The Influence of Saturated Fats, α-linolenic Acid, EPA and DHA on the Lipid Hydroperoxide Level and Fatty Acid Composition in Liver Microsomes and in Plasma Lipid of Rabbits

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Abstract

To investigate the influence of saturated fats, α-linolenic acid, EPA and DHA on the lipid hydroperoxide concentration and fatty acid composition in liver microsomes and in plasma lipid of rabbits, the animals were fed on the perilla oil rich α-linolenic acid or sardine oil rich EPA and DHA diet for four weeks were examined. The fatty acid composition of plasma lipid and liver microsomes of rabbits fed on the perilla oil diet was an accumulation of arachidonic acid(AA) 20:4 n-6, eicosapentaenoic acid(EPA) 20:5 n-3, and docosahexaenoic acid(DHA) 22:6 n-3. The fatty acid composition of plasma lipid and liver microsomes of rabbits fed on the sardine oil was an accumulation of α-linoleni acid(LNA) 18:3 n-3, and arachidonic acid(AA) 20:4.

The p/s ratio of rabbits fed on the perilla oil diet changed from 7,4 to 2,27 for plasma lipid and 2.47 for liver microsomes. The concentration of lipid hydroperoxide was 3.48 nmol MDA/m*l* and 4.35 nmol MDA/m*l* for plasma lipid and liver microsomes, respectively, in perilla oil diet. The lipid hydroperoxide liver was 4.22 nmol MDA/m*l* and 67 nmol MDA/m*l* for plasma lipid and liver microsomes in sardine oil diet.

I. introduction

The effects of dietary fats on cholesterol concentration control, heart disease and production of prostaglandins were frequently reported.[1-4]

Numerous plant seed oil which contained polyunsaturated fatty acid were examined for a cholesterol level controlling factor.[5-7]

The polyunsaturated fatty acids(fish oil) are very effective in lowering serum triglyceride and cholesterol level. Diets containing fish oil had anti-aggregatory effect on the manifestation of thrombosins, cardiovascular protective effect and is a major factor in heart attacks.[8-12]

Nutritional effects of n-3 polyunsaturated fatty acid were reviewed recently by Bud owski.[13] There has been a renewed interest in α-linolenic acid (LNA) 18:3 n-3 fatty acid and its metabolites.[14-16] In particular, the n-3 fatty acids that constitute a major portion of the polyunsaturated fatty acids in fish oils had been shown to have potential influence on lowering levels of circulating lipids and on the cardiovascular system.[17-18] Rabbits fed on the

perilla oil contained α-linolenic acid showed plasma cholesterol level lowering.[19]

There are indications that dietary lipid peroxidation products could play a role in the pathogenesis of atherosclerosis. Higher level of lipid peroxides are observed in animals and patients with atherosclerosis.[20~22] The toxicity of malondialdehyde, lipid peroxides of linoleic acid and α-linolenic acid, a secondary product could play a role in the aging.[23~24]

To investigate whether perilla oil or sardine oil affect the fatty acid composition in liver microsomes and in plasma lipid and lipid hydroperoxide, the rabbits ingesting the perilla oil rich in α-linsolenic acid or sardine oil rich in eicosapentaenoic acid(EPA) and docosahexaenoic acid(DHA) diets were examined.

II. Materials and Methods

1. Animals:

Male New Zealand White rabbits weighing 600-700g. were fed on rabbit chow for one week prior to the start of the experimental diets.
They were divided into two dietary groups of five animals. They were housed individually in wire cages at a temperature of 18~20°C and approximately 60% humidity.

2. Diets:

The basic purified diets contained corn starch 60%, soybean rind 15%, lipid sources 8%, vitamin mixtures 2%: mixture contained ascorbic acid 5.5, choline chloride 7.5,-tocopherol 15.0, inositol 0.5, niacin 0.5, pantothenic acid 0.3, riboflavin o.3, thiamine 0.3 and vit.B 12 0.1, mineral mixtures 3%: mixtures contained NaCl 11.4, K_2HPO_4 7.9, $Ca(H_2PO_4)$ $2H_2O$ 4.5, $MgSO_4 7H_2O$ 4.4, $NaH_2PO_4H_2O$ 1.5, $ZnSO_4 7H_2O$ 0.1, $MnSO_4H_2O$ 0.08, KI 0.05, $CaCl_2$ $6H_2O$ 0.05, $CuCl_2$ 0.01, Na_2SeO_3 0.01. Each experimental diet was supplemented with 10% by weight of perilla oil or sardine oil as a sources of n-3 or n-6 fatty acid. The diets were prepared every other day and fed ad libitum. The fatty acid composition of dietary fats is summarized in Tablel 1.

At the end of experimental diet period, all the animal were fasted for 24 hours and anesthetized with ether, and blood sample was obtained from arota into a test tube contained EDTA lmg/m*l* blood. Plasma was separated by a centrifuge at 2500 rpm and stored at -30°C. Livers were excised, rinsed in ice saline, weighed and frozen for lipid extraction.

3. Lipid analysis:

Total lipids were extracted from plasma and liver by the method of Bligh and Dyer.[25] The phospholipids were separated by two-step single dimension thin-layer chromatography. Plates(Merck, Art, 6721) were first developed with chloroform/methanol/acetone/acetic acid/water(100:50:100:4:10, by vol.) and dried in vacume for 30 minutes, and redeveloped in the same direction with Chloroform/methanol/acetic acid/water (180:150:30:1, by vol.).All the solvents contained 0.005% BHT(butylated hydroxytoluene). The developed plates were dried in vacuo to remove the solvent. Appropriate areas were scrapped off and lipid was transmethylated with NaOCH3-methanol at 60°C for 15 minutes. The lipids were saponified with 0.5N KOH in methanol according to the Morrison and Smith.[26]

The fatty acid methylesters were determined by gas liquid chromatography(GLC, Shimadzu GC-94) on a column packed with 10% Silar 10 C on 60~80 mesh Neopack 2A or 5% SP-2310 on 100-120 mesh Chromosorb W, with nitrogen gas flow rate 40m*l*/min. and temperature programmed from 160~240°C. Fatty acids were identified by comparing retention time with fatty acid methylester standard (Sigma, USA).

The concentration of lipid hydroperoxide in plasma and liver microsomes was determined by the

TBA method according to Marshall[27] and Frankel[28].

4. Preparation of microsomes:

The rabbits were killed and took out liver. The liver(2g resected) were excised for the preparation of microsomes. Approximately lg of liver was diced over ice, and the diced liver was homogenized in a buffered sucrose solution containing 0.1M sucrose, 0.05M KCl, 0.04M KH_2PO_4, 0.03M EDTA, pH 7.4, in a homogenizer. The whole homogenates were centrifiuged for 20 minutes at 10,000g and the resulting supernatants were centrifuged for one hour at 105,000g. The pellets were resuspended in buffer and centrifuged again at 105, 000g for one hour.

5. Statistical analysis:

Significant differences of mean values for fatty acid content between the dietary group were determined by general linear model and student't -test.[29]

Ⅲ. Results

Effect of the perilla oil or sardine oil diet on the fatty acid composition of plasma lipids and phospholipids:

The fatty acid composition of plasma lipid and phospholipid of rabbits fed on the perilla oil or sardine oil diet for 4 weeks was different from those of the respective oil(Table 1,2). In plasma lipid, there were accumulations of 18:0, 20:4, 20:5 and 22:6 for perilla oil diet subjects, but 18:3, and 20:4 for sardine oil diet subjects.

Effect of the perilla oil or sardine oil diet on the fatty acid compoition of liver microsomes:

The fatty acid composition of liver lipid and liver microsomes of rabbits fed on the perilla oil or sardine oil diet for 4 weeks was different from those of the respective oil(Table 1, 3). In liver lipid and liver microsomes, there were accumulations of 18:0, 20:4, 20:5 and 22:6 for perilla oil diet subjects, but 18:3 and 20:4 for sardine oil diet subjects. The concentration of lipid hydroperoxide of plasma lipid and liver microsomes:

The concentration of lipid hydroperoxides of plasma lipid and liver microsomes was determined, the results was Table 4. The lipid hydroperoxide level was 3.48±0.33 and 4.35±0.35nmol MDA/*ml* for plasma lipid and liver microsomes in the subjects fed on perilla oil diet. The lipid peroxides level was 4.32 ±0.34 and 5.67 ±0.56 nmol MDA/*ml* for plasma lipid and liver microsomes in the subjects fed on sardine oil diet.

Table 1. Fatty acid compostion of dieteary oils

Fatty acid	Sardine oil	Perilla oil
14:0	7.2	ND
16:1	18.2	8.2
16:1	9.4	T
18:0	3.5	1.9
18:1	13.7	14.5
18:2	4.6	15.8
18:3	T	58.9
20:1	5.4	ND
20:2	3.8	ND
20:4	T	T
20:5	20.7	ND
22:6	12.4	ND
MUFA	28.5	14.5
PUFA	41.5	74.5
n-6	8.4	15.8
n-3	33.1	58.9
n-6/n-3	0.14	0.27
p/s	1.4	7.4

ND: None detect

T: Trace amount, less than 1%

Table 2. Fatty acid composition of plasma lipid and phospholipid of rabbits fed on diet containing sardine or perilla oil

Fatty acid	Plasma lipid		Plasma phospholipid	
	Sardine	Perilla	Sardine	Perilla
14:1	3.1±0.3	ND		ND
16:0	19.2±0.2	9.4±0.2	16.5±0.2	9.4±0.2
16:1	6.9±0.1	1.5±0.1	6.6±0.2	3.2±0.1
18:0	4.4±0.2	14.5±0.2[a]	12.7±0.1	14.3±0.2[a]
18:1	14.7±0.1	19.3±0.9[a]	8.8±0.1	17.5±0.9[a]
18:2	5.2±0.1	4.5±0.2	4.3±0.1	4.7±0.2
18:3	2.3±0.3	20.4±1.4	3.7±0.2	20.4±0.9
20:1	4.2±0.2	ND	1.7±0.1	ND
20:4	6.1±0.2	7.1±0.2[b]	8.3±0.2	5.7±0.1[b]
20:5	15.7±0.1	10.2±0.2[a]	17.4±0.1	8.5±0.2[a]
22:6	17.2±0.1	12.2±0.2[a]	18.9±0.1	15.3±0.2[a]
Others	1.0	1.0	1.0	1.0
MUFA	25.8	20.8	17.1	20.7
PUFA	46.5	54.3	52.6	54.6
n-6	11.3	11.6	12.6	10.4
n-3	35.2	42.7	40.0	44.2
n-6/n-3	0.32	0.27	0.31	0.27
p/s	1.74	2.27	1.80	2.30

Values are the mean±SD of the subject (n=5)
Others, 20:2, 20:3, 22:5, each of those fatty acid was less than 1%
a: p<0.01 versus SO, b: p<0.05 versus SO.

Table 3. Fatty acid cmposition of liver microsomal lipid of rabbit fed on diet containing sardine or perilla oil

Fatty acid	liver lipid		liver phospholipid	
	Sardine	Perilla	Sardine	Perilla
14:0	T	ND	T	ND
16:0	16.8±0.2	11.7±0.2	15.8±0.2	12.4±0.2
16:1	7.2±0.1	1.8±0.1	6.5±0.1	1.3±0.1
18:0	6.5±0.2	10.4±0.1[a]	8.9±0.6	10.9±0.3[a]
18:1	12.8±0.1	20.5±0.3[a]	13.6±0.1	21.7±0.4[a]
18:2	6.7±0.1	7.2±0.1	7.5±0.5	6.8±0.1
18:3	3.1±0.2	18.5±0.2	2.9±0.1	17.2±0.2
20:1	3.7±0.2	ND	4.5±0.3	ND
20:4	4.9±0.2	4.3±0.1[b]	5.3±0.3	5.6±0.2[b]
20:5	17.8±0.1	9.4±0.4[a]	17.8±0.1	8.5±0.4[a]
22:6	19.5±0.2	15.2±0.5[a]	16.2±0.2	15.6±0.8[a]
Others	1.0	1.0	1.0	1.0
MUFA	23.8	22.3	24.6	22.0

PUFA	52.0	54.6	49.7	53.7
n-6	11.6	11.5	12.8	12.4
n-3	39.9	43.1	36.9	41.3
n-6/n-3	0.29	0.27	0.35	0.30
p/s	2.23	2.47	2.01	2.30

Values are the mean ±SD of the subject(n=5)
Others, 20:2, 20:3, 22:5, each of those fatty acids was
a: $p<0.01$ versus SO, b: $p<0.05$ verus SO.

Table 4. The concentration of lipid hydroper oxide of liver micrsomes and plasma of rabbits

	Lipid hydrperoxide (nmol MDA/ml)	
	Plasma	Microsome
PO diet group	3.48±0.33	4.35±0.35
SO diet group	4.22±0.34	5.67±0.56

Values are meam ±SD of the subject(n=5)
PO:Perilla oil SO:Sardine oil

Ⅳ. Discussions

Dietary α-linolenic acid 18:3 n-3 fatty acid was more potent than linoleic acid 18:2 n-6 fatty acid in lowering plasma chloesterol level. In the rabbit, the ingestion of perilla oil rich 18:3 resulted in plasma chloesterol level lowering.[6,7] Field, et al[30] reported that safflower oil or sunflower oil rich in n-6 linoleic acid showed plasma cholesterol level increased in the rabbits, But the diets rich in the n-3 polyunsaturated fatty acids affected plasma cholesterol level, vascular contractibility and tissue lipid compositions.[31]

In rats fed the diets containing linseed oil, 20:4 was increased and the animals fed the diets containing sardine oil exhibited a reduced conversion of 20:3 to 20:4 in liver microsomes.[32] In the animals fed on the corn oil, 22:4 n-6 and 22:5 n-6 in both PC and PE were increased.[33] Feeding the either linseed oil or fish oil diet increased the 20:4 n-6 contentin phospholipid and caused an accumulation of 20:5 n-3, and 22:6 n-3 polyunsaturated fatty acid.[31~32] When rats were fed on a diet containing sardine oil, 20:5 n-3 was incorporated into the platelet, arota and plasma lipid, 20:4 and 20:5 in the platelet phospholipids might be substituted by each other.[35]

In this study, 20:4 n-6 level in plasma and liver microsomes of the rabbits fed on the sardine oil diet subjects was similar to those on the perilla oil diet animals, despite the fact that the perilla oil contained four fold more 18:2, the precursor of 20:4. The eicosapentaenoic acid(EPA) and docosahexaenoic acid(DHA) in both plasma and liver microsomes of rabbit fed on the perilla oil did not contained 20:5 and 22:6(Table 1). There was also 20:4 fatty acid for perilla oil diet subjects. Therefore, it assumed that 18:3 n-3 α-linolenic acid was converted into the 20:5 n-3, 22:6 n-3, and 20:4 n-6 fatty acids.

Dietary fat could be played a major role in eicosanoid production and in modulation of the immune system.[36] The n-3 fatty acids of menhaden oil appeared to inhibit both cycloxygenase and lipoxygenase.[37] Feeding 20% menhaden oil diet depressed eicosanoid or because less 20:4 precursor was being fed in the diet.[38]

In this study, the n-6/n-3 ratio was 0.32 for sardine oil diet group and 0.27 for perilla oil diet group in plasma lipids, even though dietary oil showed n-6/n-3 ratio was 0.14 and 0.27. The p/s ratio showed 1.4 and 7.4 for the sardine and perilla oil, respectively. The p/s ratio of plasma lipid was 1.74 for sardine oil diet and 2.27 for perilla