

Polyunsaturated/saturated Fatty Acid Ratios and Antioxidant Supplementation under the Control of Dietary Peroxidizability Index Value: Impact on Serum Lipid Profiles in Young and Adult Rats

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An increase in serum cholesterol is directly associated with high incidences of cardiovascular diseases (CVD) and atherosclerosis. Serum lipid profiles are highly dependent on dietary fatty acids and age. The purpose of this study was to examine the age-related effects of polyunsaturated/saturated fatty acid ratios and antioxidant supplementation under the control of the dietary peroxidizability index (PI) value on serum lipid profiles in rats. While the PI level of dietary fatty acids was controlled at 81.22, the P/S ratios of fatty acids were 0.38 and 4.81 (LP and HP). The diets were supplemented with a vitamin E 1000 mg/kg diet and a selenium 2.5 mg/kg diet (LPS and HPS). Female Sprague-Dawley rats ages 3 weeks (young) and 16 weeks (adult) were fed four different experimental diets for 4 weeks. The serum triglyceride concentration of LPS was significantly higher in young rats than in adult rats. The total-cholesterol concentration of LP and HPS were higher in young rats than in adult rats. The high-density lipoprotein-cholesterol (HDL-C) concentration of LP, LPS and HP was higher in adult rats than in young rats. The low-density lipoprotein-cholesterol (LDL-C) concentration was higher in young rats than in adult rats. T-C/HDL-C and LDL-C/HDL-C ratios were much higher in young rats than in adult rats. In conclusion, P/S ratios and antioxidant supplementation did not affect T-C/HDL-C and LDL-C/HDL-C ratios as risk factors of CVD in adult rats when we controlled the PI value in the diet. Probably, the invisible and confounding effects of dietary PI value implicate the beneficial roles of dietary P/S ratios and antioxidants in CVD. Accordingly, controlling the dietary PI value may be advantageous to lower the risk of CVD in adult rats.

Key words: Age, P/S ratio, Peroxidizability index, Antioxidant supplementation, Lipid profiles

INTRODUCCION

Cardiovascular disease (CVD) is a multi-factorial disorder.^{1,2)} Total cholesterol (T-C) and high-density lipoprotein-cholesterol (HDL-C) have been shown to be independently associated with an increased CVD risk.²⁾ The increase in the CVD risk is continuous from low to high serum total-cholesterol (T-C) concentrations.¹⁾ Several investigators have found that the plasma concentration of HDL-C is a superior predictor of CVD risk compared with the blood T-C concentration.^{3,4)} Also, LDL-C is strongly and positively associated with CVD risk. Low-density lipoprotein-cholesterol (LDL-C) concentration is highly correlated with the T-C concentration.⁵⁾

The best predictors of changes indicative of CVD are changes in the ratios of T-C/HDL-C and LDL-C/HDL-C. T-C is more easily measured than LDL-C and the two are very highly correlated with one another. For these reasons, T-C/HDL-C ratio may be a more convenient in-

dex than LDL-C/HDL-C ratio but it still contains essentially the same information.²⁾

Factors such as age, sex, diet and sexual maturation may affect serum lipid profiles.⁶⁾ Some investigators have reported that metabolic risk factors tend to increase with age.^{1,6)} Others have suggested that the strongest risk factor for CVD is age.¹⁾ Epidemiological and experimental data indicate that a diet high in saturated fatty acid (SFA) is associated with high levels of serum T-C and LDL-C which in turn, is related to a high incidence of CVD.^{7,8)} The effects of dietary polyunsaturated fatty acid (PUFA) on the regulation of lipid metabolism and eicosanoid balance appear to be diverse.⁹⁾ Eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) are the major PUFA in fish oil.¹⁰⁾ Consumption of n-3 PUFA has been shown to be associated with a low incidence of atherosclerosis and CVD.^{10,11)} The extent of dietary DHA-stimulated tissue lipid peroxidation was less than expected from the relative peroxidizability index (PI) of the total tissue lipids in rats.¹⁰⁾ Although linoleic acid effectively reduces serum cholesterol, the fatty acid most re-

representative of dietary n-6 PUFA has undesirable effects.⁹⁾ Therefore, it is important to control the P/S ratio of dietary fats in regulating serum lipid profiles.^{8,9)}

In this study, we maintained the PI values of all diets at the same level to remove the effect of excessive peroxide in diets with high P/S ratios. Then, we investigated the age-related effects of P/S ratios and antioxidant supplementation on the serum lipid profiles of young and adult rats.

MATERIALS AND METHODS

1. Diets and Animals

Fatty acids analysis of fat source

We analyzed the fatty acid compositions of seven kinds of fats commonly used in Korea: Soybean oil (CJ Co., Korea), corn oil (CJ Co., Korea), palm oil (Lotte-samkang Co., Korea), perilla oil (directly extracted from perilla), sesame oil (Ottugi Co., Korea), fish oil (Dongwon Co., Korea) and beef tallow (Lottesamkang). The fatty acids were analyzed using gas chromatography (GC, Hewlett Packard model 6890).¹²⁾ The fatty acid composition was determined by area % and confirmation of each fatty acid was carried out by comparison of methyl ester with retention time.¹³⁾ Peroxidizability index was

Table 1. Fatty acid composition of lipid sources used in the experimental diets¹⁾

Fatty acid	Soybean oil	Corn oil	Palm oil	Perilla oil	Sesame oil	Fish oil	Beef tallow
C12:0	-	-	0.80	-	0.03	-	-
C14:0	0.06	0.11	1.21	0.01	0.03	3.91	3.00
C14:1	-	-	-	-	-	-	0.67
C16:0	10.49	11.25	44.34	6.04	9.33	18.90	26.50
C16:1	-	0.09	0.18	-	0.14	17.50	2.90
C18:0	3.51	2.17	4.31	1.89	5.09	5.42	17.00
C18:1(n9)	22.21	24.90	39.10	18.09	40.44	16.20	43.40
C18:2(n6)	55.23	56.67	9.12	12.37	43.88	1.51	3.40
C18:3(n3)	7.49	0.53	0.18	61.00	0.26	0.72	0.30
C20:0	0.23	0.43	0.27	0.11	0.52	-	0.29
C20:1	0.78	-	-	-	0.28	1.01	0.38
C20:4(n6)	-	-	-	-	-	1.83	-
C20:5(n3)	-	-	-	-	-	5.41	-
C22:6(n3)	-	-	-	-	-	27.31	-
Unknown	-	3.85	0.49	0.49	-	0.28	2.16
ΣSFA	14.29	13.96	50.93	8.05	15.00	28.23	46.79
ΣMUFA	22.99	24.99	39.28	18.09	40.86	34.71	47.35
ΣPUFA	62.72	57.20	9.30	73.37	44.14	36.78	3.70
P/S ratio	4.39	4.10	0.18	9.11	2.94	1.30	0.08
Σn3	7.49	0.53	0.18	61.00	0.26	33.44	0.30
Σn6	55.23	56.67	9.12	12.37	43.88	3.34	3.40
Σn6/Σn3 ratio	7.73	106.92	50.67	0.20	168.77	0.10	11.33
PI ²⁾	70.78	58.35	10.46	134.82	45.42	262.08	5.18

1) Expressed as % distribution of fatty acid methyl esters

2) PI: peroxidizability index¹⁴⁾=(% monoenoic acid×0.025)+(%) dienoic acid×1)+(%) trienoic acid×2)+(%) tetraenoic acid×4)+(%) pentaenoic acid×6)+(%) hexaenoic acid×8)

calculated using the method of Du *et al.*¹⁴⁾ Fatty acid compositions of fat sources are shown in Table 1.

Experimental diets

The experimental diets were prepared using an AIN-93G (American Institute of Nutrition-93 Growth) diet.¹⁵⁾ Experimental diets contained 15% (W/W) fat

Table 2. Amount of fat sources in the experimental diet of each group (g/kg diet)

Fat sources	Experimental diet ¹⁾			
	LP	LPS	HP	HPS
Soybean oil	1.50	1.50	121.95	121.95
Corn oil	1.50	1.50	0.15	0.15
Palm oil	16.50	16.50	0.15	0.15
Perilla oil	0.75	0.75	0.15	0.15
Sesame oil	1.50	1.50	25.20	25.20
Fish oil	42.75	42.75	0.15	0.15
Beef tallow	85.50	85.50	0.15	0.15

1) LP: low polyunsaturated / saturated fatty acid ratio (0.38),

HP: high polyunsaturated / saturated fatty acid ratio (4.81),

S: vitamin E (1000 mg/kg diet) and selenium (2.5 mg/kg diet) supplementation

Table 3. Fatty acid composition of the experimental diets¹⁾

Fatty acid	Experimental diets ²⁾			
	LP	LPS	HP	HPS
C12:0	0.09	0.09	-	-
C14:0	2.96	2.96	0.06	0.06
C14:1	0.38	0.38	-	-
C16:0	25.71	25.71	9.78	9.78
C16:1	6.66	6.66	0.02	0.02
C18:0	11.83	11.83	3.28	3.28
C18:1(n9)	34.62	34.62	21.83	21.83
C18:2(n6)	4.99	4.99	47.71	47.71
C18:3(n3)	0.78	0.78	16.34	16.34
C20:0	0.21	0.21	0.21	0.21
C20:1	0.52	0.52	0.64	0.64
C20:4(n6)	0.52	0.52	-	-
C20:5(n3)	1.54	1.54	0.01	0.01
C22:6(n3)	7.78	7.78	0.03	0.03
Unknown	1.41	1.41	0.09	0.09
ΣSFA	40.79	40.79	13.34	13.34
ΣMUFA	42.18	42.18	22.49	22.49
ΣPUFA	15.62	15.62	64.09	64.09
P/S ratio	0.38	0.38	4.81	4.81
Σn3	10.11	10.11	16.38	16.38
Σn6	5.51	5.51	47.71	47.71
Σn6/Σn3 ratio	0.55	0.55	2.91	2.91
PI ³⁾	81.22	81.22	81.22	81.22

1) Expressed as % distribution of fatty acid methyl esters

2) LP: low polyunsaturated / saturated fatty acid ratio (0.38),

HP: high polyunsaturated / saturated fatty acid ratio (4.81),

S: vitamin E (1000 mg/kg diet) and selenium (2.5 mg/kg diet) supplementation

3) PI: peroxidizability index¹⁴⁾=(% monoenoic acid×0.025)+(%) dienoic acid×1)+(%) trienoic acid×2)+(%) tetraenoic acid×4)+(%) pentaenoic acid×6)+(%) hexaenoic acid×8)

Table 4. Grouping of experimental rats at two ages

Age	Group ¹⁾	Peroxidizability index ²⁾	P/S ratio ³⁾	Vitamin E + Selenium ⁴⁾
Young (3 weeks)	LP	81.22	0.38	Without
	LPS	81.22	0.38	With
	HP	81.22	4.81	Without
	HPS	81.22	4.81	With
Adult (16 weeks)	LP	81.22	0.38	Without
	LPS	81.22	0.38	With
	HP	81.22	4.81	Without
	HPS	81.22	4.81	With

- 1) LP: low polyunsaturated/saturated fatty acid ratio (0.38), HP: high polyunsaturated/saturated fatty acid ratio (4.81), S: vitamin E (1000 mg/kg diet) and selenium (2.5 mg/kg diet) supplementation
 2) PI: peroxidizability index¹⁹⁾=(% monoenoic acid×0.025)+(% dienoic acid×1)+(% trienoic acid×2)+(% tetraenoic acid×4)+(% pentaenoic acid×6)+(% hexaenoic acid×8)
 3) P/S ratio: polyunsaturated/saturated fatty acid ratio
 4) dl- α -tocopheryl acetate 1000 mg (1100 IU)/kg diet, sodium selenite 2.5 mg/kg diet

mixed with seven kinds of commonly used fats in Korea. Table 2 shows the amount of the fat sources in the experimental diet of each group. The fatty acid composition of the experimental diets is shown in Table 3. While the peroxidizability index (PI) value was maintained at 81.22, the P/S ratios of the diets were kept at two levels (0.38 and 4.81, LP and HP) and antioxidants were supplemented (LPS and HPS).^{16,17)} Table 4 shows the grouping of the rats according to these experimental diets. Antioxidants were supplemented with vitamin E and selenium in line with the study of Horvath and Ip.¹⁸⁾ Vitamin E was supplemented with dl- α -tocopheryl acetate (Sigma Aldrich, Korea) to a total of 1000 mg per kg diet. Selenium was added as sodium selenite (Sigma Aldrich, Korea) at 2.5 mg per kg diet.

Animals

Two age groups of female Sprague-Dawley rats, ages 3 wks (weighing 50-60 g) and 16 wks (weighing 230-260 g) were used in this study and divided into four groups as shown in Table 4. The rats were given standard laboratory chow and tap water *ad libitum* for 1 wk before being fed the experimental diets. Then, the rats were fed four different experimental diets for 4 weeks. They were housed in suspended stainless steel mesh cages and kept in an environmentally controlled room at 22±2 °C, relative humidity of 50±10% and 12-h light/dark cycle. All animals were cared for in accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals*.

Food intake was recorded every day and body weight was recorded at weekly intervals. Food efficiency ratio (FER) was calculated using weight gain/food intake.

2. Analysis of Serum Lipid Profiles

After an overnight fast, the rats were anesthetized with diethyl ether. Blood samples from the heart were separated into sera by centrifugation at 3,000×g and 4 °C for 15 min in a centrifuge (MF 550, Hanil Science Industrial Co., Incheon, Korea). The serum was kept frozen at -70 °C until analyzed.

The concentrations of triglyceride, total-cholesterol and high-density lipoprotein-cholesterol in serum were measured using a commercial diagnostic kit (Shin Yang Chemical Co., Korea) at 505, 500, and 555 nm, respectively, with a spectrophotometer (DU 600, Beckman Coulter, CA).

The low-density lipoprotein-cholesterol concentration of serum was calculated using Friedewald's formula.¹⁹⁾ The T-C/HDL-C and LDL-C/HDL-C ratios as risk factors of cardiovascular disease were calculated.^{2,20)}

3. Statistical Analysis

For statistical analysis, the SPSS/PC program (Statistical Package for Social Science 11.0) was used. For each age group, data were compared among the four groups by one-way analysis of variance (ANOVA) using post hoc Duncan's multiple range test at *p* value <0.05. The difference between the young and adult rats was analyzed using Student's *t*-test at *p* value <0.05. Results were expressed as mean±S.E.M.

RESULTS

Comparison of the mean weight gain, food intake and food efficiency ratio (FER) of the young and adults rats is shown in Table 5. The results for both age groups did not differ significantly by P/S ratio and antioxidant

Table 5. Comparison of weight gain, food intake and food efficiency ratio (FER) between young and adult rats (Mean±S.E.M.)

Age	Groups ¹⁾				
	LP	LPS	HP	HPS	
Weight gain (g/day)	Young	2.87±0.09 ^{ns*}	3.25±0.24*	2.96±0.12*	2.40±0.30*
	Adult	0.93±0.14 ^{ns}	0.58±0.20	0.74±0.21	0.47±0.22
Food intake (g/day)	Young	14.03±1.18 ^{ns,NS}	14.06±0.44 ^{NS}	13.55±0.28 ^{NS}	12.67±0.68 ^{NS}
	Adult	15.58±1.01 ^{ns}	15.33±0.48	16.74±0.88	15.64±0.53
FER ²⁾	Young	0.204±0.006 ^{ns*}	0.229±0.010*	0.218±0.005*	0.185±0.019*
	Adult	0.063±0.011 ^{ns}	0.036±0.012	0.044±0.013	0.030±0.015

- 1) LP: low polyunsaturated/saturated fatty acid ratio (0.38), HP: high polyunsaturated/saturated fatty acid ratio (4.81), S: vitamin E (1000 mg/kg diet) and selenium (2.5 mg/kg diet) supplementation
 2) FER: food efficiency ratio=body weight gain for experimental period (g)/food intake for experimental period (g)
 ns: not significantly different among four groups by one-way ANOVA (*p*<0.05)
 *: significantly different between young and adult groups by Student's *t*-test (*p*<0.05)
 NS: not significantly different between young and adult groups by Student's *t*-test (*p*<0.05)

supplementation under fixed dietary PI value. Weight gain and FER of the rats were remarkably higher in the young rats than in the adult rats. Food intake by the young and adult rats did not differ significantly.

Comparison of the concentration of the serum triglyceride (TG), total-cholesterol (T-C), high-density lipoprotein-cholesterol (HDL-C) and low-density lipoprotein-cholesterol (LDL-C) concentrations of the young and adult rats is shown in Table 6. TG concentration differed significantly among the four groups of young rats. Thus, TG concentration increased using antioxidant supplementation in the young rats. However, TG concentration

Table 6. Comparison of serum lipid concentration between young and adult rats (Mean±S.E.M.)

	Age	Groups ¹⁾			
		LP	LPS	HP	HPS
TG (mg/dl)	Young	69.60±6.84 ^{a,NS}	94.48±9.16 ^{b,*}	61.59±6.17 ^{a,NS}	75.24±9.63 ^{ab,NS}
	Adult	68.24±10.79 ^{ns}	47.83±6.10	64.72±14.10	51.36±6.32
T-C (mg/dl)	Young	68.56±5.89 ^{a,*}	67.64±5.18 ^{a,NS}	81.34±4.59 ^{a,NS}	96.63±4.25 ^{b,*}
	Adult	46.04±3.30 ^a	57.61±4.24 ^a	76.48±7.12 ^b	58.73±4.27 ^a
HDL-C (mg/dl)	Young	5.57±0.76 ^{a,*}	6.74±1.83 ^{ab,*}	11.37±0.76 ^{bc,*}	16.02±2.98 ^{c,*}
	Adult	21.55±1.48 ^a	23.40±2.30 ^a	35.58±2.91 ^b	27.89±2.97 ^a
LDL-C (mg/dl)	Young	52.01±5.60 ^{a,*}	46.45±6.03 ^{a,*}	59.72±4.58 ^{ab,*}	67.31±2.69 ^{b,*}
	Adult	12.64±4.01 ^{ns}	26.28±3.37	28.51±5.57	21.80±3.95

1) LP: low polyunsaturated / saturated fatty acid ratio (0.38), HP: high polyunsaturated / saturated fatty acid ratio (4.81), S: vitamin E (1000 mg/kg diet) and selenium (2.5 mg/kg diet) supplementation
a,b: Values with different superscript (small letters) are significantly different among four groups by one-way ANOVA using post hoc Duncan's multiple range tests (p<0.05).
NS: not significantly different between young and adult groups by Student's t-test (p<0.05)
* : significantly different between young and adult groups by Student's t-test (p<0.05)
ns: not significantly different among four groups by one-way ANOVA (p<0.05)
TG: triglyceride, T-C: total-cholesterol, HDL-C: high-density lipoprotein-cholesterol, LDL-C: low-density lipoprotein-cholesterol

Table 7. T-C/HDL-C ratio and LDL-C/HDL-C ratio between young and adult rats (Mean±S.E.M.)

	Age	Groups ¹⁾			
		LP	LPS	HP	HPS
T-C/ HDL-C ratio	Young	13.22±1.70 ^{b,*}	11.97±1.48 ^{b,*}	7.30±0.50 ^{a,*}	6.98±1.13 ^{a,*}
	Adult	2.23±0.28 ^{ns}	2.59±0.23	2.16±0.13	2.19±0.17
LDL-C/ HDL-C ratio	Young	9.96±1.39 ^{c,*}	7.81±0.81 ^{bc,*}	5.37±0.48 ^{ab,*}	4.90±0.82 ^{a,*}
	Adult	0.68±0.27 ^{ns}	1.21±0.19	0.83±0.16	0.86±0.16

1) LP: low polyunsaturated / saturated fatty acid ratio (0.38), HP: high polyunsaturated / saturated fatty acid ratio (4.81), S: vitamin E (1000 mg/kg diet) and selenium (2.5 mg/kg diet) supplementation
a,b: Values with different superscript (small letters) are significantly different among four groups by one-way ANOVA using post hoc Duncan's multiple range tests (p<0.05).
* : significantly different between young and adult groups by Student's t-test (p<0.05)
ns: not significantly different among four groups by one-way ANOVA (p<0.05)
T-C/HDL-C ratio: total-cholesterol/high-density lipoprotein-cholesterol ratio, LDL-C/HDL-C ratio: low-density lipoprotein-cholesterol/high-density lipoprotein-cholesterol

tended to decrease with antioxidant supplementation in the adult rats. In addition, the TG concentration of LPS was significantly higher in the young rats than in the adult rats.

T-C concentration differed significantly in both age groups. Thus, T-C concentration of LP and LPS was lower than that of HP and HPS. The T-C concentration was higher in the young rats than in the adult rats in the LP and HPS groups.

HDL-C concentration differed significantly in both age groups. Thus, HDL-C concentration of LP and LPS was lower than that of HP and HPS. The HDL-C concentration was lower in the young rats than in the adult rats.

LDL-C concentration differed significantly in the young rats but not in the adult rats. LDL-C concentration in the young rats was higher in HP and HPS than in LP and LPS. LDL-C concentration was significantly higher in the young rats than in the adult rats.

Comparison of the T-C/HDL-C and LDL-C/HDL-C ratios of the young and adult rats is shown in Table 7. T-C/HDL-C ratio differed significantly in the young rats. Accordingly, T-C/HDL-C ratio was much higher in LP and LPS than in HP and HPS in the young rats due to the influence of P/S ratio. However, T-C/HDL-C ratio did not differ significantly in the adult rats. T-C/HDL-C ratio in the four groups was higher in the young rats than in the adult rats. LDL-C/HDL-C ratio differed significantly in the young rats. LDL-C/HDL-C ratio was affected by dietary P/S ratio. However, LDL-C/HDL-C ratio did not differ significantly in the adult rats. LDL-C/HDL-C ratio in the four groups was higher in the young rats than in the adult rats.

DISCUSSION

In the present study, weight gain did not differ significantly in the two age groups based on P/S ratios and antioxidant supplementation under a fixed dietary peroxidizability index (PI) value. In the study of Lee *et al.*,⁹⁾ it was observed that weight gain did not differ significantly among rats fed diets in which the P/S ratio and n-3/n-6 ratio of fatty acids were changed. In the study of Águila *et al.*,²¹⁾ weight gain did not differ significantly based on age in rats fed diets containing different types of lipids. But, in our results, weight gain was remarkably higher in the young rats than in the adult rats although food intake was similar. For these reasons, it seems that the food efficiency ratio (FER) is higher in young rats than in adult rats.

Lee *et al.*⁹⁾ reported that the serum triglyceride (TG) concentration tends to increase as the dietary P/S ratio increases up to 2 and it remains constant thereafter. In the present study, however, the TG concentration did not

differ based on P/S ratio. As a result, antioxidant supplementation increased serum TG concentration in the LPS group in young rats. As far as changes according to age, Yoon *et al.*²²⁾ reported that TG concentration increased as rats aged from 1 month to 4 months. This significant difference is not supported by the results of the present study. In our study, TG concentration did not differ significantly between young and adult rats, except in the LPS group. On the other hand, Águila *et al.*²¹⁾ observed that TG concentration did not differ significantly between 12- and 18-month-old rats.

The serum total-cholesterol (T-C) concentration has been known to decrease with increases in dietary P/S ratio up to 2.⁹⁾ Connor reported that a diet low in saturated fatty acids and high in n-3 fatty acids, especially EPA and DHA, has independent mechanisms of action on plasma lipid concentrations and lowers the T-C concentration.²³⁾ In the present study, the T-C concentration was higher in LP and LPS than in HP and HPS although the SFA content was lower and the n-3 fatty acid content was higher in HP and HPS. On the other hand, EPA and DHA contents in the LP and LPS diets were higher than in HP and HPS. This suggests that the effects of EPA and DHA on T-C concentration are much stronger when compared to the effects of total n-3 fatty acids and SFA contained in the diet. In many studies, it has been reported that T-C concentration increases with increasing age.^{21,22)} However, Arora *et al.* observed that T-C concentration is higher in healthy children than in middle-aged volunteers on ordinary diets.²⁰⁾ Similarly, in our study, T-C concentration tended to be higher in young rats than in adult rats when the dietary PI value was controlled at the same level. Therefore, T-C concentration does not appear to increase constantly with increasing age, but is concurrently affected by various fatty acids or the PI value of the diet.

High-density lipoprotein-cholesterol (HDL-C) has been shown to negatively correlate with the increased risk of CVD²¹⁾ whereas increased levels of low-density lipoprotein cholesterol (LDL-C) play a major contributory role in increasing the risk of CVD. Thus, LDL-C is integrally involved in the development of atherosclerosis.^{1,21)} In our previous study, HDL-C concentration increased as the P/S ratio increased.¹⁷⁾ In contrast to the n-6 fatty acid-rich vegetable oils that lower HDL-C concentrations, fish oil does not decrease HDL-C concentration.²³⁾ LDL-C concentration decreases with increases in the dietary P/S ratio.⁸⁾ There is a diversity of opinion on the influence of fish oil on LDL-C concentration.^{22,23)} High fish oil intake decreases LDL-C concentration and low intakes of fish oil increase LDL-C concentration. Replacing saturated with unsaturated fatty acids reduces LDL-C and, to some extent, also reduces HDL-C.⁷⁾ Connor²³⁾ reported that one of the effects of dietary n-3 PUFA, such as EPA

and DHA, on lipoprotein metabolism is depression of LDL synthesis. Thus, EPA and DHA have a lowering effect on LDL-C concentration. In our results, the HDL-C concentration of LP and LPS was lower than that of HP and HPS. Additionally, LDL-C concentration in the young rats was higher in HP and HPS than in LP and LPS. In the present study, n-3 fatty acid content was lowered in the diet of the high-P/S ratio groups in the process of controlling the PI value in the diet. Thus, it seems that complex influences among P/S ratio, EPA, DHA and controlled dietary PI value etc. affect HDL-C and LDL-C. Accordingly, increases and decreases in HDL-C and LDL-C concentrations are not consistent because these are not affected by a single factor but rather by a complex of factors.

T-C/HDL-C and LDL-C/HDL-C ratios are strong risk markers for coronary heart disease⁷⁾ and important factors in predicting evidence of CVD.²⁾ Thus, we calculated both indices. The T-C/HDL-C and LDL-C/HDL-C ratios of the young rats were higher in LP and LPS than in HP and HPS. Accordingly, we confirmed that in young rats the feeding of a diet with a high P/S ratio could lower the risk of CVD when the PI value was the same. However, in the adult rats, neither index changed according to P/S ratio and antioxidant supplementation when the PI value was the same. Some investigators have reported that T-C/HDL-C and LDL-C/HDL-C increase with increasing age.^{2,6)} On the other hand, others have reported that both indices are lower in adults than in those of a young age.²⁰⁾ Similarly, in the present study, T-C/HDL-C and LDL-C/HDL-C ratios were higher in the young rats than in the adult rats.

CONCLUSION

In summary, in the present study, triglyceride, total-cholesterol (T-C) and low-density lipoprotein-cholesterol (LDL-C) concentration was lower in adult rats than in young rats when the dietary peroxidizability index (PI) value was controlled at the same level. And high-density lipoprotein-cholesterol (HDL-C) was higher in adult rats. Additionally, T-C/HDL-C and LDL-C/HDL-C ratios as risk factors for CVD were lower in adult rats. The indices did not differ significantly by P/S ratio and antioxidant supplementation in adult rats when the dietary PI value was controlled at the same level.

In conclusion, P/S ratios and antioxidant supplementation did not affect the risk factors for CVD in adult rats when we controlled the PI value in the diet. Accordingly, controlling the dietary PI value may be associated with the risk factors of CVD in adult rats. However, the CVD risk was higher in young rats fed LP and LPS, although the dietary PI value was controlled

at the same level. The causes remain unclear because studies that consider both dietary PI value and age have, as yet, not been performed. Accordingly, further studies are required to consider various PI values and age.

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