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The Effect of Dietary Lipids on CVD Risk Factors in Ovariectomized Rats

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ABSTRACT

This experiment was performed to investigate the effect of different dietary lipids on the risk factors of coronary vascular disease(CVD) in ovariectomized rats. Female rats of Sprague-Dawley strain were divided into sham-operated(sham) and ovariectomized(ovx) groups and then each group was divided into a beef tallow group, a soy bean oil group and a fish oil group. After 16 weeks of feeding on experimental diets, animals were sacrificed and blood, liver, kidney and perirenal fat pad were obtained.

Food intake and weight gain of fish oil group were significantly lower than other dietary lipid groups. Food intake and weight gain tended to be higher in ovx groups than in sham groups. The weight index(g/100g body weight) of liver and kidney was higher in the fish oil group than the other groups and weight index was lower in ovx groups compared to sham groups. The weight of the perirenal fat pad was the highest in the beef tallow group and the lowest in the fish oil group. The fish oil group showed the lowest total cholesterol(TC) and triglyceride (TG) levels in serum. Serum TG levels were lower in all ovx groups than in sham groups, but serum TC levels were not influenced by ovariectomy.

Fatty acid composition of serum reflects the recent dietary intake of fat. Linoleic acid content was the highest in soy bean oil group and eicosapentaenoic acid(EPA) and docosahexaenoic acid(DHA) contents were the highest in fish oil group. Fatty acid composition of adipose tissue, especially EPA and DHA contents in perirenal fat pad, was highest in the fish oil group. Saturated fatty acid(SFA) and monounsaturated fatty acid(MUFA) in serum and adipose tissue did not reflect fatty acid intake.

The activities of glucose-6-phosphate dehydrogenase, a lipogenic enzyme, in the blood of the beef tallow and soybean oil groups showed the tendency to be high and that of the fish oil group to be low in ovx. Carnitine acetyltransferase, a lipolytic enzyme, showed the highest activity in the liver of the fish oil group and was least active in the soy bean oil group. (*Korean J Nutrition* 30(4) : 386~393, 1997)

KEY WORDS : dietary lipids · ovariectomy · glucose-6-phosphate dehydrogenase · carnitine acetyltransferase.

Introduction

Recently in Korea the incidence of cardiovascular disease(CVD) has been increasing[1] and the mortality rate from this disease reached 29.9% in 1990[2]. Although some studies[3,4] reported that menopause was not related to the risk of CVD, many studies[5-8] revealed a relationship between them. Premenopausal women have a lower incidence of CVD than men, but this characteristic disappears by natural and artificial menopause[4]. Some of case-control studies of menopause also reported that postmenopausal women have higher cardiovascular or atherosclerotic risk than

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premenopausal women[9)10)]. The changes in the endocrine system because of menopause cause changes in serum lipid profiles.

Some studies[11)12)] reported that ovariectomy causes increases in body weight and body fat accretion. Obesity, which is the most common health problem in middle-aged women after menopause, increases blood volume and cardiac output and then causes heart hypertropy, hypertension, and coronary arterial disease (CAD) directly[13)]. It also causes hypercholesterolemia, hypertriglyceridemia, low HDL-cholesterol, and then cardiovascular disease indirectly[14)]. There are a few studies about the effect of dietary lipid composition on fat accumulation. London et al.[15)] reported that they observed only weak correlations between the content of saturated and monounsaturated fatty acids in adipose tissue and the dietary lipid intake of these two fatty acids. However, they found strong correlations between polyunsaturated fatty acids and dietary lipid intake. The purpose of this study was to investigate the effect of dietary lipids on the risk factors of CVD in ovariectomized rats.

Materials and Methods

1. Experimental diets

The experimental diets were based on the AIN-76[16)] and the amount of lipids was about 15%(30% of total calories) of dietary weight. Table 1 shows the experimental diet composition. Three different kinds of dietary lipids were used ; beef tallow(Oddugi Co.), soybean oil(Dong-Bang oil Co.) and fish oil(Kohap Bio. Co.). To prevent oxidation of diets, α-tocopherol (Sigma Co. St. Louis, MO) was added to all experimental diets, which were stored under N_2 gas in a refrigerator.

2. Experimental animals and operation

Female Sprague-Dawley rats had an adaptation period with chow and their average weight was 227±3g. Animals were divided into 6 groups with 10 animals in each group. Ovariectomies were performed under ethyl ether anesthesia through under-back incisions with removal of ovaries(ovx) and sham operations consisted of only under-back incisions without removal of ovaries(sham). Animals were housed individually in stainless steel hanging cages in a temperature(22±2℃) and humidity(40~60%) regulated room. The room was lighted for 12 hours. Experimental diet and tap water were provided ad libitum.

3. Sampling

After sixteen weeks of feeding on experimental diets, animals were sacrificed. Blood samples were obtained through heart puncture and centrifuged at 950×g for 15 minutes and separated serum was stored at −75℃. Liver, kidney, and perirenal fat pad were removed and

Table 1. Composition of experimental diets (g/kg)

	Sham operation			Ovariectomy		
	BT[1)]	SB[2)]	FO[3)]	BT	SB	FO
Corn starch	470	470	470	470	470	470
Sucrose	150	150	150	150	150	150
Casein	150	150	150	150	150	150
Methionine	3	3	3	3	3	3
Choline chloride	2	2	2	2	2	2
Beef tallow	150	50	50	150	50	50
Soy bean oil		100			100	
Fish oil			100			100
Vitamin mixture[4)]	10	10	10	10	10	10
Mineral mixture[5)]	35	35	35	35	35	35
α-cellulose	30	30	30	30	30	30
α-tocopherol	0.08	0.08	0.08	0.08	0.08	0.08

1) Beef tallow, 2)Soy bean oil, 3) Fish oil

4) Vitamin Mixture(mg/100g) : VD_3 0.582, α-tocopherol-acetate 1200.0, Retinol-acetate 93.2, VK_3 6.0, Thiamin-HCl 59.0, VB_{12} 0.2, VC 588.0, Pyridoxine-HCl 29.0, D-biotin 1.0, Folic acid 2.0, Inositol 1176.0, Ca-pantothenate 235.0, Riboflavin 59.0, Nicotinic acid 294.0, Sucrose 96257.017

5) Mineral Mixture(g/100g) : $CaCO_3$ 29.29, $CaHPO_4 \cdot 2H_2O$ 0.43, KH_2PO_4 34.31, NaCl 25.06, $MgSO_4 \cdot 7H_2O$ 9.98, $Fe(C_6H_5O_7) \cdot 6H_2O$ 0.623, $CuSO_4 \cdot 5H_2O$ 0.156, $MnSO_4 \cdot H_2O$ 0.121, $(NH_4)_6Mo_7O_{24} \cdot 4H_2O$ 0.0025, $Na_2SeO_3 \cdot 5H_2O$ 0.0015, $ZnCl_2$ 0.02, KI 0.0005

weighed.

4. Analysis

1) Serum lipid concentration

Serum triglyceride, total cholesterol, and HDL-cholesterol were analyzed by enzymatic procedures using kits(Young-Dong Co.) LDL-cholesterol was calculated using Fredelwald[17] formula as follow ;

LDL-cholesterol=Total cholesterol-(HDL-cholesterol+triglyceride/5)

2) Serum and adipose tissue fatty acid composition

Serum fatty acid composition was measured using Fletcher's method[18] and Lapage and Roy's method[19] by gas liquid chromatography(GLC). The perirenal fat was homogenized with 10ml hexane : isoprophyl solution(3 : 2, v/v) and then centrifuged at 2500×g for 10 minutes. After centrifugation, a white lipid layer was removed to a plastic tube and analysed in the same manner as was the serum lipid material.

3) Glucose-6-phosphate dehydrogenase(G-6-PDH) and Carnitine acetyltransferase (CAT) activity

Activity of G-6-PDH, a lipogenic enzyme, was measured in the blood using a modification of Kornberg and Horecker's method[20] and Lohr and Waller's method[21]. In this experiment, a kit(Sigma Co. kit No. 345) was used. Enzyme activity was expressed as unit per gram of hemoglobin where one unit is defined that amount of G-6-PDH activity that will convert 1 micromole of substrate per minute.

The activity of CAT, a lipolytic enzyme, was measured spectrophotometrically by following the appearance of CoASH using the general thiol reagent DTNB(Ellmans's reagent)[22]. The mitochondria were separated from the liver for measuring CAT activity[23]. Protein content was determined by the procedures of Lowry et al[24].

5. Statistical analysis

All statistical analysis was performed using the statistical package for social science(SPSS). Data was presented as the means±SEM. Statistical evaluations were performed by ANOVA, and followed by Duncan's multiple range test for differences between means. A probability value of $\alpha=0.05$ was chosen as the level of statistical significance.

Results and Discussion

1. Food intake and Body weight gain

The food intake, body weight gain, and food efficiency ratio(FER) are shown in Table 2. The food intake of the fish oil group was slightly lower than that of other lipid groups. The reason for the low food intake in the fish oil group was thought that the fish oil diet had a fish-like smell. The food intake tended to be higher in ovx than in sham. The body weight gain of the fish oil group was the lowest and weight gain was higher in ovx than in sham. These results were consistant with a previous study[1]. The food efficiency ratio(FER) of the fish oil group was the lowest, but statistically not significant. FER of the ovariectomized groups was higher than the sham operation groups.

2. Organ weights

The organ weight and weight index, which were expressed as organ weight per 100g body weight to eliminate differences in body weight among the groups, are shown in Table 3. The liver weight and index of the fish oil group was the highest and the liver weights of the three lipid groups in ovx tended to be lower than those in sham. Perirenal fat pads were the smallest in fish oil groups for both ovx and sham animals. Results of the present experiment are consistant

Table 2. Food intake, weight gain, and FER(Food Efficiency Ratio)

Group	Food intake (g/d)	Weight gain (g/d)	FER
SBT	21.73±0.99[1)bc2)]	1.69±0.20[ab]	0.08±0.01[ab]
SSB	20.58±0.89[abc]	1.43±0.14[ab]	0.07±0.00[ab]
SFO	18.79±0.64[a]	1.23±0.15[a]	0.06±0.01[a]
OBT	22.55±0.40[c]	2.12±0.11[b]	0.09±0.00[b]
OSB	21.66±0.66[bc]	2.13±0.24[b]	0.01±0.01[b]
OFO	19.81±0.40[ab]	1.46±0.25[ab]	0.07±0.01[ab]
SF[3)]	DL	DL, OVX	OVX
DL*OVA[4)]	NS	NS	NS

1) Mean±S.E.
2) Values with different letters are significantly different among experimental groups at $p<0.05$ according to Duncan's multiple range test
3) Significant factor(SF) : Statistical significance was calculated at the $\alpha=0.05$ level by 2-way ANOVA
4) Interaction between dietary lipids(DL) and ovariectomy(OVX)

with the results of other studies comparing fish oil, coconut oil, corn oil, or olive oil[25]. Also, Parrish et al [26]. reported that the fish oil group had a lower body fat weight than the lard group. Shimomura[27] reported that long-chain fatty acids in fish oil were oxidized easily and not deposited.

3. Serum lipids

Total cholesterol, HDL-cholesterol, LDL-cholesterol, and triglyceride in serum are shown in Table 4. These results were analysed by ANACOVA(Analysis of covariance) model using food intake as a covariance variable to remove the efffect of food intake on serum lipid levels.

Total cholesterol in the fish oil group was significantly lower than other groups in both sham and ovx. It is known that saturated fatty acids tend to increase serum lipids while polyunsaturated fatty acids tend to decrease serum total cholesterol and LDL-cholesterol[28][29]. Polyunsaturated fatty acids are known to decrease total cholesterol by disturbing absorption in the small intestine[30], increasing excretion of bile acid[31], disturbing synthesis of cholesterol in the liver[30], and increasing mobilization of cholesterol through LDL-receptors to the tissue[32]. Simons et al.[33] reported that the PUFA increase the excretion of cholesterol to bile acid to decrease serum total cholesterol. Especially, n-3 PUFA decreased triglyceride synthesis in the liver and release of VLDL from the liver to the blood. The serum TG level of the fish oil group was the lowest compared to beef tallow and soybean oil groups in ovx and sham. Generally, the PUFA were known to decrease serum TG by lowering lipogenic enzyme activity[34] and VLDL synthesis in the liver[35][36]. These experimental results were consistant with those of other studies[37][38] that groups fed perilla oil or fish oil rich in n-3 PUFA showed low-

Table 3. Liver, kidney, and perirenal fat weight of experimental rats

Group	Liver (g)	Liver index (g/100g B.W.)	Kidney (g)	Kidney index (g/100g B.W.)	Perirenal fat (g)	Perirenal fat index (g/100g B.W.)
SBT	13.12±0.95[1)ab]	2.79±0.08[b2)]	2.60±0.08[abc]	0.56±0.03[cd]	20.55±0.87[cd]	4.42±0.14[bc]
SSB	13.89±1.40[ab]	2.73±0.13[ab]	2.73±0.18[c]	0.57±0.03[cd]	15.49±0.65[ab]	3.56±0.11[ab]
SFO	15.61±1.30[b]	3.68±0.16[d]	2.65±0.15[bc]	0.63±0.01[d]	12.05±0.99[a]	2.89±0.22[a]
OBT	12.83±1.09[ab]	2.52±0.17[ab]	2.34±0.05[ab]	0.47±0.01[a]	23.13±1.39[d]	4.59±0.24[c]
OSB	11.86±0.96[a]	2.37±0.14[a]	2.39±0.08[ab]	0.49±0.03[ab]	17.33±1.86[bc]	3.35±0.27[a]
OFO	13.82±0.83[ab]	3.26±0.84[c]	2.29±0.28[a]	0.55±0.02[bc]	12.36±2.11[a]	2.74±0.37[a]
SF[3]	DL, OVX	DL, OVX	DL, OVX	OVX	DL	DL
Interaction[4)]	NS[5)]	NS	NS	NS	NS	NS

1) Mean±S.E.
2) Values with different letters are significantly different among experimental groups at $p<0.05$ according to Duncan's multiple range test
3) Significant factor(SF) : Statistical significance was calculated at the $\alpha=0.05$ level by 2-way ANOVA
4) Interaction between dietary lipid(DL) and ovariectomy(OVX)
5) NS : Not significant

Table 4. Total cholesterol, Triglyceride, HDL-C and LDL-C in serum

Group	TC	TG	HDL-C	LDL-C
SBT	127.13± 4.58[1)b]	143.09±27.44[d2)]	54.92± 7.63[a]	43.59± 5.34
SSB	123.72±14.84[b]	120.73±29.0[cd]	50.91± 5.79[a]	54.51±13.04
SFO	82.08± 5.16[a]	32.01± 4.21[a]	44.27± 5.52[a]	43.91± 8.77
OBT	145.23±11.29[b]	85.06±11.94[bc]	87.06±14.87[b]	57.70± 9.66
OSB	132.00±12.28[b]	57.69± 6.20[ab]	89.96±15.73[b]	47.90± 9.69
OFO	68.61± 4.11[a]	31.73± 2.33[a]	34.54± 4.81[a]	38.94±10.30
SF[3)]	DL	DL, OVX	NS	NS
Interaction[4)]	DL*OVX	DL*OVX	DL*OVX	NS

1) Mean±S.E.
2) Values with different letters are significantly different among experimental groups at $p<0.05$ according to Duncan's multiple range test
3) Significant factor(SF) : Statistical significance was calculated at the $\alpha=0.05$ level by 2-way ANACOVA
4) Interaction between dietary lipid(DL) and ovariectomy(OVX)

er TG concentration than other lipids. In this experiment, serum HDL-cholesterol was higher in OBT and OSB groups than in other groups. Harris et al.[39] reported that serum HDL-cholesterol was higher in postmenopausal women than premenopausal women. The group fed DHA-rich fish oil showed lower HDL-cholesterol levels than other groups. Serum LDL-cholesterol concentration was not different among the experimental groups.

4. Fatty acid composition of serum and adipose tissue

Serum fatty acid composition is shown in Table 5. The soybean oil group showed the highest content of linoleic acid and the fish oil group showed the highest DHA and EPA concentrations. These results were similar to these of other studies[39]. The effect of dietary lipids on the fatty acid composition of perirenal fat is given in Table 6. The adipose tissue of the fish oil

Table 5. Fatty acid composition of serum (Relative weight %)

Fatty acid	SBT	SSB	SFO	OBT	OSB	OFO	SF[3]	Interaction[4]
C14 : 0	2.42±0.19[1)abc]	2.11±0.16[ab]	2.82±0.20[b]	2.91±0.31[c]	1.96±0.11[a]	3.26±0.29[d]	DL	NS
C16 : 0	17.69±0.71[bc]	18.02±1.03[ab]	23.97±1.13[c]	20.03±1.13[b]	17.16±0.50[a]	26.44±0.67[c]	DL	NS
C18 : 0	32.06±1.89[c]	30.22±1.30[c]	25.66±0.61[b]	18.38±2.36[a]	32.11±0.77[b]	17.44±1.01[a]	DL, OVX	NS
C18 : 1	18.74±1.80[c]	9.85±0.54[ab]	12.04±0.32[b]	22.23±0.72[d]	9.36±0.55[a]	17.96±0.95[c]	DL, OVX	DL*OVX
C18 : 2	6.76±0.51[a]	18.34±2.93[b]	6.56±0.31[a]	8.84±0.75[a]	17.75±0.87[b]	9.35±0.66[a]	DL, OVX	NS
C18 : 3	0.07±0.01[a]	0.87±0.13[d]	0.31±0.05[b]	0.09±0.03[a]	0.63±0.06[c]	0.24±0.02[ab]	DL	NS
C20 : 0	1.50±0.09[a]	1.94±0.11[a]	4.81±0.15[b]	1.77±0.27[a]	1.85±0.28[a]	4.06±0.48[b]	DL	NS
C20 : 1	0.33±0.16[b]	0.22±0.11[ab]	0.14±0.01[ab]	0.18±0.03[ab]	0.09±0.02[a]	0.15±0.02[ab]	OVX	NS
C20 : 2	0.45±0.07[b]	0.18±0.02[a]	0.15±0.03[a]	0.61±0.07[c]	0.11±0.02[a]	0.12±0.01[a]	DL	DL*OVX
C20 : 3	0.17±0.11[a]	19.65±0.79[c]	17.24±1.15[b]	ND[5)]	ND	17.50±0.83[b]	DL, OVX	DL*OVX
C20 : 4	19.58±0.31[b]	0.04±0.03[a]	0.60±0.44[a]	23.98±0.53[c]	27.99±0.89[a]	0.09±0.02[a]	DL, OVX	DL*OVX
C20 : 5	0.42±0.04[a]	1.44±0.50[a]	6.00±0.39[c]	0.89±0.13[a]	1.14±0.11[a]	3.05±0.42[b]	DL, OVX	DL*OVX
C22 : 6	0.01±0.00[a]	0.02±0.00[a]	0.10±0.05[a]	0.08±0.02[a]	0.05±0.02[a]	0.35±0.14[b]	DL	NS

1) Mean±S.E.
2) Values with different letters in a row are significantly different among experimental groups at $p<0.05$ according to Duncan's multiple range test
3) Significant factor(SF) : Statistical significance was calculated at the $\alpha=0.05$ level by 2-way ANOVA
4) Interaction between dietary lipid(DL) and ovariectomy(OVX)
5) ND : Not Detected

Table 6. Fatty acid composition of adipose tissue (Relative weight %)

Fatty acid	SBT	SSB	SFO	OBT	OSB	OFO	SF[3]	Interaction[4]
C14 : 0	11.48±0.94[1)a2)]	12.26± 2.42[a]	19.76±1.03[bc]	18.23±2.25[bc]	14.60± 1.45[ab]	23.10±2.36[c]	DL, OVX	NS
C16 : 0	12.20±4.36[a]	26.80± 9.20[ab]	11.43±5.39[ab]	31.41±5.39[ab]	31.93± 8.45[ab]	34.34±3.85[b]	OVX	NS
C18 : 0	1.56±1.56[a]	0.53± 0.51[a]	2.26±1.52[a]	4.51±1.52[a]	5.46± 1.99[a]	10.13±1.77[b]	OVX	NS
C18 : 1	5.02±5.02[ab]	14.06± 6.86[b]	ND[5)]	2.94±2.56[ab]	4.61± 3.31[ab]	7.67±4.18[ab]	NS	NS
C18 : 2	69.54±7.36[c]	44.55±12.96[bc]	58.86±6.81[bc]	40.49±9.33[abc]	37.86±11.37[ab]	14.67±1.83[a]	OVX	NS
C18 : 3	0.52±0.06[a]	4.67± 1.00[b]	1.48±0.09[a]	0.55±0.06[a]	5.57± 0.73[b]	1.44±0.18[a]	DL	NS
C20 : 0	0.02±0.01[a]	0.03± 0.02[a]	3.20±0.14[b]	0.02±0.01[a]	0.08± 0.02[a]	3.03±0.31[b]	DL	NS
C20 : 1	0.26±0.03[a]	0.40± 0.08[a]	1.06±0.09[b]	0.51±0.17[a]	0.65± 0.15[a]	1.17±0.14[b]	DL	NS
C20 : 2	0.06±0.01[a]	0.38± 0.12[b]	0.36±0.07[b]	0.15±0.07[ab]	0.38± 0.10[b]	0.37±0.07[b]	DL	NS
C20 : 3	0.62±0.09[a]	0.87± 0.24[ab]	2.30±0.47[c]	0.84±0.13[a]	1.68± 0.41[bc]	2.31±0.22[c]	DL	NS
C20 : 4	trace[5)]	0.32± 0.22[a]	0.88±0.38[b]	0.15±0.14[a]	0.15± 0.07[a]	0.08±0.01[a]	DL, OVX	DL*OVX
C20 : 5	0.09±0.00[a]	0.17± 0.04[ab]	1.04±0.07[c]	0.16±0.07[ab]	0.38± 0.08[b]	1.06±0.16[c]	DL	NS
C22 : 6	ND	0.02± 0.01[a]	0.12±0.07[b]	0.06±0.06[a]	0.02± 0.01[a]	0.09±0.04[ab]	DL	NS

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2) Values with different letters in a row are significantly different among experimental groups at $p<0.05$ according to Duncan's multiple range test
3) Significant factor(SF) : Statistical significance was calculated at the $\alpha=0.05$ level by 2-way ANOVA
4) Interaction between dietary lipid(DL) and ovariectomy(OVX)
5) ND : Not Detected
6) Trace : Amount is less than 0.01

group had higher amounts of DHA and EPA than that of the other two groups. However, there is no relationship between fatty acid intake and the content of saturated and the monounsaturated fatty acids in adipose tissue. Recently, many studies examined if the fatty acid composition of adipose tissue reflects the dietary lipid intake, especially for certain fatty acids like linoleic acid. In an early study[40], it was shown that linoleic acid percentages were higher in patients with a history of previous CHD than in patients without previous cardiac disease. Some studies[41] reported a positive correlation between the linoleic acid content of adipose tissue and CHD. But other studies [42] reported that there is negative correlation between the linoleic acid content of adipose tissue and CAD. In a clinical experiment[43], the lower the DHA content in adipose tissue, the more severe the extent of atherosclerosis. This result supports that fish oil decreases the risk of CAD, but how DHA content in adipose tissue affects the risk factors of CAD is not clearly understood.

5. G-6-PDH and CAT activity

Table 7 shows the activity of G-6-PDH and CAT in six groups. The activity of G-6-PDH in the fish oil group with ovx was significantly lower than in other groups. Yang and Williams[44] observed that DHA was more effective in suppressing fatty acid synthesis than was linoleic and linolenic acid. This present experiment showed results similar to the above experiments. The fish oil group showed the highest activity of carnitine acetyltransferase and the soybean oil group showed the lowest activity. The carnitine acetyltransferase activity of the soybean oil group was significantly lower than that of other two lipid groups in ovx. In rat liver, high-fat diets also cause an increase in peroxisomal β-oxidation[45] and marine oil-rich diets seem to increase the activity of β-oxidative enzymes. The activity of CAT in rat liver was stimulated by a high fat diet and the effect of fish oil was higher than that of soybean oil group.

Table 7. Glucose-6-phosphate dehydrogenase activity in blood and Carnitine acetyltransferase activity in liver

Group	G-6-PDH (U/g Hb)	CAT (nmol/mg protein per min)
SBT	19.46±1.78[1)bc]	0.24±0.05[ab]
SSB	16.34±1.08[ab2)]	0.16±0.08[a]
SFO	20.60±1.18[bc]	0.29±0.03[ab]
OBT	20.88±1.01[c]	0.29±0.09[ab]
OSB	19.67±1.66[bc]	0.11±0.02[a]
OFO	14.90±1.07[a]	0.42±0.10[b]
SF[3)]	NS	DL
Interaction[4)]	DL*OVX	NS

1) Mean±S.E.
2) Values with different letters are significantly different among experimental groups at $p<0.05$ according to Duncan's multiple range test
3) Sgnificant Factor(SF) : Statistical significance was calculated at the $\alpha=0.05$ level by 2-way ANOVA
4) Interaction between dietary lipid and ovariectomy

Conclusion

From the above results, fish oil had a protective effect against the risk factors of CVD such as hypercholesterolemia, hypertriglyceridemia, and obesity. Although there was no significant difference in lipogenic enzyme activity, lipolytic enzyme activity was high in the fish oil group compared with the soybean oil group and the beef tallow group.

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