Diet History Is a Reliable Predictor of Suboptimal Docosahexaenoic Acid Levels in Adult Patients with Phenylketonuria

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Abstract *Background:* Omega-3 long-chain polyunsaturated fatty acids (n3LCPUFA) levels are reduced in phenylketonuria (PKU). Recent care guidelines recommend essential fatty acid status is monitored in patients with PKU but access to such testing is limited. We hypothesized that information obtained on diet history would identify PKU adults with suboptimal levels of n3LCPUFA.

Methods: A 12-month single site prospective study was completed including 35 adults (age 18–46) attending a clinic for adults with inborn errors of metabolism. Levels of n3LCPUFA were correlated with estimated intake using a published food frequency questionnaire. n3LCPUFA levels were tested at a commercial laboratory and values > one SD below the laboratory mean value were considered suboptimal.

Results: Mean levels of docosahexaenoic acid (DHA) were lower and levels of eicosapentaenoic acid (EPA) and alpha-linoleic acid (ALA) higher in subjects with PKU than in laboratory controls. n3LCPUFA levels correlated with estimated intake ($p < 0.002$). Diet history had a positive predictive value of 93% and negative predictive value of 90% to identify subjects with suboptimal n3LCPUFA levels.

Conclusions: Diet history is sufficient to predict adult subjects who may have low DHA levels and can be used to

target testing or supplementation to those at risk. DHA levels are low despite high levels of ALA suggesting that supplementation, if indicated, should be with preformed DHA rather than with its precursors.

Introduction

Suboptimal levels of omega-3 long-chain polyunsaturated fatty acids (n3LCPUFA) have been documented in patients with phenylketonuria (PKU) (Lohner et al. 2013) related both to dietary restriction of sources of preformed n3LCPUFAs and possibly due to an inhibitory effect of phenylalanine metabolites on the endogenous synthesis of docosahexaenoic acid (DHA) (Infante and Huszagh 2001). Suboptimal levels of n3LCPUFA may be related to neurologic (Agostoni et al. 2003) and bone (Lage et al. 2010) outcomes, and supplementation may improve subtle surrogate markers of neurologic function in patients with PKU (Gutierrez-Mata et al. 2012). There are several studies which suggest a possible benefit of n3LCPUFA supplementation on neurological outcomes in children with PKU. For example, fish oil supplementation in children with PKU has been shown to improve n3LCPUFA levels and motor skills (Beblo et al. 2007) and visual evoked potential latencies (Koletzko et al. 2009; Beblo et al. 2001). In a study of female patients over the age of 12, Yi et al. found a positive relationship between red blood cell (RBC) DHA levels and performance on a verbal ability task (Yi et al. 2011).

Unfortunately, most of the data available on n3LCPUFA status in PKU are for children. A recent meta-analysis on this topic (Lohner et al. 2013) reviewed 15 studies, only two of which included patients over the age of 25 (Lage et al. 2010; Moseley et al. 2002) for a total of 47 patients

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over the age of 25 years. Despite the paucity of data on n3LCPUFA status in PKU adults, recent consensus guidelines (Singh et al. 2014) suggest that all patients given fat-free medical foods should have essential fatty acid status monitored. Some literature suggests that suboptimal n3LCPUFA status in adults may not be the same as seen in children (Moseley et al. 2002; Pöge et al. 1998), questioning the need for screening in adults and its associated costs. Recent work has also documented that adult patients with PKU have very limited access to expert care (Berry et al. 2013) so specialized laboratory testing may be even more restricted. Thus, it would be useful to identify standard nutrition screening tools to assess risk for suboptimal n3LCPUFA levels in order to reduce the need for specialized laboratory testing.

Methods

The University of British Columbia Clinical Research Ethics Board approved the study protocol and all subjects provided informed consent. Adults with PKU followed at our site and able to provide informed consent were invited to participate over a 12-month period. Plasma total fatty acid profiles are included in the VGH Adult Metabolic Clinic's annual laboratory assessment of PKU patients as this is a recommended component of the nutrition management for PKU (Singh et al. 2014). This blood work was sent to a commercial laboratory (Kennedy Krieger Institute, Baltimore, Maryland, USA), and other laboratory parameters including phenylalanine (PHE) levels were done locally using standard methods. The Kennedy Krieger laboratory has derived its normal range for plasma total fatty acids from 52 controls who reside in North America, but details of the ethnic makeup of the control population are not known (Dr. Richard Jones Ph.D., Administrative Laboratory Manager, Genetics Laboratory Kennedy Krieger Institute, personal communication, November 5, 2014). Data on docosahexaenoic acid (DHA), eicosapentaenoic acid (EPA), α -linolenic acid (ALA), and arachidonic acid (ARA) are included here. Levels within one standard deviation of the mean for laboratory controls were considered normal. Although the usual threshold for defining laboratory values as being "low" is -2 SD below the mean of healthy controls, we selected a higher threshold of -1 SD given our primary goal was to see if diet history could be used to define an at-risk population to allow appropriate targeting for n3LCPUFA screening. This lower threshold would reduce the chance that patients who might benefit from n3LCPUFA measurement and/or supplementation would be missed.

A nutrition history was obtained from each subject using the Omega-3 PUFA Food Frequency Questionnaire

(Sublette et al. 2011) modified by asking additional questions about ALA, DHA, and EPA intake from PKU medical food products and adherence to the subjects' prescription of medical food products as shown in question 5 and 6 of the Appendix. The daily amount of n3LCPUFA consumed in the diet from food, supplements, and actual (not prescribed) formula intake was used based on these patient self-reports.

The normality of plasma level distributions was tested using the Shapiro–Wilk test. Student's *t*-test was used to compare data showing normal distribution, and the Mann–Whitney *U* test was used for data which were not normally distributed. Correlations were calculated with the Pearson correlation test. All conclusions were based on a significance level of $P < 0.05$.

Results

In a 12-month period, 53 adult subjects with PKU had plasma total fatty acid profiles drawn. Of these, 10 subjects were diagnosed with PKU before newborn screening and unable to provide informed consent, 1 was pregnant, 2 consented but did not have the blood work done, 2 declined consent, and 5 could not be contacted for consent so were excluded from analysis, leaving a total of 35 subjects for inclusion in the study. Clinical characteristics and laboratory parameters of the study participants are shown in Table 1.

Plasma DHA levels in study subjects were significantly lower ($p < 0.0001$) and plasma EPA ($p = 0.021$) and ALA ($p < 0.001$) significantly higher than laboratory normal controls. ARA levels in study subjects did not differ from control values ($p = 0.2$). Estimated dietary intake of DHA and EPA using the modified questionnaire correlated with plasma levels for DHA ($p < 0.001$) and for EPA ($p = 0.002$). Blood PHE levels did not correlate with DHA ($p = 0.74$), EPA ($p = 0.4$), ALA ($p = 0.2$), or ARA ($p = 0.1$) levels in study subjects.

Fifteen subjects had normal levels of DHA, and 14 of these subjects had normal levels of DHA, EPA, and ALA. Of the 15 subjects with normal levels of DHA, 10 were consuming DHA through supplements or a supplemented PKU medical food product. Three of the 5 subjects with normal DHA levels who did not obtain DHA from medical food products or supplements reported eating fish. There were two patients who were on medical food product supplemented with DHA but had DHA levels <1 SD below the laboratory control range. Both subjects routinely consumed less than 50% of the DHA supplemented medical food product prescribed indicating limited compliance.

Predictive values were calculated from these data. If a subject was predicted on history to have adequate intake of DHA, then 93% of those subjects actually did have a

Table 1 Demographics, phenylalanine, and n3PUFA levels of adult subjects with PKU

Parameter	Subject values	Comment
N (male:female)	22:13	Data not available on sex of controls
Age (mean $\pm$ SD)	28.5 $\pm$ 7.5	
BMI (mean $\pm$ SD)	28.7 $\pm$ 6.6	
Blood PHE (μ mol/L; median (range))	703 (range 103–1,721)	$N = 33$ as two subjects did not have PHE levels at the time of PUFA analysis
Subjects on supplemented formula	10	$N = 4$ on formula supplemented with ALA alone; $N = 5$ on formulas supplemented with DHA and EPA, and $N = 1$ on formula supplemented with DHA alone
DHA – (%total; mean $\pm$ SD)	1.65 $\pm$ 0.61	$p < 0.0001$ versus laboratory control range ^a
EPA – (%total; mean $\pm$ SD)	0.79 $\pm$ 0.31	$p = 0.021$ versus laboratory control range ^a
ALA – (%total; mean $\pm$ SD)	0.96 $\pm$ 0.32	$p < 0.001$ versus laboratory control range ^a
ARA – (%total; mean $\pm$ SD)	7.12 $\pm$ 1.67	$p = 0.2$ versus laboratory control range ^a

PHE phenylalanine, n3LCPUFA omega-3 polyunsaturated fatty acids, DHA docosahexaenoic acid, EPA eicosapentaenoic acid, ALA alpha-linoleic acid, ARA arachidonic acid

^a P value compared with laboratory normal control values: control range was determined from 52 North American subjects aged (mean $\pm$ SD) 44 $\pm$ 13.8; range 18–75 years (Dr. Richard Jones Ph.D., Administrative Laboratory Manager, Genetics Laboratory Kennedy Krieger Institute, personal communication, November 5, 2014). The mean values (%total) of DHA, EPA, ALA, and ARA for the 52 controls used were 2.655%, 0.664%, 0.742%, and 6.751%, respectively, with a standard deviation of 0.968%, 0.366%, 0.307%, and 1.374%, respectively

normal plasma value of DHA, and measurement of n3LCPUFA levels in this group would be of low yield. If a subject was predicted on history to have low intake of DHA, then 90% of those subjects did have a low DHA and supplementation (with or without measurement of n3LCPUFA levels) could be considered on the basis of the diet history.

Discussion

Consistent with other authors, we have identified that levels of DHA are lower in PKU subjects although we did not identify deficiencies of other n3LCPUFA. Our intention, however, was to focus on findings useful from a patient management perspective. Firstly, information on n3LCPUFA intake obtained simply by diet history is sufficient to identify adult subjects at risk of suboptimal n3LCPUFA levels, suggesting this information can be used to target laboratory analysis or supplementation of n3LCPUFA levels only to those at risk.

Suboptimal DHA status is evident despite more than adequate levels of the precursor ALA. EPA levels were elevated, which differs from previous studies (Lohner et al. 2013), consistent with the findings of Infante and Huszagh (2001), suggesting that PHE metabolites may inhibit later stages in the pathway for DHA synthesis. This has relevance to current nutritional guidelines (Singh et al. 2014) which suggest that supplementation with the precursor ALA or with preformed DHA is appropriate in patients

with low levels of n3LCPUFA. Our subjects have low levels of DHA despite elevated levels of ALA, suggesting that precursor supplementation would not be sufficient to normalize DHA levels in our adult population.

In our study of adult patients, we did not identify a relationship between blood PHE levels and n3LCPUFA status which is contrary to reports in children (Agostoni et al. 1997) and other reports in adults (Moseley et al. 2002). We are not sure of the reason for this discrepancy but speculate that it is related to the factors underlying poor control in our population. Dietary indiscretions in our patient population tend to be related to excessive intake of carbohydrate-rich foods, dairy, and meat rather than due to intake of n3LCPUFA protein sources such as fish, so noncompliance with dietary recommendations in our patients does not mean that their intake of DHA will increase.

Our study has limitations. Subject numbers are limited although this is the one of the largest data sets to date on n3LCPUFA levels of adult patients with PKU. We used lab-based normal values as a reference rather than our own controls. However, as the purpose of our study was not to repeat the work of other authors documenting n3LCPUFA deficiency in subjects with PKU but rather to identify ways we could target our screening to individuals at risk, we feel the use of lab-based normal ranges is reasonable. We also defined patients at risk of n3LCPUFA deficiency as being below 1 SD of the mean which is higher than the usually accepted thresholds of -2 SD of the mean. The predictive values of diet history in determining subjects at risk of

having low levels of n3LCPUFAs reported in this study are expected to change if the threshold to define a low level changes. However, in the absence of data which demonstrate clinical benefits of DHA supplementation in adults, we cannot comment on what threshold level of DHA is appropriate and choose a higher threshold to minimize the risk of failing to identify patients who might benefit from supplementation. Plasma levels of n3LCPUFA were used rather than measurement of tissue levels such as in RBC membranes which may be a more reliable measure of cellular availability of n3LCPUFA (Vilaseca et al. 2010). However, as assessment of membrane lipid content is not available clinically to most centers caring for adults with PKU (due to the practicality of sample handling in that samples have to be received at the lab within 24 h of collection), we feel the information on plasma levels is still useful to clinicians. Finally, geographic differences in dietary n3LCPUFA intake which have been described in other European countries (Acosta et al. 2001) may not make our results applicable to other centers.

Conclusions

Use of a modified diet history looking at sources of n3LCPUFA intake can be used to target screening of fatty acid levels to at-risk individuals with PKU. If supplementation is considered, preformed DHA may be the most appropriate form of supplementation. Further research is required to determine the optimal dose range for DHA supplementation and the effects of such supplementation, if any, on clinical outcomes in patients with PKU.

Compliance with Ethics Guidelines

Taryn Bosdet, Jennifer Branov, Caroline Selvage, Masoud Yousefi, and Sandra Sirrs declare that they have no conflict of interest.

Contributions of Individual Authors

Taryn Bosdet and Jennifer Branov were involved in the conception, design, conduct of the study, and the interpretation of data and drafting of article including revision for content of this manuscript.

Caroline Selvage's primary contribution was in the conduct of the study and data collection as well as the drafting of the manuscript.

Sandra Sirrs was involved in the analysis and interpretation of data as well as in the drafting and revision of the manuscript.

Masoud Yousefi performed analysis and interpretation of data and contributed to the drafting of the manuscript.

Informed Consent

All procedures followed were in accordance with the ethical standards of the responsible committee on human experimentation (institutional and national) and with the Helsinki Declaration of 1975, as revised in 2000(5). Informed consent was obtained from all patients being included in the study.

Appendix

Questions useful in assessing DHA intake and predicting DHA status (Questions 1–4 selected from Sublette et al. 2011):

1. How many times have you eaten fish or shellfish in any form?
 - (1) Never
 - (2) Less than 1 time each month
 - (3) 1 time each month
 - (4) 2–3 times each month
 - (5) 1 time each week
 - (6) 2 times each week
 - (7) 3–4 times each week
 - (8) 5–6 times each week
 - (9) 1 time each day
 - (10) 2 or more times each day
2. Each time you ate fish or shellfish, how much did you eat?
 - (1) Less than 2 ounces or less than one fillet or less than 4 pieces of sushi
 - (2) 2–7 ounces or about 1 fillet or 4–14 pieces of sushi
 - (3) More than 7 ounces or more than 1 fillet or more than 14 pieces of sushi
3. In the past 6 months, about how often did you use cod liver oil?
 - (1) Never
 - (2) Less than 1 time each month
 - (3) 1 time each month
 - (4) 2–3 times each month
 - (5) 1 time each week
 - (6) 2 times each week
 - (7) 3–4 times each week
 - (8) 5–6 times each week
 - (9) 1 time each day
 - (10) 2 or more times each day

4. In the past 6 months, have you used an omega-3 fatty acid or fish oil supplement at least once each week?

- (1) No
(2) Yes – What type of an omega-3 fatty acid or fish oil supplement did you take?

Please write the name of the supplement below:

- (3) Is the omega-3 fatty acid or fish oil supplement in pill or capsule form?

- (1) No
(2) Yes – How much did you take?

- (1) 1 pill or capsule each week
(2) 2 pills or capsules each week
(3) 3–4 pills or capsules each week
(4) 5–6 pills or capsules each week
(5) 1 pill or capsule each day
(6) 2 pills or capsules each day
(7) 3–4 pills or capsules each day
(8) 5 or more pills or capsules each

- (4) Is the omega-3 fatty acid or fish oil supplement (besides cod liver oil) in liquid form?

- (1) No
(2) Yes – How much did you take?

- (1) Less than 1 tablespoon each week
(2) 1 tablespoon each week
(3) 2 tablespoons each week
(4) 3–4 tablespoons each week
(5) 5–6 tablespoons each week
(6) 1 tablespoon each day
(7) 2 tablespoons each day
(8) 3–4 tablespoons each day
(9) 5 or more tablespoons each day

- (5) Please write down the dosage of omega-3 fatty acids or fish oil supplement if you know it:

Dosage:

Pills or Capsules: _____mg DHA and _____mg EPA per pill/capsule

Liquid: _____mg DHA and _____mg EPA per tablespoon

Do not know dosage

5. Do you take a PKU Medical Food Product?

No

Yes

Please write down the name of your PKU Medical Food:

6. On average, how much of this PKU Medical Food do you take each day (please write down what you are actually taking, not what would be ideal):

Scoops/packages: I take _____scoops/packages of formula each day

Grams of powder: I take _____grams of powder per day

Tetra's/pouches: I take _____pouches of formula per day

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