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Dietary Supplementation of N-3 Fatty Acids and Hydroperoxide Levels in Rat Retinas

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Docosahexaenoic acid (DHA) plays an important role in visual and neural development in mammals. In the present study, effect of dietary supplementation with n-3 fatty acids, primarily docosahexaenoic acid (DHA) with high purity, on the fatty acid composition of photoreceptor cells of young rats (fed from 4 weeks) was investigated. DHA in rod outer segment (ROS) membranes was significantly increased in the group of high DHA feeding (9.69% total energy). Other n-3 fatty acids (α-linolenic acid (ALA) and eicosapentaenoic acid (EPA)) included in the diets with DHA (0.95% ~ 5.63% total energy) also significantly increased the proportion of DHA compared with the linoleic acid diet groups. However, the proportions of arachidonic acid (ARA) and other long chain n-6 fatty acids (22:4n6 and 22:5n6) were suppressed in these n-3 fatty acids-fed groups. Phospholipid hydroperoxides in ROS membranes were determined using a highly sensitive analytical technique, chemiluminescence-high performance liquid chromatography (CL-HPLC). There was no increasing tendency in the hydroperoxide levels of ROS membranes containing high content of DHA, and phosphatidylethanolamine hydroperoxide (PEOOH) was much lower than phosphatidylcholine hydroperoxide (PCOOH) under normal light conditions, which implies that DHA supplementation does not much affect the peroxidizability of ROS membranes *in vivo*. But UV irradiation on separated ROS membranes accelerated the formation of phospholipid hydroperoxides in high DHA feeding rats, and PEOOH was produced more efficiently than PCOOH *in vitro*.

Keywords: Rat retina, rod outer segment, fatty acid composition, DHA, peroxidation, hydroperoxide

Abbreviations: ALA, α-linolenic acid; ARA, arachidonic acid; DHA, docosahexaenoic acid; EPA, eicosapentaenoic acid; ROS, rod outer segment; CL-HPLC, chemiluminescence-high performance liquid chromatography; PL, phospholipids; PUFA, polyunsaturated fatty acids; PE, phosphatidylethanolamine; PS, phosphatidylserine; PC, phosphatidylcholine

INTRODUCTION

Docosahexaenoic acid (DHA, 22:6n3) is a major fatty acid in the neural system, especially in the retina, of mammals.[1–3] Within the retina, DHA is highly rich in the lipids of the rod outer segment (ROS) membranes. The ROS are highly specialized structures composed of stacks of

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membranous disks within a plasma membrane, which has a relatively simple lipid composition with up to 90% of the lipids being phospholipids, in which phosphatidylcholine (PC) is 27.6% and phosphatidylethanolamine (PE) is 38.3%.[4] The phospholipids of ROS are enriched in 22-carbon (C-22) polyunsaturated fatty acids, primarily DHA, which comprises about 50% of the total fatty acids in PE and phosphatidylserine (PS), respectively, and 30% in PC.[4]

Metabolic studies of both long-period feeding and short-period isotope tracing in rats and neonatal baboons have shown that DHA in the retina can be synthesized from shorter chain precursors, e.g. α-linolenic acid (ALA, 18:3n3), eicosapentaenoic acid (EPA, 20:5n3) and/or can be transported directly from blood.[5–7] A study in human adults has shown that although a certain limited conversion of high doses of ALA to EPA occurs, conversion to DHA is severely restricted. Accordingly, attention should focus on the adequate provision of DHA, which can reliably be achieved only with the supply of the preformed DHA.[8] More importantly, even though human infants have the capability to biosynthesize DHA from ALA, the quantities produced *in vivo* are inadequate to support the DHA level observed in breast-fed infants.[9] It appears that ALA may serve as an adequate substrate for the accretion of DHA in the brain, but not in the retina of the developing rat.[10] These studies indicate that the direct intake of DHA may be necessary for adequate neural DHA accretion and optimal neural development.

But little is known about the potential harmful influences of the supplementation with DHA. It is easily conceivable that DHA is very sensitive to oxidative stress due to its high unsaturation. Some reports have suggested that depletion of rat retinal DHA reduces the susceptibility of the ROS to acute light damage.[11,12] Another study, using guinea pigs reported that increase in the DHA level over an optimal amount decreases the retinal function.[13] Most of these studies carried out in retinas with different DHA levels used diets deficient in n-3 fatty acids, but fewer studies examined the direct intake of high doses of DHA. Therefore, it is very important to elucidate the effect of DHA supplementation on fatty acid composition, especially the DHA in ROS membranes during developing stages and to further clarify the relationship of DHA content and hydroperoxide levels in ROS membranes.

In this study, young rats were treated with diets of varying preformed DHA contents (ranging from negligible to approximately 9.7% of total energy) with a constant amount of linoleic acid (18:2n6) to assess the effects on fatty acid composition of the ROS membrane in rats in developing period. The additive effect of other n-3 fatty acids, ALA and EPA, were also investigated. The most potential harmful effect, i.e. oxidative stress, of high doses of DHA on the ROS membrane was also investigated. Phospholipid hydroperoxides in ROS membranes both in *in vivo* and UV irradiation-treated membranes were quantified using a highly sensitive analytical technique, chemiluminescence-high performance liquid chromatography (CL-HPLC), which can quantify PC hydroperoxide (PCOOH) and PE hydroperoxide (PEOOH) individually.

MATERIALS AND METHODS

Fats in the Diet

Docosahexaenoic ethyl (purity 91.6%) was from tuna oil; eicosapentaenoic ethyl (purity 96.6%) was from sardine oil; perilla oil was used as origin of linolenic acid directly. The peroxide values were nearly zero before use.

Animals

Four-week rats were used in the study. The composition of the experimental diet, based on the AIN-76 purified diet for rats,[14] is shown in Table I.

Forty-two male Sprague-Dawley rats were first fed the basal diet containing 5% (w/w) olive oil for 6 days, then randomly assigned to seven

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TABLE I Composition of experimental diets

Ingredient	g/100g diet
Casein	20
DL-methionine	0.3
Glucose	22.5
Cellulose	5.0
α-Cornstarch	15
Sucrose	22.5
RRR-α-tocopherol equivalent (mg)	6.7
Vitamin mix	1.0
Choline bitartrate	0.2
Mineral mix	3.5
Oil	10

diet treatments containing 10% (w/w) fat for 30 days. Animals were housed individually in a controlled temperature of 22 ± 1 °C and humidity of 50–60% with a 12-h light/dark cycle. To prevent the autoxidation of DHA in diet, the diet was prepared beforehand without adding DHA and stored at −20 °C. DHA stored at −75 °C was mixed with the diet every day immediately before feeding. Food and water were available *ad libitum*. Each diet was made available to the rats in the evening and removed the next morning. Peroxide values in residue of the diet in DHA groups were below 30–40 meq/kg. So the effect of peroxide level in the diet could be ignored. There was no significant difference in the amount of diet taken in by these rats, also in the body weight. The fatty acid composition (g/100 g) of dietary lipids is indicated in Table II. Rats were anesthetized with Nembutal by intraperitoneal injection. After removing blood and organs, rat retinas were extruded through a slit made across the entire cornea,[15] quickly frozen in dry ice, and stored at −75 °C until used.

TABLE II Fatty acid composition of dietary lipids (for 5-week rats)

Group	1	2	3	4	5	6	7
LA level (en%)[a]	4.89	13.43	5.17	4.61	4.63	4.37	3.98
ALA level (en%)	0.11	0.09	5.47	1.80	2.77	1.54	0.04
EPA level (en%)			1.04	5.13	3.12	2.19	0.26
DHA level (en%)			0.95	1.71	2.99	5.63	9.69
Fatty acid							
14:0	1.4	0.5	0.5	0.7	0.5	0.5	0.5
14:1(n-7)	0.2						
16:0	22.2	11.4	11.6	11.5	10.2	9.8	9.1
16:1(n-7)	2.1	0.6	0.8	1.0	0.8	0.9	0.9
16:2(n-7)	0.2						
18:0	11.4	4.4	5.3	5.0	5.0	4.8	4.7
18:1(n-9)	36.7	17.4	20.7	16.9	18.4	17.3	15.7
18:2(n-6)	22.6	62.1	23.9	21.3	21.4	20.2	18.4
18:3(n-3)	0.5	0.4	25.3	8.3	12.8	7.1	0.2
20:1(n-9)	0.4	0.4	0.3	0.2	0.2	0.2	0.2
20:3(n-6)	0.7	0.3	0.4	0.3	0.4	0.3	0.3
20:4(n-6)			0.2	0.7	0.6	0.7	0.9
20:4(n-3)				0.3	0.3	0.3	0.3
20:5(n-3)			4.8	23.7	14.4	10.1	1.2
22:5(n-6)					0.1	0.2	0.3
22:5(n-3)			0.1	0.2	0.3	0.6	1.0
22:6(n-3)			4.4	7.9	13.8	26.0	44.8
others	1.6	2.5	1.7	2.0	0.8	1.0	1.5
Polyunsaturates (%)	23.8	62.8	59.1	62.7	64.1	65.5	67.4
Monounsaturates (%)	39.4	18.4	21.8	18.1	19.4	18.4	16.8
Saturates (%)	35.0	16.3	17.4	17.2	15.7	15.1	14.3
P/S ratio	0.7	3.8	3.4	3.6	4.1	4.3	4.7
n-6 (%)	23.3	62.4	24.5	22.3	22.5	21.4	19.9
n-3 (%)	0.5	0.4	34.6	40.4	41.6	44.1	47.5
n-6/n-3 ratio	46.6	156.0	0.7	0.6	0.5	0.5	0.4

[a] en% – % total energy.

Preparation of ROS Membranes

All isolations were performed at 4 °C. Two retinas were pooled for separation of the ROS. ROS was isolated by discontinuous sucrose gradient centrifugation as described by Stinson *et al.* Purity of ROS was confirmed using polyacrylamide gel electrophoresis.[16]

SDS-Polyacrylamide Gel Electrophoresis (SDS-PAGE)

SDS-PAGE was conducted on a separation gel containing a 5–20% acrylamide gradient. Samples were left at room temperature for 5 min before application to the gel. Protein on the gel was stained with Coomassie Brilliant Blue.

Fatty Acid Analysis

Total lipids were extracted from the ROS membrane using the Folch method. Organic solvent (chloroform:methanol, 2:1 by volume) containing 2.25 mg/l of *t*-butyl hydroxyanisole was used as an antioxidant. Methyl esters of total

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lipids were prepared with 0.6 N methanolic-HCl as described by Kates.[17] Separation of fatty acid methyl esters was performed by gas-liquid chromatography utilizing fused silica HR-SS-10 capillary column (25 m × 0.25 mm I.D.; Shinwa, Kyoto, Japan) in a splitless mode (Shimadzu GC 18A). Helium was used as the carrier gas. The initial oven temperature was 40 °C, increased to 160 °C at 20 °C/min, followed by an increase in temperature to 210 °C at 2 °C/min and maintained for 10 min. The injector and detector temperatures were 250 °C. With these conditions, fatty acid methyl esters from the membrane of 0.5 retina could be detected.

Peroxidation Assay

Three of six samples for each group was used for direct assay of phospholipid hydroperoxides, another three samples for UV irradiation in clean bench for 5.5 h (20 cm from the UV lamp, 15 W) and then for assay of phospholipid hydroperoxides by a highly sensitive method, CL-HPLC.[18,19] Phospholipids were extracted with chloroform:methanol (2:1, v/v) containing 2.25 mg/l of *t*-butyl hydroxyanisole as an antioxidant. The chloroform layer was injected directly into a CL-HPLC unit for phospholipid hydroperoxide analysis. The CL-HPLC system included a JASCO Finepak SIL column (5 μm, 250 × 4.6 mm; Japan Spectroscopic Co., Tokyo, Japan) which was placed in a JASCO 860-CO oven at 40 °C, with a mobile phase of isopropanol:methanol:water (13.5:4.5:2, v/v/v) at a flow rate of 1.1 ml/min by a JASCO 880-PU pump. In the post-column detection system, phospholipid absorption at 210 nm was monitored with a JASCO 875-UV detector and hydroperoxide specific chemiluminescence was measured with a CLD100 detector (Tohoku Electronic Ind. Co., Sendai, Japan). The CL reagent was a mixture of 2.5 mg/l of cytochrome c and 0.2 mg/l of luminol in 50 mM borate buffer at pH 10.0. Since calibration curves of PCOOH and PEOOH almost overlap each other, the PCOOH produced from photooxidation of 1-palmitoyl-2-oleoylphosphatidylcholine was used as a standard for preparing a calibration curve for both PCOOH and PEOOH. The concentration of authentic PCOOH was determined by the ferrous oxidation-xylenol orange assay.[20]

Statistical Analysis

The effect of diet on fatty acid composition of ROS and data of the phospholipid hydroperoxides were assessed by one-way factorial ANOVA and multiple comparison tests. Significant effects of treatment were defined utilizing Scheffe's method as Post-Hoc Test. All data were expressed as mean ± standard error of the mean (SEM).

RESULTS

Fatty Acids in ROS Membranes

We attempted to obtain different contents of retinal DHA by dietary manipulation with preformed DHA in young rats. As shown in Table II, with the exception of Group 2, which had a very high linoleic acid proportion (62%), the linoleic acid was maintained at almost the same level (about 20%), with increase in DHA (0 ~ 45%). Other n-3 fatty acids (ALA and EPA) were also included in these groups. As expected, significant increases in DHA levels of ROS membranes were produced in both the high EPA feeding group (G4), the high DHA feeding group (G7) and the n-3 fatty acids-mixed groups (G5, G6) compared with the high linoleic acid group (G2, Table III). Significant differences were also obtained between the diet group with lower linoleic acid level (G1) and G4, G6, G7. However, high ALA feeding group (G3) did not enhance the DHA level significantly. By these dietary manipulations, the highest DHA level reached 42.9% (G6) compared with the lowest DHA level of 31.6% (G2). There was no significant difference in DHA proportions

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TABLE III Fatty acid composition of lipids from 9-week rat ROS membranes ($n = 6$)

Fatty acid	Diet group						
	1	2	3	4	5	6	7
16:0	18.10 ± 2.42	17.24 ± 1.28	17.18 ± 2.41	15.89 ± 1.58	16.23 ± 1.88	15.74 ± 1.63	16.59 ± 1.79
18:0	25.47 ± 1.16	26.21 ± 1.31	25.14 ± 1.78	24.69 ± 1.06	24.71 ± 1.46	25.07 ± 0.57	24.66 ± 1.92
18:1n9	7.22 ± 0.59	6.37 ± 0.75	7.28 ± 0.82	6.86 ± 0.62	6.50 ± 0.45	6.73 ± 0.67	6.40 ± 0.72
18:1n7	2.05 ± 0.11[a]	1.84 ± 0.21[ab]	1.79 ± 0.11[ab]	1.66 ± 0.24[bd]	1.45 ± 0.11[cd]	1.56 ± 0.17[bc]	1.30 ± 0.11[c]
18:2n6	0.71 ± 0.09[a]	0.65 ± 0.52[a]	1.20 ± 0.17[ab]	1.07 ± 0.11[ab]	1.11 ± 0.29[ab]	1.47 ± 0.45[b]	1.07 ± 0.16[ab]
20:4n6	8.01 ± 0.18[a]	8.04 ± 0.39[a]	6.76 ± 0.19[b]	5.54 ± 0.22[cd]	6.26 ± 0.62[bd]	5.17 ± 0.27[c]	5.70 ± 0.43[cd]
20:5n3	nd[a]	0.05 ± 0.13[a]	0.20 ± 0.22[ab]	0.84 ± 0.08[c]	0.66 ± 0.35[bc]	0.13 ± 0.31[a]	0.64 ± 0.13[bc]
22:4n6	1.81 ± 0.15[a]	1.84 ± 0.21[a]	0.80 ± 0.14[b]	0.41 ± 0.22[cd]	0.66 ± 0.12[bc]	0.06 ± 0.16[d]	0.60 ± 0.08[bc]
22:5n6	2.94 ± 0.29[a]	5.92 ± 0.68[b]	0.10 ± 0.16[c]	nd[c]	0.03 ± 0.08[c]	nd[c]	0.24 ± 0.28[c]
22:5n3	0.22 ± 0.24[a]	0.22 ± 0.39[a]	1.14 ± 0.10[bd]	1.77 ± 0.26[c]	1.52 ± 0.13[bc]	1.16 ± 0.07[bd]	1.03 ± 0.09[d]
22:6n3	33.48 ± 4.03[ab]	31.61 ± 2.09[a]	38.41 ± 4.57[ac]	41.27 ± 3.05[c]	40.86 ± 3.55[bc]	42.89 ± 2.77[c]	41.76 ± 4.14[c]

Values given are means ± SEM. For each n ($n = 6$), 2 retinas were pooled. Data were assessed by one-way factorial ANOVA and multiple comparison tests. Significant effects of treatment were defined utilizing Scheffe's method as Post-Hoc Test. Means within the same row not followed by a common letter were significantly different ($p < .05$). nd – not detected.

between the high ALA group (G3), the high EPA group (G4), the moderate ALA + EPA groups (G5 and G6) and the high DHA group (G7). Changes in 22:5n3 level showed a similar tendency to DHA, i.e. 22:5n3 was significantly increased in the high n-3 fatty acids-feeding groups (G3 ~ G7) compared with the linoleic acid groups (G1 and G2). Moreover, the highest EPA diet (G4) produced a higher EPA level in the ROS membrane, while few or no EPA were detected in the linoleic acid groups. On the other hand, addition of dietary DHA and other n-3 fatty acids (ALA and EPA) was associated with a significant decline in n-6 fatty acids, 20:4n6, 22:4n6 and 22:5n6. The lowest ARA level (5.2%) was obtained in G6, compared with the highest ARA level (8%, G1 and G2). Another n-6 fatty acid, 22:5n6, was especially reduced from 2.9% in G1 to 0.24% in G7, or almost not detected (G4, G5 and G6). However, 18:2n6 was inversely increased, especially in Group 6.

Phospholipid Hydroperoxides in ROS Membranes with Different Amount of DHA

As described above, high-doses of DHA or other n-3 fatty acids increased the DHA content in the retina. Lipid peroxidation in the retina is thought to be a major mechanism contributing to light-induced lesions. The most potentially harmful effects of DHA supplementation is enhancement of peroxidation susceptibility. Rats in the present study were reared under normal light conditions with a 12-h light/dark cycle. Phospholipid hydroperoxides were quantified by a highly sensitive technique: CL-HPLC. Figure 1 shows that there were quite high amounts

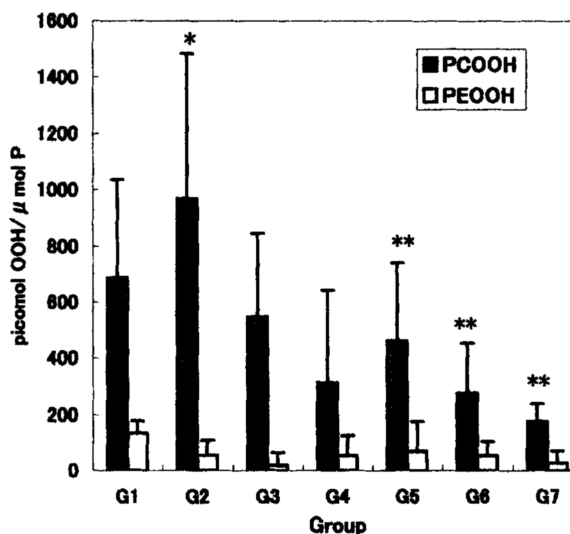

FIGURE 1 Concentrations of phospholipid hydroperoxides in rat ROS fed different amounts of DHA. Six rats were used for G2, G4 and G6. Three rats were used for G1, G3, G5 and G7. * and ** were significantly different from each other at $p < .05$ (Scheffe's method).

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of PCOOH in rat ROS membranes, compared with the value reported in plasma from healthy adults (40 ~ 80 picomol PCOOH/μmol phospholipids).[19,21] The lowest level on average was 176 picomol/μmol phospholipids (G7) and the highest level (G2) was 968 picomol/μmol phospholipids (G2). PEOOH was much less than PCOOH; the lowest level on average was 19 picomol/μmol phospholipids (G3) and the highest level was 133 picomol/μmol phospholipids (G1). There was no positive correlation between phospholipid hydroperoxides and DHA content. Conversely, significantly higher PCOOH was detected in ROS fed high linoleic acid compared with high-dose DHA. Another finding in the present study was the much lower level of PEOOH than PCOOH. The ratio of PCOOH/PEOOH was > 5.0.

UV Irradiation-induced Phospholipid Hydroperoxides in ROS

UV irradiation for 5.5 h induced a considerable increase of PCOOH in the group fed high DHA (G7), and PEOOH was especially produced in all n-3 fatty acids-fed groups tested (Figure 2).

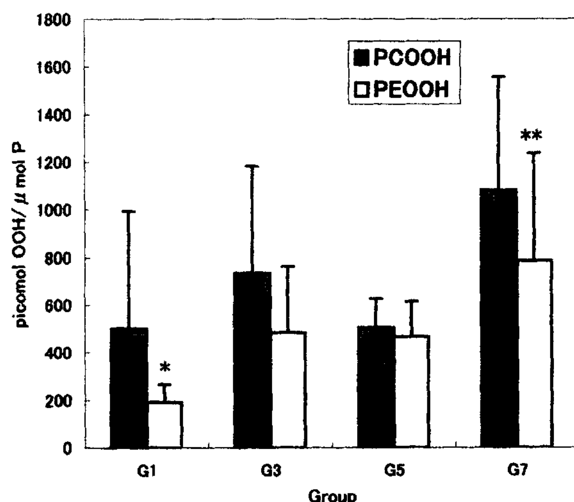

FIGURE 2 Concentrations of phopspholipid hydroperoxides in rat ROS irradiated by UV for 5.5 h. Three rats were used for each group. * and ** were significantly different at $p < .05$ (Scheffe's method).

There was a significant difference between G1 and G7 for PEOOH, indicating an enhanced peroxidative susceptibility in ROS with a high DHA content. However, UV irradiation did not enhance the levels of PCOOH and PEOOH in ROS with a low DHA content (G1), also the level of PCOOH in ROS of G5.

DISCUSSION

Fatty Acids in ROS Membranes

Many studies show that retinal fatty acid composition can be manipulated easily by diets using pregnant dams and weanling rats. For example, using unbalanced diets with high linoleic acid/ALA ratios (41 and 69) in which physiological levels of ALA are present, low and very low retinal DHA proportions (17% and 8%) were produced.[22] An increase in physiological levels of all fatty acids including small amounts of DHA (0.7%, w/w) also resulted in a significant increase of DHA contents in postweanling ROS.[23] Suh *et al.* using young rats feeding high n-3 fatty acids (5.8%, w/w) increased DHA level in retinal phospholipids by approximately 10% compared with feeding low n-3 fatty acids (1.2%, w/w) at the same linoleic acid level.[24] Nearly 10% increase in DHA content in retinal membranes was also obtained in our study by using young rats with similar age to Suh's rats. But we found that there was a limit to the increase, indicating that young rats had the capability of maintaining the necessary level of DHA in the retina even after the excessive DHA intake. On the other hand, Reme *et al.*[25] did not obtain a high DHA content with fish oil supplementation compared with soybean oil, although the diet contained 10% DHA and 15% EPA. The fatty acid composition of the fish oil diet in their study was similar to Group 5 in our present study (Table II).

Addition of dietary DHA and other n-3 fatty acids (ALA and EPA) was associated with a significant decline in n-6 fatty acids. Data on

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ARA levels in DHA and other n-3 fatty acids-supplied groups (G3 ~ G7) in our study were consistent with findings of Reme *et al.*[25] Woods *et al.* also reported that levels of ARA in the brain and retina of rat pups were significantly reduced with the feeding of a very low ratio of linoleic acid/ALA (0.08).[10] These findings have been presumed to be resulted from metabolic competition between n-6 and n-3 fatty acids. Therefore, the addition of dietary n-3 fatty acids may suppress the activity of Δ6 desaturase in developing rats.

Briefly, DHA accretion is influenced by both the ratio of n-6 to n-3 (where high level of linoleic acid inhibits the metabolism of ALA to DHA) and the direct addition of dietary DHA, the latter is more effective in increasing DHA levels.[10,26,27] The present study confirms these earlier findings that dietary DHA is an important determinant of DHA accretion in the retina of developing animals.

Phospholipid Hydroperoxides in ROS Membranes

There are many studies on the relationship of retinal lipid peroxidation between animals reared on diets high in n-3 fatty acids and low in n-3 fatty acids or n-3 fatty acids deficient. Depletion of retinal DHA by ALA deficient feeding reduces the susceptibility of the rat ROS to acute light damage,[11,12] which appears to be related to the relative levels of DHA and 22:5n6. Light-induced stress to the retina in these studies was carried out by exposing rats to intermittent light with approximately 100 times luminous intensity over the control. In normal light conditions, total hydroperoxides in the retina have been detected using an enzymatic procedure, and the concentration of hydroperoxides in the ROS fractions from dark-reared rats were found to be significantly lower than in cyclic-light-reared rats.[28] However, this method could only provide a total concentration of hydroperoxides, while our study provides data on the level of PCOOH and PEOOH individually. As a result, a much higher level of PCOOH than PEOOH was detected, although it has been known that PE in ROS is always more unsaturated than PC, and the ratio of PC/PE in rat ROS is < 1.0.[4] Another unexpected result is that significantly higher PCOOH was detected in ROS fed high linoleic acid compared with high-dose DHA. Two explanations for these findings can be suggested. First, PE might be more resistant to peroxidative stress, e.g. light; second, even if PEOOH could be produced at a similar level to PCOOH, it might be decomposed easier. Organisciak *et al.* reported that during intense light exposure of short duration, significant levels of hydroperoxides were not accumulated in rat retinas.[28] Terao found that PEOOH was much more unstable than PCOOH in the presence of the ferrous ion.[29] However, as the chelator for ferrous ion coexisted in the preparation of ROS in the present study, the decomposition of phospholipid hydroperoxides is thought to be prevented, thus, the lower accumulation of PE hydroperoxide may reflect its real content *in vivo*.

Our study with an additional experiment by UV irradiation induced a considerable increase in PEOOH content in all groups tested which contained high amount of DHA in ROS, indicating accumulation of PEOOH during UV exposure. However, significant increase in PCOOH content was only observed in the group fed very high DHA (G7), which may be derived from its more unsaturated fatty acid composition than G1. Moreover, PEOOH was formed more efficiently than PCOOH upon UV irradiation, as seen in Figure 2, which may also be derived from the more unsaturated fatty acid composition of PE than that of PC. Fatty acid analysis showed that about 70% of DHA and 50% of ARA lost during UV irradiation for 6 h, indicating that DHA was the first target to external oxidative stress such as UV irradiation (data not shown). Kobayashi reported that UV irradiation for 12 h induced decreases of 50% in DHA content and 30% in ARA content using bovine retinas. But

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much more amount of hydroperoxides of oleic acid and linoleic acid were formed, as detected by CL-HPLC method. The result implies that hydroperoxy fatty acids with more amount of double bonds such as hydroperoxide of DHA accumulate with more difficulty.[30] It should be noted that no chelator such as EDTA was used in processes of retina preparation and UV irradiation in this study. So we can not exclude the possibility of decomposition of hydroperoxides, especially DHA during UV irradiation.

In conclusion, high doses of preformed DHA increased the DHA content of ROS significantly in young rats compared with low n-3 fatty acid feeding. The increase was not infinite which indicates that a constant level of DHA is important for visual function. There was no significant difference in phospholipid hydroperoxides between ROS with high and low DHA content at normal light condition. However, there was a tendency that ROS with higher DHA content may form phospholipid hydroperoxides more efficiently by UV irradiation.

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