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Effect of Dietary Fish Oil on Lipid Peroxidation and Antiperoxidative System in Rat Liver and Brain

—Sex-related Differences—

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魚油섭취가 흰쥐의 간과 뇌조직의 지질과산화물 형성과 항산화계에 미치는 영향

—性의 차이를 중심으로—

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요 약

魚油에 포함된 C20~22(ω3) 지방산을 섭취하였을 때, 간과 뇌조직의 지질과산화물 형성 및 항산화기능을 조사하기 위하여, 고등어유를 식이함량에 10%(w/w)되게 조제한 사료를, 70g내외의 암, 수컷의 쥐에게 주어 3개월간 사육하였다. 이 결과를 ω3지방, ω6지방, ω9지방 및 포화지방과 비교하기 위하여, 다른 4군의 암, 수컷의 쥐들에게 들기름, 대두유, 채종유 및 쇠기름을 각각 첨가한 조제식이를 주어 같은 기간동안 사육하였다. 간조직의 지질과산화물 농도가 고등어유군에서 현저히 높은 반면, α-tocopherol과 환원형 glutathione (GSH)의 양은 낮았다. 이 현상은 암컷에서가 숫컷에서보다 분명히 드러났고, 고등어유군 외의 타군간의 차이는 숫컷에서 약간 보여졌으나 일관성은 없었다. 모든 식이군의 간조직 GSH농도는 숫컷에서가 암컷에서보다 낮았으나 산화형 glutathione (GSSG) 농도는 식이나 성에 따른 차이가 없었다. 뇌조직에서는 식이지방에 따른 지질과산화물과 α-tocopherol 농도의 차이가 발견되지 않았다.

간과 뇌의 양 조직에서 glutathione peroxidase나 superoxide dismutase의 활성이 식이에 따라 변화는 없었으나 glutathione peroxidase의 활성은 숫컷에서가 암컷에서보다 일관성있게 낮았다. 따라서 이 연구에서는 性에 따른 항산화 기능에 차이가 있다는 것을 밝혔으며, 魚油를 통하여 C20 이상의 ω3 다불포화지방산를 섭취할 때, 총 불포화도가 유사하거나 높은 C18:2(ω6)나 C18:3(ω3) 지방산 섭취시보다 체내에서 많은 지질과산화물을 형성하여, 그에 따른 항산화물질의 소모가 크다는 것이 주목된다고 하겠다.

Abstract

In order to examine the effect of dietary fish oil on lipid peroxide formation and antiperoxidative efficiency in liver and brain, a group of male and female rats weighing about 70 grams were fed for three months, diet containing mackerel oil(MO) at the level of 10% (w/w). Results were compared, according to sex and source of dietary fat, i.e., in addition to MO, perilla oil(PO), soybean oil(SO), rapeseed oil(RO) or beef tallow(BT). Liver lipid peroxide level was significantly higher and levels of α-tocopherol and reduced glutathione (GSH) were lower in MO group than in other groups. This phenomenon was less clear in male than in female. Liver GSH level was lower in male, compared to female, but oxidized glutathione (GSSG) level did not vary, depending on either sex or dietary fat source. Brain lipid peroxide and α-tocopherol levels were not different among five experimental groups. Activities of liver and brain glutathione peroxidase and superoxide dismutase were not changed by dietary fat source, but glutathione peroxidase activity was higher in female than in male.

The present study shows (a) that there is sex-related difference in antiperoxidative activity and (b) that fish oil containing $C_{20-22}(\omega 3)$ fatty acids, increases body lipid peroxide level and consumes more of cellular antioxidant, although it has lower total PUFA content than perilla or soybean oils.

Introduction

In recent years, there has been an increasing interest in biological effects of dietary $\omega 3$ polyunsaturated fatty acids(PUFA), which are supplied mainly from fish and marine products, because they have been assumed to have specific physiological roles[1]. The initial recognition of the role of $\omega 3$ fatty acid, came from studies on Greenland Eskimo,[2,3] who showed very little evidence of cardiovascular disease, although they ate a diet high in fat and animal protein. Main sources of their food were fish and marine animal. These finding has stimulated many further studies on the effect of $\omega 3$ fatty acid, providing evidences of hypotriglyceridemic effect,[4,5] somewhat lesser hypocholesteremic effect,[4] inhibition of platelet aggregation,[5,6] and retarding tumorigenesis.[7,8] The expected preventive role of $\omega 3$ fatty acid in the development of chronic diseases has been mostly correlated with metabolism of prostaglandins and thromboxane.[3,5,9-11] Alterations of membrane lipid composition[5,6,12-15] also appears to be one of the fundamental mechanisms by which $\omega 3$ fatty acids act in the cell, because they not only comprise large proportion of fatty acid tail of membrane phospholipid, but also incorporate into membrane lipid well.

Benefit of dietary $\omega 3$ PUFA is, however, likely to be counterbalanced by the possible harmful side effect, due to very high degree of unsaturation of the fatty acids, which easily lead the formation of various cellular peroxidation products,[16] that are related to aging and development of a few types of degenerative diseases. Neverthless, there have been strikingly a small number of studies done on cellular lipid peroxidation of $\omega 3$ PUFA diet. Kobatake et al[17,18] have shown the significant rise in lipid peroxide levels in serum and liver in rats fed $\omega 3$ fatty acid concentrates, compared to the groups fed ethyl-linoleate. These results appears to be in accordance with an increase in urinary malondialdehyde in rats fed cod liver oil.[19] Lipid peroxidation in animal tissue is considered to be identical, in regard of kinetics and mechanism, with the in vitro

autoxidation process of unsaturated fats, but lipid peroxide levels are influenced by variety of cellular defense mechanisms against oxidative damage. The enzymatic protection against superoxide anion radical (O_2^-) is provided by superoxide dismutase (SOD). It converts O_2^- into H_2O_2. The further degradation of H_2O_2 into H_2O and O_2 can be catalyzed by catalase. In addition, the Se-dependent glutathione peroxidase (GSH-Px) plays an important role in the degradation of H_2O_2 and organic hydroperoxides, especially in heart tissue where the catalase activity is very low. The Se-independent GSH-Px only metabolizes organic hydroperoxides. The reduced form of glutathione, GSH, serves as the hydrogen doner for both the Se-dependent and Se-independent GSH-Px. This leads to interconversion of GSH into its oxidized form, GSSG. GSSG is reduced to GSH again by glutathione reductase (GSH-Rd), using NADPH as a cofactor. In addition, GSH-dependent cytosolic and microsomal factors were reported which protect against lipid peroxidation.[20,21] A major contributant to the non-enzymatic protection against lipid peroxidation is vitamin E, a lipid-soluble antioxidant that can also trap oxygen radicals.[22,23] Vitamin E requirement has been related to the dietary intake of PUFA.[24] But the majority of study has used PUFA of vegetable origin. When fish oils were used, their effects were hardly compared with those of vegetable oils.

Therefore, in the present study, we aimed to investigate the effect of long chain $\omega 3$ fatty acid in fish oil on the activities of SOD and GSH-Px in liver and brain as well as tissue levels of lipid peroxide, α-tocopherol and glutathione. A special interest was focused on the comparison of the effect with those of high PUFA dietary oils(soybean and perilla oils) and of low PUFA fats(beef tallow and rapeseed oil). Sex-related difference was also examined.

Materials and Methods

Materials

Soybean oil, rapeseed oil, perilla oil and beef tallow were purchased from nearby market. Mackerel oil was prepared as described previously,[14] from fresh fishes obtained from market. Salt mix(Rogers and Harper's[25]) 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, inositiol and methionine were reagent 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

Both male and female Sprague-Dawley rats weighing 65~75g were obtained from Kyung Pook National University, Medical School. The rats of each sex were divided into five groups with five to eight rats per group and fed diets containing 10%(w/w) experimental fats. Dietary oils and fat used were mackerel oil(MO), soybean oil(SO), beef tallow(BT), perilla oil (PO) and rapeseed oil(RO), the fatty acid compositions of which were measured by gas chromatography described previously.[14] Rats were fed ad libitum for three months. Each diet prepared according to the composition shown in Table 1, was kept in refrigerator and given to rats each day freshly.

Biologicai Procedures

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

Table 1. Composition of diet

Component	Content (g/100g)	Component	Content (g/g100)
Starch	35.5	Fat	10.0
Glucose	18.8	Mineral Supplement[3]	0.08
Casein	24.4	Choline	0.05
Vitamin Mix[1]	2.0	Inositiol	0.05
Salt Mix[2]	4.5	Methionine	0.22
Cellulose	3.9		

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, 100*mg*, ascorbic acid, 0.9*g*, riboflavin, 20*mg*, thiamin HCl, 20*mg*, i-inositol, 100 *mg*, choling chlroride, 1.5*g*, menadione, 45*mg*, p-aminobenzoic acid, 100*mg*, niacin, 90*mg*, pyridoxine-HCl, 20*mg*, Ca-pantothenate, 60*mg*, biotin, 0.4*mg*, folic acid, 1.8*mg*, vitamin B_{12}, 27μg.

2) Salt mixture used had composition of Rogers and Harper's.[25]

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

and homogenized in 9*mℓ* of ice-cold 1.15% KCl by using Teflon Potter-Elvehjem homogenizer. This homogenate was kept frozen at-60℃ 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 0.25M sucrose/0.1 mM EDTA. Postmitochondrial supernatants (PMS) of liver and brain, prepared from this homogenate by successive centrifugations described before[26] 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℃ and kept at −60℃. 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.

Analytical Procedures

The quality of dietary fat and oils was checked before diet preparation by measuring peroxide value, carbonyl value and iodine value according to AOAC Official Methods.[27] Lipid peroxide in the homogenates of liver and brain was determined by the method of Ohkawa et al[28] and α-tocopherol content was measured according to Lee et al.[29] Glutathione content was determined as described by Bernt and Bergmeyer.[30] Activity of glutathione peroxidase[31] was monitored spectrophotometrically 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 cumeme 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,[32] 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.

Results and Discussions

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 iodine value (Table 2) and unsaturation index (Table 3) of beef tallow, it seems unlikely and so freshness of tallow was suspected. However, we dicided to use it because peroxide value was a little over the level (7 meg/*kg*) set for quality assuarance but carbonyl value was under the regulation level of 6.0 meg/*kg*. Besides, peroxide value of mackerel oil was

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

	Mackerel Oil	Soybean Oil	Beef Tallow	Perilla Oil	Repeseed 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					2.7
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.

** $\sum_{n=1}^{m} nx$ %(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.

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 acsribed only to dietary level of peroxide per se. Total PUFA amounts, P/S retios were calculated from fatty acid composition. Unsaturation indice[33] were well agreeable to iodine values measured.

Effect on Growth

Body weight gain was significantly fast in male rats whose average weight reached to 292±20 g, whereas that of female rats was only 192±15 g at the 7th week of feeding. After then, weight gains were slowed down, so that male weighed 320±20 g and female 230±20 g on the final day of four month feeding. There was no significant difference due to dietary fat variation.

Effect in Liver

Table 4 shows the contents of lipid peroxides and factors involved in protection against lipid peroxidation in liver. Contents of lipid peroxide was markedly high in MO fed female rats, compared to other dietary groups. This phenomenon appears to be true in male rats, but not as definite as in female, due to large variations in determined values. Since the effect of dietary PUFA on lipid peroxide level has been shown to be much more pronounced in serum than in liver.[17] the effect in male might have been shown distinctively in serum. At the same time, contents of α-tocopherol and the reduced form of glutathione (GSH) were low in MO group, although this also applies to only female, as far as GSH level was concerned. There was a significant sex difference in the level of GSH, but not in α-tocopherol, which is opposite to the reports from Julicher et al.[34] This discrepancy seems

Table 4. The contents of lipid peroxide, α-tocopherol, glutathione and the activities of glutahione peroxidase and superoxide dismutase in liver of rats fed different dietary fats

		Mackerel Oil	Soybean Oil	Beef Tallow	Perilla Oil	Repeseed Oil
Lipid peroxide (MDA nmoles/g)	F	984±267^{a}	282±75^{b}	342±93^{b}	387±30^{b}	300±102^{b}
	M	813±201^{a}	444±93^{b}	507±210^{ab}	660±105^{ab}	507±81^{ab}
α-Tocopherol (μg/g)	F	24.6±4.4^{a}	49.2±4.2^{b}	50.7±2.4^{b}	49.3±1.3^{b}	45.9±2.5^{b}
	M	23.6±5.0^{a}	32.9±2.4^{b}	76.8±1.5^{c}*	61.2±2.8^{b}*	38.5±1.3^{b}
GSH(μmoles/g)	F	3.65±0.33^{a}	4.33±0.85^{b}	4.54±0.70^{b}	4.94±0.31^{b}	4.14±0/71^{b}
	M	2.60±0.55^{a}*	1.26±0.11^{b}*	2.98±0.80^{a}*	3.49±0.40^{a}*	3.28±0.39^{a}*
GSSG(μmoles/g)	F	2.20±0.37	2.30±0.58	2.55±0.70	2.10±0.63	3.63±0.75
	M	3.86±0.45*	2.99±1.06	3.45±0.94	3.11±0.91	3.96±0.31
GSH-Px (nmoles NADPH/mg PMS protein/min)	F	213±11	196±12	171±25	202±10	159±58
	M	146±30*	149±13*	157±17	172±13*	150±11
SOD(% inhibition of pyrogallol autoxidation/mg PMS protein/min)	F	15.6±1.2	15.4±2.0	14.8±0.5	14.2±0.7	22.7±7.1
	M	15.4±0.7	17.2±0.6	17.2±1.5	15.2±0.1	13.5±1.7*

Within a row, values not sharing common superscript letters are significantly different at the $p<0.05$.
* $p<0.05$, male vs female.

mainly due to difference in animal strain(Sprague Dawley vs. LOU-M). Besides, age and diet regimen could be also responsibie. It is well known that body Vit E level responds to dietary intakes of both the vitamin and PUFA. MO diet with negligible amount of tocopherol from MO itself, had 100*mg* α-tocopherol per 100*g* diet, supplied from vitamin mix(Table 1), while vegetable oil diets were expected to have additional 10*mg* α-tocopherol equivalent from oils. But considering the amount PUFA in dietary oils(Table 3), ratios of Vit E(*mg*)/PUFA(*g*) in diets, are 2.4 in SO, 1.9 in PO and 3.0 in MO, all of which are well above the suggested ratio of 0.6.[24] Buckingham[35] has reported that Vit E at 40 I.U./*kg* diet was adequate for maximum inhibition of lipid peroxidation at the P/S ratios of 0.38 to 2.30, the range that P/S ratio(Table 3) of MO in this study, resided in. Lee *et al.* have also reported that 100*mg* Vit E/*kg* diet for chicken[36] and 400*mg* Vit E/*kg* diet for rat[37,38] were sufficient not to cause nutritional problems in animals fed 10% perilla oil in diet. They did not test the level of 110*mg* Vit E/*kg* diet, that we could not find inadequate in PO diet of the present study. Therefore, high peroxide and low Vit E levels in MO were likely to result from the susceptibility of C_{20-22} ω3 PUFA in MO to peroxidation, although dietary peroxide could have been partly responsible. We think it's important to call an attention to setting the level of dietary antioxidant, especially when large amount of fish or fish oil concentrates are taken, since current giudelines according to Vit E/PUFA or P/S ratio, do not appear appropriate to this condition.

Activities of glutathione peroxidase(GSH-Px) and superoxide dismutase(SOD) were not influenced by dietary fat manipulation. Selenium is a well known dietary factor affecting body GSH-Px activity directly. Recently, Menken *et al*[40] have reported that the enzyme activity increased with age in the heart tissue of Vit E deficient mice. Since tissue Vit E status has been found as related to amount and type of PUFA,[24,36~39] it is of interest to pursue the effect of longer term feeding with varying amount of Vit E and PUFA. GSH-Px activity was almost consistantly higher in female than in male rats, while SOD activity was not different. This is in agreement with Julicher et al.[34] The higher GSH-Px activity along with higher content GSH content is likely

Table 5. Contents of lipid peroxide, α-tocopherol and the activities of glutathione peroxidase and superoxide dismutase in brain of rats fed different dietary fats

		Mackerel Oil	Soybean Oil	Beef Tallow	Perilla Oil	Rapeseed Oil
Lipid Peroxide (MDA nmoles/*g*)	F	731±115	662±131	699±56	586±77	544±203
	M	597±224	706=163	691+86	666+118	782+34
α-Tocopherol(*μg/g*)	F	49.8+3.5	52.8—2.6	49.6+1.6	50.3+3.4	45.6+4.0
	M	50.3+3.0	48.6+3.2	48.8+0.6	52.1+3.2	47.2+1.8
GSH-Px (nmoles NADPH/*mg* PMS protien/min)	F	14.7+0.4	14.9+2.1	15.2+1.8	15.1+0.5	15.6+0.9
	M	10.8+1.0*	11.1+1.2	10.9+0.8*	13.6+1.1	11.7+1.2*
SOD (% inhibition of pyrogallol autoxidation/*mg* PMS protein/min)	F	21.4+2.2	20.5+3.0	24.0+2.8	23.2+2.2	23.7+3.0
	M	18.5+1.8	18.2+2.3	16.6+5.5	23.7+1.5	18.8+2.1

* $p>0.05$ vs female.

to afford female rats more protection against deteriorative actions of H_2O_2 and peroxidized lipids. But higher catalase activity of male liver[33] might reflect a compensation for low ability of male, although it has not been confirmed due to compartmentalization.[41]

Effects in Brain

Unlike results found in liver, changes in dietary fat did not cause any difference in the levels of lipid peroxide and Vit E. Trend in the enzyme activity changes appeared the same as shown in liver. Brain is the tissue least affected by extrinsic factors to maintain homeostasis. Therefore, it is not surprising that there was no remarkable change in parameters measured under the mild condition we employed. However, sex-related difference in GSH-Px activity was notable and imposes a new perspective on the future study, since it is first reported in the present paper.

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