $P=.03$). An initial trend in the relationship between plasma DHA and PPVT ($r=.20$, $P=.10$, $n=41$) was eliminated after controlling for plasma Phe ($r=.02$, $P=.46$, $n=41$). Plasma and RBC DHA were not found to be related to measures of cognitive processing speed, inhibition, and flexibility.

As shown in Table 4, 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, SE 4.30, $P=.004$; 95% confidence interval: 4.32–27.46). Controlling for maternal education did not change this relationship. Although we saw a trend for number of correct answers on one of the processing speed tasks (W-J Pair Cancellation), we detected no differences in performance speed and accuracy on the processing speed and executive function tasks between the four groups.

Discussion and conclusions

In this study, a combination of 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, finding that participants with the best combined Phe and DHA status had the highest mean PPVT scores and those with the poorest combined Phe and DHA status had the lowest mean PPVT scores raises a number of possible interpretations, an important one being that plasma Phe control <600 $\mu\text{mol/L}$ and improved DHA status may allow for enhanced performance on specific cognitive tasks, such as verbal ability.

Verbal ability has been connected to DHA status in other studies of participants without PKU. Similar to the present study, performance on the PPVT and whole blood DHA concentrations ($r^2 = .14$, $P = .018$) were positively related in healthy 4-year-old children supplemented with 400 mg DHA for 4 months (Ryan and Nelson 2008). There were no differences, however, in change in performance on tests of verbal ability between the DHA- and placebo-supplemented groups (Ryan and Nelson 2008). Furthermore, Birch and colleagues found that 4 year olds who were fed formula supplemented with DHA (0.36% fatty acids) and AA (0.72% fatty acids) as infants had verbal IQs similar to breastfed infants (Birch et al. 2007). Four year olds fed formula without preformed LC-PUFAs as infants, however, had significantly lower verbal IQs than those who were breastfed.

Interestingly, in this study, those with low Phe/low DHA or high Phe/high DHA displayed similarly 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.

An alternative interpretation of these results could be 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). Furthermore, although maternal education did not account for group differences in performance on the verbal ability task, other factors not assessed may also contribute to both poor dietary control and poor scholastic achievement (e.g., mental health problems or other environmental stressors).

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 (Ryan and Nelson 2008). Similarly, 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 (de Groot et al. 2004, 2007). 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, although small improvements in performance were seen in the supplemented group; note that the lack of significance may have been due to inadequate statistical power (Llorente et al. 2003).

Not all studies have shown statistically significant differences in biomarkers of DHA status, but this is likely due to inadequate statistical power (Acosta et al. 2001; LaVoie et al. 2009; Poge et al. 1998). The lower concentrations of blood DHA seen in people with PKU are comparable with those seen in other populations consuming diets lacking preformed long-chain polyunsaturated fatty acids, such as those who follow a vegan diet (Rosell et al. 2005).

or those on a low protein diet to treat maple syrup urine disease (Mazer et al. 2010); thus the lower concentrations of blood DHA are suspected to be an artifact of the restrictive diet.

Three participants failed to complete a 3-day food record, so 24-h recalls were conducted with these participants instead. The 24-h recall was chosen as an alternative to the 3-day food record since both are based on actual intake. The major limitation in using a 24-h recall instead of a 3-day food record is that it is less likely to account for within-person day-to-day variability.

Because this study was conducted with females only, these results do not necessarily extend to males. Additional study is needed for adolescent and adult males with PKU.

Conclusions

This is the first study assessing the relationship between cognitive performance and DHA status in adolescent and adult females with PKU. Here we found a positive relationship between RBC DHA and performance on a verbal ability task, but failed to detect a relationship between biomarkers of DHA and 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 that involve memory and learning.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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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
mPKU	Maternal PKU
MF	Medical food
n-3 PUFA	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

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Table 1

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

	Low Phe/High DHA ^a	Low Phe/Low DHA	High Phe/High DHA	High Phe/Low DHA
	Group 1, n=12	Group 2, n=7	Group 3, n=8	Group 4, n=14
Age, mean (SD), years	24.2 (10.7)	23.0 (9.1)	21.9 (8.1)	25.2 (12.2)
Phe prescription, mean (SD), mg ^b	384 (132)	434 (104)	265 (69)	350 (127)
Medical food prescription, mean (SD), g pro eq ^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)

PKU phenylketonuria, Phe phenylalanine, DHA docosahexaenoic acid, pro eq protein equivalent, HS high school

^aLow Phe: <600 μmol/L; High DHA: RBC DHA median by age group, <18 years: 2.23%; 18 years: 2.40%

^bPhe prescription: Group 3, n=7

^cMedical food prescription: Group 3, n=1 taking large neutral amino acids not included in mean; Group 4, n=13

^dAt 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

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

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

Table 2

Select biomarkers of plasma and red blood cell total lipid fatty acid status in females of reproductive age with PKU compared with female laboratory controls without PKU

	PKU	Lab controls	<i>F</i> test ^a	<i>P</i> value
Plasma DHA, mean (SD), % ^a	1.04 (0.33)	2.95 (0.88)	$F_{1,61}=157.8$	<.001
Plasma AA/DHA ratio, mean (SD)	6.56 (1.80)	2.32 (0.78)	$F_{1,61}=110.6$	<.001
Plasma TLFA, mean (SD), µg/mL	2,533 (942)	2,561 (363)	$F_{1,61}=0.0$	.90
RBC DHA, mean (SD), % ^b	2.41 (0.74)	3.87 (0.94)	$F_{1,64}=48.8$	<.001
RBC AA/DHA ratio, mean (SD)	5.69 (1.86)	3.85 (1.09)	$F_{1,64}=20.1$	<.001
RBC TLFA, mean (SD), µg/mL	1,393 (224)	1,347 (195)	$F_{1,64}=0.7$	.41

PKU phenylketonuria, DHA docosahexaenoic acid, LA linoleic acid, AA arachidonic acid, ALA alpha-linolenic acid, EPA eicosapentaenoic acid, RBC red blood cell, TLFA total lipid fatty acids

^a1-way ANOVA, plasma TLFA: all PKU (*n*=41) versus all lab controls (*n*=22)

^b1-way ANOVA, RBC TLFA: all PKU (*n*=41) versus all lab controls (*n*=25)

Table 3
Estimates of diet intake in females of reproductive age with PKU stratified by plasma Phe and RBC DHA status

	Low Phe/High DHA ^a		Low Phe/Low DHA		High Phe/High DHA		High Phe/Low DHA		Statistics	
	Group 1, n=11		Group 2, n=7		Group 3, n=7		Group 4, n=14		F test ^b	P value
Energy, mean (SD), kcal ^c	1,799 (362)		1,793 (631)		1,461 (373)		1,491 (357)		$F_{2,3,6}=2.8$	.07
Protein, mean (SD), g pro eq	60.4 (10.3)		66.5 (24.2)		57.6 (12.2)		52.9 (10.8)		$F_{2,3,6}=1.7$	.20
MF Protein, mean (SD), g pro eq ^d	51.9 (14.0)		53.7 (17.9)		48.2 (7.3)		40.3 (18.3)		$F_{2,3,7}=2.1$	.14
Fat, mean (SD), g	61 (24)		52 (12)		38 (16)		46 (16)		$F_{2,3,6}=2.7$	.08
Phe, mean (SD), g	.526 (.328)		.581 (.535)		.373 (.273)		.564 (.563)		$F_{2,3,6}=0.4$	.68
Tyr, mean (SD), g	5.535 (.910)		5.648 (1.834)		5.350 (1.109)		4.515 (1.515)		$F_{2,3,6}=1.9$	.16
LA, mean (SD), g	18.3 (6.9)		14.5 (4.2)		8.9 (2.6)		13.2 (6.3)		$F_{2,3,6}=3.4$	.04
AA, mean (SD), g	.002 (.002)		.014 (.023)		.006 (.008)		.008 (.014)		$F_{2,3,6}=1.3$	.28
ALA, mean (SD), g	2.49 (1.23)		1.95 (.67)		1.12 (.30)		1.57 (.62)		$F_{2,3,6}=5.0$	.01
EPA, mean (SD), g	.000 (.001)		.002 (.003)		.000 (.001)		.001 (.001)		$F_{2,3,6}=1.2$	.32
DHA, mean (SD), g	.001 (.001)		.003 (.005)		.002 (.003)		.001 (.002)		$F_{2,3,6}=0.4$	.66

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

^aLow Phe: <600 µmol/L; High DHA: RBC DHA median by age group, <18 years: 2.23%; 18 years: 2.40%

^bTwo-way ANOVA

^cDietary 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

^dGroup 1 MF Protein, n=12

Table 4
Cognitive performance in females of reproductive age with PKU stratified by plasma Phe and RBC DHA status

Domain	Low Phe/High DHA ^a		Low Phe/Low DHA		High Phe/High DHA		High Phe/Low DHA		Statistics	
	Group 1, n=12		Group 2, n=7		Group 3, n=8		Group 4, n=14		F test ^b	P value
Verbal ability, mean (SD), 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, mean (SD), 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, mean (SD), 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, mean (SD), SS ^d	9.9 (2.7)		10.2 (4.6)		10.0 (3.7)		9.2 (4.5)		$F_{2,35}=0.1$	.90

PKU phenylketonuria, Phe phenylalanine, DHA docosahexaenoic acid, SS standard score

^aLow Phe: <600 μmol/L; High DHA: RBC DHA median by age group, <18 years: 2.23%; 18 years: 2.40%

^bTwo-way ANOVA

^cPPVT 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)

^dCognitive flexibility: Group 2, n=6; Group 3, n=7; Group 4, n=13