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NORMAL FATTY ACID CONCENTRATIONS IN YOUNG CHILDREN WITH PHENYLKETONURIA (PKU)

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Abstract

The objective of this study was to determine if children with phenylketonuria (PKU) have lower fatty acid concentrations in total erythrocyte lipid due to the phenylalanine restricted diet therapy compared to healthy control subjects. Dietary intake and fatty acid concentrations in total erythrocyte lipid were measured in twenty-one subjects (≤ 6 years of age) with PKU and twenty-three control children. Subjects with PKU had significantly lower protein and significantly higher polyunsaturated fat intake compared to controls. Subjects with PKU had significantly lower concentrations in total erythrocyte lipid of the sum of the ω -3, ω -6, saturated and polyunsaturated fatty acids. Concentrations of fatty acids among subjects with PKU were lower than control subjects but no subject with PKU exhibited any signs or symptoms suggestive of essential fatty acid deficiency, thereby suggesting that subjects with PKU in this cohort have normal and adequate essential fatty acid concentrations in total erythrocyte lipid.

Keywords

Phenylketonuria; PKU; Fatty Acids; Lipids

INTRODUCTION

Phenylketonuria (PKU) is an autosomal recessive disorder affecting approximately 1:15,000 live births in North America¹ and is characterized by elevated blood phenylalanine concentration due to phenylalanine hydroxylase deficiency.^{1–2} Current literature is equivocal about the incidence of essential fatty acid (EFA) deficiency among patients with PKU. Several studies have reported lower values of EFAs, and in particular lower values of arachidonic acid (ARA; C20:4n-6) and docosahexaenoic acid (DHA; C22:6n-3) in children with PKU following a low phenylalanine diet.^{3–8} However, researchers disagree on whether the difference is clinically significant and thus suggestive of EFA deficiency.^{4,6,8–10} The possibility of an iatrogenic EFA deficiency in this population is reasonable. Treatment of PKU is centered on dietary intervention aimed at restriction of phenylalanine-containing foods (i.e. high protein foods) and supplementation with a phenylalanine-free protein substitute.^{11–12} As a result, children with PKU have a low supply of whole animal foods and a low intake of long-chain polyunsaturated fatty acids (LCPUFAs).^{7,10,13} ARA and DHA in particular are predominantly found preformed in animal foods. In cases of low or no intake of these fatty acids, humans rely on endogenous synthesis from the 18-carbon precursors, linoleic acid (LA; C18:2n-6) for ARA

and α -linolenic acid (LNA; C18:3n-3) for DHA, through desaturating and elongating reactions.^{3,7}

One possible explanation for discrepancies between studies could be the use of plasma versus red blood cells (erythrocytes) and/or the manner in which the results were expressed. Plasma fatty acids vary with feeding and fasting and are believed to be an indicator of recent fat intake. Erythrocyte fatty acids reflect longer-term intake and do not demonstrate a short-term response to dietary changes.^{14–15} The literature pertaining to the EFA status of children with PKU has traditionally expressed EFA status as a relative weight percent of fatty acids to total lipids,^{3, 8–9,13} as opposed to expressing the absolute concentration of the individual fatty acids. The ratio of fatty acids does provide valuable information on the proportion of each fatty acid as part of total lipids, but the effects of fatty acid deficiency are most likely related to absolute fatty acid concentrations.^{16–18}

We hypothesized that children with PKU consume inadequate amounts of the EFAs LA, LNA, ARA, and DHA because of a comprehensive dietary restriction of phenylalanine-containing foods. Low dietary intake of EFAs would result in significantly reduced concentrations of these fatty acids in erythrocytes. The LCPUFAs DHA and ARA have important physiological implications and are required for optimal somatic growth and brain development, particularly in the early years of life.^{3,13,19–20} Thus, the primary aim of this study was to determine if infants and young children with PKU have low EFA concentrations in erythrocytes suggesting EFA deficiency compared to sex- and age-matched controls. A secondary aim was to investigate whether the diet of subjects with PKU provided inadequate amounts of LA, LNA, ARA, and DHA as a result of the dietary therapy used to treat PKU.

METHODS

Subjects

This was an observational, case-control study conducted at a single institution (Oregon Health & Science University, OHSU, Portland, Oregon). The dietary intake of energy and macronutrients, and erythrocyte concentrations of EFAs of 21 children, nine months through seven years of age, with PKU and 23 healthy sex- and age-matched (± 1 year), unaffected control subjects were compared. Subjects with PKU were recruited from those undergoing routine follow-up at the Metabolic Clinic at the Child Development and Rehabilitation Center at OHSU. Sex- and age-matched control subjects were recruited from those undergoing minor surgery at the Day Surgery Clinic at Doernbecher Children's Hospital (Portland, Oregon). To ensure age was normally distributed within the study population, subjects were stratified into three age groups: 9–35 months of age, 3–4 years of age, and 5–7 years of age.

Blood Analysis

Blood samples were collected during a routine blood draw at the time of their regularly scheduled clinic appointment from children with PKU, and at the time of IV placement for anesthesia from control subjects. Total lipid was extracted from erythrocytes with 3:2 hexane:isopropyl alcohol. The hexane layer was transferred, dried under nitrogen and fatty acid profiles were determined by gas chromatography mass spectroscopy (GC/MS) according to the methods of Lagerstedt et al.¹⁸ The sums of the ω -3, ω -6, saturated, monounsaturated, and polyunsaturated fatty acids were calculated for each subject by adding together the absolute concentrations of the respective fatty acids within each summed group. Plasma total, HDL, LDL and VLDL cholesterol, and triglycerides were determined by standard lipid analysis.

Diet Analysis

Parents of subjects completed three, separate, twenty-four hour, multiple-pass, dietary recalls by telephone interview. Nutrient analysis was performed using The Food Processor SQL 9.7.3 (ESHA, Salem, Oregon). Dietary intake of energy, macronutrients, and saturated, monounsaturated, and polyunsaturated fat was determined for each day and the three-day average was calculated.

Statistical Analysis

Dietary intake and EFA concentrations in total erythrocyte lipid of subjects with PKU and control subjects were compared by independent sample t-test.

RESULTS

Subjects

This study was approved by the OHSU Institutional Review Board. There were no significant differences in mean age, weight, weight-for-age percentile, and weight-for-age z-score between subjects with PKU and control subjects within each age stratification.

Dietary Macronutrient Intake

Dietary intake of energy and macronutrients is presented in Table 1. Complete diet information was available for all subjects with PKU (n=21) and 20 control subjects. Subjects with PKU had a significantly lower protein intake and a significantly higher polyunsaturated fat intake compared to sex- and age-matched controls. There were no other significant differences between the two groups.

The mean contribution of the phenylalanine-free protein substitute, the low phenylalanine modified foods, and all other foods, as a percent of total nutrient intake among subjects with PKU is presented in Table 2. The phenylalanine-free protein substitute provided approximately 20% of the total energy and fat and 80% of the total protein intake among children with PKU. Low phenylalanine modified food products (i.e. low protein) provided approximately 20% of total energy and total fat. Regular foods provided the remainder of the total energy and fat intake.

Plasma Lipid Profiles and Erythrocyte Fatty Acid Concentrations

Plasma lipid profiles were compared between groups and are presented in Table 3. Subjects with PKU had significantly higher concentrations of VLDL cholesterol and triglycerides compared to sex- and age-matched control subjects. This is most likely a reflection of postprandial elevation related to recent dietary intake among subjects with PKU compared to the fasting status of control subjects undergoing routine surgery. Individual fatty acid concentrations are presented in Table 4. The blood sample for one subject with PKU was not available for analysis. Subjects with PKU had significantly lower concentrations of the sums of the ω -3 ($p=0.041$), ω -6 ($p=0.033$), saturated ($p=0.043$) and polyunsaturated ($p=0.029$) fatty acids.

CONCLUSIONS

We observed that children with PKU had lower concentrations of polyunsaturated fatty acids (PUFAs) in erythrocytes despite a higher dietary intake of PUFAs compared to sex- and age-matched control subjects. Published normal erythrocyte fatty acid concentrations are expressed as a percent of total lipid (wt%) and not as a concentration ($\mu\text{mol/L}$) so we were unable to compare our data with other established reference ranges. While our data suggests that children

with PKU have lower EFA concentrations in total erythrocyte lipid compared to control subjects, we did not observe any physical signs or symptoms of EFA deficiency in this population. Presumably, subjects with PKU had adequate concentrations of EFAs to prevent overt signs and symptoms of EFA deficiency related to adequate intake of dietary PUFAs. Our dietary analysis supports this conclusion because despite restriction of phenylalanine-containing foods, the dietary intake of children with PKU supplied more EFAs than observed in the diets of control subjects. The reason we observed a significantly higher PUFA intake with lower incorporation into erythrocytes is unknown at this time but may suggest altered utilization and incorporation of PUFAs into erythrocyte lipids among subjects with PKU.

The higher dietary intake of PUFAs among children with PKU is most likely related to the significant contribution of these nutrients by the phenylalanine-free protein substitute. Previous studies have reported that phenylalanine-free protein substitutes supply approximately 80–90% of required dietary protein and other nutrients,^{21–22} and most are a significant source of polyunsaturated fat in the form of LA and LNA. Our data suggests that within the population studied the phenylalanine-free protein substitute supplied approximately 25–35% of total dietary fat intake, and approximately 25–30% of total polyunsaturated fat intake. Natural protein foods only accounted for approximately 40–60% of total fat intake, and 45–55% of polyunsaturated fat intake. Thus, the contribution of the phenylalanine-free protein substitute and low phenylalanine modified foods to total fat intake, and in particular LA and LNA intake is critical for adequate EFA intake in this patient population. The use of low-fat or fat-free phenylalanine-free protein substitutes, particularly when not used in combination with a fat-containing protein substitute raises concern regarding the nutritional adequacy of the diet with respect to total fat and LA and LNA intake. Most of these products are not recommended for use in young children, however, many older patients desire to incorporate these low-fat or fat-free options into their nutrition therapy, either as an exclusive source of phenylalanine-free protein or in combination with some other phenylalanine-free protein substitute in order to reduce the total calorie contribution of their phenylalanine-free protein requirement. Absolute concentrations of essential fatty acids should be monitored in these individuals.

A limitation of this study is that it was conducted in young children whose dietary recall records suggested 100% compliance with the phenylalanine-free protein substitute prescription, all children were taking a fat-containing phenylalanine-free protein substitute, and all children were presumed to be in good metabolic control. Others have found the EFA status of patients with PKU who are not compliant with the diet regimen to be lower.

We also observed that cholesterol values in subjects with PKU were not significantly different compared to control subjects. This differs from previously published data indicating subjects with PKU had low plasma cholesterol values.^{23–25} These studies also documented lower total fat and lower saturated fat intake among young children with PKU.^{23–24} Despite the fact that subjects with PKU do not consume animal foods, our dietary analysis indicated the total amount of saturated fat consumed was not different between subjects with PKU and control subjects. We propose this indicates a shift in diet composition among current children with PKU compared to patients in past generations. The advent of low-phenylalanine modified foods has dramatically increased the variety and palatability of the PKU diet over the past decade but these foods may also be contributing to increased intakes of saturated fat. Our data suggest that these foods account for nearly 20% of total saturated fat intake among children with PKU. The increase in variety and palatability of the PKU diet will no doubt improve compliance and metabolic control in this patient population; however, this shift in dietary intake towards the average American diet, including the increase in saturated fat intake may have long-term health implications as it could suggest that individuals with PKU are at equal risk for cardiovascular disease as the general population.

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Table 1

Mean dietary intake of subjects with PKU compared to sex- and age-matched control subjects.

	PKU (N=21)	Control (N=20)	Sig. (2-tailed)
	Mean $\pm$ SEM		
Energy			
total kcal/day	1461 $\pm$ 91	1614 $\pm$ 132	0.342
kcal/kg/day	91 $\pm$ 7	100 $\pm$ 7	0.391
Protein			
% total energy	10.9% $\pm$ 0.8% ^a	13.3% $\pm$ 0.9%	0.048*
g/kg/day	2.4 $\pm$ 0.2	3.3 $\pm$ 0.3	0.029*
Carbohydrate			
% total energy	57.5% $\pm$ 1.6%	57.2% $\pm$ 1.7%	0.907
Total Fat			
% total energy	31.6% $\pm$ 1.5%	29.8% $\pm$ 1.4%	0.375
Saturated Fat			
% total energy	10.1% $\pm$ 1.0%	11.0% $\pm$ 0.6%	0.391
Monounsaturated Fat			
% total energy	12.0% $\pm$ 0.7%	10.9% $\pm$ 0.6%	0.266
Polyunsaturated Fat			
% total energy	7.2% $\pm$ 0.4%	5.4% $\pm$ 0.4%	0.003*

*Significant at $p < 0.05$

SEM, standard error of the mean

^aProtein intake is reported as a combination of natural protein (i.e. food sources) and synthetic amino acids (i.e. phenylalanine-free protein substitutes) among subjects with PKU.

Table 2

Mean contribution of the phenylalanine-free protein substitute, low phenylalanine modified foods, and all other foods, as a percent of total nutrient intake among 21 subjects with PKU.

	Phenylalanine-Free Protein Substitute	Low Phenylalanine Modified Foods	Other Foods
	Mean $\pm$ SEM		
Energy			
% total energy	29.7% $\pm$ 2.3%	23.8% $\pm$ 3.9%	46.5% $\pm$ 5.04%
Protein			
% total protein	76.3% $\pm$ 8.1%	1.6% $\pm$ 0.3%	22.1% $\pm$ 2.4%
Carbohydrate			
% total carbohydrate	19.7% $\pm$ 2.3%	29.5% $\pm$ 5.1%	50.7% $\pm$ 5.4%
Total Fat			
% total fat	29.7% $\pm$ 3.3%	21.2% $\pm$ 3.9%	49.1% $\pm$ 8.5%
Saturated Fat			
% total saturated fat	33.7% $\pm$ 4.7%	17.6% $\pm$ 4.0%	48.8% $\pm$ 12.5%
Monounsaturated Fat			
% total monounsaturated fat	29.3% $\pm$ 5.0%	21.0% $\pm$ 4.2%	49.7% $\pm$ 8.9%
Polyunsaturated Fat			
% total polyunsaturated fat	27.5% $\pm$ 3.8%	24.8% $\pm$ 6.0%	47.7% $\pm$ 5.9%

SEM, standard error of the mean

Table 3
Plasma lipid profiles of subjects with PKU compared to sex- and age-matched control subjects.

	Total Cholesterol	VLDL Cholesterol	LDL Cholesterol	HDL Cholesterol	Triglycerides
	Mean ± SEM (μmol/L)				
PKU (N=21)	153.8±8.5	26.1±3.7	83.1±6.1	44.5±1.8	130.3±18.3
Control (N=23)	139.3±3.6	14.6±1.1	83.2±3.3	41.5±2.0	72.7±5.6
Sig. (2-tailed)	0.112	0.003 *	0.996	0.268	0.003 *

SEM, standard error of the mean

* Significant at $p<0.05$

Fatty acid concentrations in total erythrocyte lipid of subjects with PKU and sex- and age-matched control subjects.

Table 4

Saturated Fatty Acids			Monounsaturated Fatty Acids			Polyunsaturated Fatty Acids		
PKU (N=20)	Control (N=23)	Sig. ^a	PKU (N=20)	Control (N=23)	Sig. ^a	PKU (N=20)	Control (N=23)	Sig. ^a
Mean ± SEM (μmol/L)			Mean ± SEM (μmol/L)			Mean ± SEM (μmol/L)		
C14:0	146.4±28.3	0.035*	C14:1	1.4±0.3	0.9±0.2	C18:3 (LNA)	8.8±0.2	9.5±0.4
C16:0	847.4±32.6	0.014*	C16:1	43.4±1.4	45.5±1.6	C20:5 (EPA)	51.8±2.0	53.7±1.7
C18:0	660.8±30.4	0.005*	C18:1	363.6±8.3	391.8±17.0	C22:6 (DHA)	58.0±3.7	68.6±4.2
Total	1654.7±73.6	0.043*	Total	408.4±8.0	438.3±17.5	ω-3 FA	118.6±3.9	131.8±4.7
						C18:2 (LA)	339.4±5.3	363.1±9.5
						C20:4 (ARA)	288.8±12.8	356.4±27.5
						ω-6 FA	628.2±15.2	719.5±36.3
						Total	746.8±17.9	851.3±40.2

^aSig., significance (2-tailed)

SEM, standard error of the mean

FA, fatty acids

C14:0, Myristic acid; C14:1, Myristoleic acid; C16:0, Palmitic acid; C16:1, Palmitoleic acid; C18:0, Stearic acid; C18:1, Oleic acid; LA, Linoleic acid; ALA, α-linolenic acid; EPA, Eicosapentaenoic acid; ARA, Arachidonic acid; DHA, Docosahexaenoic acid

ω-3 FA = sum of C18:3, C20:5, C22:6

ω-6 FA = sum of C18:2, C20:4

Saturated FA = sum of C14:0, C16:0, C18:0

Monounsaturated FA = sum of C14:1, C16:1, C18:1

Polyunsaturated FA = sum of C18:2, C18:3, C20:4, C20:5, C22:6

* Significant at *p*<0.05