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The effect of a formula supplemented with n-3 and n-6 long-chain polyunsaturated fatty acids on plasma phospholipid, liver microsomal, retinal, and brain fatty acid composition in neonatal piglets

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We explore in this study the influence of dietary (n-6) and (n-3) long-chain polyunsaturated fatty acids (LC-PUFA) on tissues fatty acid composition in early life. Eight newborn piglets were fed sow milk for 20 days (group SM, n = 8); another sixteen newborn piglets were initially fed an adapted pig milk formula for 3 days and then assigned to receive for 17 days either the same adapted formula (group F, n = 8), or a formula supplemented with a phospholipid source of (n-6) and (n-3) LC-PUFA (group P, n = 8). Plasma phospholipid and liver microsomes 16:1(n-7) and 18:1(n-7) were increased in animals fed maternal milk compared with those fed formulas. Feeding the LC-PUFA formula produced significantly higher plasma phospholipid and liver microsomal 22:6(n-3) than feeding the control formula. Similar plasma phospholipid and liver microsomal (n-6) and (n-3) LC-PUFA were found in animals fed SM and in those fed the control formula. The supplementation of LC-PUFA did not lead to any change in brain and retinal 20:4(n-6) and 22:6(n-3). No differences were found in brain and retinal 22:6(n-3) between animals fed the formula devoid of LC-PUFA and those fed sow milk. Retinal 22:4(n-6), 22:5(n-6) and 22:5(n-3) contents were significantly higher in maternal milk fed animals as compared with control formula fed animals. In brain, animals fed sow milk had higher (n-6) LC-PUFA than animals fed the supplemented formula. The results obtained in this study indicate that the supplementation of a phospholipid source of LC-PUFA influences plasma and liver microsomes 22:6(n-3). Under these experimental conditions, brain and retinal 22:6(n-3) were insensitive to the dietary supplementation. (J. Nutr. Biochem. 8: 217–223, 1997) © *Elsevier Science Inc. 1997*

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Introduction

Linoleic acid (18:2n-6) and α-linolenic acid (18:3n-3) are considered essential fatty acids (EFAs) for primates because of the inability to synthesize them and the resulting deficiency syndromes when they are removed from the diet.[1–4]

Mammalian tissues, specially liver, intestine, and brain can synthesize long-chain polyunsaturated fatty acids (LC-PUFA) from EFAs by reactions of desaturation and elongation.[5] LC-PUFA are the substrates for the production of many biologically active compounds, including prostaglandins, thromboxanes, and leukotrienes.[6,7]

Neural tissues are more highly enriched than most other tissues in LC-PUFA, particularly arachidonic acid (20:4n-6) and docosahexaenoic (22:6n-3).[8] During the last trimester

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of pregnancy, the fast growing fetal brain accretes (n-6) and (n-3) LC-PUFA rapidly, in contrast to small amounts of their precursors linoleic and α-linolenic acids.[9,10] Experiments in rats and monkeys showed that (n-3) LC-PUFA play an important role in developing brain and retina, influencing cognitive performance and visual function.[4,11,12] Studies in preterm infants have also documented an essential role for (n-3) fatty acids in retinal development,[13,14] visual acuity,[15,16] and neural development.[17]

A reduced (n-3) LC-PUFA content in plasma and erythrocyte of infants fed artificial formulas when compared with those fed maternal milk has been demonstrated.[18–24] The same effect has also been reported in plasma, liver, brain, and retina of newborn piglets.[25,26] These studies suggest that the reduced (n-3) LC-PUFA accretion caused by artificial formulas may be attributable to a high 18:2(n-6) content in the diet, leading to preferential desaturation and/or elongation of (n-6) fatty acids or to specific dietary requirements for (n-3) LC-PUFA.[27] Most infant formulas do not contain LC-PUFA, whereas maternal milk provides enough amounts of them to satisfy the theoretical requirement of newborn infants.[28]

Fish oil supplementation of formula can maintain cord concentrations of red blood cell phospholipids 22:6(n-3),[29,30] but could reduce 20:4(n-6), which is already low in preterm infant fed formula.[29] Moreover, addition of fish oil to adapted milk pig formula resulted in higher levels of 22:6(n-3) in brain and liver and in lower levels of 20:4(n-6) in liver compared with sow milk fed animals.[31] These authors have suggested caution in the use of fish oils, which provide (n-3) LC-PUFA without (n-6) LC-PUFA. Recent studies using infant formula supplemented with both (n-6) and (n-3) LC-PUFA have shown that this is effective in maintaining plasma and red blood cell levels of 20:4(n-6) and 22:6(n-3) as compared with breast-fed infants.[32,33] There are no data, however, about the influence of (n-6) and (n-3) LC-PUFA supplementation of formula on retina and brain fatty acid composition.

The present work was performed to study the effect of dietary (n-6) and (n-3) LC-PUFA on the fatty acid composition of plasma, liver microsomes, brain, and retina in newborn piglets at 21 days of postnatal age.

Methods and materials

Diets

Two semipurified standard diets were used in this study and prepared following the National Research Council recommendations,[34] differing only in their fat composition. The first one contained a blend of medium-chain triglycerides, olive oil, milk fat, and soy oil (2:42:42:14) (group F) and the other one was the same diet supplemented with a phospholipid concentrate of (n-6) and (n-3) LC-PUFA to resemble the LC-PUFA composition of the human milk (group P). The purified phospholipid concentrate was obtained from pig brain and its fatty acid distribution was as follows: 35% saturated fatty acids, 34% monounsaturated, 8.4% arachidonic acid, and 7.2% docosahexaenoic. The chemical composition and the fatty acid profile of both diets are shown in *Tables 1* and *2*, respectively. Sow milk (SM) fatty acid profile is also shown in *Table 2*. Pigs were fed during pregnancy and lactation a chow diet (Sanders, Madrid, Spain) whose chemical composition was as follows: 17% protein, 4.5% fat, 52% carbohydrates, 7% minerals, 6% fiber, and 11% moisture. The fatty acid distribution of the chow diet fed during pregnancy was as follows: 31% saturates, 18:1(n-9) 25%, 18:2(n-6) 33%, 18:3(n-3) 4.1%, 20:4(n-6) 0.16%, and 22:6(n-3) 0.5%.

Table 1 Composition of the artificial diets (g/100 g)♣

Ingredient	F	P
Cow's milk solids	11.8	11.8
Calcium caseinate	13.6	13.6
Demineralized whey	15.3	15.3
Cream (35% fat)	45.1	45.1
MCT oil♦	1.82	1.64
Olive oil	11.6	10.4
Soy oil	3.6	3.2
Animal fat	—	1.7
Lecithin	0.35	0.35
Lactose	20.6	20.6
Minerals¶	3.7	3.7
Vitamins♥	0.32	0.32

♣Both F and P diets were spray-dried and contained (in g per 100 g): 25.5 protein, 36.2 lipids, 29.3 carbohydrates, 0.3% minerals, and 4.5 of moisture. They were diluted to 180 g/L in distilled water at 37°C and were ready for use. ♦MCT: medium chain triglycerides
¶Salt mix (per 100 g diet): 22.2 mg $CaHPO_4.H_2O$, 5.1 mg Ca citrate.$4H_2O$, 5.1 mg $Na_2HPO_4.12H_2O$, 1.53 mg $MgSO_4$, 1.4 mg Fe lactate, 1 mg K_2HPO_4, 0.01 mg $CuSO_4$, 0.03 mg $MnSO_4$, 0.0008 mg KI, 0.005 mg $ZnSO_4.H_2O$, and 0.1 mg Na_2SeO_2.
♥Vitamin mix (per 100 g diet): 2 mg thiamin, 3 mg riboflavin, 3 mg pyridoxine, 0.03 mg nicotinic acid, 0.02 mg calcium Δ-pantothenate, 1 mg folic acid, 0.8 mg Δ-biotin, 0.025 mg cyanocobalamin, 2 mg retinol acetate, 0.4 mg cholecalciferol, 10 mg tocopherol, 0.015 mg menadione, and 1.9 mg ascorbile palmitate.

Animal and experimental design

Twenty-four newborn Yorkshire piglets were obtained from the University of Granada. Animals received 110 mg iron dextran

Table 2 Fatty acid composition (wt %) of SM, adapted pig milk formula (F), and the same diet supplemented with (n-6) and (n-3) LC-PUFAs (P)

Fatty Acid	SM[1]	F	P
8:0	n.d.	7.5 ± 0.4	7.2 ± 0.5
10:0	0.1 ± 0.0	9.3 ± 0.2	8.9 ± 0.4
12:0	0.4 ± 0.3	1.3 ± 0.3	1.2 ± 0.4
14:0	3.4 ± 0.5	3.0 ± 0.4	3.1 ± 0.2
16:0	28.7 ± 1.5	14.9 ± 1.0	15.0 ± 1.0
16:1(n-7)	6.5 ± 0.4	1.6 ± 0.2	1.6 ± 0.5
18:0	5.5 ± 1.0	4.7 ± 0.1	5.4 ± 0.2
18:1(n-9)	34.7 ± 2.3	39.7 ± 1.5	39.2 ± 1.8
18:2(n-6)	14.1 ± 0.8	15.1 ± 0.6	14.5 ± 1.0
18:3(n-3)	0.7 ± 0.2	1.3 ± 0.3	1.3 ± 0.2
20:4(n-6)	1.1 ± 0.5	n.d.[2]	0.4 ± 0.1
22:4(n-6)	0.1 ± 0.0	n.d.	0.10 ± 0.1
22:5(n-6)	0.1 ± 0.1	n.d.	0.04 ± 0.1
22:5(n-3)	0.2 ± 0.1	n.d.	0.02 ± 0.1
22:6(n-3)	0.3 ± 0.1	n.d.	0.36 ± 0.1
Ratio 18:1(n-9)/18:2(n-6)	2.5	2.6	2.7
Ratio 18:2(n-6)/18:3(n-3)	20.1	12	11

[1]The determination of sow milk fatty acid composition was performed at day seven of lactation.
[2]n.d., Not detectable. Results are expressed in weight percentages as mean ± SEM. Five determinations were performed for each diet.

complex intramuscularly the first day of the study. Sixteen animals were initially fed a pig-adapted milk formula for 3 days and then randomly assigned to receive for 17 days either the same formula (group F) ($n = 8$) or the supplemented one (group P) ($n = 8$). A group of eight animals were kept with their mothers and fed SM for 20 days. Animals within a dietary group were not littermates. Animals were killed under anaesthesia after overnight food deprivation.

The protocol of this study was approved by the Animal Care of the Biochemistry and Molecular Biology Department of the University of Granada and conformed to Spanish legal guidelines.

Sample collection

After animals were sacrificed, 5 mL of blood were collected. Plasma was obtained by centrifugation at 3000 g for 5 min and stored at −80°C until analysis. Liver samples were homogenized with 0.1 M potassium phosphate buffer, pH 7.4 (1:4, weight: volume) and microsomal fractions were obtained. Briefly, liver homogenates were centrifuged at 16,000 g for 20 min and the resulting supernatants were centrifuged again at 16,000 g for 20 min. The supernatants thus obtained were centrifuged at 105,000 g for 60 min. The pellets were washed by resuspension in the homogenizing buffer and centrifuged at 105,000 g for 30 min. The washed pellets were resuspended in 0.05 M Tris-HCl buffer, pH 7.4 and stored at −80°C until analysis. The whole brain was removed, immediately frozen with liquid nitrogen, and stored at −80°C. Whole eyes were removed and dissected to extract the retina as described.[35]

Total lipids in plasma, liver microsomes, brain, and retina were extracted according to the method of Kolarovic and Fournier.[36] Plasma phospholipids were separated from the other lipids using TLC.[37] Lipid samples were immediately transesterified using 2.03 M boron trifluoride in methanol.[38] Fatty acid methyl esters were stored at −80°C under nitrogen until analysis.

Separation and quantification of fatty acid methyl esters was done by a flame ionization detector using capillary gas-liquid chromatography and by comparing their retention times with authentic standards. We used a Hewlett-Packard gas chromatograph model 5890 (Hewlett-Packard Co, Palo Alto, CA USA) equipped with a 0.25-mm bore 30 m capillary column filled with SP-2330 as stationary phase. Nitrogen at a flow of a 2 mL/min was used as a carrier gas and the split ratio was 15:1. The column temperature program was as follows; the initial temperature of 150°C was held for 5 min, then the temperature was increased at a rate of 2°C/min to 190°C, and at 3°C/min from 190° to 211°C. Finally, the temperature was held at 211°C for 10 min. The relative concentration of individual fatty acids were expressed as percent of total fatty acid equal to or greater than 14 carbons.

Statistical analysis

One-way analysis of variance were done to evaluate the effects of diet at 21 days of age. Post-hoc Tukey tests were used to determine mean differences among the groups for all the parameters studied by using the PC-90 version of the BMDP statistical software (Berkeley, CA).[39] Statistical differences were establish at $P < 0.05$.

Results

Growth

The body weights, 4.5 ± 0.3, 4.0 ± 0.2, 4.0 ± 0.3 kg, and liver weights, 96 ± 5, 85 ± 5, 88 ± 5 g for the SM, F and P animals, respectively, were similar.

Table 3 Effect of (n-6) and (n-3) LC-PUFA dietary supplementation on the fatty acid composition[1] of plasma phospholipids in newborn piglets at 21 days of age fed SM, a pig-adapted cow's milk formula (F), and the same diet supplemented with (n-6) and (n-3) LC-PUFA

Fatty acid	SM ($n = 8$)	F ($n = 8$)	LC-PUFA Formula ($n = 8$)
14:0	0.50 ± 0.07	0.87 ± 0.13 a	0.49 ± 0.05 b
16:0	24.58 ± 0.68	22.83 ± 0.79	22.69 ± 0.47
16:1(n-9)	0.78 ± 0.08	1.01 ± 0.13	0.84 ± 0.08
16:1(n-7)	1.34 ± 0.14	0.54 ± 0.05 a	0.34 ± 0.03 a
18:0	18.83 ± 0.87	26.08 ± 0.92 a	24.91 ± 0.84 a
18:1(n-9)	15.15 ± 0.65	19.18 ± 0.64 a	18.04 ± 0.31 a
18:1(n-7)	3.41 ± 0.10	1.68 ± 0.14 a	1.96 ± 0.05 a
18:2(n-6)	13.66 ± 0.60	13.60 ± 0.61	12.83 ± 0.36
18:3(n-3)	0.32 ± 0.07	0.67 ± 0.07 a	0.44 ± 0.05 b
20:3(n-6)	0.84 ± 0.15	0.78 ± 0.09	0.79 ± 0.04
20:4(n-6)	13.43 ± 0.99	13.13 ± 1.11	13.14 ± 0.61
22:4(n-6)	0.80 ± 0.09	0.57 ± 0.06 a	0.83 ± 0.08 b
22:5(n-6)	0.67 ± 0.06	0.72 ± 0.04	0.78 ± 0.02
22:5(n-3)	2.06 ± 0.09	1.23 ± 0.13 a	1.38 ± 0.07 a
22:6(n-3)	2.60 ± 0.25	2.69 ± 0.44	4.53 ± 0.43 a,b
Total (n-9)	15.43 ± 0.80	14.66 ± 0.61	14.91 ± 0.37
Total (n-7)	4.76 ± 0.20	2.23 ± 0.12 a	2.30 ± 0.07 a
Total (n-6) LC-PUFA	15.74 ± 1.04	15.20 ± 1.23	15.53 ± 0.66
Total (n-3) LC-PUFA	4.67 ± 0.45	3.91 ± 0.55	5.91 ± 0.50 b

[1]This table includes only select fatty acids.
LC-PUFA: long-chain polyunsaturated fatty acids with more than 18 carbon atoms.
Results are expressed in weight percentages as mean ± SEM ($n = 8$/group).
a, Significantly different from group SM $P < 0.05$. b, Significantly different from group F $P < 0.05$.

Plasma phospholipids

The relative fatty acid composition of plasma phospholipids in the three experimental groups is shown in *Table 3*. The percentage of 18:0 was significantly higher in animals fed formulas (groups F and P) than in those fed SM. Animals receiving maternal milk had higher (n-7) monoenoic acids, 16:1(n-7) and 18:1(n-7), in comparison with those receiving the artificial formulas (F and P). Feeding maternal milk produced a lower content of 18:1(n-9) than feeding the artificial formulas (F and P). 22:5(n-3) content was higher in animals fed SM than in those receiving formulas. The content of 20:4(n-6) remained unchanged among the three groups. Animals given the LC-PUFA-supplemented formula showed a significantly higher content of 22:6(n-3) than those given either maternal milk or the control formula.

Liver microsomes

The influence of dietary LC-PUFA on liver microsomes fatty acids is shown in *Table 4*. 18:0 content was significantly reduced in maternal milk fed group in relation to formula fed groups. Monounsaturated fatty acids (n-7) were increased in group SM compared with groups F and P. No significant diet-induced differences were observed for linoleic 18:2(n-6). α-Linolenic acid 18:3(n-3) and 20:5(n-3) levels were increased in both formula-fed groups compared with SM group. Arachidonic acid 20:4(n-6) and the total

Table 4 Effect of (n-6) and (n-3) LC-PUFA dietary supplementation on the fatty acid composition[1] of liver microsomes in newborn piglets at 21 days of age fed SM, a pig-adapted cow's milk formula (F), and the same diet supplemented with (n-6) and (n-3) LC-PUFA

Fatty acid	SM (n = 8)	F (n = 8)	LC-PUFA Formula (n = 8)
14:0	1.06 ± 0.08	1.29 ± 0.29	1.93 ± 0.75
16:0	21.71 ± 0.38	20.04 ± 0.47	20.94 ± 1.61
16:1(n-9)	1.08 ± 0.04	1.22 ± 0.12	1.07 ± 0.05
16:1(n-7)	2.54 ± 0.14	0.53 ± 0.03 a	0.55 ± 0.11 a
18:0	18.99 ± 0.58	25.96 ± 0.42 a	25.30 ± 1.30 a
18:1(n-9)	16.98 ± 0.52	14.07 ± 0.47 a	14.29 ± 1.22
18:1(n-7)	3.00 ± 0.04	1.52 ± 0.03 a	1.60 ± 0.06 a
18:2(n-6)	12.00 ± 0.22	12.62 ± 0.58	10.50 ± 0.77
18:3(n-6)	0.40 ± 0.02	0.34 ± 0.05	0.24 ± 0.02 a
18:3(n-3)	0.40 ± 0.01	0.61 ± 0.05 a	0.57 ± 0.03 a
20:3(n-6)	0.37 ± 0.01	0.56 ± 0.02 a	0.60 ± 0.04 a
20:4(n-6)	12.52 ± 0.34	12.76 ± 0.53	12.77 ± 1.27
20:5(n-3)	0.34 ± 0.02	0.62 ± 0.02 a	0.54 ± 0.04 a
22:4(n-6)	0.81 ± 0.09	0.73 ± 0.02	0.68 ± 0.08
22:5(n-6)	0.87 ± 0.03	1.17 ± 0.12	0.96 ± 0.10
22:5(n-3)	1.70 ± 0.05	1.53 ± 0.17	1.12 ± 0.20 a
22:6(n-3)	3.09 ± 0.21	2.94 ± 0.21	4.68 ± 0.26 a,b
Total (n-9)	18.27 ± 0.51	15.58 ± 0.53 a	15.65 ± 1.24 a
Total (n-7)	5.54 ± 0.15	2.06 ± 0.02 a	2.15 ± 0.08 a
Total (n-6) LC-PUFA	15.60 ± 0.39	16.04 ± 0.50	15.54 ± 1.44
Total (n-3) LC-PUFA	5.13 ± 0.10	5.08 ± 0.19	6.35 ± 0.20 a,b

[1]This table includes only select fatty acids.
LC-PUFA: long-chain polyunsaturated fatty acids with more than 18 carbon atoms.
Results are expressed in weight percentages as mean ± SEM (n = 8/group).
a, Significantly different from group SM $P < 0.05$. b, Significantly different from group F $P < 0.05$.

Table 5 Effect of (n-6) and (n-3) LC-PUFA dietary supplementation on the fatty acid composition[1] of retina in newborn piglets at 21 days of age fed SM, a pig adapted cow's milk formula (F), and the same diet supplemented with (n-6) and (n-3) LC-PUFA

Fatty acid	SM (n = 8)	F (n = 8)	LC-PUFA Formula (n = 8)
14:0	0.53 ± 0.05	0.55 ± 0.07	0.52 ± 0.07
16:0	13.30 ± 0.37	17.27 ± 1.01 a	15.21 ± 0.76 a
16:1(n-9)	0.80 ± 0.06	0.71 ± 0.10	0.67 ± 0.08
16:1(n-7)	0.65 ± 0.01	0.63 ± 0.01	0.53 ± 0.01 a
18:0	28.87 ± 0.30	27.95 ± 0.36	28.94 ± 0.32
18:1(n-9)	16.13 ± 0.37	17.08 ± 0.24	15.59 ± 0.33 b
18:1(n-7)	3.73 ± 0.15	3.78 ± 0.08	3.61 ± 0.09
18:2(n-6)	2.38 ± 0.04	2.41 ± 0.10	2.47 ± 0.10
18:3(n-6)	0.84 ± 0.12	0.85 ± 0.10	0.25 ± 0.07 a,b
18:3(n-3)	0.88 ± 0.12	0.67 ± 0.04	0.73 ± 0.05
20:3(n-6)	0.61 ± 0.11	0.39 ± 0.01 a	0.58 ± 0.13
20:4(n-6)	10.11 ± 0.42	10.11 ± 0.50	10.39 ± 0.42
22:4(n-6)	3.15 ± 0.04	2.38 ± 0.05 a	2.86 ± 0.08
22:5(n-6)	2.26 ± 0.05	1.34 ± 0.06 a	1.85 ± 0.08
22:5(n-3)	0.79 ± 0.02	0.53 ± 0.03 a	0.66 ± 0.03
22:6(n-3)	14.04 ± 0.27	13.29 ± 0.30	14.98 ± 0.60
Total (n-9)	16.90 ± 0.33	17.78 ± 0.22	16.25 ± 0.40
Total (n-7)	4.37 ± 0.15	4.41 ± 0.08	4.14 ± 0.10
Total (n-6) LC-PUFA	16.13 ± 0.45	14.22 ± 0.46 a	15.68 ± 0.50
Total (n-3) LC-PUFA	14.83 ± 0.28	13.81 ± 0.41	15.66 ± 0.70

[1]This table only includes select fatty acids.
LC-PUFA: long-chain polyunsaturated fatty acids with more than 18 carbon atoms.
Results are expressed in percentages, as mean ± SEM (n = 8/group).
a, Significantly different from group SM $P < 0.05$. b, Significantly different from group F $P < 0.05$.

(n-6) LC-PUFA remained unchanged in all groups. Feeding LC-PUFA formula produced a significantly higher content of DHA 22:6(n-3) than feeding either the control-formula or maternal milk. In contrast, the level of 22:5(n-3) was significantly reduced in group P, compared with group SM.

Retina

The fatty acid profile of retina lipids in the experimental groups is shown in *Table 5*. Animals fed maternal milk had a significantly higher content of 22:4(n-6), 22:5(n-6) and 22:5(n-3) than animals fed the control formula (F). Animals receiving the LC-PUFA-supplemented diet had a content of these fatty acids intermediate between the maternal-fed and the control formula-fed groups (SM and F).

Brain

Table 6 shows the changes in brain fatty acids at 21 days of age among groups. Feeding maternal milk led to significantly higher (n-6) LC-PUFA [20:4(n-6), 22:4(n-6), and 22:5(n-6)] than feeding the formula providing LC-PUFA. Animals fed the control formula had an intermediate content of those fatty acids between that of animals fed SM and animals fed the LC-PUFA-supplemented formula.

Discussion

Decreased concentrations of (n-3) and (n-6) LC-PUFA in plasma and erythrocyte lipids of formula-fed compared with breast-fed term[21,22] and preterm[13,14] human infants have been reported. This was first interpreted to suggest immaturity for the conversion of EFAs to their long-chain derivatives in the neonatal period.[18] Salem et al.[40] have recently reported that human newborns as small as 2 kg are capable of forming 20:4(n-6) and 22:6(n-3), although they suggest that the amount of 22:6(n-3) produced in vivo from 18:3(n-3) may be inadequate to support the 22:6(n-3) level observed in breast-fed infants. In this study we have found similar plasma and liver microsomes phospholipids 20:4(n-6) and 22:6(n-3) contents in animals fed maternal milk and in those fed a formula devoid of LC-PUFA at 21 days of age. Previous studies have reported substantial reductions in the (n-3) LC-PUFA and parallel compensatory increases in the (n-6) LC-PUFA in plasma, erythrocytes, and liver in piglets receiving an artificial formula with a high 18:2(n-6)/18:3(n-3) ratio and a low level of 18:1(n-9) as compared with those receiving maternal feeding.[26] As the authors argued, that was probably attributable to an inhibition of 18:3(n-3) desaturation by the high 18:2(n-6) amounts of the formula. In this study an infant formula with a 18:1(n-9):18:2(n-6):18:3(n-3) ratio similar to that in human milk have led to similar plasma and liver microsomes phospholipid

Table 6 Effect of (n-6) and (n-3) LC-PUFA dietary supplementation on the fatty acid composition[1] of brain in newborn piglets at 21 days of age fed SM, a pig-adapted cow's milk formula (F), and the same diet supplemented with (n-6) and (n-3) LC-PUFA

Fatty acid	SM (n = 8)	F (n = 8)	LC-PUFA Formula (n = 8)
14:0	0.51 ± 0.02	0.54 ± 0.04	0.53 ± 0.05
16:0	19.24 ± 0.45	17.79 ± 0.76	17.08 ± 0.76
16:1(n-9)	0.96 ± 0.04	0.83 ± 0.07	0.78 ± 0.06
16:1(n-7)	1.01 ± 0.03	0.79 ± 0.04 a	0.76 ± 0.04 a
18:0	20.48 ± 0.38	20.25 ± 0.56	19.77 ± 0.31
18:1(n-9)	16.44 ± 0.53	19.19 ± 1.43	20.25 ± 1.33 a
18:1(n-7)	6.20 ± 0.31	6.41 ± 0.58	6.32 ± 0.34
18:2(n-6)	1.38 ± 0.04	1.41 ± 0.07	1.26 ± 0.06
18:3(n-6)	0.22 ± 0.05	0.29 ± 0.08	0.38 ± 0.11
20:3(n-6)	0.68 ± 0.02	0.61 ± 0.02	0.56 ± 0.02 a
20:4(n-6)	12.42 ± 0.26	11.23 ± 0.38	10.45 ± 0.24 a
20:5(n-3)	0.50 ± 0.08	0.48 ± 0.02	0.47 ± 0.03
22:4(n-6)	5.41 ± 0.12	5.20 ± 0.24	4.63 ± 0.10 a
22:5(n-6)	2.62 ± 0.05	2.49 ± 0.13	1.97 ± 0.07 a
22:5(n-3)	0.55 ± 0.07	0.48 ± 0.04	0.52 ± 0.06
22:6(n-3)	8.65 ± 0.22	8.47 ± 0.36	8.64 ± 0.36
Total (n-9)	19.10 ± 0.68	22.24 ± 1.77	23.93 ± 1.75
Total (n-7)	7.21 ± 0.29	7.20 ± 0.55	7.09 ± 0.31
Total (n-6) LC-PUFA	21.58 ± 0.34	20.01 ± 0.97	18.09 ± 0.21 a
Total (n-3) LC-PUFA	9.50 ± 0.17	9.33 ± 0.32	9.63 ± 0.64

[1]This table includes only select fatty acids.
LC-PUFA: long-chain polyunsaturated fatty acids with more than 18 carbon atoms.
Results are expressed in weight percentages as mean ± SEM (n = 8/group).
a, Significantly different from group SM $P < 0.05$. b, Significantly different from group F $P < 0.05$.

(n-6) and (n-3) LC-PUFA contents in animals fed the formula devoid of LC-PUFA and in those fed SM.

Recently, Innis et al. reported that the accumulation of 18:1(n-9) in plasma phospholipids was greater and 20:4(n-6) tended to be lower when formula saturated fat was medium-chain triglycerides (MCT) rather than 16:0.[41] The data of plasma phospholipids 20:4(n-6) contents in that study suggest that the experiment was performed in conditions in which an optimal conversion of 18:2(n-6) to 20:4(n-6) is not promoted. In our study the formulas contained higher MCT and lower 16:0 amounts than SM, however no differences in 18:1(n-9) or 20:4(n-6) were found between formula-fed and SM-fed animals. There are differences in the formula 8:0 contents in these studies; therefore, we cannot rule out the possibility that these differences may have led to different results. The results suggest that an adequate dietary 18:1(n-9):18:2(n-6):18:3(n-3) ratio may promote an optimal synthesis of (n-6) and (n-3) LC-PUFA. The data also show that the rate of synthesis of 20:4(n-6) and 22:6(n-3) in newborn piglets was probably high enough to reach the plasma and liver microsomes 20:4(n-6) and 22:6(n-3) contents observed in maternal-fed animals.

Although the content of 22:6(n-3) in the LC-PUFA supplemented diet was similar to that in human milk, group P had a significantly higher percentage of 22:6(n-3) in plasma phospholipid and liver microsomes compared with groups F and SM. Other studies with a similar level of 22:6(n-3) in the diet from fish oil triglycerides found a similar plasma and liver phospholipids 22:6(n-3) content in piglets fed maternal milk and in those fed the (n-3) LC-PUFA-supplemented diet.[31] Our source of (n-3) and (n-6) LC-PUFA is a concentrate of phospholipids, very different in its nature from fish oil. It would be of interest to determine if the amount of dietary (n-3) fatty acids required during the perinatal period is lower if newborns are fed phospholipids rather than triglycerides.

An important finding of this study is the significant increase in (n-7) monoenoic fatty acids, 16:1(n-7) and 18:1(n-7) found in plasma phospholipid and liver microsomes of piglets fed maternal milk compared with those fed artificial formulas. This result seems to be attributable to the higher content of those fatty acids in SM as compared with infant formulas.

Feeding the supplemented LC-PUFA did not lead to any significant change in retinal LC-PUFA composition. Retinal concentrations of 22:4(n-6), 22:5(n-6), and 22:5(n-3) were higher in animals given maternal milk than in those given the control formula (F). Animals receiving the LC-PUFA supplemented diet had 22:5(n-3), 22:5(n-6), and 22:4(n-6) intermediate between those of animals receiving maternal milk and those of animals receiving the formula devoid of LC-PUFA. The retina contains very LCPUFAs (VLCPUFAs) (22–40 carbon atoms) of both (n-3) and (n-6) fatty acids families that seem to derive from 22:4(n-6), 22:5(n-6), 22:5(n-3), and 22:6(n-3) by means of elongation reactions.[42] These fatty acids, incorporated in dipolyunsaturated phosphatidylcholine species, are highly concentrated in photoreceptor membranes of the retina in vertebrates.[43] A recent study has shown that these VLCPUFAs, particularly 32:4(n-6) and 34:6(n-3), were able to activate protein C (PKC) in vitro.[44] PKC is known to play a vital role in transmembrane signaling.[45] However, only changes in retina 22:6(n-3) content have been reported to be functionally relevant.[13,46] These results suggest that retina 22:6(n-3) is relatively insensitive to moderate dietary LC-PUFA manipulations.

The demonstration that brain 22:6(n-3) content did not increase in animals fed the LC-PUFA formula, despite the marked LC-PUFA formula-induced increase in plasma phospholipids and liver microsomes is highly relevant. A higher brain 22:6(n-3) contents in animals fed a fish oil supplemented diet as compared to animals fed a formula devoid of LC-PUFA has been reported.[31] However, the formula fat of that study contained a high amount of 18:2(n-6), which may have reduced the conversion of 18:3(n-3) to 22:6(n-3). Alternatively, differences in brain 22:6(n-3) content at birth could result from differences in the 22:6(n-3) content of diets fed during pregnancy. Although we do not know the fatty acid content of the diet fed during pregnancy in that study,[31] our sow diet contained large amounts of 22:6(n-3).

Piglets fed the LC-PUFA diet had lower (n-6) LC-PUFA [20:3(n-6), 20:4(n-6), 22:4(n-6), and 22:5(n-6)] than those fed SM, whereas animals fed the control formula had intermediate contents of those fatty acids between those of SM-fed animals and those of LC-PUFA formula-fed ani-

mals. Diets containing fish oil have been reported to decrease 20:4(n-6) in brain lipids of rodents[47,48] and monkeys.[4] Arbuckle et al. have shown no brain 20:4(n-6) decrease in piglets fed a fish oil-supplemented formula as compared with those fed maternal milk, despite a sharp decrease in plasma and liver phospholipids 20:4(n-6) contents.[31] In contrast with that study, our results show lower brain 20:4(n-6) in animals fed the supplemented diet and no change in liver microsomes 20:4(n-6). Differences in the metabolism of LC-PUFA-incorporated in phospholipids or triglycerides might explain the different results obtained in these studies.

In conclusion, the collected data show that the dietary supplementation of LC-PUFA influences plasma and liver microsomes 22:6(n-3) contents in newborn piglets, but retinal and brain 22:6(n-3) content were relatively insentive to the LC-PUFA supplementation. The results also show no differences in tissue 20:4(n-6) and 22:6(n-3) percentages between animals fed SM and those fed the formula devoid of LC-PUFA.

References

1 Bjerve, K.S. (1989). W-3 deficiency in man. *J. Intern. Med.* **225,** 171–175

2 Holman, R.T., Johnson, S.B., and Hatch, T.F. (1982). A case of human linolenic acid deficiency involving neurological abnormalities. *Am. J. Clin. Nutr.* **35,** 617–623

3 Martinez, M. and Ballabriga, A. (1979). Effects of parenteral nutrition with high doses of linoleate on the developing human liver and brain. *Lipids* **22,** 133–138

4 Connor, W.E. and Neuringer, M. (1988). The effects of (n-3) fatty acid deficiency and repletion upon the fatty acid composition and function of the brain and retina. In *Biological Membranes: Aberrations in Membrane Structure and Function*, pp. 275–294, Alan R. Liss, New York, NY USA

5 Brenner, R.R. (1971). The desaturation step in the animal biosynthesis of polyunsaturated fatty acids. *Lipids* **6,** 567–575

6 Wolfe, L.S. (1982). Eicosanoids: prostaglandins, thromboxanes, leukotrienes, and other derivatives of carbon-20 unsaturated fatty acids. *J. Neurochem.* **38,** 1–14

7 Sprecher, H. (1986). The metabolism of (n-3) and (n-6) fatty acids and their oxygenation by platelet cyclooxygenase and lipoxygenase. *Prog. Lipid. Res.* **25,** 19–28

8 Svennerholm, L. (1968). Distribution and fatty acid composition of phosphoglycerides in normal human brain. *J. Lipid. Res.* **9,** 570–579

9 Clandinin, M.T., Chappell, J.E., Leong, S., Heim, T., Swyer, P.R., and Chance, G.W. (1980). Intrauterine fatty acid accretion rates in human brain: implications for fatty acid requirements. *Early Hum. Dev.* **4,** 121–129

10 Clandinin, M.T., Chappell, J.E., Leong, S., Heim, T., Swyer, P.R., and Chance, G.W. (1980). Extrauterine fatty acid accretion in infant brain: implications for fatty acid requirements. *Early Hum. Dev.* **4,** 131–138

11 Yamamoto, N., Saitoh, M., Morivchi, A., Nomura, M., and Okuyama, H. (1987). Effect of dietary α-linolenate/linoleate balance on brain lipid compositions and learning ability of rats. *J. Lipid. Res.* **28,** 144–151

12 Bourre, J.M., François, M., Youyou, A., Dumont, O., Piciotti, M., Pascal, G., Durand, G. (1989). The effects of dietary α-linolenic acid con the composition of nerve membranes, enzymatic activity, amplitude of electrophysiological parameters, resistance to poisons and performance of learning tasks in rats. *J. Nutr.* **119,** 1880–1892

13 Uauy, R., Birch, D., Birch, E., and Hoffman, D.R. (1990). Effect of dietary omega-3 fatty acids on retinal function of very-low-birthweight neonates. *Pediatr. Res.* **28,** 485–492

14 Birch, D.G., Birch, E.E., Hoffman, D.R., and Uauy, R. (1992). Retinal development in very-low-birth-weight infants fed diets differing in omega-3 fatty acids. *Invest. Ophthalmol. Vis. Sci.* **33,** 2365–2376

15 Birch, E.E., Birch, D.G., Hoffman, D.R., and Uauy, R. (1992). Dietary essential fatty acid supply and visual acuity development. *Invest. Ophthalmol. Vis. Sci.* **33,** 3242–3253

16 Carlson, S.E., Werkman, S.H., Rhodes, P.G., and Tolley, E.A. (1993). Visual-acuity development in healthy preterm infants: effect of marine-oil supplementation. *Am. J. Clin. Nutr.* **58,** 35–42

17 Werkman, S.H. and Carlson, S.E. (1996). A randomized trial of visual attention of preterm infants fed docosahexaenoic acid until nine months. *Lipids* **31,** 91–97

18 Sanders, T.A.B. and Naismith, D.J. (1979). A comparison of the influence of breastfeeding and bottle-feeding on the fatty acid composition of the erytrocytes. *Br. J. Nutr.* **41,** 619–623

19 Putnam, J.C., Carlson, S.E., Devoe, P.N., and Barness, L.A. (1982). The effects of variation in dietary fatty acids on the fatty acid composition of erythrocyte phosphatidylcholine and phosphatidylethanolamine in human infants. *Am. J. Clin. Nutr.* **36,** 106–114

20 Carlson, S.E., Rhodes, P.G., and Ferguson, M.G. (1986). Docosahexaenoic status of preterm infants at birth and following feeding with human milk or formula. *Am. J. Clin. Nutr.* **44,** 798–804

21 Gil, A., Pita, M.L., Martínez, A., Molina, J.A., Sánchez-Medina, F. (1986). Effect of dietary nucleotides on the plasma fatty acids in at-term neonates. *Human Nutr. Clin. Nutr.* **40C,** 185–195

22 De Lucchi, C., Pita, M.L., Faus, M.J., Periago, J.L., and Gil. A. (1987). Effects of dietary nucleotides on the fatty acid composition of erythrocyte membrane lipids in term infants. *J. Pediatr. Gastr. Nutr.* **6,** 568–574

23 Koletzko, B., Schmidt, E., Brenner, H.J., Haug, M., and Garzer, G. (1989). Effects of dietary long-chain polyunsaturated fatty acids on the essential fatty acid status of premature infants. *Eur. J. Pediatr.* **148,** 669–675

24 Pita, M.L., DeLucchi, C., Faus, M.J., and Gil, A. (1990). Changes in the fatty acid profiles of red blood cell membrane phospholipids in human neonates during the first month of life. *Clin. Physiol. Biochem.* **8,** 91–100

25 Hrboticky, N., Mackinnon, M.J., and Innis, S.M. (1990). Effect of a vegetable oil formula rich in linoleic acid on tissue fatty acid accretion in the brain, liver, plasma and erythrocytes of infant piglets. *Am. J. Clin. Nutr.* **51,** 173–182

26 Hrboticky, N., Mackinnon, M.J., and Innis, S.M. (1991). Retina fatty acid composition in piglets fed from birth with a linoleic acid-rich vegetable-oil formula for infants. *Am. J. Clin. Nutr.* **53,** 483–490

27 Koletzko, B. and Cunnane, S. (1988). Human alpha-linolenic deficiency. *Am. J. Clin. Nutr.* **47,** 1084–1086

28 Clandinin, M.T., Chappell, J.E., Heim, T., Swyer, P.R., and Chance, G.W. (1981). Fatty acid utilization in perinatal de novo synthesis of tissues. *Early Hum. Dev.* **5,** 355–356

29 Carlson, S.E., Cooke, R.J., Rhodes, P.G., Peeples, J.M., Werkman, S.H., and Tolley, E.A. (1991). Long-term feeding of formulas high in linolenic acid and marine oil to very low birth weight infants: Phospholipid fatty acids. *Pediatr. Res.* **30,** 404–412

30 Hoffman, D.R. and Uauy, R. (1992). Essentiality of dietary ω3 fatty acids for premature infants: Plasma and red blood cell fatty acid composition. *Lipids* **27,** 886–895

31 Arbuckle, L.D., Rioux, F.M., Mackinnon, M.J., Hrboticky, N., and Innis, S.M. (1991). Response of (n-3) and (n-6) fatty acids in piglet brain, liver and plasma to increasing, but low, fish oil supplementation of formula. *Lipids* **121,** 1536–1547

32 Clandinin, M.T., Garg, M.L., Parrott, A., Van Aerde, J., Hervada, A., and Lien, E. (1992). Addition of long-chain polyunsaturated fatty acids to formula for very low birth weight infants. *Lipids* **27,** 896–900

33 Carnielli, V.P., Pederzini, F., Luijendijk, I.H.T., Bomaars, W.E.M., Boerlage, A., Degenhart, H.J., Pedrotti, D., and Sauer, P.J.J. (1994). Long chain polyunsaturated fatty (LCP) in low birth weight formula at levels found in human colostrum. *Pediatr. Res.* **3584,** 1840(abs.)

34 Miller, E.R. and Ullrey, D.E. (1987). The pig as a model for human nutrition. *Annu. Rev. Nutr.* **7,** 361–382

35 Martinez, M., Ballabriga, A., and Gil-Gibernau, J. (1988). Lipids of the developing human retina: I. Total fatty acids, plasmalogens and fatty acid composition of ethanolamine and choline of phosphoglycerides. *J. Neurosci. Res.* **52,** 484–490

36 Kolarovic, L. and Fournier, N. (1986). A comparison of extraction

methods for the isolation of phospholipids from biological sources. *Ann. Biochem.* **156,** 244–250

37 Boza, J.J., Jimenez, J., Faus, M.J., and Gil, A. (1992). Influence of postnatal age and dietary nucleotides on plasma fatty acids in the weaning rat. *J. Parent. Ent. Nutr.* **16,** 322–326

38 Morrison, M.R. and Smith, L.M. (1964). Preparation of fatty acids methyl esters and dimethylacetals from lipids with boron fluoride methanol. *J. Lipid. Res.* **5,** 600–607

39 Dixon, W.J., Brown, M.B., Engelman, L., and Jennrich, R.I. (1990). *BMDP Statistical Software Manual*, University of California Press, Berkeley, CA USA

40 Salem, N. Jr., Wegher, B., Mena, P., and Uauy, R. (1996) Arachidonic and docosahexaenoic acids are biosynthesized from their 18-carbon precursors in human infants. *Proc. Natl. Acad. Sci. USA* **93,** 49–54

41 Innis, S.M., Quinlan, P., and Diersen-Schade, D. (1993). Saturated fatty acid chain length and positional distribution in infant formula: effects on growth and plasma lipids and ketones in piglets. *Am. J. Clin. Nutr.* **57,** 382–390

42 Aveldaño, M.I. (1987). A novel group of very long chain polyenoic fatty acids in dipolyunsaturated phosphatidylcholines from vertebrate retina. *J. Biol. Chem.* **262,** 1172–1179

43 Aveldaño, M.I. and Sprecher, H. (1987). Very long chain (C_{24} to C_{36}) polyenoic fatty acids of the (n-3) and (n-6) series in dipolyunsaturated posphatidylcholines from bovine retina. *J. Biol. Chem.* **262,** 1180–1186

44 Hardy, S.J., Ferrante, A., Robinson, B.S., Johnson, D.W., Poulos, A., Clark, K.J., and Murray, A.W. (1994). In vitro activation of protein kinase C by polyenoic very-long chain fatty acids. *J. Neurochem.* **62,** 1546–1551

45 Nishizuka, Y. (1992). Intracellular signalling by hydrolysis of phospholipids and activation of protein kinase C. *Science* **258,** 607–614

46 Neuringer, M., Connor, W.E., Lin, D.S., Barstad, L., and Luck, S. (1986). Biochemical and functional effects of prenatal and postnatal w3 fatty acid deficiency on retina and brain in rhesus monkeys. *Proc. Natl. Acad. Sci. USA* **83,** 4021–4026

47 Bourre, J.M., Bonnell, M., Dumont, O., Piciotti, M., Nalbone, G., and Lafont, H. (1988). High dietary fish oil alters the brain polyunsaturated fatty acids composition. *Biochem. Biophys. Acta* **960,** 458–461

48 Swanson, J.E., Black, J.M., and Kinsella, J.E. (1988). Dietary menhaden oil: effects on the rate and magnitude of modification of phospholipid fatty acid composition of mouse heart and brain. *Brit. J. Nutr.* **59,** 535–545