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Effects of Diets on Serum and Liver Lipid Levels and Fatty Acid Composition of Liver Phospholipids in Rats

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ABSTRACT

Serum and liver lipid levels and fatty acid composition of liver phospholipids(PL) were investigated in 36 rats which consumed either one of five different dietary fats or a high carbohydrate diet for 4 weeks. As the sources of five dietary fats, concentrated eicosapentaenoic acid(EPA), fish oil(FO), perilla oil(PO), corn oil(CO) and beef tallow(BT) were provided to the rats. As a control group, corn starch(CS) replaced dietary fat. The FO group showed lower serum total cholesterol(TC), high density lipoprotein cholesterol(HDL-C) and serum PL levels than those of the CO group($p<0.05$). There were no significant differences in serum TC and serum HDL-C levels between the polyunsaturated fatty acid(PUFA) groups and the EPA, FO and PO groups. The CS group showed the highest level of serum TC. Compared with the CS group, both the EPA and CO groups showed significantly lower atherogenic indices(AI). However, there were no significant differences in AI among different dietary fat groups. No significant differences in liver triglyceride(TG), TC and PL levels were detected among the six experimental groups. Phosphatidylcholine(PC) and phosphatidylethanolamine(PE) composed 30 - 40% and 15 - 20% of total liver PL, respectively. The fatty acid composition of liver PC and PE reflected dietary fatty acid composition. Compared to the different dietary fat based diets used in our study, the high carbohydrate diet had the most adverse effects on serum lipid profiles. However, we can not conclude from this result that long chain n-3 PUFA diets such as the EPA and FO based diets have more beneficial effects on serum lipid profiles than n-6 PUFA diet such as the CO based diet or shorter chain n-3 PUFA diets like the PO based diet. (*Korean J Nutrition* 30(10) : 1140~1152, 1997)

KEY WORDS : fatty acids · serum lipids · liver lipids · phosphatidylcholine · phosphatidylethanolamine.

Introduction

The composition of diets, coupled with fatty acid metabolism in the liver, effects changes in the fatty acid composition of serum and tissue membrane phospholipids(PL), and also affects atherosclerosis-related physiological functioning of the body by influencing the liver, serum lipids, lipoproteins levels and eicosanoids synthesis[1)2)] Results of previous studies which have demonstrated the effects of dietary fatty acids on the serum and liver lipid levels have not always been consistent. Some studies have demonstrated that the n-3 polyunsaturated fatty acids

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(PUFA) reduce serum lipid levels more effectively than n-6 PUFA[3,4] However, others have shown that the degree of unsaturation of PUFA is what affects more potentially serum and liver lipid levels[5]. There have also been previous studies which did not show any significant effects of dietary PUFA on serum lipid levels, and some studies have shown that PUFA diets had adverse effects on serum lipid profiles[6,7]. Phosphatidylcholine(PC) and phosphatidylethanolamine(PE) are known to be the major fractions of membrane PL. It has been demonstrated that, in platelets, the uptake and release of eicosanoid precursors are mainly via PC and PE fractions rather than phosphatidylserine(PS) or phosphatidylinositol (PI) fractions of platelet PL[8]. In this study, we investigated serum and liver lipid levels as well as the fatty acid composition of liver PC and PE fractions and the relationship among the variables in rats which were fed one of five different fatty acid based diets or a high carbohydrate diet. The relationships among the variables affecting the lipid levels and fatty acid composition were also investigated.

Materials and Methods

1. Animals and experimental diets

Thirty six male Sprague-Dawley rats at 3 weeks in age that had an average weight of 45.3±2.3g were used in this study after having been fed commercial pellet diets for 3 days as a baseline period. Rats were randomly assigned to the six groups and fed six different diets for the 4 weeks of the experimental period. Diets were based on the AIN-76 diet(Nihon Nosan Kogyo K.K, Japan), and fat contents of the experimental diets composed 10% of the diets(22% of total energy). The five dietary fat sources were concentrated eicosapentaenoic acid(EPA), fish oil (FO), perilla oil(PO), corn oil(CO) and beef tallow (BT). As a control group, dietary fat was replaced by corn starch(CS). 2% of the control diet(4% of total energy) was composed of corn oil. To prevent the oxidation of dietary fats, all experimental diets were prepared every 3 days and stored under N_2 gas in a freezer at −20℃. The composition of the diets used in this study are shown in Table 1.

2. Serum lipid analysis

Fasting blood samples were obtained through heart puncture from anesthetized rats after 4 weeks of feeding on the experimental diets. Serum samples obtained by centrifugation were stored at −70℃ until serum lipid analysis was performed. Serum triglycerides(TG) were analyzed by an enzymatic assay using the glycerol-3-P oxidase-p-chlorophenol color development method(Wako, Japan)[9]. Serum total cholesterol(TC) was analyzed by an enzymatic assay using oxidase-p-chlorophenol(Wako, Japan)[10]. Serum total high density lipoprotein cholesterol(HDL-C) was determined with the same method used for serum TC analysis after precipitating non-HDL lipoproteins with dextran sulfate(50000MW, 0.4mmol/l Sigma) and magnesium($MgCl_2 \cdot 6H_2O$). Serum total PL was analyzed by the choline oxidase phenol method(Wako, Japan)[11]. Atherogenic Index was calculated by the following formula :

$$\text{Atherogenic Index} = \frac{\text{serum TC} - \text{serum HDL-C}}{\text{serum HDL-C}}$$

3. Liver lipid analysis

After liver was removed from sacrificed rats, liver samples were frozen with dry ice and ethanol and stored at −70℃. Total liver lipids were extracted from homogenized liver samples by the method of Bligh and Dyer[12] using chloroform-methanol(1 : 2, v/v) with 2M KCl and $CHCl_3$ in 1N HCl solution. Liver total PL were analyzed by Bartlett's methods[13] using 10N H_2SO_4 as the extraction solution, and the absorbance was read at 830nm. Analysis of liver TG was carried out by Flechter's methods[14]. Liver cholesterol was analyzed by Zak's methods[15] using H_3PO_4 and $FeCl_3 \cdot 6H_2O$ as a coloring reagent.

4. Fatty acid composition of PC and PE of liver

From extracted lipids, PC and PE fractions of PL were isolated by thin layer chromatography(TLC) using dual developing solvent systems. The first solvent system used to isolate the PL fraction was composed

of chloroform : acetone : methyl alcohol : acetic acid : d-d water(100 : 100 : 50 : 4 : 10, v/v), and the second solvent for PC and PE isolation was composed of chloroform : acetone : acetic acid : d-d water(180 : 150 : 30 : 10, v/v). Esterification of fatty acids of PC and PE in scraped silicic acid was carried out using the method of Morrison[16]. Gas chromatography(HP 5890 II) with a capillary column(SP-2340) and a flame-ionized detector were used to separate and quantitate the fatty acid esters. Nitrogen was the carrier gas and oven temperature was programmed to increase 3℃/min from 150℃ to 230℃.

5. Statistics

One way ANOVA was used to determine the significance of the differences among the variables of the six different experimental groups. Duncan's multiple range test was applied for the multiple comparisons. The level of significance was $p<0.05$. Pearson correlation coefficients were used to determine the rela-

Table 1. Composition of the experimental diets

Ingredients		Diet groups[1](g/100g)					
		EPA	FO	PO	CO	BT	CS
Casein		20	20	20	20	20	20
CHO	Corn starch	10	10	10	10	10	18
	Sucrose	50	50	50	50	50	50
Fat	EPA(63%)	10					
	Fish oil		10				
	Perilla oil			10			
	Corn oil				10		2
	Beef tallow					10	
DL-Methionine		0.3	0.3	0.3	0.3	0.3	0.3
Mineral-Mix.		3.5	3.5	3.5	3.5	3.5	3.5
Vitamin-Mix.		1.0	1.0	1.0	1.0	1.0	1.0
Fiber		5.0	5.0	5.0	5.0	5.0	5.0
Fatty acid							
14 : 0		–	7.56	–	–	2.26	–
16 : 0		–	18.86	5.95	11.35	23.58	11.35
18 : 0		1.00	3.31	1.88	1.81	16.83	1.81
18 : 1 (n-9)		1.71	10.60	16.88	25.81	39.00	25.81
18 : 2 (n-6)		–	1.58	11.84	57.39	8.07	57.39
18 : 3 (n-3)		12.12	4.76	62.99	–	–	–
18 : 4 (n-3)		–	2.93	–	–	–	–
20 : 4 (n-6)		7.60	4.91	–	–	–	–
20 : 5 (n-3)		68.06	16.36	–	–	–	–
22 : 6 (n-3)		3.77	14.90	–	–	–	–
SFA[2]		1.00	29.73	7.83	3.16	42.64	13.16
MUFA[3]		1.71	10.60	16.88	25.80	39.00	25.80
PUFA[4]		91.55	43.86	74.83	57.39	8.07	57.39
Total n-6 PUFA		7.60	6.49	11.84	57.39	8.07	57.39
Total n-3 PUFA		83.95	38.95	62.99	–	–	–
P/S ratio[5]		91.55	1.48	9.56	4.36	0.19	4.36
n-3/n-6 ratio[6]		11.05	6.00	5.32	–	–	–

1) Listed abbraviations in diet groups :
EPA : EPA group, FO : Fish oil group, PO : Perilla oil group, CO : Corn oil group, BT : Beef tallow group, CS : Corn starch group.
2) SFA : Saturated fatty acid
3) MUFA : Monounsaturated fatty acid
4) PUFA : Polyunsaturated fatty acid
5) P/S ratio : ratio of PUFA/SFA
6) n-3/n-6 ratio : ratio of n-3 PUFA/n-6 PUFA

tionships among serum and liver lipids and liver phospholipid composition. Statistical analysis was done with the SAS system(SAS Institute, Inc., Cary, NC).

Results

1. Animal body weight gain and food intakes

Body weight gain and food consumption of the rats during the experimental period are shown in Table 2. The lowest body weight gain and food efficiency ratio(FER), and the highest liver weight were observed in the EPA group. Body weight gain was the highest in the FO group. It is possible that rats in the EPA group were essential fatty acid deficient due to the lack of linoleic acid(LA, 18 : 2, n-6) in the diet. We therefore recommend that adult animals not in an actively growing state be used to investigate the effects of the diets on serum and liver lipid metabolism.

Table 2. Body weight gain, weight of liver, food consumption and food efficiency ratio(FER) of rats

Diet group[1)]	Weight gain(g/day)	Liver (g/100g BW)	Food intake	FER[2)]
EPA	$3.2\pm0.2^{a3)}$	4.1 ± 0.1^{b}	18.6 ± 1.0	0.17 ± 0.02^{a}
FO	7.4 ± 0.3^{c}	3.5 ± 0.0^{a}	21.9 ± 1.4	0.34 ± 0.02^{b}
PO	6.3 ± 0.5^{b}	3.4 ± 0.1^{a}	20.8 ± 0.8	0.30 ± 0.07^{b}
CO	6.7 ± 0.4^{bc}	3.5 ± 0.2^{a}	20.6 ± 1.2	0.33 ± 0.03^{b}
BT	5.9 ± 0.4^{b}	3.5 ± 0.2^{a}	21.1 ± 1.2	0.28 ± 0.04^{b}
CS	6.4 ± 0.0^{b}	3.7 ± 0.1^{ab}	20.8 ± 0.9	0.31 ± 0.04^{b}

1) Listed abbraviations in diet groups :
EPA : EPA group, FO : Fish oil group, PO : Perilla oil group, CO : Corn oil group, BT : Beef tallow group, CS : Corn starch group
2) FER(Food Efficiency Ratio)=Body weight gain(g/day)/Food intake(g/day)
3) Mean±SE. Values in the same column with different superscript letters are significantly different at $p<0.05$ by Duncans multiple range test

2. Serum lipid levels

Serum lipid levels of the rats in the six diet groups are shown in Table 3. Although the CS group showed the highest serum TG levels while the EPA group showed the lowest serum TG levels, the difference was not significant. The serum TC level was significantly high in the CS group and significantly low in the FO group. In this study, the n-3 PUFA diet groups(FO, EPA and PO groups) showed relatively lower serum TC levels than the n-6 PUFA diet group(CO group), but here a significant difference in serum TC was found only between the FO and CO group. Among the n-3 PUFA diet groups, the FO group showed lower serum TC levels than those of the EPA and the PO groups, but the result was not significant. Serum HDL-C levels were significantly low in the FO group and significantly high in the CO group. Despite high serum TC levels, the high HDL-C level of the CO group resulted in a low atherogenic index. The atherogenic index was thus significantly low in both the EPA and CO groups compared with the CS group. There was no significant difference in atherogenic indices among the experimental fat based groups. Serum PL concentration was significantly low in the FO and EPA groups, and significantly higher in the CS, BT and

Table 3. Serum concentrations of triglyceride, total cholesterol, HDL-cholesterol, phospholipids and atherogenic index

Diet group[1)]	TG[2)]	TC	HDL-C	PL	AI
	(mg/dl)				
EPA	45.2 ± 12.3	$68.5\pm13.4^{ab3)}$	41.2 ± 2.9^{ab}	112.2 ± 4.8^{a}	0.6 ± 0.2^{a}
FO	56.8 ± 7.7	55.3 ± 4.4^{a}	31.0 ± 4.0^{a}	108.8 ± 3.5^{a}	0.8 ± 0.2^{ab}
PO	58.4 ± 10.6	73.1 ± 6.6^{ab}	39.7 ± 3.3^{ab}	127.0 ± 7.3^{ab}	1.0 ± 0.3^{ab}
CO	63.8 ± 7.3	79.5 ± 6.4^{bc}	46.6 ± 3.8^{b}	158.9 ± 11.2^{b}	0.7 ± 0.2^{a}
BT	54.5 ± 5.4	72.3 ± 4.6^{ab}	38.6 ± 4.2^{ab}	160.1 ± 11.8^{b}	1.0 ± 0.3^{ab}
CS	84.1 ± 25.0	96.9 ± 7.7^{c}	39.5 ± 5.8^{ab}	160.7 ± 23.9^{b}	1.7 ± 0.4^{b}

1) Listed abbraviations in diet groups :
EPA : EPA group, FO : Fish oil group, PO : Perilla oil group, CO : Corn oil group, BT : Beef tallow group, CS : Corn starch group
2) Listed abbraviations in variables :
TG : Triglycerides, TC : Total cholesterol, HDL-C : High density lipoprotein-cholesterol, PL : Phospholipids, AI : Atherogenic Index
3) Mean±SE. Values in the same column with different superscript letters are significantly different at $p<0.05$ by Duncan's multiple range test

CO groups. The high serum PL concentrations in the CS and CO groups seem to be related with high serum TC and TG levels.

3. Liver lipid levels and fatty acid compositions of liver PC and PE

Liver TG, TC and total PL levels as well as the distribution of PC and PE of the rat liver are shown in Table 4. No significant differences in liver TG, TC and PL levels among the groups were found. The BT and CS groups showed insignificantly higher TG levels than other groups. The EPA group showed insignificantly higher TC and insignificantly lower TG concentrations. Although significant differences in PC and PE contents of the liver PL were not found among the groups, the PE fraction was highest in the EPA group and the PC fraction was the highest in the BT group. PC and PE composed 30-40 % and 15-25 % of total rat liver PL, respectively.

Fatty acid compositions of liver PC and PE were analyzed(Table 5, 6). Major fatty acids of which the

Table 4. Concentration of triglyceride, total cholesterol, total phospholipid and distribution of phosphatidylcholine and phosphatidylethanolamine in rat liver phospholipid

Diet group[1)]	TG[2)]	TC	Total PL	Distribution(% total phospholipid)	
	(mg/g tissue)			PC	PE
EPA	1.75±1.4[3)]	2.84±0.2	21.3±2.4	33.2	24.6
FO	2.17±0.3	2.26±0.2	20.3±2.1	38.2	17.0
PO	2.68±1.1	2.75±0.1	19.1±1.5	35.5	15.8
CO	3.51±1.3	2.55±0.4	16.5±1.7	30.9	18.4
BT	6.40±2.8	2.37±0.2	20.3±1.5	41.1	14.7
CS	5.51±2.7	2.31±0.1	18.7±0.8	31.3	19.2

1) Listed abbraviations in diet groups :
EPA : EPA group, FO : Fish oil group, PO : Perilla oil group, CO : Corn oil group, BT : Beef tallow group, CS : Corn starch group
2) Listed abbraviations in variables :
TG : Triglycerides, TC : Total cholesterol, PL : Phospholipids, PC : Phosphatidylcholine PE : Phosphatidylethanolamine

Table 5. Fatty acid composition of rat liver phosphatidylcholine(raea %)

Fatty acid[1)]	EPA[2)]	FO	PO	CO	BT	CS
16 : 0	26.8 ±0.9[ab3)]	27.6 ±2.2[a]	22.5 ±1.8[bc]	23.0 ±0.7[abc]	21.0 ±1.1[c]	26.1 ±1.2[ab]
18 : 0	22.0 ±0.2	24.3 ±1.1	23.6 ±1.7	24.8 ±0.6	23.6 ±1.2	23.2 ±1.0
18 : 1(n-9)	8.6 ±0.4[b]	8.5 ±0.6[b]	8.2 ±0.3[a]	5.9 ±0.1[c]	11.2 ±0.5[a]	9.5 ±1.2[ab]
18 : 2(n-6)	0.8 ±0.1[d]	1.5 ±0.1[d]	9.9 ±0.3[a]	5.8 ±0.5[b]	3.6 ±0.3[bc]	3.1 ±0.7[bc]
18 : 3(n-3)	1.6 ±0.7	-	0.7 ±0.0	-	-	-
18 : 4(n-3)	1.8 ±0.3[b]	2.8 ±0.4[ab]	2.4 ±0.4[ab]	3.9 ±1.2[ab]	2.5 ±0.3[ab]	5.0 ±1.1[a]
20 : 4(n-6)	17.8 ±1.0[a]	11.7 ±1.9[ab]	6.6 ±0.8[b]	14.0 ±3.8[ab]	18.1 ±1.9[a]	11.2 ±3.5[ab]
20 : 5(n-3)	3.4 ±0.3[ab]	2.7 ±0.5[b]	4.0 ±0.6[a]	-	-	-
Unknown	3.8 ±1.5	7.5 ±2.0	6.4 ±2.0	12.4 ±3.8	5.6 ±0.8	11.0 ±3.6
22 : 6(n-3)	6.6 ±0.4[a]	6.3 ±1.7[a]	6.0 ±1.3[a]	1.7 ±0.6[b]	4.6 ±0.6[a]	1.2 ±0.5[b]
SFA	48.8 ±1.0	51.9 ±3.4	46.2 ±3.5	47.8 ±0.8	44.6 ±2.2	49.3 ±2.2
MUFA	8.7 ±0.3[b]	7.4 ±1.1[ab]	8.2 ±0.3[b]	5.9 ±0.1[a]	11.2 ±0.4[c]	9.5 ±1.2[bc]
PUFA	30.3 ±1.4	24.9 ±3.8	29.0 ±2.0	25.3 ±3.8	28.7 ±2.7	20.4 ±3.5
Total n-6	18.54±1.05	13.20±2.01	16.47±1.09	19.80±4.30	1.63±2.20	14.24±4.14
Total n-3	11.76±0.34[a]	11.73±1.76[a]	12.38±1.38[a]	5.53±0.71[b]	7.07±0.51[b]	6.19±0.63[b]

1) Listed abbraviations in fatty acid :
SFA : Saturated fatty acid, MUFA : Monounsaturated fatty acid, PUFA : Polyunsaturated fatty acid, P/S ratio : ratio of PUFA/SFA, n-3/n-6 ratio : ratio of n-3 PUFA/n-6 PUFA
2) Listed abbraviations in diet groups :
EPA : EPA group, FO : Fish oil group, PO : Perilla oil group, CO : Corn oil group, BT : Beef tallow group, CS : Corn starch group
3) Mean±SE. Values in the same row with different superscript letters are significantly different at $p<0.05$ by Duncan's multiple range test

liver PC and PE were comprised were 16 : 0, 18 : 0 and 20 : 4(n-6). Compositions of saturated fatty acids(SFA), PUFA and monounsaturated fatty acids (MU-FA) were 38-50%, 20-31% and 4-11% of corresponding liver PL fractions, respectively. Fatty acid compositions of liver PC are shown in table 5. The EPA, FO and PO groups showed significantly high contents of total n-3 PUFA and 22 : 6(n-3). The BT group showed significantly high content of 18 : 1(n-9) and total MUFA. Content of 18 : 2(n-6) was significantly high in the PO group(which was followed by the CO group in content), and was significantly low in the EPA and FO groups. EPA(20 : 5(n-3)) was detected only in the n-3 FA supplement

Table 6. Fatty acid composition of rat liver phosphatidylethanolamine(area %)

Fatty acid[1)]	EPA[2)]	FO	PO	CO	BT	CS
16 : 0	25.4 ±7.6[a3)]	21.0 ±2.1[ab]	18.0 ±0.8[ab]	15.8 ±0.5[b]	13.5 ±1.3[b]	16.1 ±0.7[b]
18 : 0	27.3 ±0.2[ab]	28.7 ±2.0[ab]	31.6 ±2.7[a]	28.6 ±0.9[ab]	24.2 ±1.1[b]	28.3 ±0.7[ab]
18 : 1(n-9)	4.0 ±0.7[b]	7.5 ±1.2[a]	4.6 ±0.8[b]	4.3 ±0.4[b]	4.5 ±0.3[b]	4.8 ±1.3[b]
18 : 2(n-6)	0.8 ±0.0[c]	1.5 ±0.3[bc]	5.9 ±1.1[a]	3.0 ±0.5[b]	1.8 ±0.4[bc]	2.9 ±0.7[b]
18 : 3(n-3)	0.7 ±0.1	-	0.8 ±0.0	-	-	-
18 : 4(n-3)	3.3 ±0.7	6.7 ±1.2	4.1 ±0.8	3.5 ±1.3	5.4 ±1.1	5.4 ±1.5
20 : 4(n-6)	11.2 ±0.6[ab]	6.1 ±2.0[b]	6.6 ±1.1[b]	16.8 ±1.7[a]	11.8 ±2.4[ab]	16.0 ±1.7[a]
20 : 5(n-3)	3.8 ±0.2[ab]	2.3 ±0.9[bc]	5.4 ±1.2[a]	0.0 ±0.0[d]	0.0 ±0.0[d]	0.9 ±0.4[cd]
unknown	13.4 ±1.0[ab]	14.1 ±2.5[ab]	10.4 ±1.1[a]	12.7 ±2.8[ab]	19.4 ±2.2[b]	13.2 ±3.7[ab]
SFA	52.8 ±7.5[a]	50.0 ±2.3[a]	49.6 ±2.6[a]	44.4 ±0.6[ab]	37. 7±2.3[b]	44.4 ±1.0[ab]
MUFA	4.0 ±0.7[b]	7.5 ±1.2[a]	4.6 ±0.8[b]	4.3 ±0.4[b]	4.5 ±0.3[b]	4.8 ±1.3[b]
PUFA	31.0 ±0.9	23.1 ±6.7	29.1 ±4.3	27.5 ±3.0	24.6 ±3.3	28.3 ±1.8
total n-6	12.0 ±0.57[bc]	7.60±2.16[c]	12.48±1.91[bc]	19.79±2.05[a]	13.58±2.74[abc]	18.90±4.14[ab]

1) Listed abbraviations in fatty acid :
SFA : Saturated fatty acid, MUFA : Monounsaturated fatty acid, PUFA : Polyunsaturated fatty acid, P/S ratio : ratio of PUFA/SFA, n-3/n-6 ratio : ratio of n-3 PUFA/n-6 PUFA

2) Listed abbraviations in diet groups :
EPA : EPA group, FO : Fish oil group, PO : Perilla oil group, CO : Corn oil group, BT : Beef tallow group, CS : Corn starch group

3) Mean±SE. Values in the same row with different superscript letters are significantly different at $p<0.05$ by Duncan's multiple range test

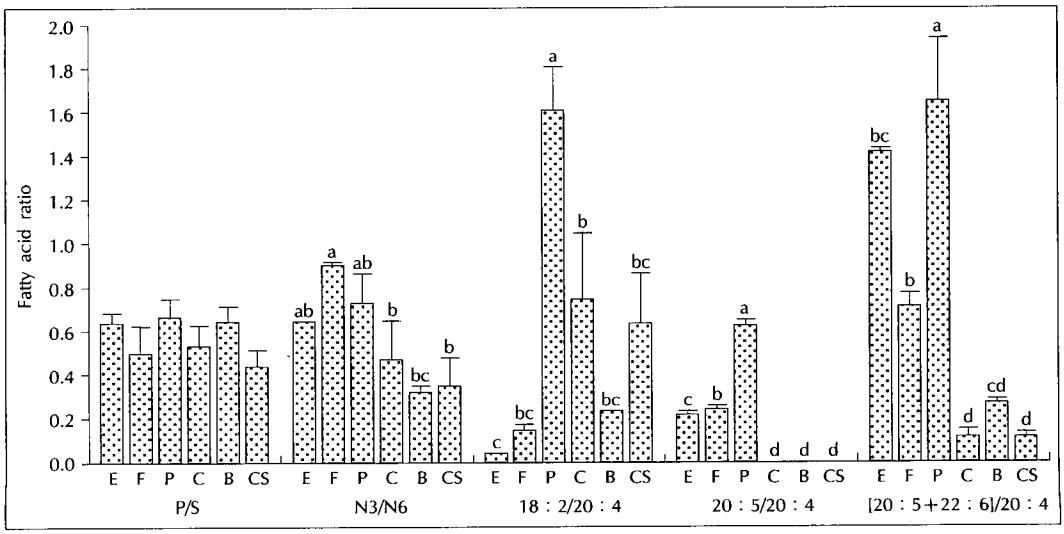

Fig. 1. Fatty acid ratios of rat liver phosphatidylcholine. Abbreviations for the diet groups ; E : EPA group, F : Fish oil group, P : Perilla group, C : Corn oil group, B : Beef tallow group, CS : Corn starch group.

diet groups. However, 22 : 6(n-3) was detected in all diet groups even though the CO and CS groups showed significantly low contents of this fatty acid compared with other groups. In the PO group, significantly high ratios of 18 : 2(n-6)/20 : 4(n-6), 20 : 5(n-3)/20 : 4(n-6) and(20 : 5+22 : 6)/20 : 4 were found(Fig. 1). A significantly low ratio of 18 : 2(n-6)/20 : 4(n-6) was found in the EPA group, and significantly low ratios of(20 : 5+22 : 6)/20 : 4 were found in the CO and CS groups(Fig. 1). Fatty acid compositions of liver PE are listed in Table 6. The EPA and the FO groups showed high levels of total n-3, n-3/n-6, SFA and 22 : 6(n-3), and low levels of total n-6 PUFA. The PO group showed significantly high content of 18 : 2(n-6) and 20 : 5(n-3). Significantly high levels of 20 : 4(n-6) and total n-6 PUFA, significantly low levels of 16 : 0, 20 : 5(n-3), 22 : 6(n-3), and total n-3 PUFA, and significantly low n-3/n-6 ratios were found in the CO and CS groups. Significantly high ratios of 18 : 2(n-6)/20 : 4(n-6) and 20 : 5(n-3)/20 : 4(n-6) were found in the PO group, and a significantly high ratio of(20 : 5+22 : 6)/20 : 4 was found in the FO group(Fig. 2). In the CO, CS and BT groups, the ratios of 20 : 5(n-3)/20 : 4(n-6) and(20 : 5+22 : 6)/20 : 4 were significantly low(Fig. 2). Contrary to our expectation, significantly low levels of SFA including 16 : 0 and 18 : 0 were found in liver PC and PE of the BT group(Table 6).

4. Correlations among the serum and liver lipids and liver phospholipid composition

Correlations among serum and liver lipids are listed in Table 7. Serum TG and serum PL were positively correlated. Serum TC was positively correlated with serum HDL-C and serum PL. Serum HDL-C was positively correlated with serum PL and liver TC.

Correlations between serum and liver lipids and fatty acids of PC and PE are listed in Table 8. Serum TC was negatively correlated with 20 : 5(n-3), 22 : 6(n-3) and total n-3 PUFA of PC. Serum TC also showed negative relationships with n-3/n-6 ratio, 22 : 6(n-3) and(20 : 5+22 : 6)/20 : 4 of PE. However, 18 : 4(n-3) of PE was positively correlated, and 16 : 0 of PE was negatively correlated with serum TC. Serum HDL-C and MUFA of PE were negatively correlated. Furthermore, serum PL was negatively correlated with various n-3 PUFAs, n-3/n-6 and MUFA of PE, and was positively correlated with 18 : 4(n-3) of PE. In this study, serum lipids showed more significant correlations with fatty acids of PE

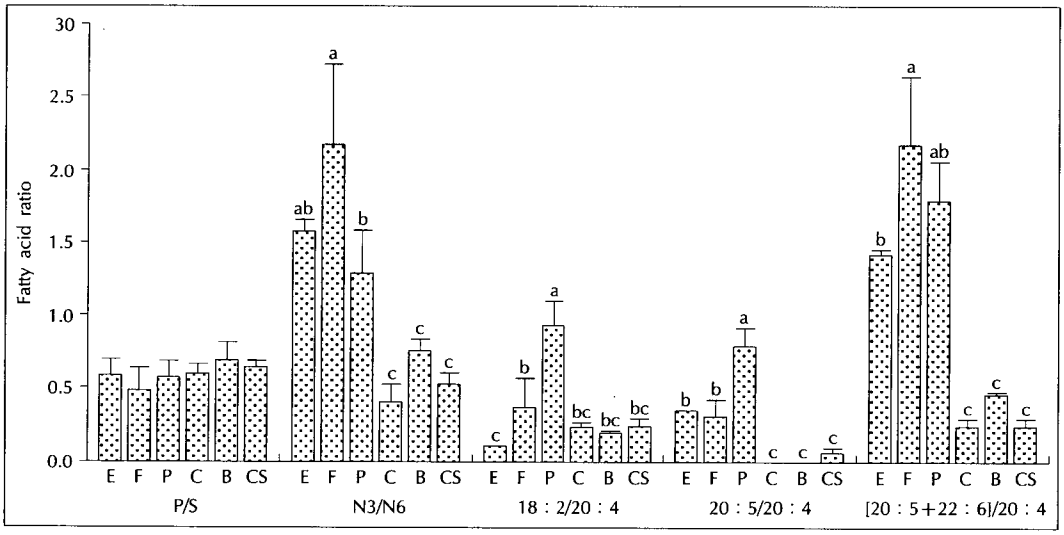

Fig. 2. Fatty acid ratios of rat liver phosphatidylethanolamine. Abbreviations for the diet groups ; E : EPA group, F : Fish oil group, P : Perilla group, C : Corn oil group, B : Beef tallow group, CS : Corn starch group.

than those of PC. Liver TG showed a positive relationship with MUFA of PC, and a negative relationship with SFA of PE. Liver PL was positively correlated with MUFA, 22 : 6(n-3) and total n-3 PUFA of PC.

Significant correlations between fatty acids of liver PC and those of liver PE are listed in Table 9. The same series of PUFAs in PC and those in PE showed positive correlations with each other. That is, n-3 PUFAs of PC were positively correlated with n-3 PUFAs of PE and negatively correlated with n-6

Table 8. Correlations between serum and liver lipids, and fatty acids of phosphatidylcholine and phosphatidylethanolamine

	Factors[1]		r[2]
Serum TC	×	PC 20 : 5(n-3)	−0.4246*
		PC 22 : 6(n-3)	−0.3718*
		PC total n-3 PUFA	−0.4379*
		PE 16 : 0	−0.3734*
		PE 18 : 4(n-3)	0.3860*
		PE 22 : 6(n-3)	−0.4242*
		PE n-3/n-6 ratio	−0.3785*
		PE(20 : 5+22 : 6)/20 : 4	−0.4443*
Serum HDL-C	×	PE MUFA	−0.4013*
Serum PL	×	PC total n-3 PUFA	−0.4148*
		PE MUFA	−0.4209*
		PE 18 : 4(n-3)	0.5369**
		PE 20 : 5(n-3)	−0.4703**
		PE 22 : 6(n-3)	−0.4996**
		PE n-3/n-6 ratio	−0.3866*
		PE(20 : 5+22 : 6)/20 : 4	−0.5205**
		PE 20 : 5/20 : 4	−0.3945*
		PE PUFA	−0.4209*
Liver TG	×	PC MUFA	0.4009*
		PE SFA	−0.3731*
Liver PL	×	PC MUFA	0.3801*
		PC 22 : 6(n-3)	0.4239*
		PC total n-3 PUFA	0.3741*

1) Listed abbraviations in Factors :
TC : Total cholesterol,
HDL-C : High density lipoprotein-cholesterol,
PL : Phospholipids, TG : Triglycerides,
PC : Phosphatidylcholine,
PE : Phosphatidylethanolamine,
SFA : Saturated fatty acid,
MUFA : Monounsaturated fatty acid
PUFA : Polyunsaturated fatty acid
n-3/n-6 ratio : ratio of n-3 PUFA/n-6 PUFA
2) Pearson correlation coefficients
*$p<0.05$ **$p<0.01$

Table 9. Correlations between fatty acids of liver phosphatidylcholine and phosphatidylethanolamine

	Factors[1]		r[2]
PC	×	PE PUFA	−0.4810**
		PE P/S ratio	−0.5549**
PC 18 : 0	×	PE total n-3 PUFA	−0.5038**
PC 18 : 2	×	PE 20 : 5/20 : 4 ratio	0.5083**
PC 20 : 5	×	PE 20 : 4(n-6)	−0.6322***
		PE 22 : 6(n-3)	0.6302***
		PE n-3/n-6 ratio	0.6528***
		PE(20 : 5+22 : 6)/20 : 4	0.8042***
		PE total n-6 PUFA	−0.5381**
		PE total n-3 PUFA	0.7437***
PC 22 : 6	×	PE 20 : 4(n-6)	−0.5000**
		PE n-3/n-6 ratio	0.5285**
		PE(20 : 5+22 : 6)/20 : 4	0.5700**
		PE total n-6 PUFA	−0.5299**
		PE total n-3 PUFA	0.5589**
		PE 20 : 5/20 : 4 ratio	0.5263**
PC 18 : 2/20 : 4	×	PE 18 : 2(n-6)	0.6633***
PC(20 : 5+ 22 : 6)/20 : 4 ratio	×	PE 20 : 5(n-3)	0.5945***
		PE 18 : 2/20 : 4 ratio	0.7216***
		PE total n-6 PUFA	−0.5475**
		PE 20 : 5/20 : 4 ratio	0.8192***
PC n-3	×	PE 20 : 4(n-6)	−0.6637***
		PE 20 : 5(n-3)	0.6273***
		PE 22 : 6(n-3)	0.5514**
		PE n-3/n-6 ratio	0.6140***
		PE(20 : 5+22 : 6)/20 : 4	0.6912***
		PE total n-6 PUFA	−0.6317***
		PE 20 : 5/20 : 4 ratio	0.7096***
PC 20 : 5/20 : 4	×	PE 18 : 2(n-6)	0.4941**
		PE 20 : 4(n-6)	−0.7032***
		PE 20 : 5(n-3)	0.8289***
		PE n-3/n-6 ratio	0.5074**
		PE 18 : 2/20 : 4 ratio	0.7775***
		PE(20 : 5+22 : 6)/20 : 4	0.7225***
		PE total n-6 PUFA	−0.4808**
		PE total n-3 PUFA	0.5193**
PC SFA	×	PE PUFA	−0.5369**
		PE P/S ratio	−0.4958**

1) Listed abbraviations in Factors :
PC : Phosphatidylcholine,
PE : Phosphatidylethanolamine,
SFA : Saturated fatty acid,
MUFA : Monounsaturated fatty acid,
PUFA : Polyunsaturated fatty acid,
P/S ratio : ratio of PUFA/SFA
n-3/n-6 ratio : ratio of n-3 PUFA/n-6 PUFA
2) Pearson's correlation coefficients
**$p<0.01$ ***$p<0.001$

PUFAs of PE. However, 18 : 2(n-6) of PC was positively correlated with 20 : 5(n-3)/20 : 4(n-6) of PE. Also, 18 : 2(n-6)/20 : 4(n-6) and 18 : 2(n-6) of PE showed positive correlations with 20 : 5(n-3)/20 : 4(n-6) and(20 : 5+22 : 6)/20 : 4 of PC. This result seems to be due to competitive effects among the fatty acids.

Discussion

Many studies have reported the effects of dietary fatty acids on the serum and liver lipid levels in both humans and animals[3)4)17)]. However, the results of these studies have not always been consistent in many respects. Numerous studies have shown the beneficial effects of n-3 PUFA diets(especially n-3 highly unsaturated fatty acid(HUFA) diets) on serum lipid profiles[3-5)16)17)]. However, a study conducted by Leaf et al.[6)] failed to show any significant effects of dietary n-3 fatty acids on serum lipid levels; it was reported that n-3 PUFA diets did not significantly change the levels of serum TC and HDL-C. There was even a study which showed that n-3 PUFA diets have adverse effects on serum lipid levels[7)]. Nelson et al.[7)] demonstrated elevated levels of LDL-C in rabbits after feeding them fish oil. As plant n-3 PUFA sources, previous researches have primarily used canola or linseed oil whose α-LNA contents are approximately 10% and 50% of total dietary fatty acids, respectively. In this study, perilla oil, a commonly used cooking oil in Korea, was used as a source for α-LNA. Perilla oil contains as much as 60 % α-LNA in its total fatty acids composition. The EPA and FO were used as n-3 HUFA sources in this study. We need to point out that the five dietary fat based diets of this study provided 22% of the total energy as fat, and thus were regarded as moderate rather than high fat diets.

Even though there was no significant difference in serum lipid levels, the highest serum TG level was observed in the CS group(Table 3). Phillipson and coworker[18)] have noted that the elevated blood glucose levels due to a high carbohydrate diet causes the secretion of insulin, which subsequently stimulates TG synthesis and VLDL secretion in the liver. The significantly high atherogenic index shown in the CS group was due to the significantly high serum TC levels of the CS group. In a previous study, rats being fed high carbohydrate diets demonstrated significantly low plasma lecithin : cholesterol acyltransferase(LCAT) activity[19)]. A decrease in the activity of plasma LCAT led to decreased HDL-C levels as well as increased VLDL-C and LDL-C levels[19)].

Considering the results of previous studies, the cholesterol lowering effects of PUFA have been and still are controversial. While some[3)16)] have reported that n-3 PUFA has higher cholesterol lowering effects than n-6 PUFA, others[5)] have demonstrated that, regardless of the position of double bonds, the more highly unsaturated fat decreases serum cholesterol levels more effectively. When Harris et al.[3)] and Kestin et al.[17)] compared the hypolipidemic effects of n-6 and n-3 PUFA, they demonstrated that the n-3 fatty acids has more serum TC and TG-lowering effects on a gram-for-gram basis. However, in a study performed by Ide and coworkers[5)], the effect of dietary fats having various degrees of unsaturation on HMG-CoA reductase activity and on cholesterogenesis in rats were investigated. It was reported that HMG-CoA reductase activity rises with the chain length of fatty acids but declines with the degree of unsaturation. Other studies have shown no significant effects of various n-3 PUFA diets including seed oil and fish oil on serum cholesterol levels[6)]. In this study, no significant difference in serum TC levels between the EPA, PO and FO groups were observed, and the only significant difference in serum TC of n-3 versus n-6 PUFA groups was between the FO and CO group. Therefore we can not conclude that n-3 PUFA is more potent than n-6 PUFA, or that long chain PUFA is more potent than shorter chain n-3 PUFA in lowering serum cholesterol levels. The significantly lower serum TC level of the BT group as compared to the CS groups of this study(Table 3) implies that all SFA do not have cholesterol elevating effects as suggested by Hayes et al.[20)]. Hayes and coworkers[20)] reported that 14 : 0 has a cholesterol

raising effect by means of decreasing LDL receptor activity and increasing the direct production of LDL. However, the 16 : 0, 18 : 0, 18 : 1, 18 : 2 and 18 : 3 were shown to exert an equal impact on the circulating cholesterol concentration in normal animals.

This study showed significantly low levels of serum HDL-C in the FO diet group and significantly high serum HDL-C levels in the CO group(Table 3). In previous studies, inconsistent serum HDL-C levels in different types of FA supplements were observed[3)6)21)]. These inconsistencies seem to be related to the absolute amount of dietary PUFA and duration of dietary treatment. As a possible mechanism explaining the decreased serum HDL-C levels in n-3 PUFA supplements, Parks[22)] demonstrated that decreased serum TC levels in n-3 diets result in decreased synthesis of nascent HDL and apoprotein A-1. In other study, however, fish oil diet was reported to increase the number of HDL receptors and turnover rate of HDL despite low levels of HDL-C[23)]. Even though this study did not show which subset of HDL contributed to the decreased level of total HDL-C in the FO group, our previous study demonstrated that the low total serum HDL-C level of the FO group was mainly due to the decreased concentration of HDL_2-C[24)]. In this study, the significantly high serum TC levels of the CO group appeared to be related to high serum HDL-C levels(Table 3, Table 7). As a result, both the EPA and CO groups showed significantly low atherogenic indices as compared to the CS group. This low atherogenic index of the CO group indicates that, despite high serum TC levels, the CO diet was not atherogenic.

Even though this study did not show any significant differences among groups, liver TG levels as well as serum TG levels were low in the EPA and FO groups(Table 4). A previous study[25)] suggested that the low serum TG concentrations in EPA and FO groups are due to the decreased activities of liver lipogenic enzymes. It is therefore suggested that the low serum TG levels in the EPA and FO groups in this study are related to the inhibition of hepatic fatty acid synthesis.

As repeatedly observed in a previous study[26)], dietary fatty acids affected the fatty acid composition of liver PL fractions in this study(Table 5, 6). The fatty acid composition of liver PC and PE fractions in this study illustrated that, besides direct incorporation of dietary fatty acids into body lipids, there must be a considerable amount of biosynthesis and catabolism of the fatty acids. In the PO diet significantly low content of 20 : 4(n-6), and higher content of 18 : 2(n-6) and 20 : 5(n-3) were detected in the liver PC and PE of the rats. It is likely that this result was due to the suppressed biosynthesis of 20 : 4(n-6) from 18 : 2(n-6). The same enzymes are involved and compete in the biosynthesis of 20 : 4(n-6) and 20 : 5(n-3) from 18 : 2(n-6) and 18 : 3(n-3), respectively, and the relative rate of enzymatic elongation and desaturation of C18 fatty acid series has been reported in the order n-3> n-6> n-9[27)]. In this study, EPA, DHA and total n-3 fatty acids were found more in the liver PE than in the liver PC (Table 5, 6). This result agrees with Gibson et al's[28)] finding that, in an EPA supplement diet, EPA was incorporated more to the erythrocyte PE than the other erythrocyte PL subfractions.

Although the P/S ratios of the diets were quite different from one another, ranging from 0.19 to 91.55, the P/S ratios of the fatty acids in liver PC and PE were not significantly different among the six diet groups. It was reported that fatty acids in the PC or PE fraction showed positional specificity with preferential location of SFA and MUFA at the 1-position and PUFA at the 2-position of the glycerol moiety[29)]. Thus, feeding rats with fish oil primarily affected the fatty acid composition on the 2-position of the PC and PE fractions of total heart PL[29)]. Astorg and coworker[30)] also reported that the P/S ratios of heart and liver PL of rats were not influenced by dietary n-3 and n-6 fatty acid composition. While EPA was detected only in the n-3 FA groups, DHA was detected in all the dietary groups, and the amount of DHA in the liver PC and PE was significantly high in the n-3 diet groups. This result supports a previous report[26)] which demonstrated that

DHA functions as a reservoir of EPA, and that DHA is synthesized from EPA in animals and humans.

Various lipoproteins transport TG, cholesterol and HDL-C in blood, and PL is known as a major component of lipoprotein membrane. Accordingly, serum PL was positively correlated with serum TG, TC and HDL-C levels in this study(Table 7). There was also a positive correlation between serum TC and HDL-C. Among cholesterol transport lipiproteins, HDL transfers cholesterol from body cells mostly to other lipoproteins for disposal. Therefore, simultaneous measurements of both serum TC and HDL-C levels allow for a more reliable prediction for the process of atherogenesis than a single measurement does. Malaspina et al.[31] have reported that the ratio of serum TC to HDL-C is more significantly related to the severity of coronary artery disease than just HDL-C or TC. The positive correlation between serum HDL-C and liver TC(Table 7) implies the possibility that high liver cholesterol uptake occurs due to high concentrations of serum HDL-C.

In this study serum TC showed to be negatively correlated with n-3 HUFAs in liver PC and in liver PE(Table 8). No significant correlation between serum lipids and n-6 PUFA of liver PL was observed. Considering our result, increased concentrations of n-3 HUFA in liver PL seem to have greater serum TC and PL lowering effects as compared to n-6 PUFA. Although we did not investigate the effects of dietary fatty acids on VLDL or LDL levels, observations of negative correlations between n-3 HUFA and serum TC or PL levels suggest that these fatty acids may play a role in inhibiting VLDL and LDL production. A previous study done by Oh et al.[32] showed significantly negative correlations between n-3 HUFA and both serum TC and LDL-C levels. Furthermore, serum lipids were more correlated with n-3 HUFA of liver PE than those of liver PC. As discussed earlier, highly unsaturated n-3 PUFA are known to be incorporated more to PE than PC[28].

The correlations between fatty acids of liver PC and PE(Table 9) found in our study support a previous result reported by Mantzioris et al.[33] who demonstrated the differences between the incorporation of α-LNA and LA to their 20-carbon fatty acids. It was reported that the conversion and subsequent incorporation of the α-LNA metabolites was several-fold higher than that of LA in various tissue fractions. In addition, more significant positive correlations between n-3 PUFAs of PC and those of PE were observed than the correlations found between n-6 PUFAs of PC and PE(Table 9). Our observation supports the study by Leaf et al.[34], which reported that significant correlations exist only between n-3 PUFAs in plasma lipid classes and their corresponding adipose tissue concentrations, and not for the fatty acids of the n-6 series.

In conclusion, the high carbohydrate diet had the most adverse effects on serum lipid profiles. Even though the FO diet showed TC lowering effects as compared to the CO diet, the FO diet showed significantly low HDL-C levels, too. Also, there were no significant differences in serum lipid levels between the EPA, FO and PO groups. Both the EPA and CO groups showed significantly low atherogenic indices compared with the CS group. Therefore we can not conclude that long chain n-3 PUFA diets have more beneficial effects on serum lipid profiles (including serum TG, TC and HDL-C) than do n-6 PUFA diets or shorter chain n-3 PUFA diets. Considering the correlation between serum lipids and fatty acid composition of liver PL, then, increased concentrations of highly unsaturated n-3 PUFA in liver PL seem to be related to serum TC and PL lowering effects.

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