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Hypolipidemic and Antithrombotic Effects of Increasing Intake of Linolenic Acid Derived from Perilla Oil in Rats

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ABSTRACT

This study investigated the hypolipidemic and antithrombotic effects of linolenic acid derived from Korean perilla oil. The experimental rats(male, Sprague-Dawley) were divided into 5 groups using a Randomized Complete Block Design and fed one of the five following diets : DO*/O#, D4/O, D4/4, D4/8, or D4/20(D*/# represents the ratio of linoleic to linolenic acid as the percentage of total dietary energy intake) for 4 or 8 months. Bleeding time and whole blood clotting time were determined and the composition of serum and platelet lipids analyzed. Comparisons from the DO/O to the D4/20 group showed that serum lipids (total lipid, triglyceride, total cholesterol, and HDL-cholesterol) gradually decreased with increasing linolenic acid intake - the hypolipidemic effect. The composition of platelet fatty acids[the ratio of eicosapentaenoic acid(EPA)/arachidonic acid(AA)] increased gradually with increasing linolenic acid intake. Higher linolenic acid intake increased bleeding time and whole blood clotting time, and decreased malondialdehyde(MDA) production in the platelets, though no significant differences. These results suggest that linolenic acid derived from perilla oil appears to suppress the conversion of linoleic acid to AA and the EPA transformed from linolenic acid appears to suppress the conversion of AA to TXA_2. Since TXA_2 is a platelet-aggregating and vasoconstricting agent, the reduction of TXA_2 released by platelets with increasing intake of perilla oil containing a lot of linolenic acid confers an antithrombotic effect.

KEY WORDS : lipid metabolism · linolenic acid · ω-3 fatty acid · perilla oil · antithrombosis.

Introduction

The circulatory system diseases such as thrombosis, atherosclerosis, infarction, and hypertension are a leading cause of death in western societies[1]. In Korea, circulatory system diseases are also a principal cause of death[2]. Many studies of possible mechanisms and prevention of these diseases have been carried out[3-7]. These diseases are closely associated with atherosclerosis, which

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reflects several deteriorative phenomena(involving interactions between plasma lipids, lipoproteins, monocytes, platelets, and the endothelium and smooth muscle of the arterial wall) that gradually result in a narrowing of the arteries terminating in thrombosis, and infarction[8-11].

Dietary fat intake changes blood lipid composition, which plays an important role in circulatory system diseases by effecting atherogenesis and thrombosis[10-14]. Saturated fatty acids and cholesterol accelerate atherogenesis whereas monounsaturated and polyunsaturated fatty acids(PUFAs), by reducing plasma lipids, generally reduce circulatory system diseases[10-12]. Recently, it has been documented that dietary ω-6 PUFAs, via their conversion to bioactive eicosanoids, influence the initiation and progress of atherogenesis and are involved in thrombosis[13][14]. Eicosanoids regulate many of the functions of platelets, arterial and endothelial cells, monocytes and macrophages which have been implicated in atherogenesis and thrombosis[8-11]. Dietary ω-3 PUFAs appear to control eicosanoid synthesis and thus may have potential for the amelioration of atherogenesis and thrombosis[12][13].

Harris et al[14] reported that the both ω-3 and ω-6 fatty acids have hypolipidemic effect and the hypolipidemic effect of PUFAs is due not to any structural differences between the ω-3 and ω-6 fatty acids but to unsaturation. The primary ω-6 fatty acid, linoleic acid(18 : 2, ω-6), contains two double bonds per molecule. The principal ω-3 fatty acids, linolenic acid(18 : 3, ω-3) has three double bonds and the eicosapentaenoic acid (EPA, 20 : 5, ω-3) and docosahexaenoic acid (DHA, 22 : 6, ω-3) contain 5.5 double bonds per molecule. Thus the ω-3 fatty acids provide about 2.74 times as much "unsaturation" as the ω-6 fatty acids, gram-for gram[14].

Fish oil containing a lot of EPA has been documented to have large hypolipidemic and antithrombotic effects[15-19], but has acute and chronic toxicity yielding hydroperoxide through its oxidation[20]. Some vegetable oils, as a substitute for fish oil, are currently being studied widely as they are a rich source of linolenic acid, the precursor of EPA[21-26] and their hypolipidemic and antithrombotic effects are being investigated.

Perilla oil, a major source of oil in the diets of Koreans, is rich in linolenic acid and has been used in the prevention of circulatory system diseases throughout much of Korea's history[27][28].

This study investigated the hypolipidemic and antithrombotic effects of rats fed a diet increasing linolenic acid for different feeding periods(4 or 8 months), using perilla oil as the source of linolenic acid.

Materials and Method

1. Rats and diet

Male weanling Sprague-Dawley rats were randomly assigned to one of five groups and were given free access to one of the following diets for 4 or 8 months ; D0*/0#, D4/0, D4/4, D4/8, or D4/20. D*/# represents the ratio of linoleic to linolenic acid as the percentage of total dietary energy intake. Protein(milk casein, Droum Cooperative Butter Co.) supplied 15% of total energy, carbohydrate(corn starch, Poong-Gin Co.) 55%, and fat(mixtures of sesame oil, perilla oil, and beef tallow) 30% (Table 1-1). The fat contained a constant amount of linoleic acid(4% of total dietary energy) derived from Korean sesame oil and different linolenic acid contents(0, 4, 8, or 20% of total dietary energy) from Korean perilla oil(Table 1-2). The fatty acids composition of oils used for the diet formulation is presented in Table 2.

2. Blood samples

Bleeding time was measured according to the procedure of Hornstra[29]. The experimental rats were anesthetized with sodium pentobrabital(40 mg/kg B.W.). Three millimeters of the animal tails were removed and soaked in 37.5℃ saline. Bleeding time was defined as the period from removal of the tail to bleeding cessation. Blood samples (8~10ml) were obtained through cardiac puncture. One milliliter of this blood was used immediately to determine the whole blood clotting time, determination of the whole blood clotting time

Table 1-1. The energy composition of diets (% of energy intake)

Groups / Nutrients	D0/0	D4/0	D4/4	D4/8	D4/20
Carbohydrate	55	55	55	55	55
Protein	15	15	15	15	15
Fat	30	30	30	30	30
Linoleic acid (18 : 2 ω-6)	(0)	(4)	(4)	(4)	(4)
Linolenic acid (18 : 3 ω-3)	(0)	(0)	(4)	(8)	(20)

Table 2. Fatty acids composition of dietary oils (Unit : %)

Oil / Fatty acid	Sesame oil	Perilla oil	Beef tallow
14 : 0	—	—	2.6
1	—	—	—
16 : 0	10.1	7.4	25.1
1	—	—	—
18 : 0	3.7	1.6	23.1
1(ω-9)	28.5	11.5	42.4
2(ω-6)	37.9*	14.5	3.6
3(ω-3)	1.5	62.7*	—
20 : 0			1.8
Unknown	2.8	2.3	1.4
P/S Ratio[1]	3.1	8.6	0.07

[1]P/S Ratio = Polyunsaturated fatty acid / Saturated fatty acid

Table 1-2. The source and amount of diet ingredients (g/kg diet)

Groups / Nutrients	D0/0	D4/0	D4/4	D4/8	D4/20
Corn Starch	635	635	635	635	635
Casein	173	173	173	173	173
Sesame oil	0	40	30	21	0
Perilla oil	0	0	30	62	152
Beef tallow	152	112	92	69	0
Salt mixture[1)]	40	40	40	40	40
Vitamin A.D.[2)] mixture	1ml	1ml	1ml	1ml	1ml
Vitamin E.K.[3)] mixture	2ml	2ml	2ml	2ml	2ml
Water soluble Vits.[4)]	—	—	—	—	—
Vitamin B_{12}[5)]	1ml	1ml	1ml	1ml	1ml

[1)]Supplied(g/kg salt mixture) : Calcium carbonate 300.0, Dipotassium phosphate 322.5, Magnesium sulfate · $7H_2O$ 102.5, Sodium chloride 167.5, Monocalcium phosphate · $2H_2O$ 97.5, Ferric citrate · $6H_2O$ 15.5, Potassium iodide 0.8, Zinc chloride 1.0, Copper sulfate · $5H_2O$ 0.6, Manganous sulfate · H_2O 5.0, Sodium selenite 0.1, Chromium potassium sulfate · H_2O 0.55

[2)]Provided(1ml/kg diet) : Vitamin A 0.1(850 I.U.), Vitamin D 0.01(85 I.U.), Corn oil 1ml

[3)]Supplied(2ml/kg Diet) : α-Tocopherol acetate(Vitamin E) 50mg, Menadion(Vitamin K) 2mg, Corn oil 2ml

[4)]Supplied(mg/kg Diet) : Choline chloride 2.000, Thiamin hydrochloride pantothenate 100, Biotin 0.05, Folic acid 4, Inositol 500, p-Amino benzoic acid 100

[5)]Provided(1ml/kg diet) : 5% Vitamin B^{12} Solution

involved gently mixing 1ml of whole blood with 200μl of 1.7% $CaCl_2 \cdot H_2O$ solution in glass tube. The whole blood clotting time[30] was defined as the period from addition of $CaCl_2$ to initial blood coagulation.

3. Preparation of washed platelets

Blood was centrifuged at 1100 rpm for 20 minutes and then at 2800rpm for 15 minutes to obtain the platelet pellet(Sorvall RT-6000 Centrifuge). The platelet-poor plasma was used in the analysis of serum lipids composition. The platelet pellet was washed once and suspended in 0.01M phosphate buffered saline(PBS, PH 7.4). Platelets in the final suspension were counted using an automatic blood cell counter(Coulter, U.S.A)[31] and diluted to obtain a final concentration of 1.5×10^9 platelets/ml. Diluted platelet suspensions were used for analysis of malondialdehyde(MDA) and fatty acids composition. MDA released by platelets after stimulation by thrombin was determined using the thiobarbituric colorimetric reaction[32].

4. Analyses of serum lipids

Serum total lipid was determined using the method of Frings and Donn[33]. Serum triglyceride and total cholesterol were determined using the method of Neri[34] and Zak[35], respectively. Serum HDL-cholesterol was determined using a commercial kit enzymatic method(International Reagents Co.)[36]. HDL-cholesterol was calculated using Fridewald formula[37] follow as ;

LDL-cholesterol=Total cholesterol−
(Triglyceride/5+HDL−cholesterol)

5. Lipid extraction of platelet pellets

Total lipid from the diluted platelet suspensions was extracted using the method of Bligh and Dyer [38]. Following extraction and removal of the water-soluble materials, the lipid partitioned into the chloroform phase was dried under N_2 gas.

6. Gas chromatographic analyses of fatty acids

The dried platelet lipid was immediately methylated by boiling with 2ml of 5% methanolic HCl for 2 hours, and fatty acid methyl esters were extracted with hexane. Extracted fatty acid methyl esters were evaporated under nitrogen gas and redissolved in small volumes of acetone. Fatty acid methyl esters were analyzed on a Carbowax 20M-fused silica capillary column at 210℃ using Hewlett-Packard 5840A gas chromatography[39]. Helium was the carrier gas at a flow rate of 20 cm/sec. Unknown fatty acid peaks were identified by comparing their retention times with those of standard fatty acids.

7. Statistics

Results were expressed as means and standard errors, and significant differences between the groups of rats maintained on different diets and feeding periods were determined using the Scheffé test. The effects of diet(Factor A), feeding period (Factor B), or any interaction of factor A and B were analyzed by two-way analysis of variance [40].

Results

1. Serum lipids

Comparisons between the groups showed that serum lipids(total lipid, triglyceride, total cholesterol, and HDL-cholesterol) decreased with increasing intake of linolenic acid derived from perilla oil(Table 3), although individual analysis of the total cholesterol and HDL-cholesterol showed no significant differences. LDL-cholesterol showed significant differences Between 4 months and 8 months feeding period. Relative cholesterol showed no significant differences by diets and feeding periods.

Table 3. Concentration of serum lipids

Groups		Total lipid (mg/dl serum)	Triglyceride (mg/dl serum)	Total cholesterol (mg/dl serum)	HDL-cholesterol (mg/dl serum)	LDL-cholesterol (mg/dl serum)	Relative cholesterol (HDL-C/TC)
Months of feeding	Diet						
4	D0/0	[1]315.15± 20.12[a2)]	124.12± 6.00[ab]	88.59± 2.79[N.S.3)]	39.79± 5.67[N.S.]	23.98± 6.87[ab]	0.44± 0.05[N.S.]
	D4/0	302.91± 20.12[a]	99.32± 3.35[cd]	88.35± 4.25	37.05± 3.25	31.44± 3.92[a]	0.43± 0.04
	D4/4	248.01± 13.82[b]	95.40± 2.58[cd]	88.41± 3.19	41.16± 6.60	28.17± 7.12[a]	0.40± 0.05
	D4/8	227.67± 11.13[bc]	104.21± 1.75[bcd]	88.39± 5.64	40.09± 3.65	27.46± 4.00[a]	0.46± 0.06
	D4/20	169.69± 5.11[c]	85.19± 4.09[d]	73.59± 4.68	28.91± 2.35	27.64± 3.17[a]	0.42± 0.06
8	D4/0	317.58± 30.56[a]	117.57± 7.88[abc]	90.60± 3.19	52.85± 3.22	14.24± 4.80[b]	0.58± 0.03
	D4/0	290.85± 12.84[a]	102.94± 4.18[bcd]	85.58± 3.19	52.35± 1.86	12.64± 2.70[b]	0.61± 0.02
	D4/4	225.99± 5.60[b]	99.70± 3.27[bcd]	84.93± 3.21	51.67± 3.27	13.32± 3.92[b]	0.61± 0.03
	D4/8	216.10± 2.66[bc]	100.31± 2.47[bcd]	81.76± 4.54	44.63± 4.59	17.07± 5.08[b]	0.54± 0.03
	D4/20	195.76± 6.33[c]	89.16± 2.67[d]	73.21± 3.95	37.74± 1.72	17.64± 2.25[b]	0.52± 0.03
Significant[4] Factor		A	A			B	

[1)]Mean± S.E.

[2)]Values with different letters within the column were significantly different at $\alpha=0.05$ by Scheffé test. If any leter combination matches, the difference between means is not significant.

[3)]Not significant at $\alpha=0.05$ by Scheffé test

[4)]Statistical significance A and B factors was calculated by 2-way ANOVA

A : Diet effect was significant at $\alpha=0.05$

B : Feeding Period effect was significant at $\alpha=0.05$

2. Platelet function

Increasing content of perilla oil containing a lot of linolenic acid in diets increased bleeding time and whole blood clotting time gradually(Fig. 1), with the exception of rats fed the diet D4/8. No significant differences were found in the feeding period, except the D4/20 group in whole blood clotting time.

MDA was determined in place of thromboxane A_2(TXA_2) released by platelets. The level of MDA generation during thrombin-induced aggregation of platelets decreased with increasing intake of linolenic acid derived from perilla oil(Fig. 2), though no significant differences. This result sug-

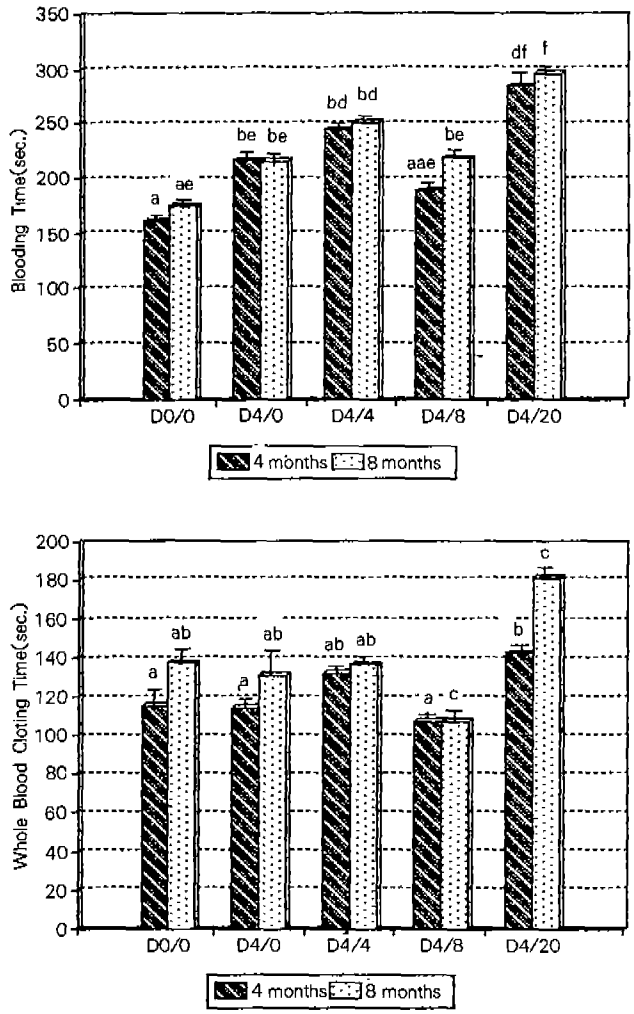

Fig. 1. The effects of diets containing different ratios of linoleic acid(18 : 2, ω-6) and linolenic acid(18 : 3, ω-3) and feeding peroid(4 or 8 months) on BLEEDING TIME and WHOLE BLOOD CLOTTING TIME. Bar graphs show mean± S.E.. Diet */# represents the ratio of linoleic acid(*) and linolenic acid(#) as a percentage of energe intake. Different letters above the error bar indicate significant differences at $\alpha=0.05$ by Scheffé test. If any letter combination matches, the difference between means is not significant.

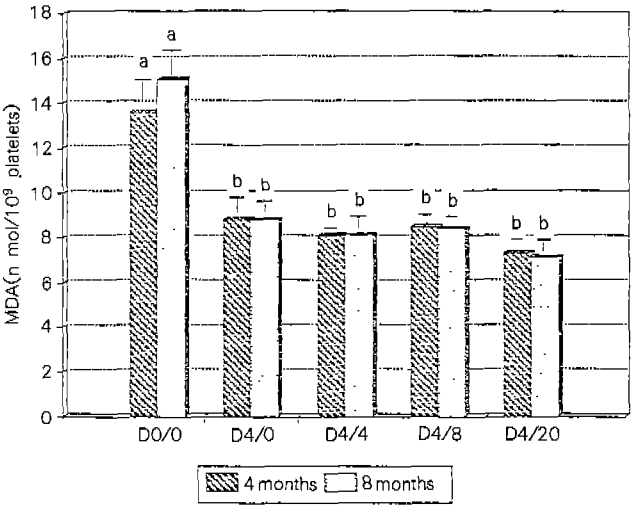

Fig. 2. The effects of diets containing different ratios of linoleic acid(18 : 2, ω-6) and linolenic acid(18 : 3, ω-3) and feeding peroid(4 or 8 months) on MALONDIALDEHYDE(MDA). Bar graphs show mean± S.E.. Diet */# represents the ratio of linoleic acid(*) and linolenic acid(#) as a percentage of energe intake. Different letters above the error bar indicate significant differences at $\alpha=0.05$ by Scheffé test.

gests that the linolenic acid derived from perilla oil suppresses the conversion of linoleic acid to arachidonic acid(AA) and then the EPA transformed from linolenic acid suppresses the conversion of AA to TXA_2. But the rats fed diet D4/0 including only linoleic acid also showed the decreased generation of MDA.

The changes observed in the fatty acid composition of platelets in response to the diets containing various ratios of linoleic to linolenic acid are sumarized in Table 4. In this experiment, GC peak areas of $C_{16:0}$, $C_{16:1}$, $C_{18:0}$, $C_{18:1}$, $C_{18:2}$, $C_{18:3}$, $C_{20:0}$, $C_{20:4}$, and $C_{20:5}$, were identified, and each individual peak area was expressed as a percentage of the total area.

It was apparent that the fatty acid composition of the platelet changed with the type of diet. The ratio of linolenic to linoleic acid(ω-3/ω-6 : 18 carbons) in the 4 month feeding period slightly increased with increasing linolenic acid intake ; 0.13 in D0/0 group, 0.15 in D4/0 group, 0.17 in D4/4 group, 0.15 in D4/8 group, and 0.20 in D4/20 group. But in the 8 month feeding period, the increase of the ω-3/ω-6 ratio was smaller than in the 4 month feeding period(Table 4).

The conversion of linolenic acid to EPA increased proportionally to the linolenic acid content of the diets ; EPA/AA ratio(ω-3/ω-6 : 20 carbons) was 0.04 in D0/0 group, 0.04 in D4/0 group, 0.10 in D4/4 group, 0.14 in D4/8 group, and 0.24 in D4/20 group for the 4 month feeding period. Rats fed diets for 8 months showed a similar result, although the EPA/AA ratio of the D4/20 group in the 4 month feeding period was higher than in the 8 month feeding period(Table 4).

In general, ω-3/ω-6 ratio of 18 and 20 carbon atoms increased proportionally to the linolenic acid content in diets ; in the 4 month feeding period, 0.07 in D0/0 group, 0.09 in D4/0 group, 0.13 in D4/4 group, 0.15 in D4/8 group, and 0.23 in D4/20 group. For the 8 month feeding period, 0.06 in D0/0 group, 0.07 in D4/0 group, 0.10 in

Table 4. Relative fatty acids composition of platelets (% Total fatty acid methyl esters)

Months	4 Months					8 Months				
Dict / Fatty acid	D0/0	D4/0	D4/4	D4/8	D4/20	D0/0	D4/0	D4/4	D4/8	D4/20
16 : 0	31.84	23.39	29.64	30.82	28.74	24.15	25.80	25.40	28.15	22.30
1	3.47	3.43	3.25	2.89	3.88	0.86	2.03	1.41	1.48	1.64
18 : 0	15.00	14.81	16.19	14.37	15.64	12.09	7.26	9.32	8.86	11.43
1(ω-9)	11.60	14.31	14.46	13.47	11.83	20.58	20.69	15.16	15.59	20.00
2(ω-6)	12.44	17.73	11.63	21.22	16.26	13.74	17.77	25.60	25.06	21.79
3(ω-3)	1.63	2.67	2.00	3.18	3.32	1.10	0.74	2.05	1.81	2.23
20 : 0	2.16	1.42	1.63	1.34	2.03	1.55	0.52	1.52	1.49	0.36
4(ω-6)	20.67	21.38	20.11	11.15	14.65	23.61	23.22	17.41	15.59	17.21
5(ω-3)	0.73	0.85	2.09	1.57	3.66	1.32	1.97	2.14	1.96	3.05
18 ω-3/ω-6	0.13	0.15	0.17	0.15	0.20	0.08	0.04	0.08	0.07	0.10
20 ω-3/ω-6	0.04	0.04	0.10	0.14	0.24	0.06	0.08	0.12	0.13	0.18
18+20 ω-3/ω-6	0.7	0.09	0.13	0.15	0.23	0.06	0.07	0.10	0.09	0.14

D4/4 group, 0.09 in D4/8 group, and 0.14 in D4/20 group. One interesting finding was that the increase of ω-3/ω-6 ratio of 18 and 20 carbon atoms in the D4/8 and D4/20 groups was higher for the 4 month feeding period than for the 8 months (Table 4).

Discussion

1. Serum lipids

Serum total lipid and triglyceride decreased gradually with increasing intake of linolenic acid dervied from perilla oil. Serum cholesterol and HDL-cholesterol showed a similar trend, but the differences were not significant.

Harris et al[14] reported that the difference in the hypolipidemic effects of ω-6 and ω-3 fatty acids is due to their level of unsaturation. This could explain the results found in this study as the gradual increment of linolenic acid content in dietary fat, yielding an increase in the degree of unsaturation, resulted in a decreased in serum lipids.

The principal ω-3 fatty acid in fish oil, EPA is known to provide protection against atherogenesis by increasing HDL transport of cholesterol from tissue to the liver for conversion to bile acids and excretion[41][42]. In this study, however, linolenic acid in perilla oil, the EPA precursor, decreased HDL-cholesterol, although the defferences were not significant. This finding corresponds with the result of Sanders and Roshanai[43]. They used 9.38g of linseed oil as the source of α-linolenic acid for two weeks, and the subjects had no changes in plasma cholesterol and plasma HDL-cholesterol. Thus the effects of dietary polyunsaturated fatty acids on HDL-cholesterol are not compeletely clear.

2. Platelet function

The fatty acids composition of the platelets was changed by the type of dietary fatty acid consumed. The ratio of linolenic acid to linoleic acid in the platelets was directly proportional to the amounts of the fatty acids administered, although the extent of the increment was affected by the feeding period. The conversion of linolenic acid to EPA gradually increased with increasing intake

of linolenic acid derived from perilla oil. Hornstra et al[44] state that α-linolenic acid, by desaturation and chain elongation, is metabolized to EPA. However, Dyerberg et al[45] and Sanders et al[43] reported that with the human subjects receiving linseed oil rich in linolenic acid, in dose higher than this study, the EPA content in the lipid fractions did not increase significantly, in contrast to the increase after ingestion of perilla oil in this study. They argue that it appears as if the capacity of the human organism to desaturate and elongate linolenic acid is limited. The result of our animal study showed that Dyerberg's result could be due to an incompelete control of dietary linoleic acid intake in the human study. The same enzymatic systems that transform linoleic acid to AA also transform α-linolenic acid to EPA by identical metabolic steps of desaturation[44]. In this study, the gradual increment of EPA/AA in the platelets with increasing intake of linolenic acid derived from perilla oil resulted not only from the conversion of linolenic acid to EPA, but also from a reduction of AA by EPA.

Several studies reported that bleeding time and whole blood clotting time were prolonged with ω-3 fatty acids intake[43][46][47]. These results from this study support that finding ; bleeding time and whole blood clotting time was longer with increasing intake of linolenic acid derived from perilla oil except the D4/8 group. The longer bleeding time and whole blood clotting time can be explained by the mechanism of the formation of the prostaglandins(TXA_2 or PGI_2) from AA[48]. In platelets, AA is converted by the enzyme cyclooxygenase to cyclic endoperoxides, which are rapidly transformed by TXA_2 synthetase to TXA_2, an extremely potent vasoconstrictor and a platelet aggregating substance. In contrast, the vascular endothelial cell converts AA via the same endoperoxides to PGI_2(prostacyclin), which is a vasodilator and inhibits platelet aggregation. EPA may compete with AA for cyclooxygenase and may therby alter platelet-vessel interactions[48]. Since assays for TXA_2 were not available at the time of this study, the platelet production of MDA was chosen as an estimate of TXA_2 synthesis in an effort to determine if ω-3 fatty acids might inhibit prostaglandin synthesis. With increasing intake of linolenic acid derived from perilla oil, the production of MDA in the platelets was lower, with the exception of rats fed diet D4/0. So, the reduction in the amount of AA and/or a diminished coversion of AA to TXA_2 by competitive inhibition of the platelet cyclooxgenase by the EPA transformed from linolenic acid could be responsible for the prolonged bleeding time and whole blood clotting time. While D4/0 group with only linoleic acid intake showed the decreased generation of MDA, other studies[49][50] showed that rats fed the diets including only linoleic acid had the increased generation of MDA.

In conclusion, this study has shown that the increasing intake of linolenic acid derived from perilla oil resulted in lowering serum lipids(hypolipidemic effect) and altering platelet function (antithrombotic effect). Especially D4/20 group containing the hightest linolenic acid has the most hypolipidemic and antithrombotic effects. Interestingly, it was demonstrated in this study that Korean perilla oil, which contains a large amount of linolenic acid, has hypolipidemic and antithrombotic effects.

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Hypolipidemic and Antithrombotic Effects of Perilla Oil in Rats

= 국문초록 =

들깨유 급원의 Linolenic Acid 섭취 증가가 흰쥐의 혈청지질 감소 및 항혈전에 미치는 효과

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본 논문에서는 linoleic acid의 주급원으로 참깨유를, linolenic acid의 주급원으로 들깨유를 사용하여, linoleic acid의 함량을 일정하게 하고(식이 총 에너지의 4%) linolenic acid의 함량을 증가시켜(각각 식이 총 에너지의 0%, 4%, 8%, 20%), 식이 총 에너지 중 지방을 30% 함유한 식이를 흰쥐에게 자유급식 시켰을 때 혈청지질감소 및 항혈전에 미치는 영향을 살펴보고자 하였다. 혈청지질 함량(총지방함량, 중성지방함량, 총 cholesterol과 HDL-cholesterol)은 들깨유 급원의 linolenic acid 함량이 높을수록 감소하였다—혈청지질감소효과(hypolipidemic effect). 혈소판의 지방산 조성에 미치는 영향을 보면 식이의 linolenic acid 함량이 증가할 수록 EPA/AA의 비율이 점차 증가하였다. 또한 linolenic acid 섭취의 증가는 출혈시간과 전혈응고시간을 연장시켰고 유의적인 차이는 없었지만 혈소판에서의 MDA 생성이 조금씩 감소했다. 이러한 결과들은 들깨유 급원의 linolenic acid가 linoleic acid의 AA로의 전환을 억제하고, 차례로 linolenic acid로부터 전환된 EPA가 AA로부터 TXA_2로의 전환을 억제한다는 것을 제시한다. TAX_2는 혈소판을 응집시키고 혈관을 수축시키는 물질이므로, 들깨유 급원의 linolenic acid 섭취 증가로 인한 TAX_2의 감소는 항혈전효과(antithrombotic effect)를 보여주어 출혈시간과 전혈응고시간의 연장을 설명해준다.

그러므로 본 연구에서 linolenic acid를 상당량 가지고 있는 들깨유는 혈청지질 감소 효과와 항혈전 효과를 갖는다고 결론내릴 수 있다.