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Influence of Dietary n3 Polyunsaturated Fatty Acids on Plasma Lipid-Lowering Effect and Peroxidation Level in Rats*

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ABSTRACT

To compare the hypolipidemic effect of n6 linoleic acid and n3 α-linolenic acid and n3 eicosapentaenoic acid plus docosahexaenoic acid, male Sprague Dawley rats weighing about 450g were fed the experimental diets for 6 weeks which composed of fat at 15% (W/W) level and were different only in dietary PUFA. Dietary fat was corn oil, perilla oil, and fish oil concentrate as a source of n6 linoleic acid, n3 α-linolenic acid, and n3 eicosapentaenoic acid+docosahexaenoic acid, respectively. Plasma total Chol and HDL-chol levels were significantly-lower in fish oil group than in corn oil and perilla oil groups. Plasma cholesterol lowering effect of PUFA was in the order of n3 EPA+DHA>n3 α-linolenic acid>n6 linoleic acid. Plasma TG was significantly lower in both fish oil and perilla oil groups than in corn oil group. Plasma TG-lowering effect was greater by n3 PUFA (EPA+DHA, α-linolenic acid) than by n6 PUFA(linoleic acid). However, there were no significant effects on lipoprotein pattern, hemolysis, and the levels of tocopherol and malondialdehyde in plasma and RBC by different dietary fat with sufficient tocopherol supplement. Liver superoxide dismutase activity was significantly increased in proportion to the degree of fat unsaturation, thereby resulted in the lower level of MDA in fish oil group.

In conclusion, fish oil and perilla oil rich in n3 PUFA may have important nutritional applications in the prevention and treatment of atherosclerotic disease.

KEY WORDS : n3 polyunsaturated fatty acids · eicosapentaenoic acid-α-linolenic acid · perilla oil · fish oil concentrate

INTRODUCTION

Although the precise causes of coronary heart disease(CHD) are still unknown, it is generally accepted that lipid metabolism, especially physiological factors such as high levels of blood cholesterol (Chol), triglyceride(TG), very low density lipoprotein(VLDL) and low density lipoprotein(LDL) represent a risk factor for the development of that disease, whereas high density lipoprotein (HDL) levels are inversely related to the incidence of that disease[1-7]. Epidemiological studies revealed unsually low incidence of CHD among Greenland Eskimos who traditionally consumed abundant amount of seal, whale and fish, rich in n3 eicosapentaenoic acid(EPA) and docosahexaenoic acid

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(DHA) and low in n6 Polyunsaturated fatty acid (PUFA) and saturated fatty acids(SFA) than Western population[4)5)]. Many studies have shown that large amount of PUFA in diet has a pronounced hypolipidemic effect. Both vegetable oils and fish oils reduced plasma total Chol and LDL-chol levels in a similar manner, but only the fish oil significantly decreased TG and VLDL levels[7-11)]. Traditionally, the apparent consumption of perilla oil is rather high in Korea. Alpha-linolenic acid(n3 PUFA), rich in perilla oil, is metabolically converted to EPA and DHA by desaturation and chain elongation and has been suggested to have some beneficial effects similar to those of EPA and DHA[12)13)], even though it was not studied as much as fish oil. N3 PUFA seemed more effective in lowering plasma lipids than n6 PUFA, but n3 PUFA could result in higher extent of lipid peroxidation because of its higher unsaturation.

Therefore, the present metabolic study was designed to observe any beneficial effect of n3 PUFA of fish oil and perilla oil over n6 PUFA of corn oil on (1) hypolipidemic effect, (2) the change of tocopherol levels and malondialdehyde(MDA) formation as a result of the lipid peroxidation and (3) superoxide dismutase(SOD) activity involved in the protection of cells against damage from lipid peroxidation in rat liver.

Table 1. Composition of basal experimental diet

Ingredient	g/100g diet
Corn starch	56
Casein	20
Oil 1)	15
DL-Methionine	0.3
Salt mixture 2)	3.2
Zinc mixture 3)	0.8
Vitamin mixture 4)	1.0
α-Cellulose	3.7

1) Oil was given by tube feeding at 17.65% (w/w) of basal diet eaten on the previous day which gives final 15g oil per 100 g diet.
2) Hubble Mendel Wakeman Mixture (per 100g) : Calcium carbonate 54.3 ; Magnesium carbonate 2.50 ; Magnesium sulfate $7H_2O$ 1.60 ; Sodium chloride 6.90 ; Potassium chloride 11.20 ; Potassium phosphate monobasic 21.20 ; Ferric phosphate 2.05 ; Potassium iodide 0.008 ; Manganese sulfate.H_2O 0.035 ; Sodium fluoride 0.01 ; Aluminium potassium sulfate 0.017 ; Copper sulfate. $5H_2O$ 0.09
3) Zinc mixture : 1.67g Zn-acetate/kg corn starch
4) Vitamin mixture (per 100g)
Thiamin-HCl 0.04 ; Riboflavin 0.08 ; Pyridoxine-HCl 0.05 ; Ca-panthothenate 0.40 ; Inositol 2.00 ; Menadione 0.04 ; Niacin 0.40 ; Choline dihydrogen citrate 42.38 ; Biotin premix (1%) 0.30 ; Vitamin B_{12} premix (0.2%) 1.00 ; Corn starch 53.27 ; Folic acid 0.04.

MATERIALS AND METHOD

Animals and diets

Male Sprague Dawley rats(37 weeks old weighing average 450g) were randomly divided into 3 groups of sixteen rats each and fed experimental diets ad libitum for 6 weeks. The composition of the basal experimental diet was shown in Table 1. Oil was given twice daily by intragastric way at the level of 17.65% of basal diet eaten on the previous day which gives the final 15 g oil per 100 g diet. Dietary fats were corn oil, perilla oil and fish oil concentrate as a source of n6 linoleic acid, n3 α-linolenic acid and n3 EPA and DHA, respectively. Beef tallow was added to perilla oil diet to give the total amount of saturated fatty acids and monoenoic fatty acid almost constant levels in all dietary groups(Table 2). Because fish oil concentrate was fortified with dl-α-tocopherol (1.2 g/100g oil) to prevent autooxidation, dl-α-tocopherol was added to CO and PO groups.

Biochemical analysis

Fasting blood was collected from inferior vena cava under anesthesia with chloroform. A small amount of blood was drawn into EDTA-containing

Table 2. Fatty acid composition of experimental diet

Fatty Acids	Dietary Groups (g/100 g oil)		
	C O	P O	F O
Linoleic acid	49.3	11.0	–
α-linolenic acid	1.3	39.5	–
EPA+DHA	–	–	37.0
SFA	16.2	22.3	25.4
MFA	32.8	25.7	28.3
PUFA	50.5	50.5	37.0
corn oil 1)	100	–	–
perilla oil 1)	–	66.7	–
beef tallow	–	33.3	–
fish oil 2)	–	–	100

1) Supplemented with 822 mg dl-α-tocopherol.
2) Fish oil concentrate contains EPA 25% and DHA 12%.
EPA : Eicosapentaenoic acid SFA : Saturated fatty acid PUFA : Polyunsaturated fatty acid
DHA : Docosahexaenoic acid MFA : Monounsaturated fatty acid

tube and hemolysis was measured immediately. A remaining blood was treated with sodium citrate to prepare plasma and the red cells were then washed three times with 5 volumes of saline-phosphate buffer (pH 7.4, 0.89% NaCl, 0.01 M Na_2HPO_4, 0.01 M HCl) and were made up to approximately 50% hematocrit. HDL fraction was immediately separated by polyanionic precipitation method of Burnstein[14] and then total Chol in serum and HDL fraction was determined with commercialized enzyme kit T-choles. 5(Dong-A Pharmacy). Liver total Chol was determined in lipid extract by the method of McDougal and Farmer[15] after lipid extracted with Bligh and Dyer method[16]. TG concentration in plasma and liver lipid extract was measured by spectrophotometrical method of Fletcher[17] after phospholipid removed with silicic acid instead of zeolite mixture. The relative percentage of lipoprotein fractions separated on cellulose acetate was determined by densitometer after electrophoresis at 180 volt for 30 min in tris-barbital buffer(pH 8.6-9.0) and stained with Oil Red O[18]. Total tocopherol content in plasma, RBC and liver was determined by the method of Desai[19] in Spectrofluorometer. Lipid peroxidation was indirectly determined by measuring MDA with 1, 1, 3, 1-tetraethoxypropane as standard. MDA level in plasma and RBC was determined by Yagi method[20] and for liver by Buckingham method[21]. Hemolysis was spectrophotometrically measured by the method of Drapper and Csallany[22]. SOD activity was determined spectrophotometrically in liver homogenate by Winterbourn et al method[23] after homogenized by Bannister and Bannister method [24]. SOD activity was expressed as units of SOD per mg of protein. One unit of SOD is defined as the amount of enzyme causing half the maximum inhibition of nitro blue tetrazolium reduction. The mean values of each dietary group in all parameters were compared for a significance at $p \leq 0.05$ level[25].

RESULTS AND DISCUSSION

Plasma cholesterol

As shown in Table 3, plasma Chol levels were significantly lower in rats fed fish oil than in those fed perilla or corn oil, whereas it was not significantly different between corn oil and perilla oil

Table 3. Effect of dietary PUFA on the levels of total cholesterol, HDL-cholesterol and triglyceride in rats

	Dietary Groups		
	C O	P O	F O
Plasma T-chol (mg/dl)	57.7 ± 9.8[a] (16)	58.5 ± 22.48[a] (13)	27.3 ± 7.2[b] (8)
Plasma TG (mg/dl)	139.0 ± 29.3[a] (14)	103.3 ± 30.3[b] (13)	99.8 ± 19.6[b] (8)
Plasma HDL-chol (mg/dl)	41.4 ± 9.1[a] (11)	40.5 ± 6.4[a] (7)	16.8 ± 4.9[b] (4)
Plasma T-lipid (mg/dl)	465.5 ± 63.1[a] (14)	463.6 ± 72.4[a] (13)	389.1 ± 24.1[b] (8)
Liver T-chol (mg/g wet liver)	2.4 ± 0.4 (16)	2.3 ± 0.3 (13)	2.1 ± 0.2 (8)
Liver TG (mg/g wet liver)	15.3 ± 5.2 (15)	14.5 ± 3.8 (12)	13.9 ± 2.1 (8)

Mean± SD
( ) : Number of rats
Superscript a or b : Values with different letter within the row were significantly different at $p \leq 0.05$.

groups. Hartog et al[9] and Hamazaki et al[26] reported that serum Chol was decreased by supplementation of sardine oil and mackerel oil. Sanders and Roshanai[27] observed that MaxEPA effectively decreased serum Chol but linseed oil rich in α-linolenic acid did not decrease serum Chol. It was reported that the cholesterol-lowering effect of PUFA seemed to be a function of their total unsaturation and not to be the PUFA structure. The primary n6 linoleic acid contains two double bonds per molecule. N3 α-linolenic acid has three bonds and n3 EPA plus DHA has an average of 5.5 double bonds per molecule. Thus, the n3 EPA and DHA provided about 2.75 times and the n3 α-linolenic acid about 1.5 times as much "unsaturation" as the n6 linoleic acid on gram-for-gram basis. In the present study, n3 EPA plus DHA was more effective in lowering plasma Chol than n3 α-linolenic acid and n6 linoleic acid. The PUFA content was 50% of total fat in CO and PO diets and 37% in FO diet, but the degree of total fat unsaturation was 2 times higher in FO and 1.4 times in PO diet than in CO diet on gram-for-gram basis. Thus, it could be assumed that plasma total Chol in FO diet was significantly decreased due to the high degree of unsaturation while plasma total Chol in PO diet was similar to that of CO diet even though the total fat unsaturation of PO diet was greater than CO diet. Therefore, plasma total Chol level was not always decreased in proportion to the degree of unsaturation and it also seemed to be related to specific fatty acid structure.

HDL-cholesterol

Plasma HDL-chol level of FO group was significantly lower than that of CO and PO groups and there was no significant differnce between CO and PO groups(Table 3). Similar findings were observed in the reports of Harris et al[13] and Sanders and Roshanai[27]. In contrast, there are some reports that HDL-chol level was increased by feeding marine oil in human[27-29] and was decreased by the addition of linseed or perilla oils, or which was not affected by vegetable oil[13,28,30,31]. Thus, the effects of different kinds of PUFA upon HDL-chol

were not consistent and seemed to be variable depending on the amount and the duration of dietary treatment.

Plasma triglyceride

TG levels of PO and FO groups were significantly lower than that of CO group(Table 3). There are many reports of significant reduction in serum TG level by supplementation of n3 EPA and DHA to the basal diet than by n6 linoleic acid[32-34]. In the present study, plasma TG levels were significantly lower in the dietary groups of both n3 EPA+DHA and n3 α-linolenic acid than in the group of n6 linoleic acid. Saynor et al[12] and Iritani et al[35] also observed that fish oil inhibited liver acetyl CoA carboxylase activity and reduced hepatic lipogenesis which resulted in lower plasma TG levels. Previous reports proposed the following mechanisms for hypotriglyceridemic effect of n3 PUFA : (1) n3 PUFA rapidly removed VLDL in blood and thereby serum TG level was reduced. (2) n3 EPA and DHA incorporated into phospholipid of lipoprotein fraction to be a better substrate for lipoprotein lipase and accelerated the removal of VLDL. (3) n3 EPA and DHA inhibited lipogenic enzyme in liver and thereby reduced fatty acid synthesis and VLDL secretion. In our study, plasma TG level was significantly decreased in PO than in CO group. α-linolenic acid rich in perilla oil is metabolically converted to EPA and DHA by desaturation and chain elongation and this might had effect on lipogenesis and VLDL secretion in liver as they suggested.

Liver cholesterol and triglyceride

There were no significant differences in Chol and TG levels in all dietary groups(Table 3), but there were some reports of significant reduction in liver Chol content by fish oil in rat[32)36]. Nossen et al[37] and others [38] reported that TG synthesis and lipogenic enzyme activities (malic enzyme, glucose-6-phosphate dehydrogenase) in liver were both inhibited when rat hepatocytes were incubated with EPA in tissue cultures.

Lipoprotein pattern

The pattern of lipoprotein fractions was not significantly different by different kinds of dietary PUFA(Table 4). Although there was a significant decrease in plasma TG level in FO group, VLDL (%) was not correspondingly responded as plasma TG level. We may assume that since the total amount of PUFA in experimental diet was rather high compared to that of chow diet, lipoprotein pattern could be changed similar way in all dietary groups. But the different kind of PUFA may give different chemical composition in lipoprotein itself rather than the relative amount of each lipoprotein fraction.

Total tocopherol

Plasma total tocopherol level was not significantly different in all three groups with similar levels

Table 4. Effect of dietary PUFA on lipoprotein pattern by electrophoresis

	Dietary Groups		
	C O(16)	P O(13)	F O(8)
VLDL(%)	50.2 ± 12.4	44.2 ± 11.3	46.1 ± 11.3
LDL(%)	14.2 ± 4.2	16.6 ± 4.8	15.4 ± 3.2
HDL(%)	36.1 ± 11.6	40.8 ± 10.3	38.5 ± 10.0

Mean ± SD
( ) : Number of rats
LDL : Low density lipoprotein
VLDL : Very low density lipoprotein
HDL : High density lipoprotein

Table 5. Effect of dietary PUFA on the levels of tocopherol, malondialdehyde, superoxide dismutase and hemolysis in rats

	Dietary Groups		
	C O	P O	F O
Plasma tocopherol (μg/ml plasma)	2.18 ± 0.09 (15)	2.17 ± 0.06 (13)	2.10 ± 0.09 (8)
Plasma MDA (nmol/ml plasma)	0.30 ± 0.14 (13)	0.26 ± 0.03 (13)	0.27 ± 0.03 (8)
P-toco/T-lipid (μg/mg)	0.49 ± 0.08 (12)	0.48 ± 0.07 (12)	0.55 ± 0.05 (7)
RBC tocopherol (μg/ml blood)	2.03 ± 0.52 (11)	2.33 ± 0.48 (7)	3.29 ± 1.55 (3)
RBC MDA (nmol/ml blood)	0.04 ± 0.01 (11)	0.04 ± 0.01 (8)	0.05 ± 0.01 (4)
Liver tocopherol (μg/g wet liver)	3.70 ± 1.90 (16)	2.90 ± 1.00 (13)	2.70 ± 1.60 (8)
Liver MDA (nmole/g wet liver)	19.9 ± 2.5[a] (12)	18.3 ± 1.6[a] (9)	16.3 ± 1.5[b] (7)
Superoxide Dismutase (10^3 units/mg protein)	4.90 ± 1.00[a] (15)	9.10 ± 2.50[b] (13)	15.3 ± 3.6[c] (8)
Hemolysis(%)	15.9 ± 12.0 (11)	15.2 ± 10.0 (8)	22.4 ± 3.8 (3)

MDA : Malondialdehyde
Tocopherol and MDA contents of RBC were recalculated on the basis of whole blood.
Superscript a,b,c : Values with different letter within the row were significantly different at $p \leq 0.05$.

of dl-α-tocopherol supplement(Table 5). Vatassery et al[39] and Lehman et al[40] reported that serum tocopherol level was increased with treatment of dietary PUFA which was supplemented with tocopherol. Serum tocopherol concentration was varied by dietary tocopherol content and supplementation period. The present study showed similar plasma tocopherol levels for all dietary groups which indicated that tocopherol intakes may have been more than sufficient and we could not observe the effect on the plasma tocopherol content by dietary PUFA. Lehman[41] and Mino et al[42] have expressed that plasma tocopherol levels are closely related to plasma lipids and plasma tocopherol level was increased as plasma lipid was increased. Thus, when we calculated the ratio of tocopherol to total plasma lipid, there was no significant correlation between plasma tocopherol and total lipid levels. Tocopherol levels in RBC and liver were not significantly different by dietary PUFA(Table 5).

Malondialdehyde

There were no significant differences in plasma and RBC MDA levels(Table 5). However, liver MDA content of FO group was significantly lower than those of CO and PO groups and there was no difference between CO and PO groups. Liver MDA level was not correlated to liver tocopherol content. Iritani et al[43] observed that when rats were fed 10% (w/w) corn oil diets containing 80 mg dl-α-tocopherol/kg diet, autooxidation occurred quickly and even though dietary dl-α-tocophe-

rol was increased up to 400 mg/kg, the autooxidation could not be prevented. Choi and Jin[44] reported that liver autooxidation in rats fed sardine oil supplemented with 200 mg α-tocopherol/kg diet could not be reduced. In the present study, we observed that there was no inverse relationship between MDA formation and tocopherol content in liver, and it could not prevent the lipid peroxidation in liver even though the tocopherol supplement was enough.

Superoxide dismutase(SOD)

Liver SOD which protects cell membranes from the deleterious effects of lipid peroxidation was significantly higher in FO group than in CO and PO groups(Table 5). This was consistent with the reports of Zindenberg-Cherr et al[45] and Keen et al[46] who showed that total SOD activity increased with lipid peroxidation. However, in this study, liver MDA content of FO diet was significantly lower than that of PO and CO diets in spite of having 1.4 to 2.0 times more unsaturated PUFA in FO diet and we expected more MDA formation in FO group. Thus, it may be assumed that the specific SOD activity of liver was significantly increased as the degree of fat unsaturation increased, and thereby which resulted in the lower level of MDA by detoxification of free radicals.

Hemolysis

There was no significant difference between dietary groups(Table 5). There have been several reports that erythrocyte hemolysis was increased as dietary PUFA content was increased and the degree of hemolysis was inversely related to plasma tocopherol level[39)47]. Han and Park[30] observed that RBC hemolysis was inversely correlated to plasma tocopherol content than RBC tocopherol level and hemolysis was increased with MDA formation in plasma. Because total tocopherol contents of plasma and RBC were not significantly different in each groups of this study, the degree of hemolysis was similar in all dietary groups.

CONCLUSION

When male Sprague Dawley rats were fed the experimental diets for 6 weeks which composed of different kinds of dietary PUFA at 15% (w/w) level with sufficient tocopherol supplement, the following results were observed.

Plasma total chol and HDL-chol levels were significantly lower in fish oil group than in corn oil and perilla oil groups. Plasma cholesterol-lowering effect of PUFA was in the order of n3 EPA+DHA>n3 α-linolenic acid>n6 linoleic acid.

Plasma TG was significantly lower in both fish oil and perilla oil groups than in corn oil group. Plasma TG-lowering effect was greater by n3 PUFA (EPA+DHA, α-linolenic acid) than by n6 PUFA(linoleic acid).

However, there were no significant effects on lipoprotein pattern, hemolysis, and the levels of tocopherol and malondialdehyde in plasma and RBC by different dietary PUFA with sufficient tocopherol supplement. Liver superoxide dismutase activity was significantly increased in proportion to the degree of fat unsaturation, which resulted in the lower level of MDA in fish oil group.

In conclusion, because of their higher hypocholesterolemic and hypotriglyceridemic effects, fish oil and perilla oil rich in n3 PUFA may have important nutritional applications in the prevention and treatment of atherosclerotic diseases.

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쥐에서 n3계 불포화지방산 식이의 혈장지질 저하효과와 과산화물형성에 미치는 영향

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국문초록

Sprague Dawley 종 수컷쥐(체중 약 450g) 48마리를 3군으로 나누어 종류가 다른 불포화지방산으로 구성된 실험식이를 6주간 투여하였다. 이때 지방을 15%(W/W)로, 불포화지방산인 n6 linoleic acid의 급원으로는 옥수수기름, n3 α-linolenic acid의 급원으로는 들기름, n3 EPA와 DHA의 급원으로는 생선유를 투여하되, 포화지방산과 monoenoic acid의 총량을 거의 같게 공급하여 불포화지방산의 영향만을 관찰할 수 있도록 하였다.

어유를 투여한 군의 cholesterol과 HDL-chol양은 옥수수기름과 들기름을 투여한 군에 비해 유의성있게 낮았으며, 불포화지방산의 혈장 cholesterol을 저하시키는 능력은 n3 EPA+DHA>α-linolenic acid>linoleic acid의 순이었다. 또 혈장 triglyceride를 저하시키는 능력은 n3계 불포화지방산군인 어유와 들기름군이 n6계 불포화지방산군인 옥수수기름군에 비해 유의성있게 더 컸다. Lipoprotein의 상대적인 함량은 식이 불포화지방산의 종류에 의해 영향을 받지않았으며, 혈장과 적혈구 및 간의 토코페롤 수준은 유사한 수준이었으며 과산화산물 형성과 용혈정도도 세군간에 큰차이가 없었다. 그러나 간조직내의 superoxide dismutase 활성은 지방산의 불포화도 정도에 비례하여 증가되었으며 SOD활성의 증가로 인해 간조직의 과산화물의 수준이 감소되었다.