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Original

Long-chain polyunsaturated fatty acid concentration in patients with inborn errors of metabolism

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Abstract

Introduction: **Long-chain polyunsaturated fatty acid (LCPUFA) can be provided by diet (fatty fish, eggs, viscera and human milk) or synthetised from essential fatty acids linoleic and α-linolenic acids through the microsomal pathway. However, endogenous LCPUFA synthesis is rather low, especially for docosahexaenoic (DHA), and seems insufficient to achieve normal DHA values in individuals devoid of preformed dietary supply. Inborn errors of metabolism (IEMs) are therefore diseases with a special risk for LCPUFA deficient status.**

Aim: **Our aim was to evaluate LCPUFA status in 132 patients with different IEMs.**

Methods: **We performed a cross-sectional study of plasma and erythrocyte LCPUFA composition of 63 patients with IEMs treated with protein-restricted diets compared with data from 69 patients with IEMs on protein-unrestricted diets, and 43 own reference values.**

Results: **Erythrocyte and plasma DHA and arachidonic acid concentrations were significantly decreased in patients treated with protein-restriction compared with those on protein-unrestricted diets and with our reference values ($p < 0.0001$). In the protein-restricted group, 45% of patients showed decreased erythrocyte and plasma DHA values (only 7% and 10%, respectively in the protein- unrestricted group) ($p < 0.0001$). Erythrocyte and plasma DHA values correlated with the natural protein intake in patients on protein-restriction ($r = 0.257$; $p = 0.045$; $r = 0.313$; $p = 0.014$, respectively).**

Conclusion: **Plasma and erythrocyte DHA concentrations are decreased in patients with IEMs treated with protein restriction. DHA concentration correlates with the patients' protein intake. Supplementation of patients with LCPUFA would have a beneficial influence on their nutritional status.**

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Key words: *Polyunsatured fatty acids, Docosahexaenoic acid. Inborn errors of metabolism. Protein-restricted diets. LCPUFA.*

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CONCENTRACIÓN DE ÁCIDOS GRASOS POLIINSATURADOS DE CADENA LARGA EN PACIENTES CON ERRORES INNATOS DEL METABOLISMO

Resumen

Introducción: **Los ácidos grasos poliinsaturados de cadena larga (LCPUFA) pueden ser suministrados por la dieta o sintetizados a partir de los ácidos grasos esenciales, linoleico y α-linolénico. La síntesis endógena de LCPUFA es escasa, especialmente la de ácido docosahexaenoico(DHA), e insuficiente para alcanzar los valores normales de DHA en individuos que carecen de un suministro dietético de dichos ácidos preformados. Por ello, los errores innatos del metabolismo (IEM) son enfermedades con riesgo especial de deficiencia de LCPUFAs.**

Objetivos: **Evaluar el estado de LCPUFA en 132 pacientes con diferentes IEMs.**

Métodos: **Estudio transversal de LCPUFA en plasma y eritrocitos de 63 pacientes con IEMs tratados con dieta restringida en proteínas comparados con 69 pacientes con IEMs con una dieta libre y 43 valores de referencia.**

Resultados: **Las concentraciones de DHA y ácido araquidónico en plasma y eritrocitos se hallaron disminuidas en pacientes con restricción proteica comparados con pacientes con dieta libre y valores de referencia ($p < 0,0001$). El 45% de pacientes con restricción proteica mostró un descenso de DHA en plasma y eritrocitos (solo un 7% y un 10%, respectivamente en aquellos con dieta libre) ($p < 0,0001$). Los valores de DHA en eritrocitos y plasma correlacionaron con la ingesta de proteínas naturales en pacientes con restricción proteica ($r = 0,257$; $p = 0,045$; $r = 0,313$; $p = 0,014$, respectivamente).**

Conclusión: **Las concentraciones de DHA en plasma y eritrocitos se hallaron descendidas en pacientes con IEMs con restricción proteica, correlacionando con la ingesta proteica de los pacientes. La suplementación de dichos pacientes con LCPUFA podría ser beneficiosa para su estado nutricional.**

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Palabras clave: *Ácidos grasos poli-insaturados. Ácido docosahexaenoico. Errores innatos del metabolismo. Dieta restringida en proteínas. LCPUFA.*

Abreviations

LCPUFAs: long-chain polyunsaturated fatty acids.
DHA: docosahexaenoic acid.
IEM: inborn errors of metabolism.
AA: arachidonic acid.
LA: linoleic acid.
ALA: α-linolenic acid.
PKU: Phenylketonuria.
CblC: Cobalamin C variant.
MSUD: Maple Syrup Urine Disease.
NKH: Non-ketotic hyperglycinemia.
HFI: Hereditary fructose intolerance.
EDTA: Etylen diamino tetraacetic acid.
PUFA: Polyunsaturated fatty acid.
SSFA: Total saturated fatty acids.
SMUFA: Total monounsaturated fatty acids.
MA: Mead acid.

Introduction

Long-chain polyunsaturated fatty acids (LCPUFAs) are essential for normal growth and development.[1,2] LCPUFAs, arachidonic acid (AA) and especially docosahexaenoic acid (DHA), are fundamental in the structure and function of the central nervous system and the retina.[3] LCPUFA can be provided by diet (fatty fish, eggs, viscera and human milk) or synthetised from essential fatty acids [linoleic (LA) and α-linolenic acids (ALA)] through the microsomal pathway. However, endogenous LCPUFA synthesis is rather low, especially for DHA, and seems insufficient to achieve normal DHA values in individuals devoid of preformed dietary supply.[4] A mitochondrial pathway has also been described for DHA synthesis where carnitine and α-tocopherol-dependent enzymes are involved.[5] The availability of LCPUFA seems therefore not only to be affected by protein malnutrition[6] but also by other nutritional factors (carnitine and a-tocopherol deficiency), and even by excess oxygen free radical production involved in some chronic diseases.[7]

Inborn errors of metabolism (IEMs) are therefore diseases with a special risk for LCPUFA-deficient status.[8] In fact, altered LCPUFA composition has been described in IEMs treated with protein-restricted diets, because the foods with the most available sources of LCPUFAs are also high in protein and eliminated in the diet. Low DHA status has been demonstrated in wide patient samples with phenylketonuria (PKU) from diverse geographical areas.[9-15] Regarding other IEMs, deficient DHA status has also been described in patients with urea cycle defects, organic acidurias, and amino acid metabolism defects,[16,17] but not in five clinically stable patients with propionic acidemia,[18] and in some, but not all, patients with fatty acid beta-oxidation defects.[19,20] Since previous studies included few patients with IEMs, except for those performed in PKU patients, their conclusions about the need for LCPUFA supplementation are controversial at present. Furthermore, altered LCPUFA status in IEMs has been related to visual and neurological problems in these patients,[3,6,16,17,20] and DHA supplementation might be necessary, and in fact has been tested in some IEM patients.[19,21]

Therefore, our aim was to evaluate LCPUFA status in a large group of patients with IEMs treated with protein-restricted diets versus patients with other IEMs treated with protein-unrestricted diets, in order to learn whether LCPUFA supplementation might be advisable.

Subjects and methods

Patients

We performed a cross-sectional study of plasma and erythrocyte LCPUFA phospholipid composition in 132 patients with IEMs, classified into 2 groups:

Group 1: 63 patients with a diagnosis of different IEMs treated with protein-restricted diets (table I). Patients with urea cycle defects (N = 27) included 2 cases with carbamoyl phosphate synthetase deficiency, 12 with ornithine transcarbamylase deficiency, 7 with argininsuccinate synthase deficiency, 3 with argininsuccinate lyase deficiency, 1 with hyperornithinemia, hyperammonemia and homocitrullinuria syndrome and 2 with lysinuria with protein intolerance. The homocystinuria patients (N = 15) included 6 cases with cystathionine b-synthase deficiency, and 9 with combined homocystinuria and methylmalonic aciduria (CblC variant). The organic aciduria group (N = 12) was composed of 4 patients with propionic aciduria, 7 with glutaric aciduria and one with isolated methylmalonic aciduria. In addition, 5 patients with maple syrup urine disease (MSUD), 2 with tyrosinemia type-1, and 2 with non-ketotic hyperglycinemia (NKH) are included in group 1 (table I).

Group 2: 69 patients with IEMs on a protein-unrestricted diet. Seventeen patients were treated with carbohydrate restriction (14 patients with galactosemia and 3 with hereditary fructose intolerance), 9 patients with beta-oxidation defects were treated with dietary fat restriction, and 43 patients with other IEMs were treated without special diets (8 patients with mitochondrial diseases, 24 patients with lysosomal diseases and a miscellaneous group of 11 patients (2 with glycogen phosphorylase deficiency, 2 with phosphomanomutase deficiency, 4 with nephropatic cystinosis, 2 with 3-phosphoglycerate dehydrogenase deficiency and one with creatine transporter deficiency).

Characteristics of these patients are summarised in table I. All patients were diagnosed at the biochemical and genetic level. Patients were treated according to classical guidelines,[22] and were periodically controlled in our hospital. Only patients with good clinical control and no metabolic decompensation were recruited for

Table I
Characteristics of the groups of patients included in the study. Results expressed as mean ± SD

Patient group	*Number*	*Age (years)*	*Natural protein (g/kg per day)*	*Energy intake (kcal/day)*	*Fat intake (g/day)*
Protein-restricted diet	63	10.4 ± 9.1	0.94 ±0.54	1,730 ± 597	68.0 ± 35.1
Urea cycle disorders	27	11.3 ± 8.3	0.91 ± 0.52	1,478 ± 553	60.2 ± 28.8
Homocystinuria	15	13.5 ± 6.4	1.13 ± 0.58	1,951 ± 817	79.7 ± 37.8
Organic acidurias	12	6.5 ± 6.4	0.93 ± 0.54	1,618 ± 393	56.6 ± 19.9
MSUD	5	6.0 ± 5.9	0.88 ± 0.37	1,510 ± 210	50.2 ± 23.9
Tyrosinemia-I	2	5.0 ± 1.4	0.95 ± 0.57	1,698 ± 141	59.0 ± 12.5
NKH	2	14.0 ± 2.1	0.81 ± 0.27	1,607 ± 150	53.6 ± 15.1
Protein-unrestricted diet	69	11.3 ± 8.3	1.33 ± 0.54	1,807 ± 390	76.1 ± 31.8

MSUD: Maple Syrup Urine Disease; NKH: Non-ketotic hyperglycinemia.

the study. Exclusion criteria were treatment with formula supplemented with PUFA.

All children or their guardians signed an informed consent agreement in accordance with the Helsinki Declaration. Our hospital ethics committee approved the study.

Reference values for PUFAs were established in 43 apparently normal children (determined by history and analytical data) who came to our laboratory for analytical control of minor surgical interventions. These children were on a normal diet for their age. Exclusion criteria were the presence of acute or chronic disease, pharmacological treatments, and special diets. No significant differences had previously been observed for PUFA values in relation to age and sex in normal individuals, so that the two groups of patients were compared with these reference values.

Methods

Nutritional examination

In patients under free diet, natural protein intake was in accordance with WHO recommendations for age and gender.[23] For IEM patients under protein-restricted diet, natural protein daily intake was allowed according to individual tolerance. A three-day food record was used for natural protein, fat, and energy, and the different nutritional parameters were calculated with the DietSouce 2.0® Sanutrin Program (Novartis Consumer Health).

Biochemical methods

Sample preparation: After an overnight fast, blood samples were collected in gel and EDTA-tubes for analytical control and PUFA analysis. Blood in EDTA-tubes was immediately centrifuged and separated into plasma and erythrocytes for PUFA analysis. The buffy coat was discarded and erythrocytes were washed twice with serum saline containing 5 g/L pyrogallol and 1 mmol/L EDTA, to prevent oxidation. The erythrocyte pellets were suspended to a hematocrit of about 50%. Erythrocytes and plasma were frozen at -40ºC until the assay was performed in the hospital laboratory, a maximum of two weeks after sample collection.

Nutritional control: Serum total and free carnitine were analysed by a spectrometric procedure adapted to the Cobas MIRA Plus Analyser.[24] Serum α-tocopherol was analysed by HPLC with UV detection as previously described.[25]

LCPUFA analysis: Plasma and erythrocyte total fatty acids were derivatized to fatty acid methyl esters in accordance with Lepage et al.[26] using tridecanoic acid as internal standard. The fatty acid methyl esters were analysed by gas chromatography with flame ionisation detection (Agilent Technologies GC 6890 N). Data were expressed as weight percentage of total fatty acids.

The fatty acids measured were grouped into saturated fatty acids (ΣSFA): (14:0, 16:0, 18:0, 22:0 and 24:0), monounsaturated fatty acids (ΣMUFA): (14:1n-5, 16:1n-7, 18:1n-9, 24:1n-9), total n-6 PUFA (Σn-6): [18:2n-6 (LA: linoleic acid), 18:3n-6, 20:3n-6, 20:4n-6 (AA), 22:4n-6, 22:5n-6], and total n-3 PUFA (Σn-3): [(18:3n-3 (ALA: α-linolenic acid), 20:5n-3, 22:5n-3, 22:6n-3 (DHA)]. We measured 20:3n-9 (MA:mead acid) as a functional parameter of biochemical essential fatty acid deficiency. 20:4n-6/22:6n-3 (AA/DHA) and 22:5n-6/22:6n-3 ratios were calculated as biochemical markers of n-3 PUFA deficiency and (n-6)/(n-3) disbalance parameter, respectively.[15,27]

Statistical analysis

Statistical analysis was performed using the SPSS package, version 17.0. The Kruskal-Wallis test was

applied to all variables among the different groups of patients previously described. Since no significant differences were observed, patients were classified into two main groups (with protein-restricted diet and protein-unrestricted diet) for further comparisons. When variables showed a Gaussian distribution (Kolmogorov-Smirnov test) and variances were homogeneous (Levene test), the ANOVA test with Bonferroni correction was used to compare the LCPUFA among patients treated and not treated with protein restriction and reference values. For skewed distributions (LA, and ALA in plasma, AA and DHA in plasma and erythrocytes, MA/20: 3n-9, 20: 4n-6/22: 6n-3, 22: 5n-6/22: 6n-3) the Kruskal-Wallis test was applied for comparisons. The Chi-square test was used to compare the proportion of patients with decreased DHA and AA concentrations in plasma and erythrocytes. The Spearman test was used to determine the correlations between the natural protein intake and DHA in plasma and erythrocytes of patients treated with protein-restricted diets. Statistical significance was accepted at $P < 0.05$.

Results

Clinical data and intake of the patients with IEM

The clinical data and natural protein, energy and fat intake of the patients are listed in table I. No significant differences were observed in natural protein intake among the different inborn errors of metabolism treated with protein restriction.

Plasma and erythrocyte phospholipids and other biochemical data of the patients with IEM with protein-restricted and protein-unrestricted diets

Plasma and erythrocyte fatty acid composition and markers of biochemical LCPUFA deficiency from the different IEM patients with natural protein-restricted diet, and protein-unrestricted diet, are summarised in tables II and III, respectively. Plasma and erythrocyte fatty acid composition and markers of biochemical LCPUFA deficiency from these two groups of IEM patients and our reference values are summarised in table IV. Plasma and erythrocyte DHA, AA, and LA, as well as (n-6)/(n-3) ratio and markers of LCPUFA deficiency, showed significant differences in patients treated with natural protein-restriction when compared with those on protein-unrestricted diets and with our reference values (table IV, fig. 1). In the protein-restricted group, 45% of patients showed decreased (below the reference range) erythrocyte and plasma DHA values (only 7% and 10%, respectively in the protein-unrestricted group) [Chi square test, $\chi = 44.98$ (erythrocytes) and $\chi = 39.9$ (plasma) $p < 0.0001$]. Only

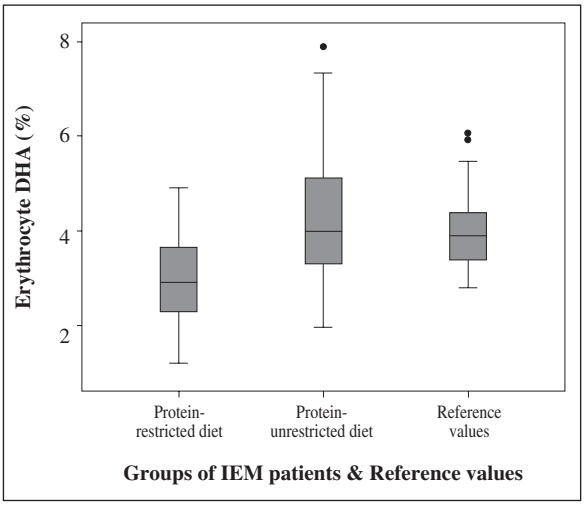

Fig. 1.—Erythrocyte DHA in the IEM patients on protein restriction compared with those on protein-unrestricted diet and with our reference values.

12% and 28% of group 1 patients showed decreased erythrocyte and plasma AA values, compared to 14% and 20% respectively for group 2 (Chi square test: $\chi = 14.13$; $p = 0.007$ only for plasma).

Concerning correlation studies, DHA values positively correlated with the natural protein intake in the patients treated with protein-restricted diets (Spearman test, DHA in erythrocytes: $r = 0.257$; $p = 0.045$; DHA in plasma: $r = 0.313$; $p = 0.014$).

Serum-free carnitine and α-tocopherol concentrations were not significantly different among the three studied groups, while total carnitine levels were significantly higher in patients treated with protein-restricted diets than in the other two groups ($p = 0.012$) (data not shown).

Discussion

LCPUFA composition has been extensively reported in PKU patients but there are few data regarding other IEM,[16-20] probably due to the difficulty of gathering a large number of patients with each disease. In our Metabolic Unit, we included the LCPUFA composition measurement in the periodic control of patients with IEM so as to optimize their nutritional management.[28] Owing to the complexity of interpretation of the multiple results obtained when considering the diseases individually, we decided to arrange them into two main groups, according to the treatment strategy (no significant differences were observed among the fatty acid composition of the different diseases included in each group).

The LCPUFA study was performed in plasma and erythrocytes since the latter yields more information about long-term PUFA status and is related to brain fatty acid composition.[18,27] Moreover, erythrocyte fatty acids are less influenced by fasting and appear to be

Table II
Composition of plasma (p) and erythrocyte (e) fatty acids [% of total fatty acids expressed as median (range)] in patients with different IEMs treated with protein-restricted diets

Fatty acid	*Urea cycle def.*	*Homocystinurias*	*Organic acidurias*	*MSUD*	*Tyrosinemia Type-I*	*NKH*
Number	27	15	12	5	2	2
SSFA (e)	366 (162-950)	362 (266-1172)	335 (183-784)	321 (188-426)	361-293	304-383
SMUFA (e)	140 ± (66-245)	149 (67-212)	140 (94-281)	161 (86-187)	149-117	131-146
LA 18:2n-6 (p)	31.0 (18.9-40.3)	28.9 (19.2-42.9)	24.9 (18.1-34.5)	28.5 (14.1-38.2)	30.2-19.4	25.2-28.5
LA 18:2n-6 (e)	10.7 (5.9-14-5)	9.2 (5.1-11.8)	8.8 (5.6-15.7)	9.8 (8.8-11.3)	9.5-7.8	8.4-11.0
ALA18:3n-3 (p)	0.28 (0.14-0.97)	0.28 (0.16-0.53)	0.39 (0.11 ± 0.31)	0.33 (0.01-0.50)	0.26-0.51	0.17-0.33
ALA18:3n-3 (e)	0.06 (0.01 ± 0.44)	0.06 (0.1-0.38)	0.07 (0.03-0.40)	0.05 (0.01-0.08)	0.04- 0.03	0.11-0.12
AA 18:3n-3 (p)	**6.2 (4.1-10.4)**	**6.9 (4.8-9.2)**	**5.8 (3.6-9.1)**	**5.4 (4.3-5.6)**	**6.9-7.1**	**8.8-4.4**
AA 18:3n-3 (e)	**13.8 (8.8-15.8)**	**14.6 (7.4-15.9)**	**14.0 (9.3-17.5)**	**12.5 (11.2-14.6)**	**14.8-17.1**	**16.8-15.1**
DHA22:6n-3(p)	**1.2 (0.6-2.3)**	**1.4 (0.7-3.0)**	**1.2 (0.5-1.8)**	**0.9 (0.6-1.9)**	**1.01-1.02**	**1.2- 0.8**
DHA22:6n-3(e)	**2.7 (1.5-4-8)**	**2.9 (1.5-4.9)**	**3.15 (1.2-4.5)**	**2.3 (1.4-4.5)**	**2.6-2.8**	**2.8-2.9**
20:3n-9(e)[a]	0.04 (0.01 ± 0.156)	0.05 (0.04-0.15)	0.09 (0.01-0.19)	0.02 (0.01-0.05)	0.0-0.07	0.1- 0.1
Σ total FA (e)	865 (570-1,160)	908 (590-1,226)	824 (492-1,156)	694 (472-916)	806-644	692-902
Σ n-6/S n-3 (e)	6.04 (3.8-8.59)	5.14 (3.08-9.01)	5.10 (4.06-8.10)	7.1 (4.5-9.1)	6.3-6.7	6.2- 6.5
20:4n-6/22:6n-3[b]	5.05 (2.54-7.75)	4.62 (2.19-9.26)	4.63 (3.15-11.60)	5.43 (2.5-10.5)	5.59-6.19	5.95-5.13
22:5n-6/22:6n-3[c]	0.26 (0.16-2.57)	0.41 (0.11-1.70)	0.32 (0.15-0.66)	0.33 (0.21-0.82)	0.38-0.56	0.38-0.45

IEM: Inborn errors of metabolism.
MSUD: Maple Syrup Urine Disease; NKH: Non-ketotic hyperglycinemia.
FA: Fatty acids. **Bold,** fatty acids of main interest.
SSFA: Total saturated fatty acids: 14:0, 16:0, 18:0, 22:0, 24:0.
SMUFA: Total monounsaturated fatty acids: 14:1n-5, 16:1n-7, 18:1n-9, 24:1n-9.
LA: Linoleic acid; ALA: a-Linolenic acid; AA: Arachidonic acid; DHA: docosahexaenoic acid; MA (mead acid: 20:3n-9).
Σ n-6 FA: 18:2n-6, 18:3n-6, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6.
Σ n-3 FA: 18:3n-3, 20:5n-3, 22:5n-3, 22:6n-3.
Σ total FA : S n-6 FA + S n-3 FA.
[a]MA: 20:3n-9 (marker of essential fatty acid deficiency).
[b]AA/DHA (marker of n-3 deficiency): 20:4n-6/22:6n-3.
[c]DHA deficiency index [n-6)/(n-3) imbalance parameter] 22:5n-6/22:6n-3.
Kruskal-Wallis test: no significant differences among the different IEM patient values.

more valuable for assessing nutritional status.[20]

Regarding the results of the present study, we found decreased concentrations, especially of DHA, in plasma and erythrocytes of patients on protein-restricted diets compared with patients on protein-unrestricted diets and with reference values. These results are in accordance with those of some authors,[16,17] but not of others.[18,21] However, the greater number of patients included in our study lends support to the hypothesis that impaired DHA status is a common situation among patients with IEM under dietary treatment.

It is interesting to note the differences between our present data and those from a previous study performed by our unit in PKU patients.[15] Although both groups of diseases are treated with natural protein-restricted

Table III
Composition of plasma (p) and erythrocyte (e) fatty acids [% of total fatty acids expressed as median (range)] in patients with different IEMs on protein-unrestricted diets

Fatty acid	*Galactosemia*	*HFI*	*β-oxidat. acidurias*	*Mitochon. diseases*	*Lysosomal diseases*	*Various*
Patient number	14	3	9	8	24	11
ΣSFA (e)	372 (148-618)	338 (278-343)	431 (247-596)	323 (112-439)	375 (190-789)	328 (206-421)
ΣMUFA (e)	152 (32-227)	122 (65-139)	132 (46-232)	128 (86-194)	127 (52-229)	104 (82-176)
LA 18:2n-6 (p)	32.1 (26.1-40.7)	28.5 (21.9-32.8)	27.2 (16.5-36.3)	26.6 (15.1-33.5)	30.00 (20.0-38.9)	30.2 (24.8-43.4)
LA 18:2n-6 (e)	10.2 (8.5-13.6)	9.1 (6.4-10.2)	8.2 (5.8-10.9)	7.7 (6.5-11-8)	9.5 (6.2-13.4)	9.1 (6.8-12.8)
ALA18:3n-3 (p)	0.24 (0.01-0.53)	0.21 (0.01-0.31)	0.33 (0.01-0.69)	0.31 (0.08 ± 0.74)	0.27 (0.01-0.57)	0.23 (0.01-0.53)
ALA18:3n-3 (e)	0.05 (0.01-0.13)	0.03 (0.01-0.06)	0.04 (0.01-0.09)	0.04 (0.01-0.13)	0.04 (0.01-0.16)	0.03 (0.01-0.13)
AA 18:3n-3 (p)	**6.1 (5.0-10.3)**	**6.6 (6.1-9.7)**	**7.2 (5.2-8.9)**	**8.0 (4.1-11.6)**	**6.6 (4.4-10.2)**	**6.8 (5.5-13.6)**
AA 18:3n-3 (e)	**13.9 (10.7-17.0)**	**14.0 (13.3-17.7)**	**15.1 (12.2-17.6)**	**14.6 (10.1-16.6)**	**13.5 (10.7-16.8)**	**14.1 (11.8-17.3)**
DHA22:6n-3(p)	**1.8 (1.4-4.0)**	**1.6 (1.5-3.0)**	**1.6 (1.0-3.9)**	**1.7 (1.1-4.2)**	**1.7 (0.9-5.6)**	**1.5 (0.8-2.8)**
DHA22:6n-3(e)	**4.3 (2.9-6.8)**	**4.4 (3.5-4.9)**	**3.7 (2.3-7.3)**	**4.0 (2.6-5.3)**	**4.9 (2.6-7.9)**	**3.1 (1.9-4.8)**
20:3n-9(e)[a]	0.03 (0.001 ± 0.07)	0.05 (0.01-0.08)	0.04 (0.01-0.15)	0.03 (0.01-0.12)	0.04 (0.01-0.16)	0.06 (0.01-0.13)
Σ total FA (e)	830 (562- 1,098)	694 (611-777)	837 (570-1,104)	665 (418-914)	839 (610-1,068)	680 (512-848)
Σ n-6/S n-3 (e)	4.38 (1.80-6.21)	4.01 (2.96-5.06)	4.59 (2.68-7.12)	4.90 (2.90-6.10)	3.77 (1.85-7.00)	5.60 (3.37-7.06)
20:4n-6/22:6n-3[b]	3.15 (1.76- 5.10)	3.61 (2.98-3.94)	3.58 (2.40-6.46)	3.95 (2.20-5.40)	2.65 (1.67-5.99)	4.62 (2.79-7.94)
22:5n-6/22:6n-3[c]	0.18 (0.09-1.75)	0.13 (0.11-0.20)	0.23 (0.12-0.52)	0.21 (0.06-0.31)	0.25 (0.04-2.36)	0.19 (0.13-0.76)

IEM: Inborn errors of metabolism
HFI: Hereditary fructose intolerance; β-oxidat. defects: b-oxidation defects; Mitochon. Diseases: Mitochondrial diseases.
FA: Fatty acids. **Bold,** fatty acids of main interest.
ΣSFA: Total saturated fatty acids: 14:0, 16:0, 18:0, 22:0, 24:0.
ΣMUFA: Total monounsaturated fatty acids: 14:1n-5, 16:1n-7, 18:1n-9, 24:1n-9.
LA: Linoleic acid; ALA: a-Linolenic acid; AA: Arachidonic acid; DHA: docosahexaenoic acid; MA (mead acid: 20:3n-9).
Σ n-6 FA: 18:2n-6, 18:3n-6, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6.
Σ n-3 FA: 18:3n-3, 20:5n-3, 22:5n-3, 22:6n-3.
Σ total FA : S n-6 FA + S n-3 FA.
[a]MA: 20:3n-9 (marker of essential fatty acid deficiency).
[b]AA/DHA (marker of n-3 deficiency): 20:4n-6/22:6n-3.
[c]DHA deficiency index [n-6)/(n-3) imbalance parameter] 22:5n-6/22:6n-3.
Kruskal-Wallis test: no significant differences among the different IEM patient values.

diets, in PKU patients we only found low values of DHA in plasma (0.94 ± 0.35) and erythrocytes (2.13 ± 0.72%), and altered markers of DHA status. However, in the present study the DHA status was not so greatly decreased [(1.27 ± 0.51 and 2.96 ± 0.95) in plasma and erythrocytes, respectively], yet low concentration of AA was also observed in comparison with the reference values. Since both groups of patients (PKU and other IEMs) were treated by the same nutrition team, possible explanations for the lower DHA concentration in PKU patients include the more homogeneous composition of this group, the lower protein tolerance (and thus, lower natural protein intake), and the better compliance of PKU patients easily controlled through blood phenylalanine-level monitoring. In fact, a limitation of our study is the heterogeneity of the patient

Table IV

Composition of plasma (p) and erythrocyte (e) fatty acids [% of total fatty acids expressed as median (range)] in the groups of IEM patients with protein restriction and protein-unrestricted diets compared with our reference values

Fatty acid (%)	*Protein restriction*	*Protein unrestricted diets*	*Reference values*	*Significance ANOVA (B)* (K-W test)***
Patient number	63	69	43	
ΣSFA (e)	360 (162-1,172)	361 (112-789)	307 (134-767)	P = 0.017*
ΣMUFA (e)	143 (66-281)	129 (32-232)	116 (58-654)	NS*
LA 18:2n-6 (p)	29.3 (14.1-42.9)	30.2 (15.1-43.4)	31.5 (24.4-40.2)	P = 0.016**
LA 18:2n-6 (e)	9.44 (5.09-15.70)	9.23 (5.85-13.63)	10.3 (7.7-27.8)	P = 0.023*
ALA 18:3n-3 (p)	0.29 (0.11-0.97)	0.27 (0.01- 0.74)	0.26 (0.12 -0.53)	NS**
ALA 18:3n-3 (e)	0.06 (0.01-0.44)	0.04 (0.01-0.16)	0.04 (0.01-0.07)	NS*
AA 18:3n-3 (p)	**6.0 (3.6 ± 10.4)**	**7.0 (4.1 ± 13.6)**	**7.5 (5.5 -9.9)**	**P < 0.0001****
AA 18:3n-3 (e)	**14.0 (7.4-17.5)**	**14.0 (10.1-17.7)**	**14.6 (12.3-17.1)**	**P = 0.034****
DHA 22:6n-3(p)	**1.20 (0.49- 3.01)**	**1.69 (0.85-5.58)**	**1.7 (1.1 -3.2)**	**P < 0.0001****
DHA 22:6n-3(e)	**2.90 (1.20-4.91)**	**3.96 (1.95-7.90)**	**3.96 (2.79-6.07)**	**P < 0.0001****
MA:20:3n-9(e)[a]	0.047 (0.01-0.191)	0.043 (0.01-0.162)	0.033 (0.02-0.131)	NS**
Σ total FA (e)	848 (472-1,226)	780 (418-1,104)	711 (396-1,026)	P = 0.046*
Σ n-6/S n-3 (e)	**5.66 (3.09-9.09)**	**4.59 (1.80-7.12)**	**4.78 (3.21-6.86)**	**P < 0.0001****
20:4n-6/22:6n-3[b]	**4.97 (2.19-11.60)**	**3.58 (1.67-7.94)**	**3.69 (2.35-5.33)**	**P < 0.0001****
22:5n-6/22:6n-3[c]	**0.31 (0.11-2.57)**	**0.20 (0.04-2.36)**	**0.19 (0.09-0.31)**	**P < 0.0001****

Bold, fatty acids and ratios of main interest.
*ANOVA with Bonferroni correction; ** Kruskal-Wallis test.
FA: Fatty acids. **Bold,** fatty acids of main interest.
ΣSFA: Total saturated fatty acids: 14:0, 16:0, 18:0, 22:0, 24:0.
ΣMUFA: Total monounsaturated fatty acids: 14:1n-5, 16:1n-7, 18:1n-9, 24:1n-9.
LA: Linoleic acid; ALA: a-Linolenic acid; AA: Arachidonic acid; DHA: docosahexaenoic acid; MA (mead acid: 20:3n-9).
Σ n-6 FA: 18:2n-6, 18:3n-6, 20:3n-6, 20:4n-6, 22:4n-6, 22:5n-6.
Σ n-3 FA: 18:3n-3, 20:5n-3, 22:5n-3, 22:6n-3.
Σ total FA : S n-6 FA + S n-3 FA.
[a]MA: 20:3n-9 (marker of essential fatty acid deficiency).
[b]AA/DHA (marker of n-3 deficiency): 20:4n-6/22:6n-3.
[c]DHA deficiency index [n-6)/(n-3) imbalance parameter] 22:5n-6/22:6n-3.

groups, which could lead to a bias in the interpretation of the results. However, achieving homogeneous groups of patients (according to disease, age, and gender) is very difficult when dealing with IEMs, except for PKU.

The moderately decreased AA concentration revealed in the present study was also observed by Sanjurjo et al.[16] in patients with urea cycle defects and methylmalonic aciduria, although only in plasma. These authors attributed the decreased concentration of AA to the effect of high linoleic acid (essential n-6 precursor) intake of these patients, who also showed increased concentration in plasma and erythrocytes. Elevated LA intake would constitute an effective impairment of compensatory DHA synthesis from α-linolenic acid, the n-3 precursor.[17] This is not the case in our patients,

in whom LA concentration was slightly decreased in comparison with our reference values.

Low DHA status has also been attributed to impaired DHA mitochondrial synthesis, which might be affected by low serum carnitine and α-tocopherol.[7] This is not the case with our patients, many of them supplemented with both cofactors, either through the special formula (α-tocopherol) or as therapy (carnitine in urea cycle defects, organic acidurias, mitochondrial diseases, and some β-oxidation defects).

Dietary intake of natural proteins correlated with DHA in erythrocytes and in plasma in our study. This is an interesting observation because it reinforces the hypothesis that the most important determinant of DHA status is the preformed LCPUFA intake through protein-rich foods (fish, meat, eggs, nuts, liver and milk products).[16]

Supplementation of PKU patients with decreased DHA concentrations has been carried out in the first year of life,[29] and in childhood.[30] DHA supplementation normalised plasma and erythrocyte DHA levels, while supplementation with essential fatty acids (LA and ALA) only increased DHA values by 19%.[31] Beneficial effects of DHA supplementation were reported not only in visual function[29] but also in fine motor skills.[32,33] Further studies are necessary to evaluate the effect of supplementation in other IEMs with protein-restricted diets. However, the heterogeneity of the sample composition (age, disease, clinical symptom) makes it difficult to conclude that there is a clinical improvement. Larger randomized multicenter studies performed in PKU children would help to evaluate the appropriate composition and dosage of LCPUFA supplementation and to document safety.[33]

In conclusion, DHA concentration is decreased in patients with IEMs treated with natural protein restriction, and thus, with low LCPUFA intake. Moreover, plasma and erythrocyte DHA composition is related to the patients' natural protein intake. Supplementation of patients with LCPUFA normalises decreased DHA concentrations and might have a beneficial influence on the nutritional status and, probably, the neurological outcome of these patients.

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References

1. Innis SM. Essential fatty acids in growth and development. *Prog Lipid Res* 1991; 30: 39-103.
2. Sellmayer A, Koletzko B. Long-chain polyunsaturated fatty acids and eicosanoids in infants- physiological and pathophysiological aspects and open questions. *Lipids* 1999; 34: 199-205.
3. Neuringer M, Anderson GJ, Connor WE. The essentiality of n-3 fatty acids for the development and function of the retina and brain. *Annu Rev Nutr* 988; 8: 517-41.
4. Rosell NS, 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* 2005; 82: 327-34.
5. 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* 2001; 72: 185-98.
6. Decsi T, Molnár D, Koletzko B. The effect of under- and overnutrition on essential fatty acid metabolism in childhood. *Eur J Clin Nutr* 1998; 52: 541-8.
7. Infante JP, Huszagh VA. Secondary carnitine deficiency and impaired docosahexaenoic (22: 6n-3) acid synthesis: a common denominator in the pathophysiology of diseases of oxidative phosphorylation and beta-oxidation. *FEBS Lett* 2000; 468: 1-5.
8. Giovannini M, Biasucci G, Agostoni C, Luotti D, Riva E. Lipid status and fatty acid metabolism in phenylketonuria. *J Inherit Metab Dis* 1995; 18: 265-72.
9. Galli G, 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* 1991; 119: 562-7.
10. Pöge AP, Bäumann K, Müller E, Leichsenring M, Schmidt H, Bremer HJ. Long chain polyunsaturated fatty acids in plasma and erythocyte membrane lipids of children with phenylketonuria after controlled linoleic acid intake. *J Inherit Metab Dis* 1998; 21: 373-381.
11. Sanjurjo P, Perteagudo L, Rodriguez Soriano J, Vilaseca MA, Campistol J. Polyunsaturated fatty acid status in patients with phenylketonuria. *J Inherit Metab Dis* 1994; 17: 704-709.
12. 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* 1995; 53: 401-404.
13. 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* 2002; 25: 56-64.
14. Koletzko B, Sauerwald T, Demmelmair H, et al. Dietary long-chain polyunsaturated fatty acid supplementation in infants with phenylketonuria: a randomized controlled trial. *J Inherit Metab Dis* 2007; 30: 326-32.
15. Vilaseca MA, Lambruschini N, Gómez-López L, Gutiérrez A, Moreno J, Tondo M, Artuch R, Campistol J. Long-chain polyunsaturated fatty acid status in phenylketonuric patients treated with tetrahydrobiopterin. *Clin Biochem* 2010; 43: 411-15.
16. Sanjurjo P, Ruiz JI, Montejo M. Inborn errors of metabolism with a protein-restricted diet: effect on polyunsaturated fatty acids. *J Inherit Metab Dis* 1997; 20: 783-9.
17. Vlaardingerbroek H, Hornstra G, de Koning TJ et al. Essential polyunsaturated fatty acids in plasma and erythrocytes of children with inborn errors of amino acid metabolism. *Mol Genet Metab* 2006;88:159-65.
18. Decsi T, Sperl W, Koletzko B. Essential fatty acids in clinically stable children with propionic acidaemia. *J Inherit Metab Dis* 1997; 20: 778-82.
19. Harding CO, Gillingham MB, Van Calcar SC, Wolff JA, Verhoeve JN, Mills MD. Docosahexaenoic acid and retinal function in children with long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *J Inherit Metab Dis* 1999; 22: 276-80.
20. Lund AM, Dixon MA, Vreken P, Leonard JV, Morris AA. Plasma and erythrocyte fatty acid concentrations in long-chain 3-hydroxyacyl-CoA dehydrogenase deficiency. *J Inherit Metab Dis* 2003; 26: 410-2.
21. Aldámiz-Echevarría L, Sanjurjo P, Elorz J, Prieto JA, Pérez C, Andrade F, Rodríguez-Soriano J. Effect of docosahexaenoic

acid administration on plasma lipid profile and metabolic parameters of children with methylmalonic acidaemia. *J Inherit Metab Dis* 2006; 29: 58-63.
22. Physician's Guide to the Treatment and Follow-up of Metabolic Diseases. Nenad Blau, Georg F. Hoffmann, James Leonard, Joe T. R. Clarke (Eds.) Springer-Verlag Berlin Heidelberg 2006.
23. Report of a joint FAO/WHO/UNU Expert Consultation (WHO Technical Report Series; no. 935). Protein and amino acid requirements in human nutrition. [monograph on the Internet]. Geneva: World Health Organization; 2007 [cited 2009 Aug 23]. Available at: http://whqlibdoc.who.int/trs/WHO_TRS_935_eng.pdf
24. Artuch R, Quintana M, Moyano D, Moreno J, Puig R, Vilaseca MA. Determinación de carnitina en plasma por un procedimiento espectrométrico. Valores de referencia para una población pediátrica. *Química Clínica* 1997; 16: 397-400.
25. Moyano D, Vilaseca MA, Artuch R, et al. Tocopherol in inborn errors of intermediary metabolism. *Clin Chim Acta* 1997; 263: 147-155.
26. Lepage G, Levy E, Ronco N, Smith L, Galéano N, Roy CC. Direct transesterification of plasma fatty acids for the diagnosis of essential fatty acid deficiency in cystic fibrosis. *J Lipid Res* 1989; 30: 1483-90.
27. Fokkema MR, Smit EN, Martini IA, Woltil HA, Boersma ER, Muskiet FA. Assessment of essential fatty acid and omega3-fatty acid status by measurement of erythrocyte 20:3omega9 (Mead acid), 22:5 omega6/20:4 omega 6 and 22:5omega 6/22:6omega 3. *Prostaglandins Leukot Essent Fatty Acids* 2002; 67: 345-56.
28. Giovannini M, Verduci E, Salvatici E, Fiori L, Riva E. Phenylketonuria: dietary and therapeutic challenges. *J Inherit Metab Dis* 2007; 30: 145-52.
29. Agostoni C, Harvie A, McCulloch DL, Demellweek C, Cockburn F, Giovannini M, Murray G, Harkness RA, Riva E. A randomized trial of long-chain polyunsaturated fatty acid supplementation in infants with phenylketonuria. *Dev Med Child Neurol* 2006; 48: 207-12.
30. 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* 2001; 57: 1488-91.
31. Cleary MA, Feillet F, White FJ, Vidailhet M, Macdonald A, Grimsley A, Maurin N, de Baulny HO, Rutherford PJ. Randomised controlled trial of essential fatty acid supplementation in phenylketonuria. *Eur J Clin Nutr* 2006; 60: 915-20.
32. 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* 2007; 150: 479-84.
33. Koletzko B, Beblo S, Demmelmair H, Müller-Felber W, Hanebutt FL. Does dietary DHA improve neural function in children? Observations in phenylketonuria. *Prostaglandins Leukot Essent Fatty Acids* 2009; 81: 159-64.