J. Food Sci. Nutr.
Vol. 6, No. 3, p. 170~175 (2001)

The Plasma Fatty Acid Composition and Cholesterol Levels of Rats Fed Different Sources of ω3 Fatty Acid and Excess DHA during Gestation, Lactation, and Growth

Hongmie Lee†, Juhee Lee*, Jiwon Kim* and Haymie Choi*

Department of Food and Nutrtion, Daejin University, Pocheon 487-711, Korea
**Department of Food and Nutrition, Seoul National University, Seoul 151-742, Korea*

Abstract

This study was designed to investigate the effect of different sources of ω3 fatty acid in the diet with a similar polyunsaturated/saturated (P/S) fatty acid ratio and ω6/ω3 fatty acid ratio as well as excess DHA on the plasma fatty acid composition and cholesterol level of rats. Three experimental diets contained 10% (w/w) dietary lipids. The control diet and one treatment diet were corn oil-based diets with different ω-3 fatty acid sources: perilla (CO) or fish oil (CF), respectively. In order to examine the effect of excess DHA, the other treatment diet (FO) was a fish oil-based diet with corn oil to supply essential fatty acids at the level of 1.8%(w/w) of the diet. Female Sprague Dawley rats were fed the experimental diets for 2 weeks prior to mating and throughout gestation and lactation. Pups were weaned to the same diet of dams at 21 days of age. Plasma fatty acid compositions and cholesterol contents were analyzed for pups at 3th, 7th and 10th week after birth. Plasma DHA concentrations increased significantly as the level of fish oil supplementation increased. Three-, seven- and ten-week old rats fed on CO diet which contained only α-linolenic acid as a ω-3 fatty acid source had plasma DHA levels of 4.85%, 3.15% and 2.47%, respectively, suggesting that rats at this period of development can convert α-linolenic acid to DHA. But the ability to form DHA might be limited, since dietary DHA showed to be more effective in raising the plasma level of DHA. There was a significant negative correlation between DHA and cholesterol concentration of the rat plasma at 7th week (r=0.34, p<0.05) and 10th week after birth (r=036, p<0.05), proving the hypocholesterolemic effect of DHA.

Key words: α-linoleic acid, DHA, plasma fatty acid composition, cholesterol

INTRODUCTION

Several sources of information suggest that man evolved on a diet with a ratio of ω6 to ω3 fatty acid of approximately 1, whereas today this ratio is approximately 10~25 (1). This indicates that our diets, becoming increasingly westernizing these days, may be deficient in ω3 fatty acids compared with the diet on which humans evolved and their genetic patterns were established.

Plasma fatty acid composition has been shown to alter in several physiological and pathological conditions such as the decreased levels of EPA in rheumatoid arthritis (2) and increased total polyunsaturated fatty acids with aging (3). Also, a negative correlation was reported between plasma DHA concentration and cerebraospinal fluid 5-hydroxyindoleacetic acid, a serotonin metabolite in impulsive violence (4). Moreover, consumption of a low-fat diet alters fatty acid patterns in a manner similar to that observed with feeding of ω3 long chain fatty acids (5).

Different physiological actions according to type of ω3 polyunsaturated fatty acid (PUFA) was also studied by Yamada et al. (6). They suggested that DHA be a more effective ω3 PUFA for suppression of platelet aggregation and for modulating lipid metabolism in plasma and liver and the active component of fish oil of which intake has been shown to have negative association with various cardiovascular diseases and tumor cell proliferation is DHA.

One important point of controversy that remains is the degree to which adequate levels of DHA can be acquired from endogenous synthesis in infants vs. what should be provided as dietary DHA. Recent studies using stable-isotope-labelled tracers demonstrated that even preterm infants are able to synthesize DHA but the level of synthesis is extremely low (7). Cunnane et al. (8) suggested that formula-fed infants not consuming DHA may not be able to convert the necessary 5.22% of α-linoleinic acid intake to DHA to match the DHA accumulation of breast-fed infants. Moreover, breast-fed infants score better on visual and developmental tests than formula-fed infants and this has been related to higher concentration of DHA in brain cortex (9).

On the other hands, Cheon et al. (10) suggested that if the diet contained appropriate amounts and balance of linoleic acid and linolenic acid, it may be possible for rats to synthesize an appropriate amount of DHA and have normal behavioral activity without DHA supplementation.

Although DHA is added to various infant formula, dairy

†Corresponding author. E-mail: hmlee@road.daejin.ac.kr
Phone: 82-31-539-1862, Fax: 82-31-539-1860

product or snacks in an effort to provide positive effects on the brain development and learning ability of the children, the effectiveness and safety of DHA supplementation remain unestablished yet. There have been numerous studies confirming beneficial effects of DHA supplementation to an ω3 fatty acid deficient diet (11-13). However, it was considered that the experiments on the effect of DHA supplementation should be done on diets which can be called normal, namely not deficient in ω3 fatty acids or DHA for the results of the study to be applied to human nutrition in our society.

Therefore, this study was designed to examine the effects of different ω3 fatty acid sources of the diets with similar ω3 fatty acid content and excess dietary DHA on the plasma fatty acid composition and cholesterol level of the rats according to developmental age and the level of DHA supplemented.

MATERIALS AND METHODS

Animals and diets

Thirty female Sprague Dawley rats donated by Yuhanyanghaeng were mated with the same breed for 10 days and fed in separate cage. Litter size was controlled to 8 at birth and pups were weaned to the same diet of dams at 21 days of age. Temperature, humidity and light were controlled to 20±1°C, 55±10%, 12 hours (07 : 00~19 : 00). Diet and water were provided adlibitum. Body weight was recorded weekly.

Three experimental diets contained 10% (w/w) dietary lipids (Table 1). Control diet and one treatment diet were corn oil based diet with different ω-3 fatty acid source: α-linolenic acid rich perilla (CO) or DHA rich fish oil (CF), respectively. The P/S ratio and ω6/ω3 ratio of CO and CF diets were made close to 1/1 and 4/1, respectively (Table 2). In order to examine the effect of excess DHA, the other treatment diet (FO) was a fish oil based diet with corn oil to supply essential fatty acids at the level of 1.8% (w/w) of the diet. The fatty acid composition of experimental diet was shown in Table 3. Sprague Dawley female rats were fed with experimental diets for 2 weeks prior to mating and throughout gestation and lactation. α-Tocopherol was added to prevent the peroxidation of the lipid in the diets at the level of 0.2% (w/w). Beef tallow, corn oil and perilla oil were

Table 1. Composition of experimental diets

Component	g/100 g diet
Corn starch	59.7
Casein	20
Fat	10
Vitamin mixture[1)]	1
Salt mixture[2)]	4
α-cellulose	5
DL-methionine	0.3
α-tocopherol	200 mg/100 g fat

[1)]Nutritional Biochemicals, ICN Life Science Group, Cleveland, Ohio. Vitamin mixture is composed of: vit. A acetate (500,000 IU/g) 1.8 g, vit. D_2 (850,000 IU/g) 0.125 g, DL-α-tocopherol (250 IU/g) 22.0 g, ascorbic acid 45.0 g, inositol 5.9 g, choline chloride 75.0 g, menadione 2.25 g, ρ-aminobenzoic acid 5.0 g, niacin 4.25 g, riboflavin 1.0 g, pyridoxine hydrochloride 1.0 g calcium pantothenate 3.0 g, biotin 0.02 g, folic acid 0.09 g, vit. B_{12} 0.00135 g, and dextrose to 1 kg.

[2)]Composition of salt mixture, g/kg mixture: $CaHPO_4$ 500 g, NaCl 74 g, K_2SO_4 52 g, potassium citrate monohydrate 220 g, MgO 24 g, manganase carbonate (42~48% Mn) 3.5 g, ferric citrate (16~17% Fe) 6.0 g, zinc carbonate (70% ZnO) 1.6 g, cupric carbonate (53~55% Cu) 0.3 g, KIO_3 0.01 g, chromium potassium sulfate 0.55 g, $Na_2SeO_3 \cdot 5H_2O$ 0.01 g, sucrose finely powdered 118.0 g.

Table 2. Fat composition of experimental diet

Dietary fat \ Group[1)]	CO	CF	FO
Beef tallow	4	3.5	-
Corn oil	5	5	1.8
Perilla oil	1	-	-
Fish oil	-	1.5	8.2
P/S[2)]	1.34	1.18	1.34
ω6/ω3[3)]	4	4.31	0.4

[1)]CO: corn oil based diet with perilla oil
CF: corn oil based diet with fish oil
FO: fish oil based diet

[2)]Calculated polyunsaturated fatty acid/saturated fatty acid ratio values

[3)]Calculated ω-6/ω-3 ratio values

Table 3. Fatty acid composition of experimental diet (% of total fatty acids)

Fatty acid	CO[1)]	CF[2)]	FO[3)]
C12 : 0	0.916	-	-
C14 : 0	2.082	3.567	3.234
C15 : 0	0.412	-	-
C14 : 1	0.388	-	0.475
C16 : 0	16.464	13.360	12.576
C16 : 1	1.612	3.099	4.893
C17 : 0	1.237	-	-
C18 : 0	10.618	24.208	6.987
C18 : 1	27.430	10.878	17.229
C18 : 2	29.748	31.582	13.330
C18 : 3 (ω-6)	0.153	-	-
C18 : 3 (ω-3)	7.614	0.992	0.854
C20 : 0	0.397	-	0.733
C20 : 1	0.433	0.643	1.015
C20 : 2	0.235	0.193	0.339
C20 : 4	-	0.673	2.510
C20 : 5	-	2.171	6.612
C22 : 0	0.154	0.424	0.632
C22 : 6	-	7.830	27.316
C24 : 0	0.147	0.377	1.267
ΣSFA	32.427	41.936	25.429
ΣMUFA	29.861	14.620	23.612
ΣPUFA	37.750	43.441	50.961
P/S	1.16/1	1.04/1	2.00/1
Σω-6	30.136	32.448	16.179
Σω-3	7.614	10.993	34.782
ω-6/ω-3	3.927	2.95	0.47
DHA	-	7.830	27.316

[1)~3)]Refer to the legend in Table 2.

purchased from Lotte Samkang, Heinz, and Nonghyup, respectively. Tuna oil which was extracted from the tuna eye was donated by Dongwonsanup. Casein, α-cellulose, DL-methionine and α-tocopherol were purchased from Sigma and a vitamin/salt mixture was obtained from ICN.

The diets were kept in a freezer under nitrogen gas and given to the animals daily.

Plasma fatty acid composition and cholesterol level

Plasma samples obtained from 3-, 7-, and 10-weeks old rats. Six offsprings from each experimental group were killed at the age of 3 weeks to collect plasma. Plasma samples from five female offsprings in each group were analyzed at the age of 7 weeks. Six male offsprings were killed from CO group and eight male offsprings were killed from each of the other two groups at the age of 10 weeks.

The method of Lepage and Roy (14) was modified to determine the composition of plasma fatty acid composition. One hundred mL of plasma was put in screw-capped tube and 2 mL of 2 : 1 (v/v) methanol : benzene solution was added. With stirring slowly, 200 μL of acetylchloride was added for 1 minute and capped tightly. Right after boiling at 100°C for 60 minutes, it was cooled in water and the reaction was stopped by adding 5 mL of6% K_2CO_3. The hexane layer was separated by centrifugation and used for determination of fatty acid composition. Gas chromatography was used to determine the content of the fatty acid under the condition in Table 4, and each fatty acid was identified by comparing the retention time with that of standard fatty acid ester. The content of each fatty acid was shown as the percentage of peak area to total area of all the identified fatty acids.

Plasma cholesterol concentration was determined using a kit from Yungdong Jeyak (Seoul, Korea).

Statistical analysis

SAS program was used to obtain mean and standard deviation of results of each experimental group. After analysis of variance, differences between groups were determined by Duncan's multiple range test at $p<0.05$. Pearson's correlation coefficient was used to analyze the association between plasma concentrationsof cholesterol and DHA.

Table 4. Conditions of gas chromatography for fatty acid determination

Instrument	Hewlett Packard 5973
Column	30 m×0.25 mm ID capillary column
Packing material	SP™-2330, 0.20 μm thickness
Detector	Mass Detector (Hewlett Packard 6890)
Carrier gas	He
Oven temperature	
Initial temperature	120°C
Initial time	2 min
Rate 1	10°C/min
Intermediate temperature	180°C
Intermediate time	15 min
Rate 2	10°C/min
Final temperature	200°C
Final time	5 min
Sample injected temperature	250°C
Sample detected temperature	260°C

RESULTS AND DISCUSSION

Feed intake and weight gain

The body weight changes and feed intake of the rats in each group are shown in Fig. 1 and Table 5, respectively. There was no significant difference in feed intake and weight gain of female rats between groups, while male rats in the CF group ate significantly less and tended to have slower weight gain than those of CO group from the 3 weeks of age. However, the final body weight of three groups did not differ (Fig. 1).

These results are consistent with previous reports. Feed intake and growth were not changed by the feeding an oil rich in DHA without EPA in the study of Atkinson et al. (15) and no significantly affected body weights was shown by the dietary fish oil in the study of Yonekubo et al. (16) or by the supplementation of different levels of fish oil in that of Bourre et al., too (17). However, Yuan et al. observed that rats fed menhaden oil exhibited smaller body weight

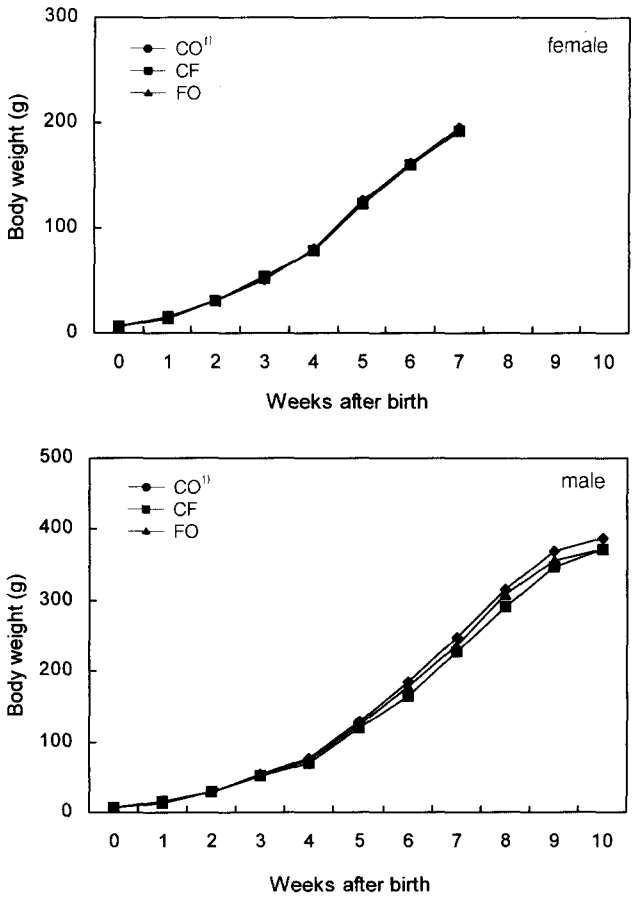

Fig. 1. The body weight change in male and female rats fed the experimental diets. Female rats sacrificed at 7 weeks and male rats sacrificed at 10 weeks after birth.
[1)]Refer to the legend in Table 2.

Table 5. Food intake and calorie intake in rats fed with the experimental diets

Group[1]	Female		Male	
	Food intake (g/rat/day)	Calorie intake (kcal/rat/day)	Food intake (g/rat/day)	Calorie intake (kcal/rat/day)
CO	12.1±3.84	49.3±15.69	18.9±6.41[a2)]	77.2±26.21[a]
CF	12.0±3.98	49.0±16.28	15.7±5.57[b]	64.4±22.76[b]
FO	10.9±3.48	44.5±14.23	17.3±5.44[ab]	70.8±22.24[ab]

[1)]Refer to the legend in Table 2.
[2)]Values are mean±SD from 5 rats and means with the different superscripts are significantly different at p<0.05 by Duncan's multiple range test.

gain and lower feed efficiency ratio (18).

Fatty acid composition of plasma

The plasma fatty acid composition of 3, 7, and 10-week old rats were shown in Table 6-1, 6-2 and 6-3. The most abundant fatty acids in the plasma were palmitic aicd, stearic acid, oleic acid, linoleic acid and arachidonic acid except for FO group in which DHA became at least third most abundant fatty acid.

The levels of linoleic acid and α-linolenic acid which were present in diets by the amount of 29.75% and 7.61%, 31.58% and 0.99%, and 13.33% and 0.85% in CO, CF, and FO diet, respectively (Table 3). But these values were much reduced in the plasma of 3-week old rats to 23.07% and 1.08%, 23.77% and 0.46%, and 12.65% and 0.16% in CO, CF, and FO, respectively (Table 6-1). Comparing to fatty acid composition of the diet, linoleic acid and α-linolenic acid levels in plasma became lower while arachidonic acid level became higher regardless of the diets. The same tendency was observed in 7 and 10 week-old rats, indicating that these two essential fatty acids can only be converted to other necessary compounds but not be synthesized in the rat body.

On the other hand, arachidonic acid which was absent in the CO diet and present only in very little quantities (0.67% and 2.51% in CF and FO, respectively) became at least the fourth most abundant fatty acid in the rat plasma (Table 6-1, 6-2, 6-3). This result indicates that arachidonic acid could be formed from linoleic acid enough in the rat.

Age also affected fatty acid composition and the most consistent change with developmental age was the decreases in EPA and DHA levels (Table 6-1, 6-2, 6-3). An increased PUFA has also reported with age of the Fischer 344 rats from 4-, 15- and 24-months old (3). The discrepancy between the two results may be attributed to the age (3 to 10 weeksvs. 4 to 24 months) or type of PUFA.

There are also some inconsistent tendencies found in the plasma fatty compositions with developmental stages, name-

Table 6-1. Fatty acid composition of plasma in rats fed with the experimental diets (3 weeks) (% of total fatty acids)

Fatty acids	CO[1)]	CF[2)]	FO[3)]
14 : 0	1.63±0.604	1.56±0.806	2.08±0.395
15 : 0	0.25±0.019[c4)]	0.31±0.052[b]	0.58±0.055[a]
16 : 0	17.58±0.923	17.64±0.374	17.27±0.804
16 : 1	1.12±0.154[b]	1.12±0.200[b]	2.08±0.246[a]
17 : 0	0.44±0.040[b]	0.59±0.111[b]	1.27±0.261[a]
18 : 0	13.76±0.397[a]	12.74±0.747[b]	11.04±0.539[c]
18 : 1	12.85±1.502[a]	11.18±1.958[a]	7.83±1.055[b]
18 : 2	23.07±0.300[a]	23.77±1.776[a]	12.65±0.585[b]
18 : 3 (ω6)	0.23±0.045[a]	0.19±0.051[a]	0.12±0.025[b]
18 : 3 (ω3)	1.08±0.230[a]	0.46±0.470[b]	0.16±0.046[b]
20 : 0	0.10±0.008[b]	0.14±0.026[b]	0.22±0.052[a]
20 : 1	0.23±0.069	0.21±0.047	0.27±0.089
20 : 2	0.35±0.033[a]	0.31±0.050[a]	0.17±0.059[b]
20 : 3	0.96±0.261[a]	0.84±0.057[a]	0.42±0.093[b]
22 : 0	0.11±0.069	0.15±0.050	0.08±0.010
20 : 4	19.85±2.459[a]	15.90±2.637[b]	17.68±1.389[ab]
20 : 5	0.78±0.264[b]	1.45±0.447[b]	5.79±0.890[a]
22 : 6	4.85±0.503[c]	10.51±3.382[b]	19.74±1.32[a]
24 : 0	0.46±0.123	0.45±0.121	0.37±0.107
24 : 1	0.39±0.298	0.54±0.180	0.69±0.045
ΣSFA	34.31±1.327[a]	33.49±1.006[ab]	32.88±0.580[b]
ΣMUFA	14.53±1.365[a]	13.06±2.160[a]	10.41±1.334[b]
ΣPUFA	51.16±2.486[b]	53.45±2.766[b]	56.71±1.527[a]
P/S	1.50±0.130[b]	1.60±0.123[ab]	1.73±0.066[a]
ω6	44.44±2.29[a]	41.02±1.845[b]	31.01±1.179[c]
ω3	6.72±0.488[a]	12.43±3.050[b]	25.70±1.029[c]
ω6/ω3	6.64±0.524[a]	3.52±1.150[b]	1.21±0.070[c]

[1)-3)]Refer to the legend in Table 2.
[4)]Values are mean±SD from 6 rats and means with the different superscripts are significantly different at p<0.05 by Duncan's multiple range test.

Table 6-2. Fatty acid composition of plasma in rats fed with the experimental diets (7 weeks) (% of total fatty acids)

Fatty acids	CO[1)]	CF[2)]	FO[3)]
14 : 0	0.90±0.196	0.69±0.026	0.82±0.102
15 : 0	0.30±0.049[b4)]	0.30±0.081[b]	0.46±0.065[a]
16 : 0	20.41±0.955	21.62±2.482	20.56±1.161
16 : 1	1.49±0.287[b]	1.35±0.321[b]	2.14±0.282[a]
17 : 0	0.78±0.184[b]	0.66±0.074[b]	1.19±0.129[a]
18 : 0	28.43±5.791[a]	21.48±1.174[b]	20.17±1.781[b]
18 : 1	11.58±2.794	11.05±1.946	10.21±1.256
18 : 2	11.68±2.157[b]	14.00±1.326[a]	6.59±0.846[c]
18 : 3 (ω-6)	0.42±0.102[a]	0.23±0.044[b]	0.10±0.033[c]
18 : 3 (ω-3)	0.72±0.233[a]	0.13±0.013[b]	0.16±0.010[b]
20 : 0	0.30±0.089	0.19±0.020	0.24±0.071
20 : 1	0.13±0.070	0.14±0.044	0.08±0.022
20 : 2	0.10±0.036	0.10±0.035	-
20 : 3	0.37±0.116[b]	0.55±0.065[a]	0.29±0.059[b]
20 : 4	17.72±2.914	17.47±2.885	14.04±1.867
20 : 5	0.64±0.223[c]	1.49±0.385[b]	5.13±0.719[a]
22 : 0	0.29±0.024[a]	0.19±0.047[b]	0.18±0.030[b]
22 : 6	3.15±0.600[c]	7.41±0.704[b]	16.92±1.256[a]
24 : 0	0.50±0.147	0.56±0.117	0.40±0.090
24 : 1	0.16±0.137[b]	0.40±0.068[a]	0.40±0.104[a]
ΣSFA	51.91±6.801[a]	45.69±2.017[b]	44.01±2.704[b]
ΣMUFA	13.32±3.051	12.93±2.191	12.74±1.309
ΣPUFA	34.77±3.895[b]	41.38±3.391[a]	43.24±3.217[a]
P/S	0.69±0.1605[b]	0.91±0.113[a]	0.99±0.124[a]
ω-6	30.27±3.610[a]	32.35±2.655[a]	21.03±2.166[b]
ω-3	4.50±0.750[c]	9.03±1.067[b]	22.22±1.358[a]
ω-6/ω-3	6.84±1.186[a]	3.61±0.370[b]	0.95±0.078[c]

[1)-3)]Refer to the legend in Table 2.
[4)]Values are mean±SD from 5 rats and means with the different superscripts are significantly different at p<0.05 by Duncan's multiple range test.

Table 6-3. Fatty acid composition of plasma in rats fed with the experimental diets (10 weeks) (% of total fatty acids)

Fatty acids	CO[1)]	CF[2)]	FO[3)]
14 : 0	0.46±0.130	0.51±0.122	0.54±0.137
15 : 0	0.23±0.045[c4)]	0.30±0.056[b]	0.47±0.073[a]
16 : 0	19.61±1.012[b]	23.82±3.434[a]	24.12±2.617[a]
16 : 1	2.32±0.309	1.82±0.680	1.96±0.176
17 : 0	0.40±0.061[b]	0.48±0.101[b]	0.80±0.339[a]
18 : 0	10.42±1.194	10.63±3.037	12.45±3.520
18 : 1	21.15±1.997[a]	20.33±3.795[a]	12.14±0.831[b]
18 : 2	20.74±1.743[a]	20.53±5.047[a]	10.06±0.979[b]
18 : 3 (ω-6)	0.29±0.065[a]	0.21±0.065[b]	0.06±0.006[c]
18 : 3 (ω-3)	1.55±0.294[a]	0.19±0.082[b]	0.14±0.028[b]
20 : 0	0.13±0.076	0.13±0.025	0.18±0.043
20 : 1	0.39±0.128[b]	0.52±0.276[ab]	0.82±0.371[a]
20 : 2	0.26±0.128	0.24±0.100	0.17±0.114
20 : 3	0.40±0.237[b]	0.69±0.167[a]	0.30±0.143[b]
20 : 4	18.26±2.684[a]	10.71±3.808[b]	17.41±1.740[a]
20 : 5	0.41±0.247[b]	0.89±0.343[b]	2.68±0.596[a]
22 : 0	0.13±0.040	0.10±0.028	0.17±0.048
22 : 6	2.47±0.370[c]	7.59±1.036[b]	15.26±1.516[a]
24 : 0	0.26±0.119	0.26±0.097	0.38±0.181
24 : 1	0.24±0.103	0.15±0.017	0.18±0.079
ΣSFA	31.62±2.068[b]	36.20±6.130[ab]	39.02±5.126[a]
ΣMUFA	24.00±2.222[a]	22.75±6.130[ab]	15.05±1.217[b]
ΣPUFA	44.38±1.794[a]	41.05±1.770[b]	45.93±4.134[a]
P/S	1.41±0.122	1.16±0.220	1.20±0.248
ω-6	39.95±1.589[a]	32.38±2.100[b]	27.85±2.854[c]
ω-3	4.43±0.388[c]	8.67±1.037[b]	18.08±1.856[a]
ω-6/ω-3	9.07±0.717[a]	3.81±0.716[b]	1.55±0.153[c]

[1)~3)]Refer to the legend in Table 2.

[4)]Values are mean±SD from 5 rats and means with the different superscripts are significantly different at $p<0.05$ by Duncan's multiple range test.

ly 3 weeks through to 7 and 10 weeks of age. For example, the mean ratios of $\omega 6/\omega 3$ were 6.64, 3.52 and 1.21 for the 3 week old rats of CO, CF and FO group, respectively while those were 6.84, 3.61 and 0.95 for 7 week old rats and 9.07, 3.81 and 1.55 for 10 week old rats in CO, CF, and FO group, respectively. These inconsistent tendency may be attributed to the fact that the plasma was collected from only female 7 week old rats and only male 10 week old rats. Therefore, the effect of different sources of $\omega 3$ fatty acid and excess DHA of the diet on the plasma fatty acid composition should be studied further in the aspect of gender difference.

Plasma DHA concentrations increased significantly as the level of fish oil supplementation increased. Rats on the CO diet which did not contain any DHA showed to have 4.85%, 3.15%, and 2.47% of plasma DHA at 3, 7 and 10 weeks of age, respectively (Table 6-1, 6-2, 6-3). This proves that the ability for rats at this age can synthesize DHA, although it is unknown if this amount can match bodily need for this fatty acid. While Table 6-1, 6-2, 6-3 also shows that EPA could also be formed from linolenic aicd and the levels increased significantly with fish oil supplementation, the levels of EPA was much lower, namely 1/8.5 to 1/3 of that of DHA.

Rats on the FO diet which has three times (27.32% vs 7.83% in Table 3) more DHA had only two times (19.74% vs 10.51%, 16.92% vs 7.41% and 15.26% vs 7.59% for 3-, 7- and 10-weeks old rats in Table 6-1, 6-2, 6-3) more plasma DHA, compared to those on CF diet. The results of this study also suggested that rats have some mechanisms to defense against dietary changes.

Fish oil supplementation significantly increased $\omega 3$ fatty acid as significantly decreased $\omega 6$ fatty acids in plasma from CO and CF to FO rats. Plasma linoleic acid level of rats supplemented fish oil was significantly lower than that of CO and CF, which supported the other researchers' studies (19).

However, the concentration of another ω-6 fatty acid, arachidonic acid level of plasma did not reduced by fish oil supplementation in this study (Table 6-1, 6-2, 6-3), which was not consistent with the previous studies (19). The discrepancy may be attributed to the difference between the dietary fatty acids. FO diet in this study was added with 1.8% (w/w) corn oil to prevent essential fatty acid deficiency, resulting in 13.33% linoleic acid of FO diet. Since FO diet contained little linolenic acid and much EPA and PUFA (6.61% and 27.32%, respectively), enzymes for elongation and desaturation must have been easily available for linoleic acid to be converted to arachidonic acid in the rats on FO diet.

Plasma cholesterol concentration

Plasma cholesterol concentration was significantly lower in CF group at 3 weeks after birth and the same effect was observed in FO group at 7 weeks after birth, while 10 week old rats in CF and FO group had significantly lower plasma cholesterol level than those in CO group (Fig. 2).

There was a significant negative correlation between PUFA and cholesterol concentration of the rat plasma at 7 weeks ($r=0.34$, $p<0.05$) and 10 weeks after birth ($r=036$, $p<0.05$).

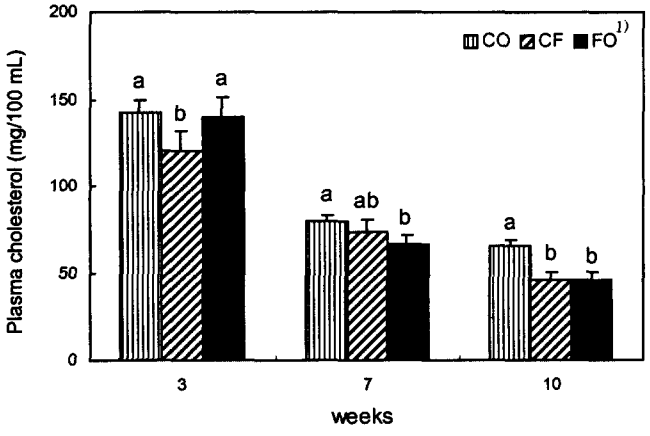

Fig. 2. Plasma cholesterol content in rats fed the experimental diets. Female rats sacrificed at 3 and 7 weeks and male rats sacrificed at 10 weeks. Values are mean±SD and means with the different letters (a,b) are significantly different at $p<0.05$ by Duncan's multiple range test.

[1)]Refer to the legend in Table 2.

This is consistent to the study of Yaqoob et al. (20) and Yuan et al. (18) reporting that plasma cholesterol as well as triglyceride level by dietary fish oil supplementation. Chicks have been used as an animal model in this regard. Castillo et al. (19) reported significantly reduced cholesterol in LDL, HDL and VLDL and suggested a strong hypocholesterolemic effect of menhaden oil supplemented to hypercholesterolemic chicks.

ACKNOWLEDGMENTS

This study was supported by a grant from the Korean Ministry of Health and Welfare, HMP-98-F-4-0012.

REFERENCES

1. Simopoulos, A.P. : Human requirement for N-3 polyunsaturated fatty acids. *Poult. Sci.*, **79**, 961 (2000)
2. Navarro, E., Esteve, M., Olive, A., Klaassen, J., Cabre, E., Tena, X., Ferandex-Banares, F., Pastor, C. and Gassull, M.A. : Abnormal fatty acid pattern in sheumatoid arthritis. A rationale for treatment with marine and botanical lipids. *J. Rheumatol.*, **27**, 298 (2000)
3. Engler, M.M., Engler, M.B. and Nguyen, H. : Age-related changes in plasma and tissue fatty acid composition Fischer 344 rats. *Biochem. Mol. Biol. Int.*, **46**, 1117 (1998)
4. Hibblen, J.R., Umhau, J.C., Linnoila, M., Geeorge, D.T., Ragan, P.W., Shoaf, S.E., Vaughan, M.R., Rawlings, R. and Salem, N.Jr. : A replication study of violent and nonviolent subjects : cerebrosopinal fluid metabolites of serotonin and dopamine are predicted by plasma essential fatty acids. *Biol. Psychiatry*, **15**, 243 (1998)
5. Raatz, S.K., Bibus, D., Thomas, W. and Kris-Etherton, P. : Total fat intake modifies plasma fatty acid composition in humans. *J. Nutr.*, **131**, 231 (2001)
6. Yamada, N., Shimizu, J., Wada, M., Takita, T. and Innami, S. : Changes in platelet aggregation and lipid metabolism in rats given dietary lipids containing different n-3 polyunsaturated fatty acids. *J. Nutr. Sci. Vitaminol.*, **44**, 279 (1998)
7. Uauy, R., Mena, P. and Rojas, C. : Essential fatty acids in early life : structural and functional role. *Proc. Nutr. Soc.*, **59**, 3 (2000)
8. Cunnane, S.C., Francescutti, V., Brenna, J.T. and Crawford, M.A. : Breast-fed infants achieve a higher rate of brain and whole body docosahexaenoic acid accumulation than formula-fed infants not consuming dietary docosahexaenoate. *Lipids*, **35**, 105 (2000)
9. Makrides, M., Neumann, M.A., Byard, R.W., Simmer, K. and Gibson, R.A. : Fatty acid composition of brain, retina, and erythrocytes in breast and formula-fed infants. *Am. J. Clin. Nutr.*, **60**, 189 (1994)
10. Cheon, S.H., Huh, M.H., Lee, Y.B., Park, J.S., Sohn, H.S. and Chung, C.W. : Effect of dietary linoleate/linolenate balance on the brain lipid composition, reproductive outcome and behavior of rats during their prenatal and postnatal development. *Biosci. Biotechnol. Biochem.*, **64**, 2290 (2000)
11. Carrie, I., Clement, M., De Javel., D., Frances, H. and Bourre, J.M. : Phospholipid supplementation reverses behavioral and biochemical alterations induced by n-3 polyunsaturated fatty acid deficiency in mice. *J. Lipid Res.*, **41**, 473 (2000)
12. Um, Y.S., Chung, E.J., Lee, Y.C. and Kim, Y.C. : Effects of dietary ω3 and ω6 fatty acid on the fatty acid composition of RBC and brain synaptosomal, microsomal and mitochondrial phospholipids and on behavioral development of rats. *Kor. J. Nutr.*, **29**, 849 (1996)
13. Yokota, A. : Relationship between polyunsaturated fatty acid and learning ability in the brain of rat fetus and newborn. *Nippon Sanka Fujinka Gakkai Zasshi*, **45**, 15 (1993)
14. Lepage, G. and Roy, C.C. : Direct transesterification of all classes of lipids in a one-step reaction. *J. Lipid. Res.*, **27**, 114 (1986)
15. Atkinson, T.G., Barker, H.J. and Mechking-Gill, K.A. : Incorporation of long-chain n-3 fatty aids in tissues and enhanced bone marrow cellularity with docosahexaenoic acid feeding in post-weanling Fischer 344 rats. *Lipids*, **32**, 293 (1997)
16. Yonekubo, A., Honda, S., Okano, M., Takahashi, K. and Yamamoto, Y. : Dietary fish oil alters rat milk composition and liver and brainfatty acid composition of fetal and neonatal rats. *J. Nutr.*, **123**, 1703 (1993)
17. Bourre, J.M., Bonneil, M., Dumont, O., Piciotti, M., Nalbone, G. and Lafont, H. : High dietary fish oil alters the brain polyunsaturated fatty acid composition. *Biochem. et Biophys. Acta*, **960**, 458 (1988)
18. Yuan, Y.V., Kitts, D.D. and Godin, D.V. : Variations in dietary fat and cholesterol intakes modify antioxidant status of SHR and WKY rats. *J. Nutr.*, **128**, 1620 (1998)
19. Castillo, M., Amlik, F., Linares, A. and Garcia-Peregrin, E. : Fish oil reduces cholesterol and arachidonic acid levels in plasma and lipoproteins from hypercholeserolemic chicks. *Mol. Cell Biochem.*, **210**, 121 (2000)
20. Yaqoob, P., Sherrington, E.J., Jeffery, N.M., Sanderson, P., Harvey, D.J., Newsholme, E.A. and Calder, P.C. : Comparison of the effects of a range of lipids upon serum and tissue lipid composition in rat. *Int. J. Biochem. Cell. Biol.*, **27**, 297 (1995)

(Received July 20, 2001)