Annals of Nutrition & Metabolism

Ann Nutr Metab 1998;42:195–201

Received: February 28, 1998
Accepted: March 24, 1998

T. Decsi[a, b]
I. Burus[a]
B. Koletzko[b]

[a] Department of Pediatrics, University Medical School of Pécs, Hungary;
[b] Kinderpoliklinik, Klinikum Innenstadt, Ludwig-Maximilians-Universität, München, Germany

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 (C18:3ω3) may enhance oxidative damage of essential amino acids. We investigated whether the addition of the long-chain polyunsaturated fatty acids (LCPUFA) arachidonate (C20:4ω-6; AA) and docosahexaenoate (C22:6-ω3; 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 μmol/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.

KARGER
Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

0250–6807/98/0424–0195$15.00/0

Accessible online at:
http://BioMedNet.com/karger

Berthold Koletzko, MD, Kinderpoliklinik
Klinikum Innenstadt, Ludwig-Maximilians-Universität
Pettenkoferstrasse 8a, D–80336 München (Germany)
Tel. +49 89 5160 3967/3511, Fax +49 89 5160 4733
E-Mail Berthold.Koletzko@kk-i.med.uni-muenchen.de

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 (C18:3ω-3) that was subjected to storage for several week 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 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 (C20:4ω-6, AA) and docosahexaenoate (C22:6ω-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 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.

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 ± SEM)

	Age, days				
	5	30	60	90	120
Arachidonate, μmol/l					
Conventional formula	484 ± 60	295 ± 32	280 ± 27***	306 ± 31	266 ± 28**
LCPUFA formula	529 ± 74	385 ± 33	448 ± 29***	428 ± 94	384 ± 18**
Docosahexaenoate, μmol/l					
Conventional formula	95 ± 9	62 ± 8**	46 ± 7***	49 ± 9***	44 ± 12*
LCPUFA formula	114 ± 69	106 ± 10**	137 ± 12***	133 ± 26***	122 ± 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 μl plasma was deproteinized with 200 μl absolute ethanol, and the internal standard (10 μl of 1 mmol/l norleucine solution) was added. The supernatant was alkalinized 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 ± 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 ± SEM)

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

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

Acknowledgements

This study was financially supported in part by Deutsche Forschungsgemeinschaft, Bonn, Germany (Ko 912/4-3), and by Milupa, Puch, Austria. The authors thank Dr. Volker Veitl for his dedicated help and support.

References

1 Koletzko B, Thiel I, Abiodun PO: The fatty acid composition of human milk in Europe and Africa. J Pediatr 1992;20(suppl):62–70.
2 Koletzko B, Bremer HJ: Fat content and fatty acid composition of infant formulae. Acta Paediatr Scand 1989;78:513–521.
3 Decsi T, Behrendt E, Koletzko B: Fatty acid composition of Hungarian infant formulae revisited. Acta Paediatr Hung 1994;34:107–116.
4 Demmelmair H, Schenck U, Behrendt E, Sauerwald T, Koletzko B: Estimation of arachidonic acid synthesis in full term neonates using natural variation of ^{13}C content. J Pediatr Gastroenterol Nutr 1995; 21:31–36.
5 Decsi T, Thiel I, Koletzko B: Essential fatty acids in full term infants fed breast milk or formula. Arch Dis Child 1995;72:F23-F28.
6 DeLucchi C, Pita ML, Faus MJ, Molina JA, Uauy R, Gil A: Effects of dietary nucleotides on the fatty acid composition of erythrocyte membrane lipids in term infants. J Pediatr Gastroenterol Nutr 1987;6: 568–574.
7 Farquharson J, Cockburn F, Patrick WA, Jamieson EC, Logan RW: Effect of diet on infant subcutaneous tissue triglyceride fatty acids. Arch Dis Child 1993;69:589–593.
8 Makrides M, Neumann MA, Byard RW, Simmer K, Gibson RA: Fatty acid composition of brain, retina and erythrocytes in breast- and formula-fed infants. Am J Clin Nutr 1994;60:189–194.
9 Farquharson J, Cockburn F, Patrick WA, Jamieson EC, Logan RW: Infant cerebral cortex phospholipid fatty acid composition and diet. Lancet 1993;340:810–813.
10 Koletzko B: Importance of dietary lipids; in Tsang R, Zlotkin SH, Nichols B, Hansen JW (eds): Nutrition during Infancy. Principles and Practice. Cincinnati, Digital Educational Publishing, 1997, pp 123–153.
11 Koletzko B: Lipid supply for infants with special needs. Eur J Med Res 1997;2:69–73.
12 Nielsen HK, Löliger J, Hurrell RF: Reactions of proteins with oxidizing lipids. 1. Analytical measurements of lipid oxidation and of amino acid losses in a whey protein-methyl linoleate system. Br J Nutr 1985;53:61–73.
13 Nielsen HK, Finot PA, Hurrell RF: Reactions of proteins with oxidizing lipids. 2. Influence on protein quality and on the bioavailability of lysine, methionine, cyst(e)ine and tryptophan as measured in rat assays. Brit J Nutr 1985;53:75–86.
14 Decsi T, Koletzko B: Growth, fatty acid composition of plasma lipid classes, and plasma retinol and α-tocopherol concentrations in full-term infants fed formula enriched with ω-6 and ω-3 long-chain polyunsaturated fatty acids. Acta Paediatr 1995;84:725–732.
15 Decsi T, Veitl V, Szász M, Pintér Z, Méhes K: Plasma amino acid concentrations in healthy, full-term infants fed hydrolysate infant formula. J Pediatr Gastroenterol Nutr 1996; 22:62–67.
16 Decsi T, Koletzko B: Fatty acid composition of plasma lipid classes in healthy subjects from birth to young adulthood. Eur J Pediatr 1994;153:520–525.
17 Turnell DC, Cooper JDH: Rapid assay for amino acids in serum and urine by precolumn derivatization and reversed phase liquid chromatography. Clin Chem 1982;28:527–531.
18 Roubal WT, Tappel AL: Damage to proteins, enzymes, and amino acids by peroxidizing lipids. Arch Biochem Biophys 1966;113:5–8.
19 Horigome T, Miura M: Interaction of protein and oxidized ethyl linoleate in the dry state and nutritive value of reacted protein. J Agric Soc Jpn 1974;48:437–444.
20 Yanagita T, Sugano M, Cho S, Wada M: Changes in available lysine and in vitro digestibility of casein accompanied with oxidation of ethyl linoleate: Effect of oxidized fats on nutritive value of proteins. J Agric Chem Soc Jpn 1973;47:73–78.
21 Chiba H, Doi H, Yoshikawa M, Sugimoto E: Deterioration of casein components by malondialdehyde. Agric Biol Chem 1976;40:1001–1010.

22 Braddock RJ, Dugan LR: Reaction of autoxidating linoleate with coho salmon myosin. J Am Oil Chem Soc 1973;50:343–347.
23 Nair V, Cooper CS, Vietti DE, Turner GA: The chemistry of lipid peroxidation metabolites: Cross-linking reactions of malondialdehyde. Lipids 1986;21:6–10.
24 Kanazawa K, Danno G, Nataka M: Lysozyme damage caused by secondary products during the autoxidation process of linoleic acid. J Nutr Sci Vitaminol (Tokyo) 1975; 21:373–382.
25 Koletzko B, Braun M: Arachidonic acid and early human growth: Is there a relation? Ann Nutr Metab 1991;35:128–131.
26 Danesch U, Weber PC, Sellmayer A: Differential effects of n-6 and n-3 polyunsaturated fatty acids on cell growth and early gene expression in Swiss 3T3 fibroblasts. J Cell Physiol 1996;168:618–624.