# Physiological Activity of ω3 Polyunsaturated Fatty Acids in Dark Fleshed Fishes

## II. Antioxidative Effect on Lipid Peroxidation in Rats

Jin-Ho CHOI and Dae-Seok BYUN

*Department of Nutrition and Food Science, National Fisheries University of Pusan, Pusan 608-737, Korea*

To compare antioxidant action of ω3 polyunsaturated fatty acid (PUFA) on lipid peroxidation in rats, the formation of malondialdehyde (MDA) and membranes of liver and brain and activities of antioxidant-related enzymes such as catalase, glutathione peroxidase, and superoxide dismutase (SOD) in blood, were studied. Malondialdehyde contents of ω3 PUFA and sardine oil groups were significantly decreased compared with lard group as control ($p<0.05$). Catalase and superoxide dismutase showed higher activities in ω3 PUFA and sardine oil groups than those of lard control group. These findings suggest that fish oil has a inhibitory effect on formation of lipid peroxides in blood and membranes of rats.

## INTRODUCTION

Up to date, numerous theories have been offered to explain the causes and biological changes of aging, but the principal mechanism of aging has not been elucidated because of difficulties in obtaining experimental evidence and related complicated factors.

In 1956, Harman (1) proposed a hypothesis that a major cause of the aging process is deleterious free radical reactions occurring in normal aerobic metabolism. Since these free radicals are known to preferentially attack lipids and lipid molecules in cell membrane structures (2).

In many human diseases, there often occurs membrane damage in some organ or tissue, which provokes lipid peroxidation in the membrane and accelerates the disorder in structure and function of the membranes. When the lipid peroxides formed accumulates to a certain degree, they leak from the organ or tissue into the blood stream and increase the lipid peroxide level in the serum and plasma. Thus, the increased level of lipid peroxide obviously indicates the degree of some membrane damage in cells provoked by diseases (3). For this reason, the blood peroxide level often indicates the severity of diseases.

The presence of a large amount of unsaturated lipids in cells, high susceptibility of these lipids to peroxidation and the toxicity of resultant lipid peroxide make us suggest the possible role of lipid peroxidation in various pathological states. It is known that many works on lipid peroxidation in biological system have primarily focused on various pathological conditions and aging process (4).

Superoxide dismutase (SOD) levels in various diseases are attracting the attention of clinicians. SOD was directly related to the scavenging of free radical such as superoxide anion (5).

Because of the diversity of age-related phenomena, this paper mainly deals with the following two points: The first is to compare the lipid peroxidation in blood, liver and brain by malondialdehyde. The second is to compare the antioxidant-related enzyme activities such as glutathione peroxidase, catalase and superoxide dismutase in blood and liver cytosol.

* This study was supported by the research grant from The Korean Science and Engineering Foundation.

## MATERIALS AND METHODS

Animal and Diets: Male Sprague-Dawley rats (160~180 *g*) were maintained in our animal facility and fed semi-synthetic diets for 6 weeks as previously described (6). The semi-synthetic diets were prepared to be the same calorie level.

Isolation of Serum and Membranes: Serum from blood, mitochondrial and microsomal fractions from liver and brain were isolated according to the previous paper (6). Cytosol fraction was isolated from supernatant which obtained after centrifugation at 105,000×*g* for 60 min to yield microsomal fraction. Purity of mitochondria and microsomes was assessed with the following marker enzymes: glucose-6-phosphatase (7), citrate synthase (8) and NADPH cytochrome C (P 450) reductase (9). Protein was determined by the method of Lowry et al. (10).

Determination of Malondialdehyde(MDA): The MDA contents (n mole/*mg* protein) on lipid peroxidation in serum, mitochondria and microsomes were measured by modification of the TBA-positive material reported by Ohkawa et al. (11) using malonaldehyde bisdiethyl acetal as a standard. 1.5 *ml* of 20% acetic acid, 200 *μl* of 8.1% SDS and 1.0 *ml* of 1.2% TBA were added to 20 *μl* of serum and membranes, and boiled for 30 min. MDA contents by TBA value were measured with spectrophotometer at 532 nm.

Determination of Enzyme Activity: Specific activity (unit/*mg* protein) of enzymes such as glutathione peroxidase, catalase and superoxide dismutase in serum, mitochondria and microsomes were measured by the method of Oyanagui (12), Rigo et al. (13) and Hafeman et al. (14), respectively.

Data were statistically analyzed by Student t-test.

## RESULTS

Effects of Dietary Lipid on Lipid Peroxidation: All lipid peroxides produced from blood and membranes in liver, kidney, brain and lung undergo reaction with malondialdehyde (15). Malondialdehyde also has been used as the most popular marker for lipid peroxidation.

Table 1 shows the changes in MDA contents in serum, liver mitochondria and microsomes, and brain homogenate of rats. MDA contents of ω3 PUFA and sardine oil groups in liver microsome were remarkably decreased to 27.3% and 22.4%, and followed by perilla oil group (13.3%) compared with control group. Similar data also were obtained in liver mitochondria. MDA contents of ω3 PUFA and sardine oil groups in liver mitochondria also were decreased to 23.6% and 19.0% compared with control group ($p<0.05$), whereas MDA contents of plant oil group such as perilla and corn oils showed a slight decreasing effect compared with control group. MDA contents in brain homogenates were decreased in order of ω3 PUFA (30.3%), squalene (24.5%), sardine oil (13.8%) and perilla oil (8.3%) compared with control group. It is interested that fish oil such as ω3 PUFA and sardine oil is more effective than plant oil and/or animal oil for inhibitory effects on lipid peroxidation in liver and brain of rats.

From the inhibitory effects on lipid peroxidation in serum, fish and plant oil groups were more effective than control group ($p<0.05$~$0.001$). But there is no significant difference between fish and plant oils.

Effect of Dietary Lipid on Enzyme Activity: It is known that glutathione reductase activity may be related to the redox state of GSH and NADP, and the effects on the concentration of free CoA which has a possible action on ketogenesis and lipogenesis (16).

As shown in Table 2, catalase activity (%) of perilla oil group in liver cytosol was the highest (32.7%), and followed by ω3 PUFA (20.1%) and sardine oil (15.4%) compared with control group (0.05~0.001). It is observed that catalase activities of fish oil and plant oil groups were higher than that of control group. Similar data on glutathione peroxidase activity also were obtained in liver cytosol of rats (Data not shown). This is in accord with earlier report that GSH peroxidase activity increased in tissues of rats fed with diet containing 15.7% of codliver oil (17).

Table 1. Changes in malondialdehyde contents in blood, liver and brain

(n mole/*mg* protein)

| Diet group | Liver | | Brain | Blood |
|---|---|---|---|---|
| | Microsome | Mitochondria | Homogenate | Serum* |
| Lard (control) | 2.86± 0.38 | 2.42± 0.35 | 6.04± 0.42 | 4.74± 0.52 |
| Corn oil | 2.52± 0.56 | 2.36± 0.20 | 7.21± 0.56 | 3.82± 0.38b |
| Perilla oil | 2.48± 0.28 | 2.26± 0.16 | 5.54± 0.26c | 3.03± 0.42a |
| Sardine oil | 2.22± 0.26c | 1.96± 0.12b | 5.25± 0.23b | 2.99± 0.45a |
| Squalene | 2.72± 0.36 | 2.32± 0.24 | 4.56± 0.32a | 3.43± 0.40b |
| ω3 PUFA | 2.08± 0.22c | 1.85± 0.25b | 4.21± 0.18a | 3.62± 0.32b |

* MDA content: n mole/*ml* serum. a: $p<0.001$, b: $p<0.05$, c: $p<0.01$.

Table 2. Changes in catalase activity of liver cytosol

| Diet group | Catalase (unit/*mg* protein) | % Activity |
|---|---|---|
| Lard (control) | 155.54± 18.20 | — |
| Corn oil | 171.50± 16.82c | 10.3 |
| Perilla oil | 206.40± 19.56a | 32.7 |
| Sardine oil | 179.45± 16.82b | 15.4 |
| Squalene | 165.03± 10.20 | 6.1 |
| ω3 PUFA | 186.77± 12.62b | 20.1 |

a: $p<0.001$, b: $p<0.05$, c: $p<0.01$

Table 3 shows the changes in superoxide dismutase (SOD) activities in serum and liver cytosol of rats. SOD activities (%) of ω3 PUFA, sardine and perilla oil groups in serum were increased to 18.9 %～29.8% compared with control group ($p<0.05$～0.001). SOD activities in liver cytosol showed almost same the trend as in serum; SOD activities (%) of ω3 PUFA, sardine and perilla oil groups in liver cytosol were increased to 15.6%～20.4% compared with control group ($p<0.05$).

Table 3. Changes in superoxide dismutase activity in serum and liver cytosol

| Diet group | Serum (unit/*ml*) | % Activity increased | Liver cytosol (unit/*mg* protein) | % Activity increased |
|---|---|---|---|---|
| Lard (control) | 187.0± 26.0 | — | 14.1± 2.2 | — |
| Corn oil | 198.1± 18.2 | 5.8 | 16.1± 1.9 | 13.9 |
| Perilla oil | 242.3± 23.5a | 29.2 | 16.8± 1.8b | 19.2 |
| Sardine oil | 230.1± 15.7b | 23.2 | 17.0± 2.7b | 20.4 |
| Squalene | 192.2± 16.8 | 2.8 | 14.5± 2.3 | 3.3 |
| ω3 PUFA | 222.2± 15.4b | 18.9 | 16.3± 1.6b | 15.6 |

a: $p<0.001$, b: $p<0.05$.

## DISCUSSIONS

Numerous studies on lipid peroxidation in biological system have primarily been focused on various physiological conditions and aging processes. Aging studies so far published suggested that aging processes were closely connected with lipid peroxidation in membranes. Malondialdehyde (MDA) formation in cell membranes has been held positive position as very important marker for lipid peroxidaiton.

The addition of vitamin E as antioxidant affected the hemolysis in red blood cell, and malondialdehyde productions in liver homogenates(18). To reduce the effect of antioxidant on lipid peroxidation, we examined the effect on formation of lipid peroxides in absence of antioxidant.

In both mitochondria and microsomes, MDA contents of fish oil groups such as ω3 PUFA and sardine oil were consistently lower than those of plant oil groups such as corn oil and perilla oil and/or animal oil control group ($p<0.05$). MDA contents in brain homogenates of fish oil groups including squlalene were decreased to 13.8%～30.3%

compared with control group ($p<0.05\sim0.005$). But in serum, MDA contents of fish oil and plant oil groups were consistently lower than that of control group ($p<0.05\sim0.001$).

It was found that ω3 PUFA group was the most effective for inhibition on lipid peroxidation in mitochondria and microsome of liver and brain homogenates. Therefore, ω3 PUFA such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) may be related to inhibitory action on formation of lipid peroxides in liver and brain.

MDA contents of fish oil and plant oil groups in serum were consistently lower than that of control group. Plant oil as well as fish oil may be possibly related to inhibition of lipid peroxidation in serum. Therefore, new finding in our study is that fish oil has a strong inhibitory effect on formation of lipid peroxides in cell membranes. There is a possibility that ω3 PUFA in diets directly might affect the decrease of lipid peroxidation in serum and membranes. It is known that polyunsaturated fatty acid is converted into lipid peroxidation of hydroperoxide and/or endoperoxide by lipooxigenase and cyclooxigenase, and then prostaglandins and thromboxane are produced from endoperoxide by their synthetase. Therefore, another possibility may be speculated that the decrease of lipid peroxidation affected by formation of prostaglandins and thromboxane (19~21). It is also believed that the lower MDA content by fish oil may be related to anti-aging effects in cell membranes.

It is observed that catalase activity in liver cytosol of perilla oil group was the highest ($p<0.001$), and followed by sardine oil and ω3 PUFA groups ($p<0.05$). Similar data also were obtained for glutathione peroxidase activity in liver cytosol. In serum, SOD activity of perilla group was the highest ($p<0.001$), and followed by those of sardine oil and ω3 PUFA groups ($p<0.05$). On the other hand, in liver cytosol, SOD activity of sardine oil group was the highest, and followed by perilla oil and ω3 PUFA groups ($p<0.05$).

Therefore, it could not see that antioxidant-related enzyme activities such as glutathione peroxidase, catalase and SOD are exactlies in accord with the changes in lipid peroxide contents formed in serum, and membranes of liver and brain.

## REFERENCES


1. Harman, D. 1956. Aging: A theory based on free radical and radiation chemistry. J. Gerontol. 11, 298~300.
2. Del Maestro, R. F., Thaw, H. H., Bjork, J., Planker, M. and Arfors, K-E. 1980. Free radicals and mediators of tissue injury. Acta Physiol Scand(Suppl), 492, 43~57.
3. Yagi, K. 1987. Lipid peroxides and human diseases. Chemistry and Physics of Lipids. 45, 337~351.
4. Hirai, S., Okamoto, K. and Morimatsu, M. 1982. Lipid peroxides in biology and medicine. Academic Press Inc. (N. Y.) 305~315.
5. Marklund, S. 1976. Spectrophotometric study of spontaneous disproportion of superoxide anion radical and sensitive direct assay for SOD. J. Biol. Chem. 251(23), 7504~7510.
6. Choi, J. H. and Byun, D. S. 1989. Studies on physiological activity of ω3 Polyunsaturated fatty acid in dark freshed fishes I. The effects of ω3 polyunsaturated fatty acid on cholesterol levels in rats. Bull. Korean Fish. Soc. 22(2), 102~108.
7. Wills, E. D. 1971. Effects of lipid peroxidation on membrane-bound enzymes of the endoplasmic reaction. Biochem. J. 123, 983~991.
8. Shepherd, D. and Garland, P. B. 1966. ATP controlled acetoacetate and citrate synthesis by rat liver mitochondria oxidizing palmitoylcarnitine, and the inhibition of citrate synthase by ATP. Biochem. Biophys. Res. Commun. 22(1), 89~93.
9. Phillips, A. H. and Landon, R. G. 1962. Hepatic triphosphopyridine nucleotide-cytochrome C reductase: Isolation, characterization and kinetic studies. J. Biol. Chem. 237(8), 2652~2660.
10. Lowry, O. H., Rosebrough, N. J., Farr, A. L. and Randall, R. J. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193, 265~275.
11. Ohkawa, H., Ohishi, N. and Yasi, K. 1979. Assay for lipid peroxides in animal tissues by thiobarbituric acid reaction. Anal. Biochem. 95, 351~358.
12. Oyanagui, Y. 1984. Reevaluation of assay me-

thods and establishment of kit for superoxide dismutase activity. Anal. Biochem. 142, 290~296.

13. Rigo, A. and Rotilio, G. 1977. Simultaneous determination of superoxide dismutase and catalase in biological materials by polarography. Anal. Biochem. 81, 159~166.
14. Hafeman, D. G., Sunde, R. A. and Hoekstra, W. G. 1974. Effect of dietary selenium on erythrocyte and liver glutathione peroxidase in the rats. J. Nutr. 104, 580~587.
15. Falkay, G., Herczeg, J. and Sas, M. 1971. Microsomal lipid peroxidation in human pregnant uterus. Biochem. Biophys. Res. Commun. 79(3) 843~851.
16. Pinto, R. E. and Bartley, W. 1969. The effect of age and sex glutathione reductase and glutathione peroxidase activities and aerobic glutathione oxidation. Biochem. J. 112, 109~115.
17. Chow, C. K., Reddy, K. and Tappel, A. A. 1972. Effect of dietary vitamin E on the activities of the glutathione peroxidase system in rat tissues. Lipids 7, 618~624.
18. Buckingham, K. W. 1985. Effect of dietary polyunsaturated/saturated fatty acid ratio and dietary vitamin E on lipid peroxidation in the rat. J. Nutr. 115, 1425~1435.
19. Choi, J. H. and Yu, B. P. 1988. Antioxidant action of food restriction on membrane alteration of aged kidney mitochondria and microsomes. 72nd Annual Federation of American Societies for Experimental Biology (FASEB) Meeting(May 1~5), Abstracts 5,300.
20. Murota, S. I., Okugi, M. and Morita, I. 1982. Prostaglandins and liver aging. Liver and Aging (Elscvier Biomedical Press), 231~244.
21. Chang, W. C. and Tai, H. H. 1984. Changes in prostacyclin and thromboxane biosynthesis and their catabolic enzyme activity in kidneys of aging rats. Life Sciences, 34, 1269~1280.

Received March 20, 1989
Accepted April 19, 1989

# ω3 고도불포화지방산의 생리활성에 관한 연구

## II. 과산화지질에 대한 항산화 작용

최진호 · 변대석
부산대학교 식품영양학과

요 약

ω3 고도불포화지방산의 투여가 생체내의 과산화지질 생성 및 항산화관련 효소들의 활성에 미치는 영향을 비교하기 위하여 어유에서 분리한 ω3 고도불포화지방산, 식물유 및 동물성 지방(대조군)을 10%가 되도록 첨가한 사료로써 실험동물에 6주간 투여하였다.

혈청, 뇌 및 간장에서의 과산화지질 생성은 ω3 고도불포화지방산과 정어리 기름 투여군이 대조군에 비해 유의성 있게 감소하였다. 카탈라아제 및 슈퍼옥사이드 디스뮤타제의 항산화활성은 이들 어유 투여군이 대조군에 비해 15%~20% 정도 높음을 알 수 있었다.

따라서 ω3 고도불포화지방산을 포함한 어유는 과산화지질 생성을 효과적으로 억제하고 또 노화관련 효소들의 활성을 증가시켜주므로써 생체의 노화 방지에 효과가 있을 것으로 추정된다.