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Dietary supplementation with long-chain polyunsaturated fatty acids increases susceptibility of weanling rat tissue lipids to in vitro lipid peroxidation

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The intake of (n-3) long chain polyunsaturated fatty acids (LCPs) have beneficial effects on cardiovascular diseases, renal function, and physiology of retina and brain in human neonates. Several authors recently reported a correlation between tissue 20:4(n-6) status and neonatal growth. Incorporation of highly unsaturated fatty acids into tissue phospholipids may enhance peroxidation of cellular membranes. We fed weanling rats with a 10% fat diet that provided 18:1(n-9), 18:2(n-6) and 18:3(n-3) in a similar ratio to that of rat milk (group A), and with a diet supplemented with (n-3) LCPs (group B), or with (n-6) and (n-3) LCPs (group C), and studied the effects of diet on lipid peroxidation of erythrocyte membranes, liver microsomes and brain homogenates, and hepatic and cerebral activities of antioxidant enzymes. Alterations in tissue fatty acid composition were not paralleled by significant changes in activities of antioxidant enzymes or vitamin E content in liver microsomes. Total and reduced glutathione levels in liver homogenates were significantly higher in groups B and C compared with group A. Tissue lipids in groups B and C were more susceptible to induced peroxidation than in group A. Maximal formation of lipid peroxidation products was observed in erythrocyte membranes and liver microsomes in group C. These results may have implications on the optimal design of infant formulas based on (n-3) and (n-6) LCP supplementation. (J. Nutr. Biochem. 7:252–260, 1996.)

Keywords: lipid peroxidation; antioxidant defense; long-chain polyunsaturated fatty acids; weanling rats; brain; liver

Introduction

Long-chain polyunsaturated fatty acids (LCPs) are now recognized as important components of the human diet. Intense research during the past decade showed that intake of (*n*-3) LCPs have beneficial effects on cardiovascular diseases, renal function, and the development of normal function of the retina and brain in neonates.[1–6]

During early postnatal life, docosahexaenoic acid (22:6(*n*-3)) is essential for the development of neural tissues. After birth, human milk provides linoleic acid (18:2(*n*-6)), linolenic acid (18:3(*n*-3)), and 0.5 to 3% of total fatty acids as (*n*-3) and (*n*-6) LCPs. Limited 22:6(*n*-3) accretion to tissue lipids has been related to alterations in retinal function in preterm infants[7,8,9] and alterations in visual response and learning behavior in rats.[10–13] Farquharson et al.[14] showed that cerebral cortex 22:6(*n*-3) was higher in breast-fed than in formula-fed term infants. Supplementation of diets with fish oil, which contains high levels of 22:6(*n*-3), increases 22:6(*n*-3) content in tissue lipids and affects early retinal function[7] and later visual acuity in infants[8,9,15] and visual function and learning ability in rats.[16,17] Thus, membrane (*n*-6) fatty acids, particularly arachidonic acid (20:4(*n*-6)), were replaced with competing (*n*-3) fatty acids in tissue lipids in neonates[18] and rats fed diets supplemented with (*n*-3) LCPs.[19–22] However, several reports documented

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a correlation between 20:4(*n*-6) and neonatal growth.[23–27] These results support the hypothesis that preterm infant formulas should provide optimal amounts of both 20:4(*n*-6) and 22:6(*n*-3) to allow tissue development during early postnatal life. Feeding formulas supplemented with blends of semipurified fat containing (*n*-3) and (*n*-6) LCPs increased 20:4(*n*-6) and 22:6(*n*-3) in plasma and erythrocyte lipids in preterm infants,[28–30] and in plasma, erythrocyte, and liver lipids in rats[31] in comparison with nonsupplemented controls.

However, the incorporation of highly unsaturated fatty acids into tissue phospholipids may enhance peroxidation of cellular membranes.[32,33] Many studies showed that dietary supplementation with (*n*-3) LCPs was associated with increased susceptibility of tissue lipids to peroxidation in vivo[34–38] and in vitro.[39–47] However, no studies have assessed the effect of dietary supplementation with (*n*-6) LCPs on the susceptibility of tissue lipids to peroxidation. We investigated the effect of dietary supplementation with (*n*-3) LCPs and (*n*-6) LCPs on plasma, erythrocyte, liver and brain fatty acid composition, on the susceptibility of erythrocyte membranes, liver microsome phospholipids, and brain homogenates to in vitro lipid peroxidation, and on hepatic and cerebral activities of antioxidant enzymes in rats at weaning. Diets were designed to provide amounts of the saturated fatty acids 18:1(*n*-9), 18:2(*n*-6), and 18:3(*n*-3) similar to those provided by the dam's milk.[48,49]

Methods and materials

Experimental design

The protocol of this study was approved by the University of Granada Committee of Animal Welfare. Male Wistar rats at weaning were purchased from Interfauna Iberica S.A. (Barcelona, Spain). Animals were randomly divided into three groups of seven rats each, and were housed seven per cage in a room with controlled temperature (21 ± 1°C) and light (08.00–20.00 h).

Diets were prepared and packaged by the R&D department of PULEVA (Granada, Spain) and stored at 4°C under nitrogen. All groups received a 10% (wt:wt) fat semipurified diet with different sources of dietary fat. The overall composition of diets is shown in *Table 1*. The fatty acid composition of each dietary fat is given in *Table 2*. Group A fat consisted of a mixture of olive oil (62.5%), soy oil (11.1%) and refined coconut oil (26.4%). Group B received 7% group A fat and 3% deodorized Spanish sardine oil, kindly supplied by Dr. Valenzuela (INTA, University of Chile); group C was fed with 7% wt:wt group A fat, 1.5% wt:wt of the same fish oil concentrate, and 1.5% wt:wt of a purified animal tissue phospholipid concentrate obtained from pig brain by PULEVA (Granada, Spain). The lipid distribution in the phospholipid concentrate was: phosphatidylcholine 27.4%, phosphatidylethanolamine 21%, phosphatidilserine 14.5%, phosphatidylinositol 3.3%, sphingomyelin 11.4%, phosphatidic acid 5.0%, lysophosphatidyl choline 0.6%, gangliosides 4.6%, sulphatides 5.1%, cerebrosides 6.0%, and minor amounts of cholesterol. Obtention process for the phospholipid concentrate and its detailed composition have been previously reported.[31] All animals were given free access to fresh diet and water daily.

After 4 weeks of feeding, animals were deprived of food for 24 hr, lightly anesthetized with diethyl ether and killed. Blood was obtained by cardiac puncture and collected into tubes containing calcium heparin as an anticoagulant. Plasma was immediately removed by centrifugation and stored at −80°C until analysis. Erythrocytes were washed three times with PBS buffer (150 mmol/L NaCl, 5 mmol/L sodium phosphate, pH 8) and resuspended in an equal volume of the saline solution. Membrane ghosts were prepared according to Burton et al.[50] and stored at −80°C until analysis. The brain was removed and stored at −80°C. A midline abdominal incision was made and a portion of the liver (portion I) was ligated, transected between ligatures, removed, weighed, and processed immediately for the determination of total and reduced GSH. The blood remaining in the liver was eliminated by infusing 25 mL of sterile saline solution at 37°C into the aorta at a constant flow of 5 mL/min. Livers were drained with filter paper and divided into two portions (II and III). Portion II was processed to obtain liver microsomes. Portion III was immediately frozen with liquid nitrogen and stored at −80°C for protein determinations according to Bradford.[51]

Table 1 Composition of the diet

Ingredient	Amount (g/kg)
Casein	189.6
Starch	481.6
Oil[1]	100
Saccharose	150.5
Cellulose	50.2
DL-methionine	3
Choline chloride	1.1
Mineral supplement[2]	24.1
Vitamin supplement[3]	0.1

[1]Group A fat consisted of a mixture of olive oil (62.5%), soy oil (11.1%), and refined coconut oil (26.4%); Group B received 7% wt:wt Group A fat and 3% wt:wt deodorized Spanish sardine oil; Group C was fed 7% wt:wt Group A fat, 1.5% wt:wt of the same fish oil concentrate, and 1.5% wt:wt of a purified animal brain phospholipid concentrate.
[2]American Institute of Nutrition (1977).
[3]Composition of the vitamin supplement was as follows (g/100 g): Thiamin HCl, 0.51; riboflavin, 0.51; pyridoxine HCl, 0.59; para-aminobenzoic acid, 2.53; calcium pantothenate, 1.35; folic acid, 0.17; vitamin B_{12}, 0.008; retinol acetate, 0.12; vitamin D_3, 0.002; vitamin K, 0.004. The energy density was 4186 kcal/kg.

Preparation of liver microsomes

Liver microsomes were obtained as described by Albro et al.[52] Essentially, liver portions designated II were diced in washing solution (0.25 mol/L mannitol) and homogenized in four volumes of freshly prepared homogenizing medium (0.25 mol/L mannitol, 0.2 mmol/L TET, and 0.025 mol/L MOPS buffer pH 7.4) using a Teflon-glass homogenizer driven by a stirring motor at a constant speed in a saline water bath. The homogenates were centrifuged at 12,000 × g for 10 min at 4°C. The supernate was filtered and centrifuged again at 12,000 × g for 10 min at 4°C. It was then diluted in four volumes of dilutant solution (0.0125 mol/L mannitol, 0.1 mmol/L TET and 8 mmol/L calcium chloride pH 7.5) and centrifuged at 1000 × g for 10 min at 4°C. The liver microsomes were suspended in 1 mL 150 mmol/L Tris-HCl pH 7.5 and stored at −80°C.

Fatty acid analyses

To measure the fatty acid composition of plasma, erythrocyte, liver, and brain lipids, total lipids were extracted from all tissues.[53] Butylated hydroxytoluene (2 mg/L) was used as antioxidant. Liver microsome phospholipids were obtained by thin layer chromatography.[54] Fatty acids were saponified and methylated simulta-

Table 2 Fatty acid composition of diets[1]

	Diet		
Fatty acid	A	B	C
8:0	15.5	10.7	12.1
10:0	9.7	6.8	7.9
12:0	0.8	0.6	0.6
14:0	0.2	2.3	1.5
15:0	ND	0.4	0.2
16:0	7.6	10.3	9.7
16:1n-7	0.6	2.6	1.9
17:0	ND	0.8	0.4
18:0	2.8	3.0	3.4
18:1n-9	51.1	39.7	42.7
18:2n-6	9.8	7.5	8.4
18:3n-6	ND	0.2	0.1
18:3n-3	1.6	1.0	1.1
18:4n-3 + 20:1n-9	ND	1.4	0.9
20:2n-6	ND	0.1	0.1
20:3n-6	ND	ND	0.1
20:4n-6	ND	0.4	0.6
20:5n-3	ND	3.6	2.2
22:1n-9	ND	0.5	0.4
22:4n-6	ND	0.2	0.2
22:5n-6	ND	0.2	0.3
22:5n-3	ND	0.7	0.4
22:6n-3	ND	5.0	3.5
ND[2]	ND	2.5	1.2
n-3[3]	ND	9.3	6.1
n-6[3]	ND	0.9	1.3
n-6/n-3[3]	ND	0.10	0.21
P/S[3]	0.31	0.80	0.50

[1]Fatty acids are expressed as mol/100 mol of total fatty acid methyl esters; A, B and C refer to *Table 1*.
[2]ND, not detected.
[3]n-3, sum of n-3 polyunsaturated fatty acids longer than 20 carbon atoms; n-6, sum of n-6 polyunsaturated fatty acids longer than 20 carbon atoms. P/S, polyunsaturated-to-saturated ratio.

neously with 2.06 mol/L boron trifluoride in methanol.[55] Separation and quantitation of fatty acid methyl esters was done by capillary gas chromatography on a Hewlett-Packard model no. 5890 gas chromatograph (Hewlett-Packard Co., Palo Alto, CA) equipped with a flame ionization detector and a 30 m length, 0.25 mm internal diameter capillary column filled with DB-2330-N stationary phase (J & W Scientific, Folsom, CA). Fatty acid methyl esters were identified by comparing their retention times with authentic standards (Sigma Chemical, St. Louis, MO). The relative concentration of each fatty acid was expressed as the molar percentage of total fatty acids equal to or greater than 8 carbon atoms for diets, and equal to or greater than 16 carbon atoms for tissue samples. The peroxidizability index (PI) was calculated as follows: PI = (% dienoic × 1) + (% trienoic × 2) + (% tetraenoic × 3) + (% pentaenoic × 4) + (% hexaenoic × 5).[47] To evaluate the effects of diets we performed a one-way analysis of variance and posteriori comparisons of means were done using the Bonferroni test. The level of statistical significance was determined at $P < 0.05$.[56]

Analysis of total glutathione, reduced glutathione and vitamin E

Total glutathione and GSH were determined in liver portions designated I. These portions were homogenized at 4°C in five volumes of 50 g/L 5-sulfosalicylic acid (previously flushed with N_2) using a Teflon-glass homogenizer driven by a stirring motor at constant speed. The homogenates were centrifuged in a microfuge at 12,000 × g for 10 min at 4°C. Total glutathione was determined at 30°C by the DTNB-GSSG reductase recycling assay as described by Anderson et al.[57] GSH was assayed at room temperature by the reaction with DTNB according to Akerboom and Sies.[58] Vitamin E content of liver microsomes was determined by HPLC according to Bieri et al.[59]

Antioxidant enzyme activities

Liver portions designated III and brain portions were homogenized at 4°C in 1 mmol/L EDTA, 0.01% digitonin, 0.1 mmol/L phosphate buffer solution (pH 7.0) using a Teflon-glass homogenizer driven by a stirring motor at constant speed. The homogenate was centrifuged at 13,000 × g for 15 min at 4°C. The supernatant was transferred to an Eppendorf tube and stored at 4°C until analysis. Total superoxide dismutase (SOD) activities in the supernatant fraction were measured by the inhibition of cytochrome 3c reduction mediated via superoxide anions generated by the xanthine/xanthine oxidase system and monitored at 550 nm. Manganese-superoxide dismutase (MnSOD) activities were determined with the same procedure in the presence of 10 μmol/L potassium cyanide. Activities are expressed as units/mg protein^{-1} where one unit of SOD, was defined as the amount required to cause half-maximal inhibition of cytochrome 3c reduction.[60] Catalase (CAT) activity was determined according to Aebi[61] by following the decomposition of hydrogen peroxide at 240 nm. Glutathione peroxidase (GSH-Px) activity was determined at 31°C by NADPH oxidation in a coupled reaction system consisting of tert-butyl hydroperoxide and oxidized glutathione.[62] Glutathione peroxidase (GST) activity was measured with 1-chloro-2,4-dinitrobenzene at 30°C according to Warholm et al.[63]

Induction of lipid peroxidation and determination of thiobarbituric acid reactive substances (TBARS)

Lipid peroxidation was induced as described by Garrido et al.[41] In erythrocyte membranes and liver microsomes, lipid peroxidation was initiated by adding ferric sulfate and sodium ascorbate to a final concentration of 50 μmol/L and 400 μmol/L, respectively. Lipid peroxidation was also induced in liver microsomes by adding a NADPH-Fe^{3+}-ADP solution (400 μmol/L NADPH, 50 μmol/LM $FeCl_3$, 4 mmol/L ADP). In brain homogenates, lipid peroxidation was induced by incubating samples at 37°C in a shaker water bath. Susceptibility of tissues to lipid peroxidation was assessed by TBARS formation at different time points after induction. The production of TBARS was determined according to Esterbauer and Cheeseman and expressed as nmol malondialdehyde per mg of protein.[64]

Results

Molar percentage contributions of selected fatty acids to total plasma lipids, erythrocyte membrane lipids, liver microsome phospholipids and brain lipids as well as the sum of saturated fatty acids (ΣSats), monounsaturated fatty acids (ΣMonos), (*n*-6) and (*n*-3) LCPs and PI are shown in *Table 3*. Activities of hepatic CAT, GSH-Px, GST, total SOD and MnSOD activities as well as brain GSH-Px, GST, total SOD and MnSOD activities are shown in *Table 4*. Total glutathione and GSH contents in rat livers and vitamin E contents in liver microsomes are given in *Table 5*. *Figure 1* shows the production of TBARS by erythrocyte membranes after induction with Fe^{2+}-ascorbate. *Figures 2* and *3* show the production of TBARS by liver microsomes after induction

Table 3 Influence of dietary lipids on tissue arachidonic and docosahexaenoic acids and on several fatty acid parameters in rats at weaning fed experimental diets[1]

		Diet		
Tissue	Fatty acids	A	B	C
Plasma	20:4(n-6)	23.93 ± 1.70	8.71 ± 0.58	15.11 ± 0.79*†
	22:6(n-3)	3.69 ± 0.23	9.90 ± 0.71†	9.07 ± 0.33†
	Σ Sats[2]	33.42 ± 1.05	31.54 ± 0.98	30.83 ± 0.60†
	Σ Monos[2]	23.98 ± 1.22	24.92 ± 1.20	25.41 ± 0.69
	Σ(n-6)	25.38 ± 1.71	9.32 ± 0.34†	16.11 ± 0.77*†
	Σ(n-3)	4.31 ± 0.30	15.63 ± 0.35†	14.40 ± 0.76†
Erythrocyte	20:4(n-6)	27.88 ± 0.67	18.42 ± 0.29†	21.74 ± 0.44*†
	22:6(n-3)	4.23 ± 0.22	8.74 ± 0.20†	7.29 ± 0.25*†
	Σ Sats[2]	41.25 ± 0.83	42.51 ± 0.40	43.68 ± 0.34
	Σ Monos[2]	16.56 ± 0.51	14.90 ± 0.42†	14.46 ± 0.25†
	Σ(n-6)	30.59 ± 0.54	19.47 ± 0.43†	22.83 ± 0.39*†
	Σ(n-3)	6.05 ± 0.31	17.55 ± 0.21†	12.97 ± 0.41*†
	PI[1]	125.40 ± 2.94	13.50 ± 1.60†	133.40 ± 2.50*†
Liver microsomes	20:4(n-6)	17.93 ± 1.50	11.25 ± 0.45†	15.47 ± 0.88*
	22:6(n-3)	7.64 ± 1.04	10.44 ± 1.21†	14.31 ± 1.18†
	Σ Sats[2]	47.82 ± 2.27	49.29 ± 2.38	45.81 ± 1.54
	Σ Monos[2]	14.84 ± 1.67	14.07 ± 0.55	11.01 ± 0.59*†
	Σ(n-6)	19.44 ± 1.71	12.10 ± 0.44†	16.59 ± 0.85*
	Σ(n-3)	8.56 ± 1.16	14.61 ± 1.73†	17.57 ± 1.40
	PI[3]	106.80 ± 8.7	114.00 ± 9.00	141.80 ± 8.00†*
Brain	20:4(n-6)	10.94 ± 0.34	8.63 ± 0.37†	9.72 ± 0.32†*
	22:6(n-3)	17.32 ± 0.74	21.83 ± 0.78†	19.81 ± 0.74†
	Σ Sats[2]	38.13 ± 1.15	38.25 ± 1.46	37.60 ± 1.29†
	Σ Monos[2]	24.54 ± 0.79	23.39 ± 1.15	24.29 ± 1.61
	Σ(n-6)	16.90 ± 0.42	13.28 ± 0.38†	15.32 ± 0.55†*
	Σ(n-3)	17.38 ± 0.72	22.38 ± 0.63†	20.29 ± 0.73†
	PI[3]	140.00 ± 3.1	151.30 ± 4.50	148.10 ± 2.60

[1]Fatty acids are expressed in mol/100 mol ± SEM of total fatty acid methyl esters; and peroxidizability index (PI) as Σ [unsaturated fatty acids × (no. double bonds-1)]; 7 animals for each group. A, B and C refer to Table 1. †Significantly different from the value for animals fed diet A. *Significantly different from the value for animals fed diet B.
[2]Σ Sats, total saturated fatty acids (calculated as Σ % of each saturated fatty acid); Σ Monos, total monounsaturated fatty acids (calculated as Σ % each of monounsaturated fatty acid). PI, peroxidizability index.

with Fe^{2+}-ascorbate and NADPH-Fe^{3+}-ADP respectively. *Figure 4* shows the production of TBARS by brain homogenates after thermal induction at 37°C.

Fatty acid changes

Saturated fatty acids comprised the major class of tissue fatty acids in all groups. Levels of total monounsaturated fatty acids in erythrocyte membranes in group A were significantly higher than in groups B or C. In liver microsomes, group C had significantly lower total monounsaturated fatty acids than groups A or B. Feeding rats with diet A, which provided a 18:2(*n*-6)/18:3(*n*-3) ratio of 6:1, led to significantly higher 20:4(*n*-6) and total (*n*-6) LCPs values in all tissues except for liver microsomes in comparison with LCP-supplemented groups (diets B and C). Rats fed diet C exhibited similar values for 20:4n-6 and total (*n*-6) LCPs to those of rats fed diet B in liver microsomes. 22:6(*n*-3) and total (*n*-3) LCPs were significantly lower in group A as compared to B and C group for all tissues. Supplementation of diet B with (*n*-3) LCPs resulted in the highest 22:6(*n*-3) and total (*n*-3) LCPs values in all tissues. The increase in (*n*-3) LPCs in group B was accompanied by a general decrease in (*n*-6) LCP content in tissues. In rats fed diet C, levels of 20:4(*n*-6) and total (*n*-6) LCPs in all tissues were higher than in group B, and percent 22:6(*n*-3) and total (*n*-3) LCPs, increased in all tissues compared with group A. Dietary LCPs in groups B and C resulted in higher PI in erythrocyte membranes than in group A. Brain PI responses were not significantly different among the diets and only liver microsomes from rats fed diet C were different from rats fed diets A or B.

Effect on antioxidant enzymes

Alterations in the fatty acid profile of liver and brain were not paralleled by significant changes in antioxidant enzyme activities in the different groups. Rats fed diet B had significantly lower total SOD activity in liver homogenates than rats fed diet C. GST in liver homogenates of LCP-supplemented groups (diets B and C) decreased in comparison with the nonsupplemented group (diet A). Differences in liver GSH-Px activities were statistically significant between groups A and C. Total SOD activity in brain tend to increase ($P < 0.1$) in LCP-supplemented groups (diets B and C) in comparison with group A.

Effect on reduced glutathione, total glutathione, and vitamin E

Rats fed diet C had the highest liver GSH and total glutathione levels. Reduced and total glutathione were signifi-

Table 4 Influence of dietary lipids on liver and brain antioxidant enzymes[1]

		Diets[2]		
Tissue	Enzymes	A	B	C
Liver	CAT[1]	257.0 ± 32.0	260.0 ± 25.0	255.0 ± 16.0
	Total SOD[1]	279.0 ± 6.6	261.6 ± 7.3	284.0 ± 2.9*
	MnSOD[1]	42.7 ± 0.6	40.2 ± 1.1	40.1 ± 0.9
	GSH-Px[1]	133.5 ± 29.3	94.4 ± 21.2	65.9 ± 6.5†
	GST[1]	69.0 ± 2.0	73.0 ± 2.2	71.0 ± 1.8
Brain	Total SOD[1]	188.5 ± 7.5	197.0 ± 9.5	201.3 ± 13.5
	MnSOD[1]	32.6 ± 1.6	33.8 ± 1.7	31.5 ± 1.7
	GSH-Px[1]	5.5 ± 0.5	4.9 ± 0.3	5.1 ± 0.4
	GST[1]	25.0 ± 1.2	25.0 ± 1.5	26.0 ± 1.0

[1]Results are expressed as mean ± SEM; seven animals for each group. A, B, and C refer to *Table 1*. Activities of antioxidant enzymes are expressed as: Catalase (CAT), μmol H_2O_2 decomposed. min^{-1} mg $protein^{-1}$. Total superoxide dismutase (SOD), manganese-superoxide dismutase (MnSOD) and glutathione-S-transferase (GST), $units.min^{-1}mg\ protein^{-1}$; Glutathione peroxidase (GSH-Px), $units.min^{-1}.mg\ protein^{-1}$. †Significantly different from the value for animals fed diet A. *Significantly different from the value for animals fed diet B.

cantly higher in group C than in group A. Liver homogenates in rats fed diet C had a significantly higher total glutathione content than in rats fed diet B. No significant differences between groups were observed in vitamin E contents in liver microsomes.

Effect on tissue susceptibility to in vitro lipid peroxidation

After the induction of lipid peroxidation in erythrocyte membranes, liver microsomes and brain homogenates, TBARS production was significantly higher in rats fed diets supplemented with LCPs (diets B and C) than in rats fed diet A. In liver microsomes treated with NADPH-Fe^{3+}-ADP, in vitro susceptibility to induced lipid peroxidation was significantly higher in rats fed diet C than in rats fed diet B. In brain homogenates, 1-hr time-point values of TBARS production were significantly higher in LCP-supplemented groups (diets B and C) than in group A.

Table 5 Influence of dietary lipids on hepatic total glutathione, reduced glutathione, and vitamin E[1]

	Diets		
	A	B	C
GSH[1]	3.34 ± 0.30	3.78 ± 0.43	4.60 ± 0.26†
Total Glutathione	1.90 ± 0.07	2.12 ± 0.23	3.25 ± 0.27*†
Vitamin E	0.14 ± 0.06	0.21 ± 0.07	0.21 ± 0.01

[1]Results are expressed as mean ± SEM; seven animals for each group. A, B, and C refer to *Table 1*. Reduced glutathione (GSH) and total glutathione are expressed as μmol.g^{-1} of liver, and vitamin E as $\mu g.mg\ protein^{-1}$. †Significantly different from the value for animals fed diet A. *Significantly different from the value for animals fed diet B.

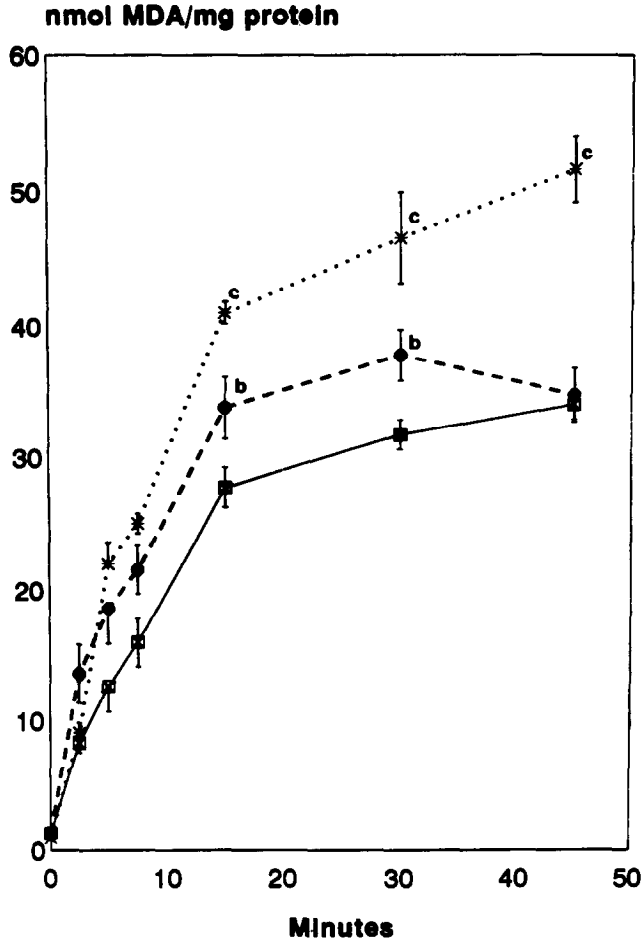

Figure 1 Erythrocyte membrane peroxidation after induction with Fe^{2+}-ascorbate in rats at weaning. Diet A (□), Diet B (●), Diet C (*). Results are expressed in nmol of malondialdehyde (MDA).mg of $protein^{-1}$ as mean ± SEM; seven animals for each group. [b]Significantly different from diet A and [c]significantly different from diet B.

Discussion

Our data show that the in vitro peroxidizability of erythrocyte membranes and liver microsomes is related to diet-induced changes in LCP content of tissue lipids during early postnatal life in this species. The most interesting finding was that dietary supplementation with both fish oil and a phospholipid concentrate (Group C) significantly increased the susceptibility of erythrocyte membranes and liver microsomes to in vitro lipid peroxidation compared with a diet supplemented with only fish oil (Group B). Increased peroxidizability of liver microsomes in group C was accompanied by significant increases in GSH and total glutathione contents in liver homogenates compared with groups A (nonsupplemented diet) and B. In this experiment, diet-induced changes in LCP content of liver and brain lipids were not paralleled by substantial alterations in the activities of antioxidant enzymes in liver and brain homogenates.

Several authors have shown that the quantity as well as the type of dietary fat affect the activities of antioxidant enzymes in animals.[40,45,65–67] In our study, feeding rats with

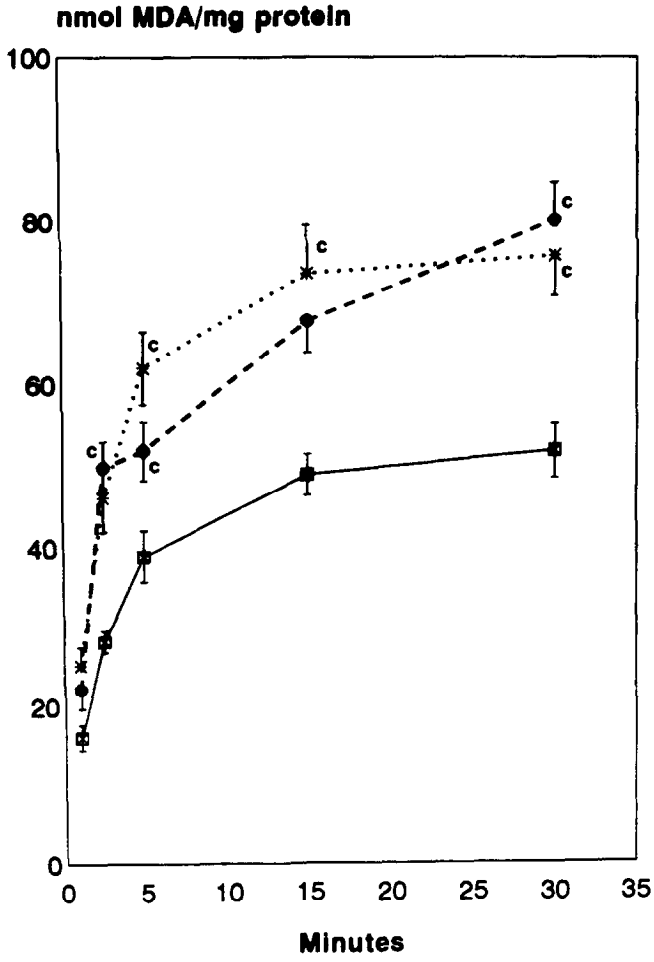

Figure 2 Liver microsomes peroxidation after induction with Fe^{2+}-ascorbate in rats at weaning. Diet A (□), Diet B (●), Diet C (*). Results are expressed in nmol of malondialdehyde (MDA). mg of protein^{-1} as mean ± SEM; seven animals for each group. [b]Significantly different from diet A and [c]significantly different from diet B.

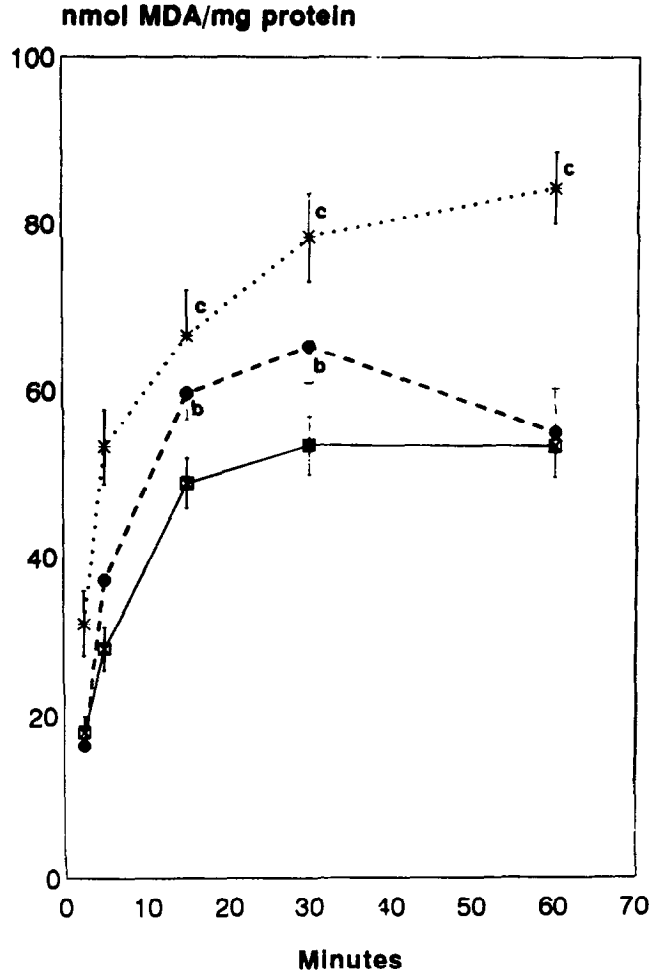

Figure 3 Liver microsomes peroxidation after induction with NADPH-Fe^{3+}-ADP. Diet A (□), Diet B (●), Diet C (*). Results are expressed in nmol of malondialdehyde (MDA).mg of protein^{-1} as mean ± SEM; seven animals for each group. [b]Significantly different from diet A and [c]significantly different from diet B.

experimental diets for 4 weeks did not significantly affect the activities of antioxidant enzymes in liver and brain. The only significant differences were found in hepatic GSH-Px activity between groups A and C, and in total SOD activities in liver between groups B and C. The absence of marked changes brain in liver and antioxidant defense may be due in part to the low amount of LCP added to diets B and C (3 wt%) in relation to other studies (10 to 20 wt%). Moreover, dietary fat in this study contained a relatively high quantity of saturated and monounsaturated fatty acids. Recently, Chen, et al.[66] showed that a diet rich in (*n*-9) fatty acids did not modify CAT and selenium GSH-Px activities in rat livers.

Protection of tissue lipids against free radical damage is also dependent on the availability of endogenous antioxidants such as vitamin E and GSH.[68–70] Previous studies showed that the hepatic contents of vitamin E and GSH are highly influenced by the intake of fish oil, rich in (*n*-3) LCPs.[36,44,71,72] In our study, whereas vitamin E content in liver microsomes was similar in all three groups, total glutathione and GSH levels in liver homogenates were significantly higher in both LCP-supplemented diets (groups B and C) compared with the nonsupplemented diet (group A). Detoxification of lipid hydroperoxides by GSH-Px and inactivation of the aldehyde products of lipid peroxidation by GST require the presence of GSH. Increased synthesis of glutathione by hepatocytes in the LCP-supplemented groups was probably a response to increased detoxification of lipid peroxidation products,[71] or to increased rates of consumption as a result of eicosanoid metabolism.[72]

According to previous data,[73,74] feeding rats at weaning for 4 weeks with a diet containing parent essential fatty acids at a ratio of 6:1 increased 20:4(*n*-6) and total (*n*-6) LCP, markedly reduced 22:6(*n*-3) and total (*n*-3) LCPs in tissue lipids. As a result, decreased PI values in tissue lipids and reduced tissue susceptibility to lipid peroxidation were observed in erythrocyte membranes, liver microsomes and brain homogenates in group A compared with groups B and C. In group B a replacement of tissue (*n*-6) LCPs by (*n*-3) LCPs occurred. These findings agree with previous reports[18,20,21] of an increase in (*n*-3) LCPs and a decrease in

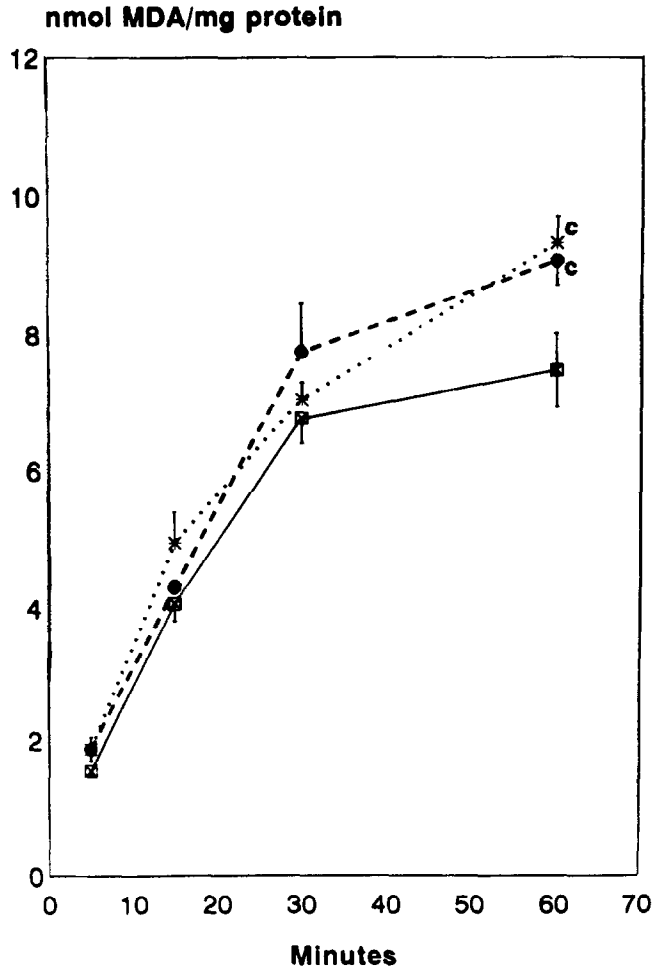

Figure 4 Spontaneous brain peroxidation at 37°C. Diet A (□), Diet B (●), Diet C (*). Results are expressed in nmol of malondialdehyde (MDA).mg of protein^{-1} as mean ± SEM; seven animals for each group. [b]Significantly different from diet A and [c]significantly different from diet B.

(*n*-6) LCPs in tissue lipids in preterm infants and in rats given (*n*-3) LCP-supplemented formulas. The incorporation of highly peroxidizable (*n*-3) LCPs into tissue lipids in rats fed diet B caused higher PI values as well as increased susceptibility to in vitro lipid peroxidation in erythrocyte membranes, liver microsomes and brain homogenates, compared with rats fed diet A. Similar increases in the susceptibility of tissue lipids to in vitro lipid peroxidation have been reported previously.[41,43,75]

Diet C increased tissue 22:6(*n*-3) in lipids compared with diet A, and reduced the replacement of 20:4(*n*-6) with competing (*n*-3) LCPs. The most interesting result was that rats fed diet C, which provided both (*n*-3) and (*n*-6) LCPs, showed higher rates of TBARS formation after induction of in vitro lipid peroxidation in erythrocyte membranes and liver microsomes than rats fed diets A or B. Although the in vitro lipid peroxidation susceptibility results do not necessarily reflects the in vivo effects our data suggest that dietary (*n*-3) and n-6 LCP supplementation could have potential harmful effects particularly in early life. Further studies to ascertain whether infant formulas supplemented with LCP affect in vivo lipid peroxidation processes are needed.

Our data also suggest that a low amount of (*n*-3) and (*n*-6) LCPs may account for the accretion of 20:4(*n*-6) and 22:6(*n*-3) to tissue lipids without substantial alterations in the activities of antioxidant enzymes. However, higher amounts of antioxidants such as vitamin E than those presently recommended may be necessary to protect dietary (*n*-3) and (*n*-6) LCPs incorporated into tissue lipids from damage by free radicals.

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