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# The influence of dietary perilla oil or sardine oil on the fatty acid composition in liver microsomes and in plasma lipid of rabbits

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## Ⅰ. Introduction

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

Diets containing fish oil had anti-aggregatory effects on the manifestation of thrombosins, cardiovascular protective effect and is a major factor in heart attacks.[8~12] Because the fish oil contains a lot of n-3 polyunsaturated fatty acid.

Nutritional effects of n-3 polyunsaturated fatty acid were reviewed recently by Budowski.[13] There has been a renewed interest in $\alpha$-linolenic acid(LNA) 18 : 3 n-3 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 cardievascular system.[17~18] Perilla oil rich $\alpha$-linolenic acid showed cholesterol level lowering.[19]

To investigate whether perilla oil rich $\alpha$-linolenic acid or sardine oil rich eicosapentaenoic and docosahexaenoic acid affect the fatty acid composition in liver microsomes and in plasma lipid, the rabbits ingesting the perilla oil or sardine oil diets were examined.

## Ⅱ. Materials and Methods

**Animals :**

Male New Zealand White (MNZW) rabbits weighing 600-800 g 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℃ and approximately 60% humidity.

**Diets :**

The basic purified diets contained corn starch 60%, soybean rind 25%, lipid sources 10%, vitamin mixtures 2% : mixture contained ascorbic acid 3.5, choline chloride 5.5, $\alpha$-tocopherol 9.0, inositol 0.5, niacin 0.5, pantothenic acid 0.3, riboflavin 0.3, 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, $MgSO_47H_2O$ 4.4, $NaH_2PO_4H_2O$ 1.5, $ZnSO_47H_2O$ 0.1, $MnSO_4H_2O$ 0.08, KI 0.05, $CoCl_26H_2O$ 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 lipid source. The diets were prepared every other day and fed ad libitum. The fatty acid composition of the dietary fats is summarized in Table 1.

At the end of experimental diet period, all the animals were fasted for 24 hours and anesthetized with ether, and blood sample was obtained from arota into a test tube contained EDTA 1mg / ml blood. Plasma was separated by a centrifuge at 3000 rpm and stored at -10℃. Liver were excised, rinsed in ice cold saline, weighed and frozen for lipid extract.

**Lipid analysis :**

Total lipids were extracted from plasma and liver by the method of Bligh and Dyer. (20) 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 vacue 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 plate were dried in vacue to remove the solvent. Appropriate areas were scrapped off and lipid was transmethylated with $NaOCH_3$-methanol at 60℃ for 15 minutes. The lipids were saponified with 0.5N KOH in methanol according to the Morrison and Smith.(21)

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

**Preparation of microsomes :**

The rabbits were killed and took out liver. The liver (2g resected) were excised for the preparation of microsomes. Approximately 1g. of liver was diced over ice, and the diced liver

was homogenized in a buffered sucrose solution containing 0.1 M sucrose, 0.05 M KCl, 0.04 M $KH_2PO_4$, 0.03M EDTA, PH 7.4, in a homogenizer. The whole homogenates were centrifuged for 20 minutes at 10,000 g and the resulting supernatants were centrifuged for one hour at 105,000 g. The pellets were resuspended in buffer and centrifuged again at 105,000 g for one hour.

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.

## Ⅲ. Results

Effect of the PO or SO diet on the fatty acid composition of plasma lipids and phospholipids:

The fatty acid composition of plasma lipid and phospholipid in rabbits fed on the PO (perilla oil) or SO (sardine oil) diet for 4 weeks was different from those of the respective oils (Table 1, 2, and 4). In plasma lipids, phosphatidylcholine(PC), and phosphatidylethanolamine (PE), there were accumulations of 18 : 3, 20 : 4, and 22 : 6 in rabbits fed on the SO or PO diet.

Effect of the PO or SO diet on the fatty acid composition of liver

**microsomes** :

The fatty acid composition of liver microsomes in rabbits fed on the PO (perilla oil) or SO (sardine oil) diet for 4 weeks was different from those of the respective oil (Table 1 and 3). In liver microsomes, there were acumulations of 18 : 3, 20 : 4, 20 : 5, and 22 : 6 in rabbits fed on the PO diet or SO diet.

According to those results, $\alpha$-linolenic acid 18 : 3 n-3 in perilla oil was converted into both eicosapentaenoic acid 20 : 5 and docosahexaenoic acid 22 : 6 in rabbits.

## Ⅳ. Discussions

Dietary $\alpha$-linolenic acid 18 : 3 n-3 was more potent than linoleic acid 18 : 2 n-6 in lowering plasma cholesterol level. In the rabbits, the ingestion of perilla oil rich $\alpha$-linolenic acid resulted in plasma cholesterol level lowering.[6~7] Field, et al[22] reported that safflower oil or sunflower oil rich in linoleic acid n-6 showed plasma cholesterol level increased in the rabbits, But the

diets rich in the n-3 polyunsaturated fatty acids affected plasma cholesterol level, vascular contractivility and tissue lipid compostions.[23]

In rats fed the diets containing linseed oil, arachidonic acid 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.[24]

In the animals fed on the corn oil, 22 : 4 n-6 and 22 : 5 n-6 in both PC and PE were increased.[25] Feeding the either linseed oil or fish oil diet increased the arachidonic acid 20 : 4 n-6 content in phospholipid and caused an accumulation of eicosapentaenoic acid 20 : 5 n-3, and docosahexaenoic acid 22 : 6 n-3 fatty acid. (23-24) When rats were fed on a diet containing sardine oil, EPA 20 : 5 n-3 was incorporated into the platelet, arota and plasma lipid, AA 20 : 4 and EPA 20 : 5 in the platelet phospholipids might be substituted by each other.[27]

In this study, AA 20 : 4 level in plasma and microsome of the rabbits fed on the SO diet was similar to those on the PO diet, despite the fact that the PO contained 4 fold more linoleic acid 18 : 2, the precursor of arachidonic acid 20 : 4. The $\alpha$-linolenic acid 18 : 3 in perilla oil was very high (Table 1), but EPA and DHA did not contained. In both plasma

**Table 1. Fatty acid composition of dietary oils (%)**

| Fatty acid | sardine oil | perilla oil |
|---|---|---|
| 14 : 0 | 7.2 | ND |
| 16 : 0 | 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 |
| p / s | 1.4 | 7.4 |
| n-6 / n-3 | 0.14 | 0.27 |
| PUFA | 41.5 | 74.5 |
| MUFA | 28.5 | 14.5 |

T : Trace amounts (less than 1%)
ND : Not detect

and microsome of rabbits fed on the PO diet showed the EPA and DHA. It is able to say that $\alpha$-linolenic acid 18 : 3 was converted into EPA and DHA (Table 2 and 3).

Dietary fat could be played a major role in eicosanoid production and in modulation of the immune system.[28] The n-3 of menhaden oil appeared to inhibit both cycloxygenase and lipoxygenase.[29] Feeding 20% menhaden oil diet depressed eicosanoid or because less 20 : 4 precursor was being fed in the diet.[30] Therefore, eicosanoid synthesis could be selectively modified by feeding n-3 or n-6 precursors and the n-6 / n-3 ratio was an important factor.[31]

In this study, the n-6 / n-3 ratio was 0.3 and 0.2 in plasma lipid and phospholipid of rabbits fed on the SO or PO diet. In liver microsomes, the n-6 / n-3 ratio was 0.3 and 0.4 for SO diet group and 0.3 and 0.2 for PO diet group. The 20 : 5 / 20 : 4 ratios of phospholipid were 3.2 and 2.0 in plasma of rabbits fed on the SO diet, 2.3 and 3.3 in plasma of rabbits fed on the PO diet.

**Table 2. Fatty acid composition of plasma lipid from rabbits fed on diet containing sardine or perilla oil. (%)**

| Fatty acid | Plasma lipid | | Phospholipid | |
|---|---|---|---|---|
| | SO[a] | PO[a] | SO | PO |
| 14 : 0 | 3.1±0.3 | ND | T | 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[c] | 12.7±0.1 | 14.3±0.2[c] |
| 18 : 1 | 14.7±0.1 | 23.3±0.9 | 8.6±0.1 | 17.5±0.9 |
| 18 : 2 | 5.2±0.1 | 4.5±0.2 | 4.3±0.1 | 4.7±0.2 |
| 18 : 3 | 2.3±0.3 | 20.3±1.4[c] | 3.7±0.2 | 20.4±0.9[c] |
| 20 : 1 | 4.2±0.2 | ND | 1.7±0.1 | ND |
| 20 : 4 | 6.1±0.2 | .31±0.2 | 8.3±0.2 | 3.7±0.1 |
| 20 : 5 | 15.7±0.1 | 7.2±0.2[c] | 17.4±0.1 | 8.5±0.3[c] |
| 22 : 6 | 17.2±0.1 | 15.2±0.2[c] | 18.9±0.1 | 17.3±0.2[c] |
| Others[b] | 1.0 | 1.0 | 1.2 | 1.0 |
| P / S | 1.8 | 2.1 | 1.8 | 2.3 |
| n-6 / n-3 | 0.32 | 0.18 | 0.31 | 0.18 |
| PUFA | 46.5 | 50.3 | 52.7 | 54.6 |
| MUFA | 25.8 | 25.8 | 16.9 | 20.7 |

Values are the mean±SD of five samples (n=5) in each diet group.
a : SO : Sardine oil ; PO : Perilla oil
b : Others 20 : 2, 20 : 3, 22 : 5, each of these fatty acids was less than 1%.
c : $P<0.01$ versus SO

While 18 : 2 and 18 : 3 did not affect 20 : 4, 20 : 5, and 22 : 6 displaced 20 : 4 from spleen phospholipids.[31] Consumption of the safflower oil diet resulted in an increase in plasma 20 : 4 level, while in liver, the concentration of 20 : 4 was decreased in comparison with animals fed the diet high in beef tallow.[32]

**Table 3. Fatty acid composition of liver microsomes from rabbits fed on diet containing sardine or perilla oil. (%)**

| Fatty acid | liver lipid | | Phospholipid | |
|---|---|---|---|---|
| | SO[a] | PO[a] | SO | PO |
| 14 : 0 | T | ND | T | ND |
| 16 : 0 | 16.9±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[c] | 8.9±0.6 | 10.9±0.3[c] |
| 18 : 1 | 12.9±0.1 | 20.5±0.3 | 13.1±0.1 | 23.7±0.4 |
| 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[c] | 2.9±0.1 | 17.2±0.2[c] |
| 20 : 1 | 3.7±0.2 | ND | 4.5±0.3 | ND |
| 20 : 4 | 4.9±0.2 | 4.3±0.1 | 5.3±0.3 | 2.6±0.2 |
| 20 : 5 | 17.3±0.1 | 9.4±0.4[c] | 17.8±0.1 | 8.5±0.4[c] |
| 22 : 6 | 19.5±0.2 | 15.2±0.5[c] | 16.2±0.2 | 15.6±0.8[c] |
| Others[b] | 1.3 | 1.0 | 1.5 | 1.0 |
| P / S | 2.2 | 2.5 | 2.1 | 2.1 |
| n-6 / n-3 | 0.29 | 0.27 | 0.35 | 0.23 |
| PUFA | 51.5 | 54.6 | 48.9 | 50.7 |
| MUFA | 23.8 | 22.3 | 24.1 | 25.0 |

Values are the mean±SD of five samples (n=5) in each diet group.
a : SO : Sardine oil ; PO : Perilla oil
b : Others 20 : 2, 20 : 3, 22 : 5, each of these fatty acids was less than 1%.
c : $P<0.01$ versus SO

**Table 4. Fatty acid composition of phosphatide from rabbits fed on diet containing sardine or perilla oil. (%)**

| Fatty acid | Phosphatidylcholine | | Phosphatidylethanolamine | |
|---|---|---|---|---|
| | SO[a] | PO[a] | SO | PO |
| 16 : 0 | 20.3±0.1 | 9.5±0.2 | 15.4±0.3 | 6.4±0.1 |
| 16 : 1 | 3.5±0.1 | 1.5±0.1 | 1.5±0.1 | T |
| 18 : 0 | 16.5±0.3 | 14.2±0.2[c] | 23.5±0.1 | 12.5±0.2[c] |
| 18 : 1 | 8.3±0.2 | 19.1±0.5 | 7.9±0.1 | 18.2±0.1 |
| 18 : 2 | 3.5±0.1 | 1.5±0.1 | 2.7±0.2 | 2.5±0.2 |
| 18 : 3 | 2.7±0.2 | 25.2±0.2[c] | 1.5±0.2 | 28.3±0.3[c] |
| 20 : 1 | 1.5±0.1 | ND | 1.7±0.2 | ND |
| 20 : 4 | 8.5±0.3 | 2.2±0.3 | 6.3±0.2 | 2.5±0.1 |
| 20 : 5 | 15.2±0.9 | 9.3±0.3[c] | 14.9±0.6 | 10.2±0.2[c] |
| 22 : 6 | 18.5±0.5 | 16.5±0.5[c] | 23.4±0.3 | 18.4±0.3[c] |
| Others[b] | 1.5 | T | 1.2 | T |
| P / S | 1.36 | 2.31 | 1.29 | 3.28 |
| n-6 / n-3 | 0.37 | 0.07 | 0.26 | 0.08 |
| PUFA | 49.9 | 54.7 | 50.0 | 61.9 |
| MUFA | 13.3 | 20.6 | 11. | 18.2 |

Values are the mean±SD of five samples (n=5) in each diet group.
a : SO : Sardine oil ; PO : Perilla oil
b : Others 20 : 2, 20 : 3, 22 : 5, each of these fatty acids was less than 1%.
c : P<0.01 versus SO

## V. Abstract

To investigate the influence of dietary perilla oil or sardine oil on the 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 eicosapentaenoic acid and docosahexaenoic acid 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 stearic acid 18 : 0, arachidonic acid 20 : 4, eicosapentaenoic acid 20 : 5, and docosahexaenoic acid 22 : 6. The fatty acid composition of plasma lipid and liver microsomes of rabbits fed on the sardine oil was an accumulation of α-linolenic acid 18 : 3, and arachidonic acid 20 : 4. The p / s ratio of rabbits fed on the perilla oil diet changed from 7.4 to 2.3 for plasma lipid and to 2.5 for liver microsomes. The n-6 / n-3 ratio of rabbits fed on the perilla oil diet changed from 0.3 to 0.18 for plasma lipid and to 0.2 for liver microsomes. It was assumed that rabbits fed on the perilla oil diet rich α-linolenic acid was converted into the eicosapentaenoic and docosahexaenoic acid in the living

cell. The rabbits fed on the sardine oil diet rich eicosapentaenoic acid and docosahexaenoic acid showed $\alpha$-linolenic and arachidonic acid increasing.

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# 토끼의 혈장지질과 간장 마이크로솜의 지방산 조성에 미치는 들깨기름과 정어리기름의 영향

남 현 근

〉요 약〈

토끼의 혈장과 간장지질의 지방산 조성에 미치는 들깨기름의 영향을 조사하기 위하여 정어리기름과 비교하여 실험하였다.

실험식이로 동물들을 4주간동안 사육하고 실험식이 기간이 끝난 후 금식시키고 채혈한 다음 해부하여 간장을 적출하여 실험에 사용하였다.

혈장과 간장조직으로 부터 지방을 추출하고 인지질을 분리하고 지방산 조성을 조사하여 혈장지질과 인지질에 들깨기름을 급여한 동물에서 arachidonic acid (AA) 3~3.7%, eicosapentaenoic acid (EPA) 7~8.5%, docosahexaenoic acid (DHA) 15~17%를 보였고 간장지질과 인지질에서 arachidonic acid 2.6~4.3%, eicosapentaenoic acid 8~9% docosahexaenoic acid 15% 정도를 보여 w-3 $\alpha$-linolenic acid가 풍부한 들깨기름이 체내에서 AA, EPA, DHA로 전환됨을 알 수 있다.

그리고 P/S 비는 2.1~2.5, w-6/w-3비는 0.18~0.27을 보였다.