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Effect of Dietary Fish Oil on Lipid Peroxidation in Rat Liver and Brain During Postnatal Development*

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魚油섭취가 출생후 발달과정의 흰 쥐의 간과 뇌조직의 지질과산화와 그 관련기능에 미치는 영향*

박명희 · 최경원 · 장경숙 · 조성희

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□ 국 문 초 록 □

魚油를 섭취하였을 때, 체내 과산화물 생성과 항산화 기능을 조사하며, 그 결과를 다른 식이지방과 비교하기 위하여, 고등어유, 대두유, 쇠기름, 돌기름, 채종유의 5종의 지방을 먹이의 10%되게 식이를 조제하여 70g 내외의 암, 수컷의 흰쥐에게 섭취시켰다. 출생후 초기 성장시에 적응도를 관찰하기 위하여, 위의 쥐들을 34일간 조제식이로 사육한 후 교배시켜 출생한 2대째 쥐들을 수유시기(17일, 26일)과 이유시기(39일)에 희생시키고, 출산, 수유를 마친 어미쥐(나이 123일, 총 식이일수 81일)를 희생하여 간과 뇌조직의 지질과산화물, α-tocopherol, glutathione양을 정량하고, glutathione peroxidase, superoxide dismutase의 활성을 측정하였다. 간조직의 지질과산화물 값은 고등어유를 섭취한 어미쥐와 수유기(17, 26일)의 새끼쥐들에게서 타군에 비해 높았으나 39일의 새끼쥐에서는 채종유군 보다는 높았으나 대두유, 돌기름군과 같은 수준이었다. 뇌조직에서는 군간에 차이가 거의 없었다. 간조직의 α-tocopherol 농도와 환원형의 glutathione(GSH)의 농도가 어미쥐에서 타군에 비해 현저히 낮았고, 새끼쥐에서도 α-tocopherol 경우는 같은 경향이나 그 정도가 덜 현저하며, GSH의 경우는 타군들과 차이가 없었다. 산화형 glutathione(GSSG)의 농도는 어미쥐, 새끼쥐모두에서 식이지방에 따른 차이가 일관성 있게 나타나지 않았다. Glutathione peroxidase활성은 간장과 뇌조직에서 성장과정 중의 새끼쥐들에서 모두 약간씩 증가하였고, 채종유군에서 타군에 비해 증가정도가 낮아 활성이 39일에 현저히 낮았다. Superoxide dismutase의 활성은

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성장기간에 따른 변화는 적었고, 고등어유군이 타군에 비해 비교적 낮은 값을 유지하였다. 어미쥐와 17일째 새끼쥐에서와는 달리 26일과 39일째 새끼쥐의 간조직 glutathione peroxidase의 활성이 지질과산화물 농도와 정의 상관관계가 보여져, 출생 후 초기 성장과정에서 체내 적응성이 존재함을 나타내주었다.

INTRODUCTION

Current interest in ω3 fatty acids of fish oil developed largely from studies on Greenland Eskimos[1], who had a diet high in fat and protein of marine origin, with very little incidence of cardiovascular disease. During last decade, a large volume of evidence[2] has indicated that beneficial effects of fish oil have been related to almost all chronic diseases, i.e., not only cardiovascular disease but also cancer, hypertension, multiple sclerosis and rhematoid arthritis. Essentiality of long-chain ($C \geqq 20$) ω3 fatty acid has been shown, separately in the brain and retinal function[3], which appeared to be mediated by membrane lipid. However, an imposing problem is that high degree of unsaturation of eicosapentenoic and docosahesaenoic acids in fish oil could easily lead the formation of various lipid peroxidation products[4] in the cell, being causes of aging and development of atherogenesis[5]. A major contribution to the non-enzymatic protection against lipid peroxidation is Vit E, dietary requirement of which has been related to intake of polyunsaturated fatty acids[6] or to P/S ratios[7),8]. But there seems to be difference in peroxidative potentials between ω6 and ω3 PUFA[9),10], indicating that the undistinguished P/S ratio may not afford to be used universally. The reduced form of glutathione(GSH) is a cellular constituent protecting against lipid peroxidation either as the hydrogen donor for both the Se-dependent[11] and Se-independent[12] glutathione peroxidase or as a dispenser for cytosolic[13] and microsomal[14] GSH-dependent factors. Unlike the studies on Vit E, cellular status of GSH has been rarely examined, in relation to lipid peroxidation caused by dietary PUFA. Besides, the influence of PUFA on the enzymatic defense reaction has been shown only in the limited study of Thomassen et al[15] that cardiac catalase activity decreased by feeding partially hydrogenated fish oil. Therefore, in the present study, we aimed to investigate the effect of fish oil on the activities of superoxide dismutase and glutathione peroxidase in rat liver and brain as well as on the tissue contents of lipid peroxide, α-tocopherol and glutathione. Comparisons were made with the effects of other types of high PUFA containing dietary oils, i.e., soybean and perilla oils and low PUFA fats, i.e., rapeseed oil and beef tallow. To see the possible adaptation occur in development stage, the examination was followed up through early postnatal period in the progeny of rats fed the five different dietary oils and fat.

MATERIALS AND METHODS

Materials

Soybean, repeseed and perilla oils and beef tallow were purchased from nearby market. Mackerel oil was prepared from fresh fishes obtained from market, as described previously.[16] Salt mix (Rogers and Harper's[17]) and vitamin mix were purchased from TEKLAD Inc.(Madison, Wisconsin, U.S.A.) Casein, starch and glucose were obtained from Pooing Jin Chemical Co. Minor dietary components such as choline, inositol and methionine were regent grade. Cellulose, 1.1.3.3-tetramethoxypropane, NADPH,

TRIS, cumene hydroperoxide, EDTA and bovine serum albumin (Fraction V) were obtained from Sigma Chemical Co. and all other chemicals were analytical grade.

Animals and Diet

Twenty male and forty female Sprague-Dawley rats weighing 65-75 g, were divided into five groups and fed diets containing 10%(w/w) fats for 34 days, before they were mated. The experimental fats used were mackerel oil(MO), soybean oil(SO), beef tallow (BT), perilla oil(PO) and rapeseed oil(RO). Peroxide value, carbonyl value and iodine value of each fat were measured[18] and fatty acid composition was analyzed by gas chromatography[16]. Female rats were kept on experimental diets during pregnancy and lactation, so that total feeding period was 81 days (age of about 4 months), after which they were sacrified for biological analysis. Six to eight pups from different litters of each dietary group were sacrified during (17 days) and at the end (26 days) of suckling and after weanling (39 days).

Sample Preparation

At completion of the feeding period, the rats were killed by decapitation and the liver and brain were immediately excised. Each gram of both tissues were washed, cut in small pieces and homogenized in 9ml of ice-cold 1.15% KCl by using Teflon Potter-Elvehjem homogenizer. This homogenate was kept frozen at -60°C until use for measurement of tissue peroxide. To determine tissue α-tocopherol, saline homogenates were prepared from both tissues as in the same way as KCl homogenate. Another gram from each tissue was washed and homogenized in ice-cold 0.25 M sucrose/0.1 mM EDTA. Postmitochondrial supernatants (PMS) of liver and brain, prepared from this homogenate by successive centrifugations described before[19] were used for assay of glutathione peroxidase and superoxide dismutase activities. Remaining portions of liver were freeze-clamped with aluminum press which was precooled in dry ice at -20°C and kept at -60°C. Frozen tissue was pulverized with precooled aluminum mortar and extracted with 3 volumes of ice-cold 2N-$HClO_4$. Acid soluble parts were obtained by centrifugation of protein precipitate at 3000 rpm for 10 min and neutralized with 4M K_2CO_3. This neutralized acid extracts of tissue were used for determination of glutathione. Lipid peroxide in the homogenates of liver and brain was determined by the method of Ohkawa et al[20] and α-tocopherol content was measured according to Lee et al[21] Glutathione content was determined as described by Bernt and Bergmeyer[22]. Activity of glutathione peroxidase[23] was monitored

Table 1. Composition of diet

Component	Content(g/100g)
Starch	35.5
Glucose	18.8
Casein	24.4
Vitamin mix[1]	2.0
Salt mix[2]	4.5
Cellulose	3.9
Fat	10.0
Mineral supplement[3]	0.08
Choline	0.05
Inositiol	0.55
Methionine	0.22

1) Vitamin fortification mixture obtained from Bio Serv. Inc. Frenchtown, N.J., U.S.A., provided the following(per kg diet); vitamin A, 4000 I.U., vitamin D, 2000 I.U., alpha-tocopherol, 100mg, ascorbic acid, 0.9g, riboflavin, 20mg, thiamin HCl, 20mg, i-inositol, 100mg, choline chlroride, 1.5g, menadione, 45mg, p-aminobenzoic acid, 100mg, niacin, 90mg, pyridoxine-HCl, 20mg, Ca-pantothenate, 60mg, biotin, 0.4mg, folic acid, 1.8mg, vitamin B_{12}, 27 μg.

2) Salt mixture used had composition of Rogers and Harper's[17].

3) Na_2SeO_3 0.258g, MnO_2 37.19 g, Fiber 62.66 g in 100 g.

spectro-photometrically by the disappearance of NADPH at 340 nm in total reaction volume of 2.38 ml containing 0.11 M Tris-HCl (pH 7.0), 0.13 mM NADPH, 0.29 mM GSH, 0.21 mM cumene hydroperoxide, 3.36 mM EDTA and 5 units of glutathione reductase, with the addition of 0.05 ml of liver and brain PMS. Superoxide dismutase activity was assayed as described by Marklund[24], using inhibition of pyrogallol autoxidation in 3 ml of 50 mM Tris-carcodylic acid buffer (pH 8.2) by the addition of 0.1 ml of liver and brain PMS.

Data Analysis

The results were analyzed using analysis of variance and significant differences between treatments were evaluated by Student's t-test.

Table 2. Peroxide value, carbonyl value and iodine value of dietary oil

	Mackerel oil	Soybean oil	Beef tallow	Perilla oil	Rapeseed oil
Peroxide value(meq/kg)	9.25	2.18	10.50	3.98	5.19
Carbonyl value(meq/kg)	3.81	2.83	5.95	2.34	3.77
Iodine value	168	147	37	193	134

Table 3. Fatty acid composition of dietary fat

Fatty acid*	Mackerel oil	Soybean oil	Beef tallow	Perilla oil	Rapeseed oil
			%(w/w)		
14:0	1.0		0.8		
16:0	19.6	10.8	23.9	6.6	3.7
16:1	7.3		4.1	0.4	0.2
18:0	5.4	4.0	26.9	2.4	1.6
18:1	25.1	24.3	41.4	14.8	27.1
18:2	2.0	53.1	2.2	16.3	17.8
18:3		7.8	0.6	59.5	
18:4	1.9				
20:0					3.7
20:1	5.6				15.6
20:4	6.3				
20:5	7.6				
22:1					30.2
22:5	1.1				
22:6	15.9				
Total PUFA (%)	32.8	60.9	2.2	75.8	17.8
P/S ratio	1.26	4.11	0.04	8.42	3.36
Unsaturation index**	216	154	52	226	109

*: Carbon number : number of double bonds.

**: $\sum_{i=1}^{m}$ nx %(w/w) of ith fatty acid, where m is total number of fatty acids detected in each dietary fat and n is number of unsaturated bonds in each ith fatty acid.

RESULTS

Characteristics of Dietary Fat and Oils

Analytical values of five dietary fat and oils are listed in Table 2 and the fatty acid compositions of these fat and oils are shown in Table 3. Peroxide value and carbonyl value of beef tallow were highest of all. Considering low lodine value (Table 2) and unsaturation index (Table 3) of beef tallow, it seems unlikely and so freshness of tallow could be suspected. However, we decided to use it, because peroxide value was a little over the level (7 meq/Kg) set for quality assurance but carbonyl value was under the regulation level of 6.0 meq/Kg. Besides, peroxide value of mackerel oil was close to that of tallow. Therefore, using the tallow in this experiment, was regarded to have an advantage in interpretation of results, since any difference between effects of mackerel oil and beef tallow (or other types of oils) could not be ascribed only to dietary level of peroxide *per se* Total PUFA amounts, P/S ratios were calculated from fatty acid composition. Unsaturation indice[25] were well agreeable to iodine values measured. There was no significant difference in weight gains of both mother rats and pups due to dietary fat variation (data not shown).

Lipid Peroxide, α-Tocopherol and Glutathione Contents

Fig. 1 shows lipid peroxide contents in livers and brains of mother rats and their offsprings of early postnatal period. Mother rats fed MO had significantly higher level of lipid peroxide than the rest of groups. Their pups also had the highest level before weanling, but afterwards, it dropped to the level of PO and SO. During lactation, there seemed to be among groups except MO, differences positively related to unsaturation indice (Table 3) of dietary fat the mother rats had. Brain peroxide levels of mother rats of

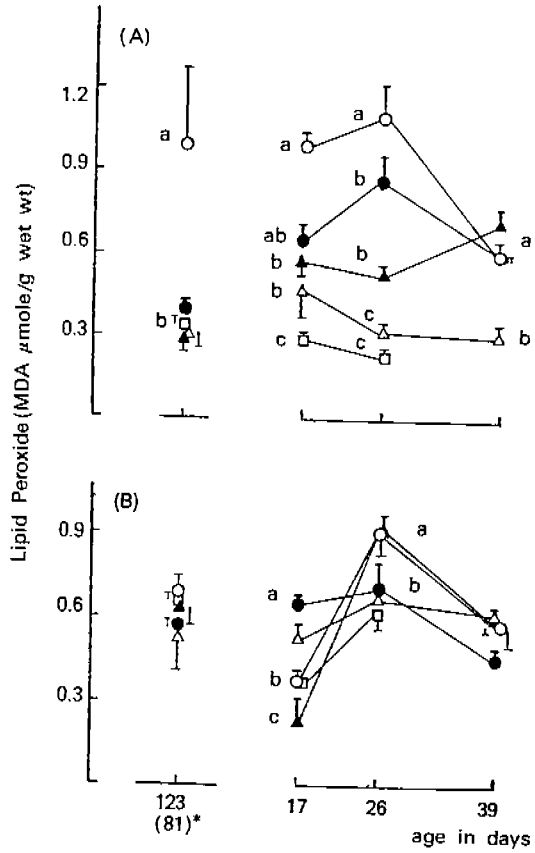

Fig. 1. Lipid Peroxide Contents in(A) Liver and(B) Brain of Rats Fed Different Dietary Fats(Mo —○—, PO —●—, RO —△—, SO —▲—, BT —□—) as Function of Age. Values for the same age groups not sharing common letters are significantly different at the $p<0.05$.
*Number in the parenthesis represents days of dietary treatment of mothes rats.

all groups were virtually the same. Those of their progeny were somewhat different each other, but there was no consistent trend. However, an overall tendency was that weanling decreased the peroxide level in both liver and brain.

α-Tocopherol levels, on the other hand, were the lowest in serum and liver of mother rats fed MO. In their progeny, the result was same in liver but not in serum. Liver level in MO group maintained low up to 39 days. Changing pattern

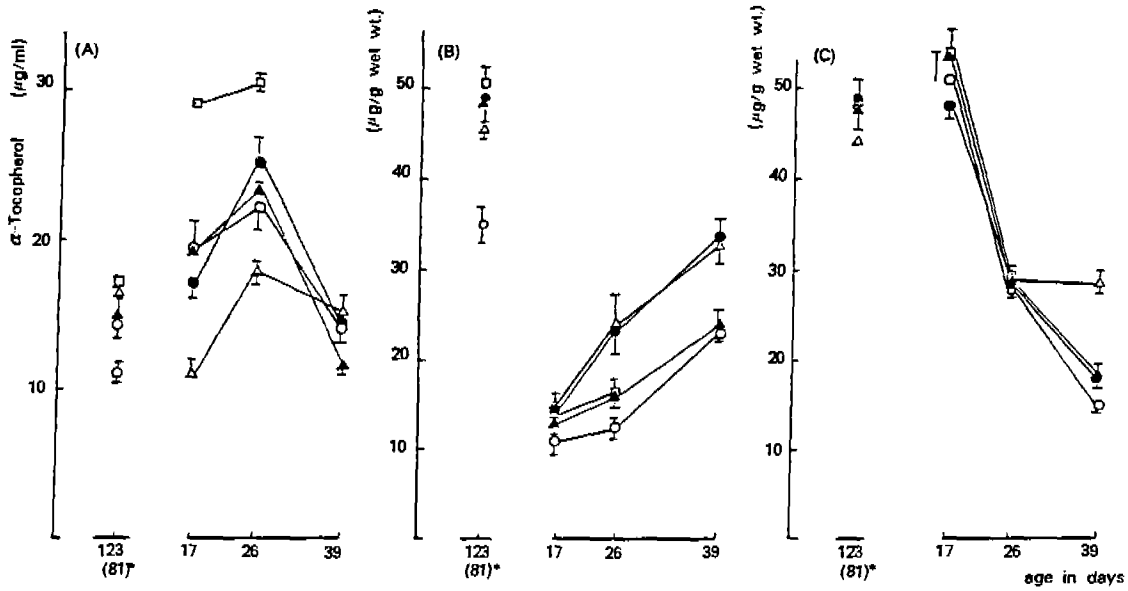

Fig. 2. Contents of α-Tocopherol in (A) Serum, (B) Liver and (C) Brain of Rats Fed Different Dietary Fats (MO —○—, PO —●—, RO —△—, SO —▲—, BT —□—) as Function of Age.
Conditions are same as described in Fig. 1.

during young ages of serum and liver in the present study, coincided well with those of other's[21]. Discrepancy found in brain level may be partly due to units calculated (μ /g wet wt.vs μg/DNA[21]). In accordance with α-tocopherol level, liver GSH content was lower in MO fed mother rats, while GSSG level was not different from the rest groups. In pups, GSSG levels became apparently higher and GSH levels lower in MO and PO groups than SO group at 39 days of age.

Activities of Glutathione Peroxidase and Superoxide Dismutase

Glutathione peroxidase activities in both liver and brain were not affected in mother rats by alterations in dietary fats, but in their progeny, were lowest in RO group (Fig. 4). The activity increased rapidly in liver and steadily in brain until 39 days of age, except in RO group. It can not be, however, speculated here whether or not the liver activity would increase further after 39 days, although the selenium-dependent enzyme activity has been reported to increase in rat heart mitochondria in old age[26]. Liver superoxide dismutase activity was highest in RO fed mother rats and their pups of 17 days old, but as pups grew, difference by dietary fat disappeared (Fig. 5). There was no significant change in brain enzyme activity either by age or by the type of dietary fat. To examine closely the involvement of these enzymes in prevention of accumulation of lipid peroxide, we have carried out regression analysis with the enzyme activities and the level of lipid peroxide. Positive correlations (Fig. 6) were found between liver lipid peroxide levels and glutathione activities in 26 and 39 days old pups.

DISCUSSIONS

Underlying causes for the high lipid peroxide

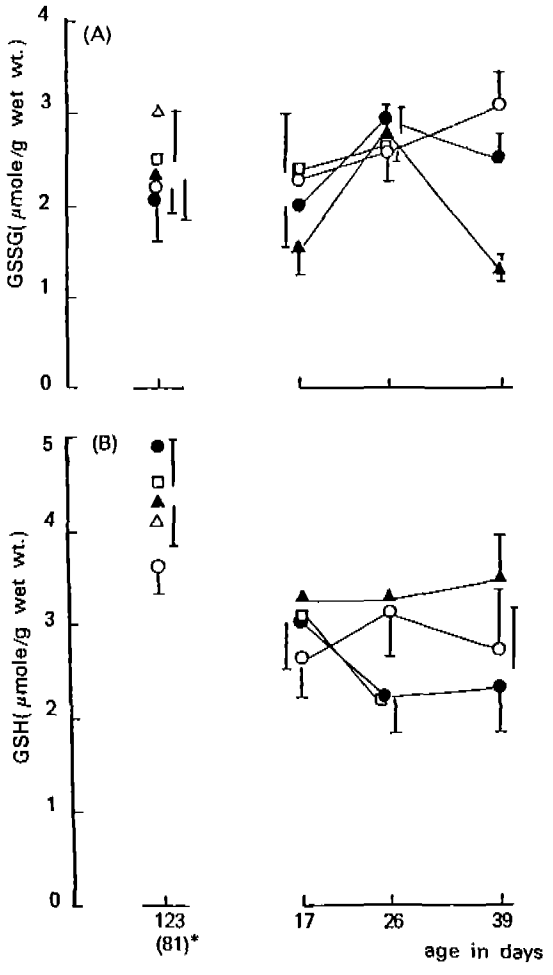

Fig. 3. Levels of (A) Oxidized and (B) Reduced Glutathione in Livers of Rats Fed Different Dietary Fats(MO –○–, PO –●–, RO –△–, SO –▲–, BT –□–) as Function of Age.
Conditions are same as described in Fig. 1.

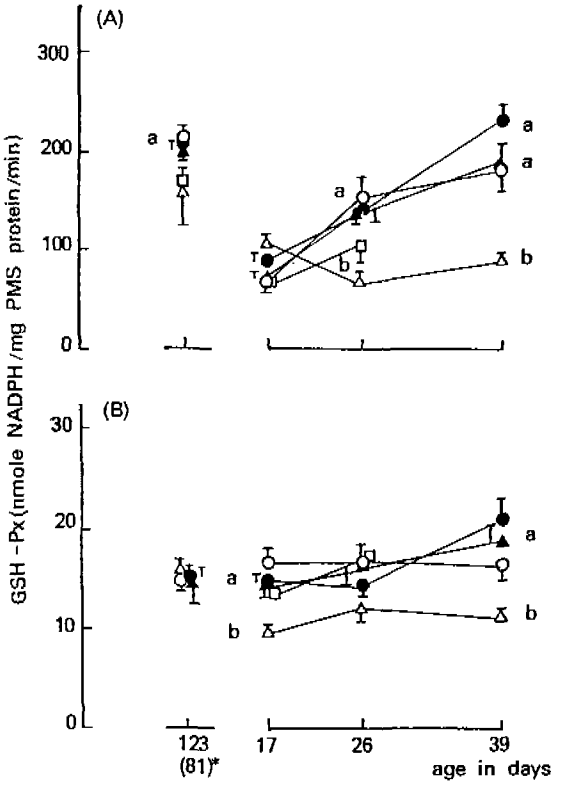

Fig. 4. Glutathione Peroxidase Activities in(A) Liver and(B) Brain of Rats Fed Different Dietary Fats(MO –○–, PO –●–, RO –△–, SO –▲–, BT –□–) as Function of Age.

level in MO group, can be delineated in several possible ways. First one may be high dietary peroxide level of MO (Table 2), but as mentioned earlier, it can not be the only reason due to low tissue level found in group fed BT with same peroxide value. Moreover, it has been reported[27] that liver TBA-reactive substance did not increase in rats fed heated oil of same range of peroxide value and even higher carbonyl value. On the other hand, Iritani et al[28] have reported that TBA-reactive substance increased with heated corn oil feeding, but lipoperoxide was not detected by iodometry. Second one is PUFA contents or P/S ratio of dietary fat, which is a clear explanation for the difference found between MO and BT groups. However, when compared to SO and PO, MO has much lower PUFA content and P/S ratio. At this point, it should be considered that vegetable oils contain approximately 100 mg of total tocopherol per 100g, one tenth of which is α-form[29]. Therefore, provided that other forms of tocopherol than α-type in vegetable oils were all β-form, the next potent one to α-tocopherol, PO and SO diets supplied at most, 100mg α-tocopherol equivalent/Kg diet, while MO and BT diets had

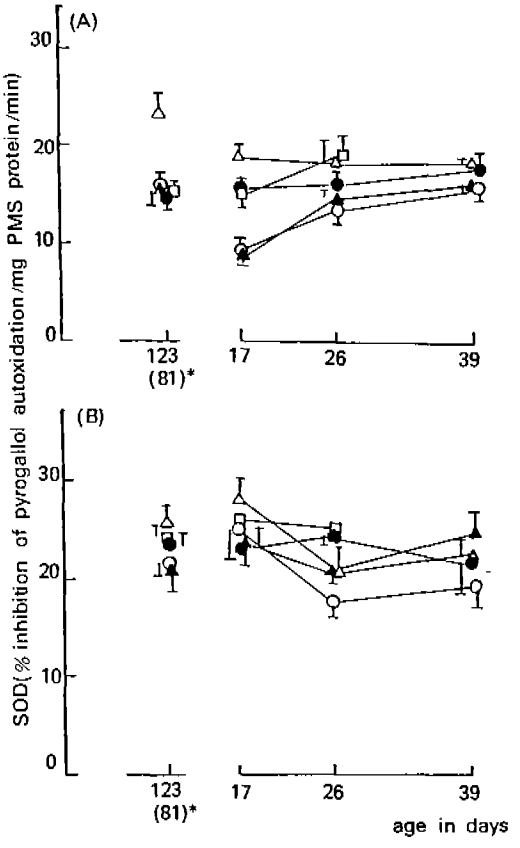

Fig. 5. Superoxide Dismutase Activities in (A) Liver and (B) Brain of Rats Fed Different Dietary Fats (MO —○—, PO —●—, RO —△—, SO —▲—, BT —□—) as Function of Age

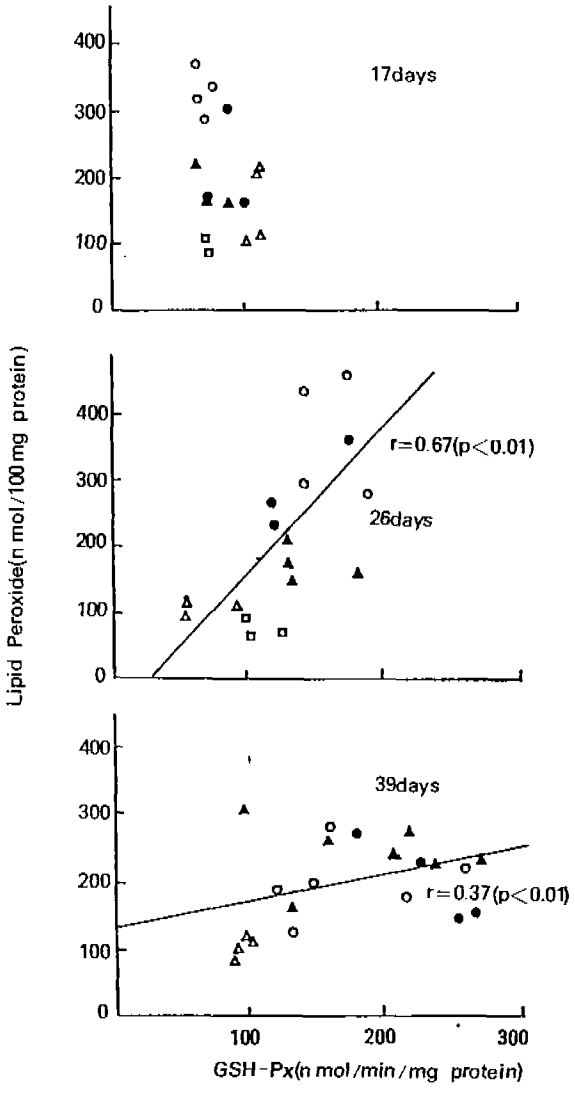

Fig. 6. Lipid Peroxide Content of Postnatal Rat Liver as a Function of Glutathione Peroxidase Activity. Symbols are same as in preovious figures.

100 mg α-tocopherol/Kg diet, which was originated externally from vitamin mix added. When Vit E(mg)/PUFA(g) ratios were calculated, they could not be higher than 2.4 (SO), 1.9(PO) and 8.2(RO). In MO diet it is 3.0, the value that is rather higher than those of SO and PO diet. Therefore, it does not appear to be a little low content of Vit E in MO diet, that caused high lipid peroxidation. Third one is very high degree of unsaturation of C_{20-22} fatty acids, present only in MO, that contributed to a major portion of unsaturation index (Table 3). As suggested in other reports,[9),10)] the suceptability of C_{20-22} fatty acid increased in cellular pool by MO feeding, is believed to be one of main factors causing increased formation of lipid peroxide, although the above two other reasons can not be totally neglected. Regardless of the mechanisms involved, a special attention needs to be paid to high liver peroxide level in MO group. Serum level, although not measured in this study, is reportedly more sensitively changed[31)] and so

would have shown a more significant increase, which seems to be directly related to atherogensis[5)]

In an effort to protect against increased formation of the lipid peroxide, Vit E and GSH in liver were consumed. The Vit E value was also reflected in serum. During lactation, maternal diet has been shown to influence milk composition[32)]. Therefore, C_{20-22} PUFA in milk from mother rats fed MO, is regarded to be a major factor in lipid peroxidation in suckling pups, especially when liver Vit E was not fully accumulated. Less clear effect of MO after weanling, can be ascribed to Vit E accumulation in liver. But another plausible reason is the adaptation that occured via increase in glutathione peroxidase activity in accordance with peroxide level. It is not surprising that it was glutathione peroxidase, not superoxide dismutase, because the former is the enzyme catalyzing directly reduction of lipid peroxide. Besides, superoxide dismutase in heart mitochondria was found to be unchanged with age[25)]. However, since the correlation found in the present study, lessened after weanling, this could be only a transient phenomena. To clarify this, a well designed study is required in the future. Compared to liver results, smaller or no change was observed in brain, most likely due to special blood-brain barrier, maintaining brain cellular homeostasis.

SUMMARY

Lipie peroxide formation, antiperoxidative system and body adaptability for handling lipid peroxide were examined in the first and second generations of rats fed fish oil. Mackerel oil(MO) was used and four other dietary oils and fat, i.e. soybean oil(SO), perilla oil(PO), rapeseed oil(RO) and beef tallow(BT) were also employed to compare the effect of fish oil. Synthetic diets containing these five dietary fats at the level of 10%(w/w), were given to the corresponding groups of male and female rats weighing about 70 grams. After 34 days of feeding, male and female rats were mated and their offsprings were raised throughout suckling (17, 26 days) and weanling (39 days) periods. Liver lipid peroxide level was highest in MO group of both first (mother rats after lactation) and second generations of 17 and 26 days old, but not of 39 days old. During suckling period, liver lipid peroxide level was well matched to total unsaturation of dietary fat. Brain lipid peroxide levels were not different among five groups. Liver α-tocopherol and reduced glutathione (GSH) levels were lowest in MO fed first generation. In second generation, α-tocopherol level was also low in MO group, although the effect was less pronounced, but GSH level was not different from other groups. Oxidized glutathione (GSSG) level did not consistently vary by change in dietary fat. Glutathione peroxidase activity increased as young rats grew up to 39 days. Superoxide dismutase activity change was insignificant by age, but was shown as lowest in MO group. At the age of 26 and 39 days, liver glutatione peroxidase activity was increased as was level of lipid peroxide, suggesting that this is the one of the mechanisms responsible for body adaptability for protection against the accumulation of lipid peroxide.

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