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Brain Docosahexaenoic Acid Status and Learning in Young Rats Submitted to Dietary Long-Chain Polyunsaturated Fatty Acid Deficiency and Supplementation Limited to Lactation

SALVADOR GARCÍA-CALATAYUD, CARLOS REDONDO, EVA MARTÍN, JOSÉ IGNACIO RUIZ, MIGUEL GARCÍA-FUENTES, AND PABLO SANJURJO

Department of Pediatrics [S.G.-C., C.R., E.M., M.G.-F.], University of Cantabria, 39011 Santander, Cantabria, Spain; and Unit of Infant Metabolism [J.I.R., P.S.], Department of Pediatrics, Hospital de Cruces, 48903 Baracaldo, Vizcaya, Spain

ABSTRACT

N-3 fatty acid deficiency has been related to decreased docosahexaenoic acid (DHA) and increased docosapentaenoic acid (DPA) levels in brain and to learning disadvantages. The influence of n-3 deficiency and supplementation on brain fatty acids and learning were investigated in young rats. Newborn Wistar rats were assigned to three groups of cross-foster mothers. The control group (C) was nursed by mothers that received essential fatty acids during pregnancy and lactation, and the deficient group (D) was nursed by mothers that did not receive those fatty acids. The supplemental group (S) had the same conditions as D, receiving an additional DHA and arachidonic acid supplement during lactation. Cerebral cortex and hippocampus fatty acid composition was examined using thin-layer and capillary column gas chromatography, and learning was measured by passive-avoidance procedure. D brains showed low DHA and high DPA levels, but S brain composition was similar to C. Learning in the S group was unaffected, but in the D group, it was poorer than C. Learning was directly correlated with DHA levels and inversely with DPA levels in brain. Low DHA and high DPA brain levels both were correlated with poor learning. DPA seems not to be a suitable brain functional analogue of DHA, and DHA supplementation reversed both biochemical and learning adverse effects observed in n-3 deficiency. (***Pediatr Res*** **57: 719–723, 2005)**

Abbreviations

AA, arachidonic acid
DHA, docosahexaenoic acid
DPA, docosapentaenoic acid
EFA, essential fatty acid
FA, fatty acid
IQR, interquartile range
LCPUFA, long-chain polyunsaturated fatty acids
PC, phosphatidylcholine
PE, phosphatidylethanolamine
PI, phosphatidylinositol
PS, phosphatidylserine
SFA, saturated fatty acids
STL, step-through latency

Arachidonic acid (AA; 20:4 n-6) and docosahexaenoic acid (DHA; 22:6 n-3) are the major components of brain phospholipids and play a role in maintaining structural and functional integrity of membranes (1,2). Mammalian brain accretes these long-chain polyunsaturated fatty acids (LCPUFA) derived from diet, especially during intrauterine and early postnatal life, and afterward, the adult brain has been described to be resistant to alter its fatty acid (FA) composition and tenaciously retains AA and DHA during n-3 and n-6 FA dietary deficiency (3,4).

Adult animals that were submitted to long-term dietary n-3 deficiency shared a similar FA profile in phospholipids of different tissues (5–7). This biochemical pattern has also been defined in young animals that were submitted to dietary n-3 deficiency in the prenatal and early postnatal periods (8,9). This dietary n-3 FA deficiency profile consists of decreased levels of DHA and increased levels of 22:4 n-6 and 22:5 n-6 and the maintenance of the total n-3 plus n-6 FA in membrane phospholipids (10). Contrariwise, the FA profile of dietary DHA supplementation over a previous n-3 deficiency had also been described in different tissues (11–13). DHA deficiency is related to learning disturbances in humans (14) and rats (15), and, on the contrary, DHA supplementation is related to learning improvement in some animal models (16). The neurobiologic bases of learning and memory have been allocated to the neuronal network

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Correspondence: Salvador García Calatayud, M.D., Ph.D., General Dávila 306, portal 1°, 9° C 39007 Santander, Spain; e-mail: garciacs@ono.com.
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connecting the hippocampal formation to frontal cerebral cortex (17,18).

The purpose of the present study was to assess the effects of dietary LCPUFA deficiency restricted to lactation on cerebral cortex and hippocampus biochemical FA profile and on retention memory in young rats. The hypothesis of reversing the adverse effects of LCPUFA deficiency during this period by means of dietary LCPUFA supplements was also tested.

METHODS

Animals and dietary schedules. Female Wistar rats (CRIFFA, SA, Barcelona, Spain) that weighed 80–100 g were housed in standard macrolon cages with controlled temperature (21–23°C) and a 12-h light/dark cycle. The animals were fed with a standard rodent diet that contained essential FA (EFA), before mating and throughout pregnancy and lactation. At parturition, litters were culled to eight pups, and newborn male rats were nursed for 3 d by their own mothers and then randomly assigned to be nursed by three groups of cross-foster mothers.

The control (C) group ($n = 12$) was nursed by non–EFA-deficient lactating rats that produced control milk, the deficient (D) group ($n = 12$) was nursed by EFA-deficient lactating rats that produced EFA-deficient milk, and the supplemented (S) group ($n = 12$) was nursed by EFA-deficient lactating rats and received additional LCPUFA supplement by gavage. The milk FA content of control and deficient milk was described previously (19). The LCPUFA supplement (Ordesa SL, Barcelona, Spain) composition was as follows: 16:0, 27%; 18:0, 13%; 18:1 n-9, 38%; 18:2 n-6, 19%; 20:4 n-6, 2%; and 22:6 n-3, 1%. Animals from the C and D groups were also gavaged and received the same volume of distilled water. The gavage procedure in the three groups was started at day 8 of life to ensure newborn pups' survival. The daily amount of LCPUFA supplement was progressively increased according to a previous model (19).

The dietary schedule was maintained for 3 wk until the end of lactation, and then all groups received a standard rodent diet that contained EFA. The protocol was approved by the Institutional Animal Research Committee, and the care of the animals was in accordance with ethical guidelines for animal research.

Tissue dissection and sample extraction. Animals were killed by decapitation at 42 d of age, and then cortex and hippocampus were dissected at 4°C. For membrane preparation, these brain areas were homogenized in buffer [10 mM Na_2PO_4/HKH_2PO_4 (pH = 7.4), 1:10 wt/vol) and then centrifuged (2000 $\times$ *g*, 15 min at 4°C; Sorval, Kendro, Langenselbold, Germany, RC-5B). The pellets, which contained cerebral membranes, were resuspended in buffer. All samples were stored at −80°C until lipid extraction was performed.

Lipid determination. Lipid fractions of different samples were extracted according to Folch (20). Phosphatidylethanolamine (PE), phosphatidylinositol (PI), phosphatidylserine (PS), phosphatidylcholine (PC), and sphingomyelin phospholipids were separated by thin-layer chromatography as described by Ruiz and Ochoa (21). Briefly, plates were developed in a step-wise manner in chambers that were saturated with *1*) chloroform-methanol-water 65:40:5 (vol/vol/vol) to 4 cm; *2*) ethyl acetate-isopropyl alcohol-ethanol-chloroform-methanol-0.25% KCl 35:5:20:22:15:9 (vol/vol/vol/vol/vol/vol) to 11 cm; and *3*) n-heptane to 14 cm. After the plate was sprayed with an ethanol solution of 2',7'-dichlorofluorescein, lipids were made visible under UV light. The bands were scraped and the FA were transmethylated following Lepage and Roy (22). Fatty acid methyl esters were separated and quantified on a Hewlett Packard GC 5890 gas chromatograph using a flame-ionization detector on a capillary column SP 2330 (30 m $\times$ 0.25 mm, 0.20 mm; Supleco Company, Bellefonte, PA). The oven temperature was 80°C at injection, and this was maintained for 1 min, then raised by 50°C/min to 140°C, 5°C/min to 190°C and maintained for 5 min, and finally raised by 5°C/min to 215°C and isothermal maintained for 15 min. Injector and detector temperature was 250°C. Helium was used as the carrier gas under a pressure of 0.5 bars. FA identification was performed by comparison with commercial standards (Nu Chek, Elysian, MN). Quantification of each FA was done by electronic integration. Results for FAs are expressed as weight %.

Passive-avoidance test. The passive-avoidance test has been used widely to investigate learning and memory processes (23). The experiment was carried out using a step-through–type passive-avoidance apparatus (Letica S.A., Barcelona, Spain), consisting of an illuminated compartment (20 $\times$ 20 $\times$ 20 cm) and a dark compartment with a grid floor (20 $\times$ 20 $\times$ 20 cm), both separated by a guillotine door (8 $\times$ 8 cm). In the training session, each rat was placed in the illuminated compartment and allowed 1 min for habituation. The guillotine door was opened and closed immediately when the animal entered the dark compartment. After 10 s for habituation, electroshocks (0.3 mA-AC) were delivered through the grid floor for 3 s by a shock scrambler (LI100-26 Shocker; Letica S.A., España). In the test session, 24 h after the training session, each animal again was placed in the illuminated compartment. The step-through latency (STL) to enter the dark compartment was measured in both sessions, and the cut-off time was 600 s.

Statistical analysis. The statistical analysis was performed by means of *t* test and ANOVA for FA analysis and Mann-Whitney and Kruskall Wallis for data from the passive-avoidance test. The significance level was set at $p < 0.05$. Correlations among FAs of different phospholipids fractions and between brain FAs and STL in the passive-avoidance test were made on the basis of Spearman correlation coefficients.

RESULTS

Cerebral cortex FA composition. In PE, the D group showed higher docosapentaenoic acid (DPA) and saturated fatty acid (SFA) levels than the C and S groups but lower levels of PUFA, n-3 PUFA, and DHA. 20:3 n-9 levels in the S group were lower than in the D group, but no differences were found compared with the C group. The S group presented lower SFA than the D group and presented DHA and total n-3 PUFA higher than the D and C groups (Table 1).

In PI fraction, the D group presented higher 20:3 n-9 than the C and S groups. The S group presented higher levels of PUFA, n-6 PUFA, and 20:4 n-6 than the C and D groups.

In PS fraction, the D group presented higher levels of 20:3 n-9, n-6 PUFA, and 22:5 n-6 and lower levels of n-3 PUFA and DHA than the C group. The S group was similar to the C group with higher levels of total n-3 PUFA and DHA and lower levels of total n-6 PUFA and DPA than the D group.

Hippocampal FA composition. In PE fraction, the D group showed higher levels of SFA, n-6 PUFA, 22:4 n-6, and DPA and lower levels of n-3 PUFA and DHA compared with the C group. The S group showed higher n-3 PUFA and DHA and lower SFA than the C group. Compared with the D group, the S group showed higher DHA and total n-3 PUFA but lower SFA and total n-6 PUFA (Table 2).

In PI fraction, the D group presented lower levels of n-6 PUFA and AA but higher levels of 22:5 n-6. The S group presented lower levels of SFA and higher levels of 20:3 n-9 and 22:4 n-6. Compared with the D group, the S group showed higher AA, total PUFA, and total n-3 PUFA levels but lower DPA and SFA levels.

In PS fraction, the D group presented higher levels of n-6 PUFA and 22:5 n-6 and lower levels of n-3 PUFA and DHA. The S group presented higher levels of 20:3 n-9 compared with the C group and higher DHA and lower DPA levels compared with the D group.

Passive-avoidance test. STL were similar among the three groups in the training test, but the retention trial, carried out 24 h later, showed significant differences (Kruskal-Wallis test, $\chi^2 = 7.22$, $p = 0.027$). Analyzing medians and interquartile ranges (IQRs), the D group (66.0 s; IQR, 131) showed shorter STL than the C group (314.0 s; IQR, 472.7), and the S group (380.0 s; IQR, 430.7) showed no differences with the C group. All of these data state poorer memory in the D group than in the C and S groups and no differences between these last two groups (Fig. 1).

Correlations. The analysis of correlations between LCPUFA from cerebral cortex and hippocampus, and memory

Table 1. *Fatty acid composition of cerebral cortex (%, wt/wt)*

Fatty acid	C group	D group	S group	ANOVA	*p*	C-D	C-S	D-S
PC								
SFA	60.11 ± 2.04	59.37 ± 2.41	57.64 ± 1.73	4.51	0.02	0.40	0.01	0.05
Σ PUFA	13.61 ± 1.35	13.91 ± 1.08	14.58 ± 0.92	2.27	0.12			
20:3n9	ND	0.07 ± 0.06	0.04 ± 0.04	12.75	0.00	0.00	0.03	0.04
n6-PUFA	9.86 ± 1.01	10.34 ± 0.75	10.10 ± 0.53	1.04	0.36			
20:4n6 (AA)	6.40 ± 0.65	6.83 ± 0.58	6.93 ± 0.49	2.75	0.08			
22:4n6	0.87 ± 0.12	0.90 ± 0.13	1.03 ± 0.19	3.66	0.04	0.60	0.01	0.05
22:5n6 (DPA)	1.40 ± 0.38	1.45 ± 0.54	1.04 ± 0.52	2.54	0.10			
n3-PUFA	3.75 ± 0.47	3.50 ± 0.47	4.43 ± 0.60	10.09	0.00	0.26	0.00	0.00
22:6n3 (DHA)	3.59 ± 0.49	3.38 ± 0.42	4.30 ± 0.55	11.11	0.00	0.32	0.00	0.00
PE								
SFA	29.42 ± 2.57	31.65 ± 2.65	27.93 ± 1.05	8.27	0.00	0.02	0.10	0.00
Σ PUFA	57.26 ± 2.23	54.98 ± 2.70	57.95 ± 1.54	5.70	0.01	0.02	0.45	0.00
20:3n9	0.22 ± 0.05	0.69 ± 0.47	0.31 ± 0.13	7.89	0.02	0.00	0.47	0.00
n6-PUFA	31.25 ± 1.67	32.27 ± 1.87	30.60 ± 1.06	3.32	0.04	0.13	0.31	0.01
20:4n6 (AA)	18.28 ± 0.80	17.80 ± 1.23	18.28 ± 0.85	0.52	0.77			
22:4n6	6.80 ± 0.30	6.25 ± 0.29	6.57 ± 0.33	9.53	0.00	0.00	0.07	0.01
22:5n6 (DPA)	4.29 ± 0.88	6.69 ± 1.24	4.07 ± 0.64	27.02	0.00	0.00	0.57	0.00
n3-PUFA	25.79 ± 1.10	22.03 ± 1.60	27.05 ± 1.17	45.97	0.00	0.00	0.02	0.00
22:6n3 (DHA)	25.42 ± 1.05	21.67 ± 1.61	26.73 ± 1.17	47.29	0.00	0.00	0.02	0.00
PI								
SFA	47.06 ± 2.71	49.27 ± 3.50	46.35 ± 2.11	3.33	0.04	0.07	0.54	0.01
Σ PUFA	37.23 ± 3.57	37.58 ± 3.68	41.68 ± 3.42	5.75	0.01	0.81	0.00	0.01
20:3n9	ND	0.29 ± 0.25	0.03 ± 0.11	15.12	0.00	0.00	0.62	0.00
n6-PUFA	33.05 ± 4.64	32.92 ± 5.36	37.90 ± 3.21	4.78	0.01	0.94	0.01	0.01
20:4n6 (AA)	26.40 ± 5.86	27.02 ± 7.22	31.93 ± 3.90	3.28	0.05	0.80	0.02	0.05
22:4n6	1.32 ± 0.95	1.38 ± 0.90	1.74 ± 1.01	0.67	0.52			
22:5n6 (DPA)	3.81 ± 1.55	3.34 ± 1.93	2.92 ± 3.23	0.43	0.65			
n3-PUFA	4.17 ± 3.56	4.37 ± 5.97	3.75 ± 1.00	0.07	0.93			
22:6n3 (DHA)	3.86 ± 3.51	4.13 ± 5.98	3.47 ± 0.77	0.08	0.92			
PS								
SFA	46.62 ± 5.84	51.07 ± 10.91	44.64 ± 2.52	2.38	0.11			
Σ PUFA	40.54 ± 7.55	37.71 ± 9.97	43.28 ± 2.61	1.69	0.20			
20:3n9	ND	0.25 ± 0.17	0.05 ± 0.08	15.91	0.00	0.00	0.31	0.00
n6-PUFA	14.43 ± 1.98	17.19 ± 3.81	14.69 ± 1.15	4.03	0.03	0.02	0.80	0.02
20:4n6 (AA)	3.74 ± 0.69	3.31 ± 0.62	3.85 ± 0.59	2.22	0.13			
22:4n6	3.73 ± 0.42	3.07 ± 1.20	3.89 ± 0.50	3.51	0.04	0.05	0.64	0.02
22:5n6 (DPA)	6.13 ± 1.77	10.09 ± 2.64	6.13 ± 0.92	16.33	0.00	0.00	0.99	0.00
n3-PUFA	26.11 ± 5.78	20.26 ± 6.81	28.54 ± 2.51	7.35	0.00	0.01	0.28	0.00
22:6n3 (DHA)	25.87 ± 5.70	20.05 ± 6.70	28.22 ± 2.45	7.40	0.00	0.01	0.29	0.00

Values represent mean ± SD ($n = 12$ per group). Σ PUFA, sum of all polyunsaturated fatty acids; n6-PUFA, sum of all n6 polyunsaturated fatty acids; n6-PUFA, sum of all n6 polyunsaturated fatty acids; ND, not detected.

showed that memory at 24 h was inversely correlated with 20:3 n-9 in PC, PE, and PS and positively with 22:6 n-3 in PC, PE, and PI fractions and inversely correlated with DPA in PE fraction, all of them in cerebral cortex. In hippocampus, STL was inversely correlated with DPA from all phospholipid fractions and positively with DHA in PS fraction (Table 3).

DISCUSSION

This experiment, carried out in rats, studied both biochemical and behavioral consequences of dietary restriction of LCPUFA limited to lactation and the possible reversal effects of a LCPUFA supplementation. Two different dietary schedules were analyzed and compared with a control group. The first group was nursed with a LCPUFA-deficient lactation, and the second group was nursed with a LCPUFA-deficient lactation supplemented with LCPUFA.

Decreased DHA and increased 22:4 n-6 and 22:5 n-6 levels are the classic biochemical FA profile observed in long-term n-3 dietary deficiency, both in adult animals submitted to n-3 FA dietary deficiency in different tissues and brain areas (5,6) and in neonatal and young rats that are born to mothers submitted to this dietary deficiency (8,9). However, in our experimental conditions, a shorter time of n-3 deficiency during part of lactation reflects the same FA profile. In reverse, deficient animals with LCPUFA supplementation showed levels of DHA and DPA similar to the C group, showing the biochemical reversal effect of DHA supplementation on a previous n-3 deficiency FA pattern (24). All of these data suggest that lactation is as important as the prenatal period in LCPUFA brain accretion.

Learning and visual disabilities have been associated with an n-3–deficient FA profile in postnatal long-term deficiency (25) and prenatal deficiency (26). Memory of the D group was poorer than the C group, whereas memory in the S group was unaffected, translating that LCPUFA deficiency during lactation also affects learning. It is remarkable that this former

Table 2. *Fatty acid composition of hippocampus (%, wt/wt)*

Fatty acid	C group	D group	S group	ANOVA	*p*	C-D	C-S	D-S
PC								
SFA	58.34 ± 1.14	59.40 ± 5.42	58.97 ± 1.92	0.65	0.72			
Σ PUFA	14.24 ± 1.02	14.42 ± 5.24	14.36 ± 1.12	0.48	0.78			
20:3n9	0.01 ± 0.02	0.08 ± 0.14	0.10 ± 0.05	3.49	0.04	0.07	0.02	0.54
n6-PUFA	11.71 ± 1.01	11.55 ± 4.06	10.97 ± 0.78	0.89	0.64			
20:4n6 (AA)	8.34 ± 0.38	8.33 ± 3.36	8.37 ± 0.61	0.37	0.83			
22:4n6	0.83 ± 0.07	0.77 ± 0.28	1.13 ± 0.11	18.15	0.00	0.48	0.00	0.00
22:5n6 (DPA)	0.98 ± 1.02	1.36 ± 0.86	0.53 ± 0.13	12.33	0.00	0.25	0.18	0.02
n3-PUFA	3.06 ± 0.41	2.79 ± 1.18	3.28 ± 0.35	2.40	0.30			
22:6n3 (DHA)	2.93 ± 0.33	2.71 ± 1.16	3.10 ± 0.33	1.42	0.49			
PE								
SFA	28.31 ± 1.13	29.76 ± 2.23	26.75 ± 0.96	14.25	0.00	0.03	0.02	0.00
Σ PUFA	53.38 ± 1.62	53.84 ± 2.39	55.22 ± 1.22	5.71	0.06			
20:3n9	0.39 ± 0.25	0.39 ± 0.72	0.63 ± 0.07	1.21	0.31			
n6-PUFA	30.84 ± 1.12	32.88 ± 1.62	30.94 ± 0.62	12.17	0.00	0.00	0.84	0.00
20:4n6 (AA)	18.25 ± 0.46	18.03 ± 0.53	18.46 ± 0.42	2.39	0.11			
22:4n6	7.61 ± 0.23	6.77 ± 0.39	7.63 ± 0.41	21.33	0.00	0.00	0.90	0.00
22:5n6 (DPA)	3.29 ± 0.89	6.46 ± 1.26	3.35 ± 0.43	44.60	0.00	0.00	0.87	0.00
n3-PUFA	22.15 ± 1.05	20.57 ± 1.79	23.65 ± 1.14	14.84	0.00	0.01	0.01	0.00
22:6n3 (DHA)	21.75 ± 1.05	20.19 ± 1.80	23.17 ± 1.13	13.78	0.00	0.01	0.02	0.00
PI								
SFA	52.38 ± 2.39	52.46 ± 4.20	49.19 ± 2.45	4.30	0.02	0.95	0.02	0.02
Σ PUFA	35.90 ± 1.71	33.87 ± 4.50	38.18 ± 2.74	9.17	0.01	0.13	0.08	0.00
20:3n9	ND	0.16 ± 0.29	0.24 ± 0.25	7.18	0.03	0.09	0.01	0.38
n6-PUFA	32.34 ± 1.58	29.36 ± 3.98	33.92 ± 3.84	5.62	0.01	0.04	0.25	0.00
20:4n6 (AA)	28.11 ± 2.38	22.57 ± 6.79	29.20 ± 5.49	5.37	0.01	0.01	0.61	0.00
22:4n6	1.31 ± 0.61	1.16 ± 0.73	3.40 ± 1.41	19.02	0.00	0.72	0.00	0.00
22:5n6 (DPA)	1.86 ± 1.97	3.73 ± 2.98	0.56 ± 0.47	14.41	0.00	0.04	0.13	0.00
n3-PUFA	3.55 ± 1.63	4.35 ± 3.67	4.03 ± 2.22	0.27	0.76			
22:6n3 (DHA)	3.08 ± 1.51	4.10 ± 3.53	3.47 ± 2.00	0.50	0.61			
PS								
SFA	48.79 ± 2.05	51.07 ± 5.07	46.34 ± 4.20	4.14	0.02	0.17	0.14	0.00
Σ PUFA	36.84 ± 2.50	34.59 ± 6.16	38.05 ± 4.18	1.75	0.19			
20:3n9	0.03 ± 0.07	0.14 ± 0.25	0.27 ± 0.13	6.45	0.00	0.11	0.00	0.07
n6-PUFA	14.19 ± 1.57	16.21 ± 2.92	14.59 ± 1.73	7.39	0.02	0.03	0.65	0.08
20:4n6 (AA)	4.65 ± 0.55	4.66 ± 2.45	4.90 ± 0.76	0.11	0.89			
22:4n6	4.14 ± 0.40	3.28 ± 0.45	4.28 ± 0.84	9.09	0.00	0.00	0.56	0.00
22:5n6 (DPA)	4.57 ± 1.26	7.05 ± 2.10	4.42 ± 0.90	11.25	0.00	0.00	0.81	0.00
n3-PUFA	22.61 ± 2.06	18.24 ± 4.54	23.18 ± 3.02	7.51	0.00	0.00	0.68	0.00
22:6n3 (DHA)	22.31 ± 1.97	17.61 ± 4.65	22.66 ± 3.00	10.88	0.00	0.00	0.80	0.00

Values represent mean ± SD (n = 12 per group).

adverse effect is reversed when a dietary supplement that contains DHA and AA is supplied to D animals. The functional reversibility of n-3 deficiency by adding DHA to the diet was described previously (27,28). Taking into account that retina and brain FA profiles have similar changes according to n-3 content of diet (29), the learning differences found in our study could be explained on the basis of presumed visual disturbances. However, the exploratory behavior in the training session of the passive-avoidance test did not show differences among groups. Therefore, visual differences would not explain the poorer retention memory found in D animals, and, therefore, memory differences could be more related to brain FA composition.

Considering that PS and PE fractions are those with higher levels of DHA (30), it could be hypothesized that these phospholipids could be involved in cognitive functions assigned to cerebral cortex and hippocampus such as learning. Learning was correlated directly to DHA in PS from hippocampus and in PC, PE, and PI from cerebral cortex and inversely correlated to both 20:3 n-9 and DPA in PC and PE fractions from cerebral cortex. All of these data confirm the importance of these brain areas in memory but, moreover, the phospholipid fractions involved, PE probably being more important than PS fraction. The physiologic bases of learning deficits described in n-3 deficiency have been related to changes in lipid matrix properties and neurotransmission. The DPA, lacking a double bond near the chain methyl end, induces some differences in lipid matrix properties that contain DPA instead of DHA. The DHA-containing lipid matrix is more flexible at the methyl end and has a higher electron density near the lipid-water interface (31). Alterations in dopaminergic (32) and cholinergic (33) neurotransmission have been described in n-3–deficient animals related to brain functional deficits. The possible relationships among DHA content in different phospholipid fractions from different brain areas and the alterations in brain neurotransmission remain unclear.

CONCLUSION

In conclusion, DPA, a known biochemical index of n-3 dietary deficiency, is not a suitable functional substitute of

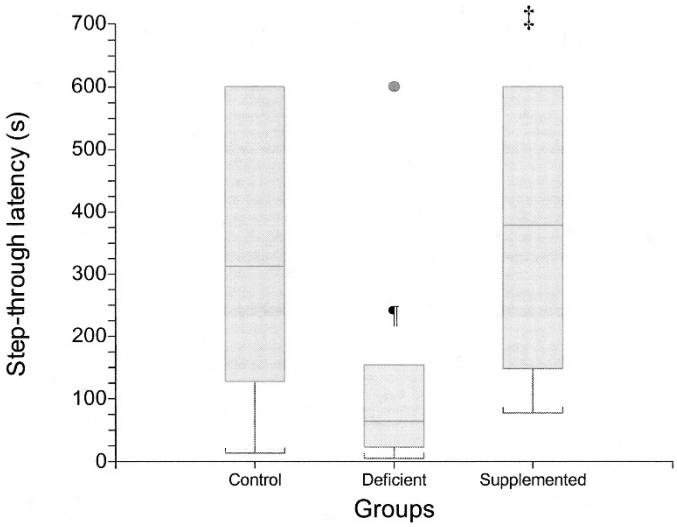

Figure 1. Effects of LCPUFA deficiency and supplementation on learning measured by passive-avoidance test in rats 24 h after the aversive event during lactation. Values represent median ± IQR. Kruskal-Wallis test, $\chi^2 = 7.22$, $p = 0.027$ ($n = 36$). ¶Deficient vs Control) $p = 0.07$), ‡Supplemented vs Deficient ($p = 0.02$).

Table 3. *Correlations between brain LCPUFA from different phospholipids fractions and learning measured by STL in the passive-avoidance test*

Brain area	Lipid fraction	Fatty acid	*r*	*p*
Cerebral cortex	PC	20:3n9	−0.340	0.046
		22:6n3 (DHA)	0.349	0.040
	PE	20:3n9	−0.480	0.003
		22:5n6 (DPA)	−0.446	0.007
		22:6n3 (DHA)	0.485	0.003
	PI	22:6n3 (DHA)	0.384	0.023
	PS	20:3n9	−0.399	0.020
Hippocampus	PC	22:5n6 (DPA)	−0.519	0.002
	PE	20:5n3	−0.418	0.012
		22:5n6 (DPA)	−0.468	0.005
	PI	22:5n6 (DPA)	−0.354	0.037
	PS	22:5n6 (DPA)	−0.353	0.038
		22:6n3 (DHA)	0.329	0.054

Values represent the Spearman correlation coefficients (*r*) and the level of significance (*p*); $n = 12$ per group.

DHA. In this work, young rats with a deficient supply of n-3 FA limited to the lactation period presented poor memory retention in passive-avoidance test directly correlated to DHA and inversely correlated to DPA brain levels. Both biochemical and functional consequences of this dietary deficiency were reversed when a supplement of DHA was administered to deficient animals.

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