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Effects of Dietary Long-Chain Polyunsaturated Fatty Acids on Plasma Amino Acids and Indices of Protein Metabolism in Infants: Results from a Randomized Clinical Trial

Key Words

Long-chain polyunsaturated fatty acids
Amino acids
Protein metabolism
Infant nutrition
Term infants

Abstract

Background/Aim: Previous studies in vitro and in animals in vivo found that α -linolenic acid (C18:3 ω 3) may enhance oxidative damage of essential amino acids. We investigated whether the addition of the long-chain polyunsaturated fatty acids (LCPUFA) arachidonate (C20:4 ω -6; AA) and docosahexaenoate (C22:6 ω 3; DHA) in the form of egg phospholipids to infant formula affects plasma amino acid concentrations and indices of protein metabolism in term infants. **Methods:** In a double-blind, randomized clinical trial, healthy infants were fed from day 5 of life formula with or without preformed LCPUFA (n = 10 and 12, respectively). At the age of 5 days and 1, 2, 3 and 4 months, blood samples were obtained and analyzed for plasma amino acids by high-performance liquid chromatography and for plasma phospholipid fatty acid composition by gas chromatography. **Results:** At the age of 3 months, plasma threonine concentrations were significantly lower in infants receiving dietary LCPUFA than in controls (124 ± 16 vs. 216 ± 28 μ mol/l, $p < 0.05$). Values of other plasma essential amino acids, total protein, albumin, creatinine and urea nitrogen did not differ between the two feeding groups throughout the study. At the age of 5 days, plasma phospholipid AA and DHA concentrations were inversely correlated with histidine concentrations (AA: $r = -0.60$, $p = 0.01$; DHA: $r = -0.53$, $p < 0.05$). At the age of 3 months, DHA concentrations were inversely related to plasma histidine, methionine and threonine concentrations ($r = -0.66$, -0.62 , and -0.64 , respectively, $p < 0.05$). **Conclusions:** The dietary LCPUFA supplementation of infant formula used in this study has no adverse effects on infant plasma amino acid concentrations and indicators of protein metabolism. Nonetheless, the apparent interaction of LCPUFA with some amino acids in formula-fed infants warrants further investigation.

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Introduction

Breast-fed infants receive considerable amounts of preformed long-chain polyunsaturated fatty acids (LCPUFA) in human milk [1], while infants fed conventional infant formula based on vegetable oils do not [2, 3]. Due to the limited capacity of endogenous LCPUFA synthesis in infancy [4], the differences in LCPUFA intakes between infants fed human milk or conventional infant formula are reflected in the fatty acid composition of blood plasma, erythrocytes, subcutaneous tissue, retina and brain [5–9]. In view of the possible importance of ω -6 and ω -3 LCPUFA for optimal infant growth and development, LCPUFA have been added to several infant formulae in Europe [10, 11].

Results of investigations both in vitro [12] and in animals in vivo [13] showed that enrichment of a whey protein-water solution with α -linolenate (C18:3 ω -3) that was subjected to storage for several weeks may decrease the availability of essential amino acids, apparently due to oxidative degradation. It has not been studied previously whether the supplementation of infant formula with LCPUFA may affect the availability of essential amino acids in human infants. Here we report our results on plasma amino acid concentrations and indices of protein metabolism in healthy, term infants fed formulae supplemented with both ω -6 and ω -3 LCPUFA in a randomized clinical trial.

Subjects and Methods

At the age of 5 days, healthy, full-term, appropriate-for-gestational-age infants whose parents decided not to provide breast-feeding were enrolled in this study. The primary aim of this trial, on which the calculation of sample size was based, was to evaluate dietary effects on essential fatty acid status [14]. The study protocol was approved by the Ethical Committee

Table 1. Amino acid spectrum of conventional infant formula and formula supplemented with ω -3 and ω -6 LCPUFA (data provided by the manufacturer, Milupa GmbH, Austria)

Amino acid (g/100 g protein)	Conventional formula	LCPUFA formula
Alanine	4.3	4.0
Arginine	3.5	4.0
Asparagine ^a	9.6	8.7
Glutamate ^b	17.4	18.7
Glycine	1.7	2.0
Histidine	3.5	3.3
Isoleucine	4.3	4.7
Leucine	9.6	9.3
Lysine	7.0	7.3
Methionine	2.6	2.7
Phenylalanine	3.5	4.0
Serine	5.2	5.3
Threonine	6.1	5.3
Tryptophan	1.7	2.0
Tyrosine	3.5	3.3
Valine	5.2	5.3

^a Asparagine and aspartate together.

^b Glutamine and glutamate together.

of the University Medical School of Pécs, and informed written parental consent was obtained. With the use of a computer-generated random number table, the infants were assigned randomly and double-blind to receive either a conventional infant formula (n = 10, Pre-Aptamil; Milupa, Salzburg, Austria) based on whey protein enriched cow's milk protein with a casein/whey ratio of 40/60 and vegetable fat or the same formula supplemented with egg lipids and evening primrose oil (n = 12).

Macronutrient composition of the two formulae was identical (36 g/l fat, 15 g/l protein and 72 g/l carbohydrate). The conventional formula contained <0.1% (wt/wt) LCPUFA without detectable amounts of arachidonate (C20:4 ω -6, AA) and docosahexaenoate (C22:6 ω -3, DHA), the supplemented formula 1.1% LCPUFA including 0.5% AA and 0.3% DHA. The amino acid contents of the two formulae were similar (table 1). Data on intakes of formula volumes could not be obtained in the healthy infants studied under ambulatory conditions.

Table 2. Plasma concentrations of AA and DHA in healthy, term infants fed conventional formula (n = 10) or formula supplemented with ω -6 and ω -3 LCPUFA (n = 12; mean \pm SEM)

	Age, days				
	5	30	60	90	120
<i>Arachidonate, μmol/l</i>					
Conventional formula	484 \pm 60	295 \pm 32	280 \pm 27***	306 \pm 31	266 \pm 28**
LCPUFA formula	529 \pm 74	385 \pm 33	448 \pm 29***	428 \pm 94	384 \pm 18**
<i>Docosahexaenoate, μmol/l</i>					
Conventional formula	95 \pm 9	62 \pm 8**	46 \pm 7***	49 \pm 9***	44 \pm 12*
LCPUFA formula	114 \pm 69	106 \pm 10**	137 \pm 12***	133 \pm 26***	122 \pm 11*

* $p < 0.05$; ** $p < 0.005$; *** $p < 0.0005$ between dietary groups.

The infants' parents were advised to use exclusive feeding of breast milk or the study formula only during the study period. At the age of 5 days and 1, 2, 3 and 4 months, venous blood samples were obtained prior to feeding from all participating infants. Plasma total protein, albumin, urea nitrogen and creatinine concentrations were determined immediately as described previously [15]. Blood samples for fatty acid and amino acid analyses were collected into tubes containing ethylenediaminetetraacetate and heparin, respectively, and stored at -20°C until analysis. The fatty acid composition of plasma phospholipids was analyzed with high-resolution capillary gas-liquid chromatography as previously reported [16].

Plasma amino acid concentrations were determined with high-performance liquid chromatography following *o*-phthalaldehyde derivatization [17]. Briefly, 50 μ l plasma was deproteinized with 200 μ l absolute ethanol, and the internal standard (10 μ l of 1 mmol/l norleucine solution) was added. The supernatant was alkalized with sodium-tetraborate buffer (400 mmol/l, pH 9.5). Primary amines were derivatized with 2-mercaptoethanol. A Beckman System Gold chromatograph with gradient pump, ultraviolet detection and a Hewlett-Packard RP AA-572 C_{18} microbore column was used for analysis.

Results were evaluated with Minitab for Windows, release 9 (Minitab, State College, Pa., USA) and are presented as means \pm SEM. Student's unpaired *t* test was used for comparison of data between dietary groups. Differences were regarded as statistically significant at $p < 0.05$.

Results

Data on growth and the relative contributions of fatty acids to plasma lipid classes have been described elsewhere [14]. Growth did not differ between the two groups. Plasma phospholipid AA and DHA concentrations are shown in table 2. At the age of 2 and 4 months, AA concentrations were significantly higher in infants receiving formula enriched with LCPUFA than in controls. From the age of 1 month onwards throughout the study, DHA concentrations were significantly higher in infants receiving dietary LCPUFA than in those fed conventional formula.

Total protein, albumin, creatinine and urea nitrogen concentrations did not differ between the two groups at any time point of the study (table 3). At the age of 3 months, plasma threonine concentrations were significantly lower in infants fed formula supplemented with LCPUFA than in controls (table 4). Neither plasma histidine and methionine concentrations nor the sum of essential amino acid (histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine) concentrations differed between the two groups at any point of the study (table 3).

Table 3. Plasma total protein, albumin, creatinine and urea nitrogen concentrations in healthy, term infants fed conventional formula (n = 10) or formula supplemented with ω -6 and ω -3 LCPUFA (n = 12; mean \pm SEM)

	Age, days				
	5	30	60	90	120
<i>Total protein, g/l</i>					
Conventional formula	58.8 \pm 1.4	57.7 \pm 1.9	59.9 \pm 1.2	60.5 \pm 1.8	62.4 \pm 2.5
LCPUFA formula	61.3 \pm 2.2	56.9 \pm 0.7	59.7 \pm 1.0	61.0 \pm 2.0	62.4 \pm 1.3
<i>Albumin, g/l</i>					
Conventional formula	41.5 \pm 1.0	42.4 \pm 1.4	44.5 \pm 1.4	46.1 \pm 2.1	48.9 \pm 1.2
LCPUFA formula	44.1 \pm 1.8	42.8 \pm 0.6	44.4 \pm 1.4	47.0 \pm 1.2	44.3 \pm 1.9
<i>Creatinine, μmol/l</i>					
Conventional formula	77.1 \pm 9.2	57.9 \pm 5.1	57.7 \pm 4.4	78.9 \pm 12.8	67.5 \pm 10.6
LCPUFA formula	84.0 \pm 5.0	58.0 \pm 4.2	57.4 \pm 5.4	55.3 \pm 4.4	63.7 \pm 5.9
<i>Urea nitrogen, mmol/l</i>					
Conventional formula	2.82 \pm 0.30	4.19 \pm 0.46	4.57 \pm 0.35	4.32 \pm 0.26	3.80 \pm 0.24
LCPUFA formula	3.04 \pm 0.66	3.26 \pm 0.18	3.53 \pm 0.37	4.43 \pm 0.27	3.69 \pm 0.21

Table 4. Plasma concentrations of histidine, methionine and threonine and the sum of essential amino acid concentrations in healthy, term infants fed conventional formula (n = 10) or formula supplemented with ω -6 and ω -3 LCPUFA (n = 12; mean \pm SEM)

	Age, days				
	5	30	60	90	120
<i>Histidine, μmol/l</i>					
Conventional formula	51 \pm 6	67 \pm 4	77 \pm 11	60 \pm 7	58 \pm 12
LCPUFA formula	59 \pm 9	72 \pm 8	67 \pm 9	41 \pm 8	71 \pm 10
<i>Methionine, μmol/l</i>					
Conventional formula	38 \pm 3	48 \pm 7	50 \pm 9	37 \pm 4	35 \pm 7
LCPUFA formula	43 \pm 7	57 \pm 6	38 \pm 2	24 \pm 6	37 \pm 7
<i>Threonine, μmol/l</i>					
Conventional formula	156 \pm 11	253 \pm 27	241 \pm 40	216 \pm 28*	171 \pm 33
LCPUFA formula	148 \pm 18	234 \pm 20	167 \pm 12	124 \pm 16*	202 \pm 24
<i>Sum of essential amino acids, μmol/l^a</i>					
Conventional formula	854 \pm 64	1,216 \pm 58	1,303 \pm 130	1,057 \pm 85	1,128 \pm 161
LCPUFA formula	987 \pm 115	1,379 \pm 96	1,085 \pm 57	959 \pm 141	1,121 \pm 116

^a Sum of histidine, isoleucine, leucine, lysine, methionine, phenylalanine, threonine and valine concentrations.

* $p < 0.05$ between dietary groups.

Plasma concentrations of aspartate, the sum of both glutamine and glutamate, glycine, isoleucine, leucine, phenylalanine, serine, tyrosine and valine did not differ between the two groups throughout the study (data not shown). In infants fed formula enriched with LCPUFA, we found higher concentrations ($\mu\text{mol/l}$, $p < 0.05$) of asparagine at the age of 5 days (29 ± 2 vs. 17 ± 2) and of arginine at the age of 1 month (68 ± 7 vs. 46 ± 7) and lower concentrations of alanine at the age of 3 months (287 ± 33 vs. 467 ± 31). No other differences in alanine, asparagine and arginine values were seen (data not shown).

Plasma phospholipid AA and DHA concentrations were used as predictive variables in linear regression analyses with plasma amino acid concentrations entered as dependent variables. Significant inverse correlations were found at the age of 5 days between AA and DHA values and histidine ($r = -0.60$, $p = 0.01$, and $r = -0.53$, $p = 0.04$, respectively) and at the age of 3 months between DHA and alanine ($r = -0.64$, $p = 0.02$), histidine ($r = -0.66$, $p = 0.03$), methionine ($r = -0.62$, $p = 0.03$), serine ($r = -0.65$, $p = 0.03$) and threonine ($r = -0.64$, $p = 0.02$). No other statistically significant correlation was found.

Discussion

Reactions of protein-bound amino acids with oxidizing lipids have been reported by several investigators. Although most amino acids were reported to be degradable by polyunsaturated fats [18, 19], the most sensitive amino acids appear to be histidine [20], lysine [12, 13, 21], methionine [12, 13, 22], tyrosine [23] and to a lesser extent tryptophan [12, 24]. In an *in vitro* model using whey protein and methyl linolenate in water, significant losses of lysine (up to 71%), histidine (up to 57%) and tryptophan (up to 31%) were observed

during a storage period of 4 weeks [12]. In rat assays, the bioavailability of lysine and tryptophan were reduced, and net protein utilization and true nitrogen digestibility were significantly lowered by oxidizing lipids [13]. These findings indicate that modification of the fat blend of food products may influence the availability and metabolism of amino acids.

In the infants studied here, we found no effect of formula supplementation with LCPUFA on the plasma concentrations of the amino acids considered to be most sensitive to the effect of oxidizing lipids, i.e., histidine, lysine, methionine, tryptophan, and tyrosine, throughout the first 4 months of life. Moreover, biochemical indices of protein metabolism considered to reflect protein utilization and metabolism (i.e., plasma total protein, albumin, urea nitrogen, and creatinine concentrations) did not differ between the two feeding groups, nor was there any consistent trend. Hence, we did not find any indication for an adverse effect of the LCPUFA enrichment of infant formula used here on the bioavailability of amino acids in healthy term infants. However, significant inverse correlations were found between plasma concentrations of AA and DHA and concentrations of five amino acids, including the essential amino acids histidine and methionine. It appears possible that high availability of LCPUFA in the infant organism enhances the probability of oxidative damage of some amino acids by polyunsaturated fatty acids or by lipid peroxidation metabolites, such as malondialdehyde [23]. An alternative explanation for these findings is a possible interaction of LCPUFA and metabolic turnover of amino acids. In view of the observed relationship between arachidonic acid availability and infant growth [25] and the mitogenic and growth promoting effect of prostaglandin E_2 derived from arachidonic acid in cell systems *in vitro*

[26], it is conceivable that high tissue concentrations of LCPUFA might be associated with an enhanced protein synthesis rate and, thereby, might induce lower plasma concentrations of some essential amino acids. It appears worthwhile to investigate further the potential relationship between LCPUFA supply and amino acid metabolism both in experimental studies and in future clinical trials.

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