ELSEVIER

The essential fatty acid status in phenylketonuria patients under treatment

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Phenylketonuric patients are on a special diet that lacks certain essential fatty acids. This study evaluates the essential fatty acid status of a group of phenylketonuric patients in the Netherlands undergoing dietary treatment. To this end, the essential fatty acid status of nine phenylketonuria patients was studied. On the basis of age and gender, two control subjects were selected for each patient. The essential fatty acid composition of duplicate food portions and the essential fatty acid status of plasma and erythrocytes were analyzed. Phenylketonuria subjects had a different essential fatty acid profile from their peers, especially concerning the n-3 fatty acids. N-6 and n-3 fatty long-chain polyenes were hardly consumed by phenylketonuria subjects, in contrast to the control subjects. Linoleic acid, on the other hand, was consumed in significantly higher amounts by phenylketonuria subjects and made up about 40% of their daily fat consumption. The essential fatty acid consumption pattern of the phenylketonuria subjects is mirrored by the essential fatty acid concentrations in blood. The essential fatty acid status of the phenylketonuric diet should be improved in order to prevent deficiency in n-3 fatty acids. (J. Nutr. Biochem. 11:543–547, 2000)

Keywords: phenylketonuria; diet; fatty acids; essential; alpha-linolenic acid; linoleic acid

Introduction

Phenylketonuria (PKU) is a disease caused by absent or reduced activity of the enzyme phenylalanine hydroxylase. It is an autosomal recessive hereditary disease, the incidence of which varies among ethnic groups. The prevalence of PKU in the Netherlands is 1:18,000,[1] which is comparable to the incidence in other European countries and the United States.[2] The consequences of PKU, if untreated, are elevated blood concentrations of the amino acid phenylalanine (Phe), which causes mental retardation, behavioral disturbances, seizures, and eczema. Treatment consists of a low-Phe diet that has to be started after diagnosis, and has to be continued through adolescence and, preferably, lifelong. Low dietary Phe intake is achieved by reduction of natural protein intake, which means that the intake of animal products in particular is low.[3] Food derived from animal sources, however, is a major source of long-chain polyenes (LCPs) of the n-3 and n-6 families.

It has been reported that children with classical PKU have different essential fatty acid (EFA) profiles compared to healthy controls.[4–6] The present study was designed to assess the EFA status of phenylketonuric patients and to explore the relationship with their diet.

Methods and materials

Study population

Ten PKU patients with classical PKU and/or their parents were approached by the dietician of the Department of Pediatrics of the Maastricht University Hospital. Age varied from 6 months to 25 yr. Patients were regarded eligible if their Phe plasma values were between 200 and 500 μmol/L and were stable for over a year. In the case of the infants younger than 1 yr, plasma values had to have been stable during treatment. These plasma values are a good indication of compliance with the diet. The parents of nine children were willing to participate; one refused.

For each patient, two matching control subjects were selected, based on age and gender. Control subjects were recruited via patients (school friends), primary schools, and primary health care

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centers. In the case of children younger than 3 yr, the age difference had to be less than 5%; in the case of children 3 yr and older, the difference had to be less than 10%. Weight and height of the patients was recorded.

The procedures followed were in accordance with the ethical standards of the local ethics committee and patients and/or their parents gave written informed consent.

Duplicate portion collection

Parents were asked to collect a duplicate portion of the food their child consumed during 48 hr, and to keep a detailed diary of food intake including solid and liquid foods. To this end, parents were provided with two plastic buckets, two plastic bottles, a balance, and written instructions. The parents were instructed to keep buckets and bottles containing food in the refrigerator until the second visit. Buckets and bottles containing food and drinks, the diary, and the balance were picked up during the second visit, the day after food collection had been completed. After thorough mixing, two samples of 30 g were taken and frozen at −20°C until analysis.

Food analysis

Approximately 5 g of food was freeze dried overnight and the energy content of the sample was measured by direct calorimetry (Janke & Kunkel, IKA Calorimeter C-400; adiabatic, Staufen, Germany). Lipids were extracted from freeze-dried food using a combination of the Bligh and Dyer[7] method and the Folch method.[8] Preparation of a total lipid extract of 125 mg of freeze-dried food samples was performed with a modified Folch extraction.[8,9] Three drops of HCl (Merck, Darmstadt, Germany), 37%, were added to precipitate proteins. After addition of an internal standard, trinonadecanoin (SIGMA, Zwijndrecht, The Netherlands) (19:0), total lipid extracts of food suspensions were prepared according to the method of Bligh and Dyer.[7] The lipid extracts were saponified and the fatty acids converted to the corresponding methyl esters by reaction with 10% boron trifluoride in methanol at 100°C for 30 min.[10] Butylated hydroxytoluene (BHT) (50 mg/L) (SIGMA) was added as an antioxidant. The fatty acid composition of the phospholipids (PLs) was determined by capillary gas liquid chromatography as described before.[11] Fatty acid amounts present in the PL fraction were quantified based on the amount of 19:0 internal standard fatty acid methyl ester recovered. Fatty acid composition of the lipids was determined by gas liquid chromatography as described by Rand et al.[12]

From the diaries, all foods and drinks were coded according to the system of the Netherlands Nutrient Databank developed in the Netherlands, and converted into energy and nutrients by using the extended computerized version of the Netherlands Food Table.[13]

Blood sampling and analysis

Blood was collected in EDTA-containing tubes (Becton Dickinson, NJ USA) by a finger prick during the second visit. Plasma was separated from the blood cells by centrifugation (at 3,000 *g* during 10 min at 4°C) and after plasma collection, the erythrocytes were washed with EDTA containing saline (SIGMA). The tubes containing the plasma or erythrocytes were closed under a stream of nitrogen to prevent oxidation and stored at −20°C until analysis. The maximum storage time was 3 months. The fatty acid composition of plasma PLs was determined as described by Foreman van Drongelen et al.[11] Briefly, preparation of a total lipid extract of 100-μL samples was performed with a modified Folch extraction.[8,9] After addition of an internal standard, 1,2-alpha-dinonadecanoyl (SIGMA) phosphatidylcholine, total lipid extracts of plasma or erythrocyte suspensions were prepared according to the method of Bligh and Dyer.[7] Heptadecaenoic acid (17:1) (SIGMA) was added to the samples to check for any carry-over of free fatty acids during the isolation of PLs. The PL fractions were isolated by solid phase extraction from the total lipid extracts on aminopropyl-silica columns.[14] The resulting PLs were saponified and the fatty acids converted to the corresponding methyl esters by reaction with 14% boron trifluoride (SIGMA) in methanol (Merck) at 100°C for one hr.[10] To all organic solvents, BHT (50 mg/L) was added as an antioxidant. The fatty acid composition of the PLs was determined by capillary gas liquid chromatography as described before.[11] Fatty acid amounts present in the PL fraction were quantified based on the amount of 19:0 internal standard fatty acid methyl ester recovered. Thirty-five fatty acids were characterized, but only a selection of these are presented in this article, expressed as μg/mL plasma or erythrocyte suspension, and as percentage of total fatty acids by weight. The sum of the saturated and mono-unsaturated fatty acids as well as the sum of the n-6 LCP (20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6) and the n-3 LCP (20:5n-3, 22:5n-3, 22:6n-3) are presented. All blood samples of the PKU patients and the matching controls were analyzed simultaneously.

Statistical analyses

All data are presented as mean ± SEM. The data of the two matching controls were averaged and the average was taken into analyses to compare the controls with the PKU patients. The Student's *t*-test for paired samples was used to compare the two groups. P-values < 0.05 were considered to be significant.

Results

Clinical parameters

In general, the body weight of PKU subjects was lower than that of the control group (*Table 1*). No differences were observed for height and body mass index.

Macronutrient and EFA consumption

As *Table 2* shows, insignificant differences were observed between the energy intake as calculated from the diaries and as measured by duplicate portion analyses. PKU subjects had a significantly lower fat intake, and tended toward a lower protein consumption. This was compensated by a significantly higher carbohydrate intake. A disagreement exists between calculated results and laboratory analyses of the fat consumption in the PKU group, resulting in a lower fat consumption as compared to the control group.

As shown in *Table 3*, the proportion of n-6 fatty acids consumed was much higher in the PKU group. Total n-6 fatty acid intake of the PKU subjects made up almost 40% of their daily fat consumption, whereas for the control subjects, this figure was only 20%. Furthermore, PKU subjects did not consume any n-6 LCPs, in contrast to control subjects who did so to a small extent.

PKU subjects consumed half the level of n-3 fatty acids compared with the controls. No LCPs of the n-3 family were consumed by the PKU group, whereas the control peers did consume small but significant amounts of n-3 LCPs. The control group consumed more saturated fatty acids compared to the PKU patients.

Table 1 Study population

	PKU patients (n = 9)				Control subjects (n = 18)			
Gender	Year of birth	Weight (kg)	Length (m)	BMI (kg/m^2)	Year of birth	Weight (kg)	Length (m)	BMI (kg/m^2)
Male	1967	65	1.8	20.06	1966	77	1.87	22.02
					1966	75	1.68	26.57
Male	1978	47	1.66	17.06	1978	42	1.60	16.41
					1978	67	1.86	19.37
Male	1986	19	1.16	14.12	1986	23	1.23	15.20
					1986	27	1.36	14.60
Female	1987	17	1.18	12.21	1987	19	1.14	14.62
					1987	21	1.36	11.35
Female	1988	19.5	1.06	17.35	1988	15	1.03	14.14
					1988	18	1.20	12.50
Male	1988	15.3	0.99	15.61	1988	18	1.11	14.61
					1988	20	1.11	16.23
Male	1991	9.8	0.74	17.90	1991	12.5	0.84	17.72
					1991	11.5	0.83	16.69
Male	1992	7	0.7	14.29	1992	11	0.78	18.08
					1992	9	0.72	17.36
Female	1992	8.7	0.71	17.26	1992	8	0.73	15.22
					1992	8.2	0.69	17.22

PKU–phenylketonuria. BMI–body mass index.

Fatty acid composition of plasma and erythrocyte phospholipids

The fatty acid composition of the plasma PLs shows clear differences between both groups (*Table 4*). The proportions of total n-3 and n-3 LCPs are significantly lower in the PKU group, whereas total n-6 and n-6 LCPs are significantly higher. Comparable differences were observed for the fatty acid composition of the erythrocyte PLs (*Table 5*).

Discussion

The main conclusions of this study are that total fat, alpha-linolenic acid (18:3n-3; ALA), LCPs, and saturated fatty acids were consumed significantly less by the PKU subjects as compared to matched controls, whereas linoleic acid (18:2n-6; LA) was consumed more. In plasma and erythrocyte profiles of PKU subjects, the total n-6 as well as the n-6 LCPs were significantly higher, whereas total n-3 and n-3 LCPs were significantly lower. At the time of our study, patients were on a strict diet and known to be compliant. It is very likely that the observed differences in these fatty acid profiles between patients and controls are a result of this diet.

The fat intake of PKU subjects, determined from duplicate portion analyses, was lower than that calculated from the computer program. A cause of this difference might be that the computer program was limited in its sources of food because the special PKU products are not incorporated into this program. To overcome this problem, the composition of these products had to be added to the program. For this, we were dependent on the information provided by the producers of the products as well as on the recipes provided by the parents.

Clearly, the differences in composition of the diets between PKU subjects and controls were the main cause for the different total LCPs. ALA consumption was reduced by half in the PKU group compared with the healthy group. Consequently, the amounts in plasma and erythrocyte PLs were significantly lower. Also n-3 LCPs were reduced in the plasma and erythrocytes of PKU subjects. The higher LA intake by the PKU subjects in this study is due to the higher consumption of mayonnaise, LA-enriched margarine, and

Table 2 Contribution of the three main food components to total energy

	PKU patients n = 9)	Control subjects (n = 18)	P-value
Energy intake calculated (kJ/day)	6382 ± 1292	6267 ± 877	n.s.
Energy intake analyzed (kJ/day)	6534 ± 1208	6051 ± 841	n.s.
Total fat consumption calculated (en%)	24.68 ± 2.45	29.63 ± 1.93	n.s.
Total fat consumption analyzed (en%)	18.92 ± 2.00	30.80 ± 2.80	0.005
Carbohydrate consumption calculated (en%)	64.96 ± 2.57	56.77 ± 2.27	0.0227
Protein consumption calculated (en%)	10.38 ± 1.62	13.03 ± 0.88	n.s.

Values are mean ± standard error of the mean.
PKU–phenylketonuria. n.s.–not significant.

Table 3 Fatty acids in duplicate portions (percentage (wt/wt) of total fatty acids)

Fatty acid	PKU patients (n = 9)	Control subjects (n = 18)	P-value
18:2 n-6	39.65 ± 2.75	20.44 ± 1.23	0.0004
20:2 n-6	n.d.	0.02 ± 0.01	0.0392
20:4 n-6	n.d.	0.12 ± 0.02	0.0009
18:3 n-3	1.76 ± 0.17	2.39 ± 0.19	0.0022
20:4 n-3	n.d.	0.01 ± 0.01	n.s.
20:5 n-3	n.d.	0.04 ± 0.04	0.0138
22:5 n-3	n.d.	0.02 ± 0.01	0.0138
22:6 n-3	n.d.	0.07 ± 0.07	n.s.
n-6	39.65 ± 2.75	20.58 ± 1.23	0.0004
n-6 LCPs	n.d.	0.14 ± 0.03	0.0011
n-3	1.76 ± 0.17	2.53 ± 0.03	0.0058
n-3 LCPs	n.d.	0.14 ± 0.13	n.s.
n-7 + n-9	28.19 ± 1.14	30.94 ± 1.05	0.0003
MUFA	31.18 ± 1.70	34.44 ± 1.84	n.s.
SAFA	28.44 ± 1.56	44.61 ± 1.97	0.0004
Total fatty acid intake (g/day)	68.46 ± 17.17	108.54 ± 27.66	n.s.

Values are mean ± standard error of the mean.
PKU–phenylketonuria. n.s.–not significant. n.d.–not detectable. LCPs–long-chain polyenes. MUFA–monounsaturated fatty acids. SAFA–saturated fatty acids.

(in the case of the infants) sunflower oil. Osbond acid (22:5n-6) is significantly higher in plasma and erythrocytes of PKU subjects compared to the healthy controls. This is an indication for a relative deficiency of docosahexanoic acid (DHA) in the diet of these patients.[15,16]

Galli et al.[5] found a reduction of 50% of arachidonic acid (20:4n-6; AA), eicosapentanoic acid (EPA), and DHA in plasma and erythrocyte fatty acid profiles of PKU patients compared with controls of the same age and gender. These results were later confirmed in another study by Sanjurjo et al.[6] Poge et al.[4] did not find different AA amounts in plasma or erythrocytes between PKU patients and healthy controls in blood but confirmed lower contents of DHA in erythrocytes. Our results corroborate the lower DHA contents in plasma and erythrocyte PLs as reported in these three studies. In contrast, we did not find lowered AA concentrations in plasma of PKU subjects. In their erythrocytes, however, a significantly lower concentration of AA was observed. It is not unlikely that LA and ALA intakes in other countries with different consumption patterns are different from those in our subjects. It is important, though, to keep a close watch on the essential fatty acid consump-

Table 4 Fatty acid composition (% of total fatty acids) of plasma phospholipids in PKU patients compared with healthy controls

Fatty acid	PKU patients (n = 9)	Control subjects (n = 18)	P-value
18:2 n-6	23.65 ± 1.14	23.63 ± 0.63	n.s.
18:3 n-6	0.10 ± 0.04	0.05 ± 0.01	n.s.
20:2 n-6	0.47 ± 0.02	0.33 ± 0.01	<0.0001
20:3 n-6	3.32 ± 0.27	2.72 ± 0.07	0.0374
20:4 n-6	9.34 ± 0.43	8.90 ± 0.68	n.s.
22:2 n-6	0.03 ± 0.00	0.01 ± 0.00	n.s.
22:4 n-6	0.88 ± 0.12	0.46 ± 0.03	0.0061
22:5 n-6	0.70 ± 0.08	0.40 ± 0.04	0.0023
24:2 n-6	0.32 ± 0.03	0.18 ± 0.01	0.0017
18:3 n-3	0.09 ± 0.01	0.20 ± 0.02	0.0001
20:4 n-3	0.07 ± 0.01	0.12 ± 0.01	0.0023
20:5 n-3	0.18 ± 0.02	0.48 ± 0.07	0.0036
22:5 n-3	0.68 ± 0.03	0.94 ± 0.02	0.0001
22:6 n-3	1.41 ± 0.11	2.74 ± 0.17	0.0002
n-6	38.81 ± 0.64	36.68 ± 0.49	0.0223
n-6 LCPs	14.59 ± 0.68	12.67 ± 0.34	0.0406
n-3	2.45 ± 0.14	4.51 ± 0.21	0.0001
n-3 LCPs	2.36 ± 0.13	4.31 ± 0.22	0.0001
n-7 + n-9	12.13 ± 0.49	11.86 ± 0.25	n.s.
MUFA	11.97 ± 0.92	11.64 ± 0.48	n.s.
SAFA	43.08 ± 0.08	43.44 ± 0.30	n.s.
Total fatty acids (mg/L)	1,018.50 ± 35.70	1,152.14 ± 62.57	n.s.

Values are mean ± standard error of the mean.
PKU–phenylketonuria. n.s.–not significant. LCPs–long-chain polyenes. MUFA–monounsaturated fatty acids. SAFA–saturated fatty acids.

Table 5 Fatty acid composition (% of total fatty acids) of erythrocyte phospholipids in PKU patients compared with healthy controls

Fatty acid	PKU (n = 9)	Controls (n = 18)	P-value
18:2 n-6	12.99 ± 0.33	12.21 ± 0.21	0.0229
18:3 n-6	0.04 ± 0.01	0.02 ± 0.00	0.0175
20:2 n-6	0.47 ± 0.03	0.28 ± 0.01	0.0001
20:3 n-6	1.50 ± 0.13	1.34 ± 0.03	n.s.
20:4 n-6	10.89 ± 0.18	11.33 ± 0.22	0.0304
22:2 n-6	0.15 ± 0.02	0.06 ± 0.00	0.0005
22:4 n-6	3.97 ± 0.16	2.82 ± 0.13	0.0002
22:5 n-6	0.70 ± 0.05	0.45 ± 0.04	0.0003
24:2 n-6	1.19 ± 0.09	0.70 ± 0.03	0.0002
18:3 n-3	0.07 ± 0.01	0.11 ± 0.01	0.0031
20:4 n-3	0.04 ± 0.01	0.06 ± 0.01	0.0152
20:5 n-3	0.10 ± 0.01	0.40 ± 0.05	0.0006
22:5 n-3	1.17 ± 0.07	1.74 ± 0.03	0.0005
22:6 n-3	1.26 ± 0.01	2.53 ± 0.13	0.0001
n-6	31.90 ± 0.28	29.21 ± 0.25	0.0003
n-6 LCPs	18.40 ± 0.26	16.70 ± 0.35	0.0007
n-3	2.66 ± 0.16	4.87 ± 0.18	0.0001
n-3 LCPs	2.59 ± 0.15	4.76 ± 0.18	0.0001
n-7 + n-9	17.36 ± 0.40	17.03 ± 0.25	n.s.
MUFA	17.16 ± 0.50	16.75 ± 0.43	n.s.
SAFA	39.55 ± 0.50	39.94 ± 0.20	n.s.
Total fatty acids (mg/L)	1,066.48 ± 108.30	875.87 ± 73.70	n.s.

Values are mean ± standard error of the mean.
PKU–phenylketonuria. LCPs–long-chain polyenes. MUFA–monounsaturated fatty acids. SAFA–saturated fatty acids. n.s.–not significant.

tion of PKU patients, as DHA is directly involved in brain structure and function.[17–19] PKU patients, in particular, form a vulnerable group in this matter, as the purpose of their diet is to maintain normal intellectual development, and every effort should be made to keep this development optimized.

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