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The effects of polyunsaturated:saturated fatty acids ratios and peroxidisability index values of dietary fats on serum lipid profiles and hepatic enzyme activities in rats

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Raising the dietary PUFA:saturated fatty acids (SFA) ratio has been recommended for the prevention of CVD. However, a high PUFA:SFA (P:S) ratio diet enhances oxidative stress because PUFA are highly susceptible to lipid peroxidation. Thus, we focused on the role of the dietary P:S ratio and peroxidisability index (PI) value on serum and liver tissue and investigated the effects of dietary P:S ratios (0·4, 1·0, and 4·8) with a fixed PI value (81) on serum lipid parameters and hepatic enzyme activities (experiment 1). To elucidate whether those phenomena were unique to the P:S ratio, we examined the effects of dietary PI values (36, 81, 126, and 217) with a constant P:S ratio (1·0) (experiment 2). Female Sprague–Dawley rats weighing 240–280 g were fed experimental diets for 4 weeks. When dietary PI value was maintained at 81, serum HDL-cholesterol (HDL-C) increased with increasing dietary P:S ratio. When the P:S ratio was fixed at 1·0, HDL-C was the lowest with mid–low PI (MLPI) (PI value of 81). In both experiments, serum LDL-cholesterol:HDL-C ratio kept in the range of 0–2. The hepatic superoxide dismutase, catalase, and glutathione peroxidase (GSH-Px) activities and thiobarbituric acid reactive substance (TBARS) concentrations were the highest in the lowest dietary P:S ratio group (experiment 1). GSH-Px, glutathione-*S*-transferase, and TBARS were the lowest in rats fed the MLPI diet (experiment 2). In conclusion, these results indicate that a P:S ratio of 1·0–1·5 and a PI value of 80–90 in the diet are within a favourable range to reduce the risk of CVD.

Dietary fatty acids: Polyunsaturated:saturated fatty acids ratio: Peroxidisability index: Lipid profiles: Antioxidant enzyme activity

The high intake of saturated fatty acids (SFA) is associated with a high level of serum cholesterol and strongly correlated with coronary death rates. Also, the hypercholesterolaemic effect is influenced by the level of SFA rather than by the amount of total fat in the diet (Muller *et al.* 2003). However, the effects of dietary PUFA on the regulation of lipid metabolism and on the prevention of CVD appear to be diverse (Lee *et al.* 1989; Muller *et al.* 2003; Saito & Kubo, 2003). In many studies, a balanced intake of dietary PUFA and SFA was thought to be very important in regulating serum cholesterol (Lee *et al.* 1989; Du *et al.* 2003; Muller *et al.* 2003). However, excessive intake of PUFA has undesirable effects such as oxidative stress (Lee *et al.* 1989; Park *et al.* 1999) because of high susceptibility to lipid peroxidation (Lee *et al.* 1989; Saito & Kubo, 2003). Because the peroxidisability index (PI) value represents the degree of unsaturation of dietary lipids (Hu *et al.* 1989; Saito & Kubo, 2003), it has been used as an indicator of PUFA peroxidation (Nagyova *et al.* 2001).

Oxidative stress, which is associated with the formation of lipid peroxides, has been suggested as contributing to pathological processes in ageing and many diseases, including atherosclerosis (Jiang *et al.* 1992; Qujeq *et al.* 2004). Small amounts of reactive oxygen species, including hydroxyl radicals ($^{\bullet}OH$), superoxide anions ($O_2^{\bullet}$), and H_2O_2 are constantly formed in all cells and tissues (for aerobic organisms) in response to both external and internal stimuli (Hurst *et al.* 1997; Chopra & Wallace, 1998; Jornot *et al.* 1998; Mills *et al.* 1998; Kamanli *et al.* 2004; Qujeq *et al.* 2004). Under normal conditions, enzymic antioxidant activities exist as a safeguard against the accumulation of reactive oxygen species (Kamanli *et al.* 2004; Qujeq *et al.* 2004). Superoxide dismutase (SOD; *EC* 1.15.1.1), catalase (CAT; *EC* 1.11.1.6), glutathione peroxidase (GSH-Px; *EC* 1.11.1.9), glutathione-*S*-transferase (GST; *EC* 2.5.1.18), and glutathione reductase (*EC* 1.6.4.2) as well as glutathione are part of cellular antioxidant defences (Kuratko & Pence, 1991; Lutoslawska *et al.* 2003). As an enzymic antioxidant system, SOD catalyses dismutation of the superoxide anion into H_2O_2. At the same time, GSH-Px and CAT detoxify H_2O_2 (Czernichow & Hercberg, 2001; Kamanli *et al.* 2004) and catalyse the reduction of H_2O_2 using glutathione for converting lipid H_2O_2 into non-toxic alcohols (Czernichow & Hercberg, 2001; Kamanli *et al.* 2004; Qujeq *et al.* 2004). The GST removes glutathione by conjugation. And glutathione is reduced via glutathione reductase (Kuratko & Pence, 1991).

Abbreviations: CAT, catalase; GSH-Px, glutathione peroxidase; GST, glutathione-*S*-transferase; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol; PI, peroxidisability index; P:S, PUFA:saturated fatty acids; SFA, saturated fatty acids; SOD, superoxide dismutase; TBARS, thiobarbituric acid reactive substance; T-C, total cholesterol; TG, triacylglycerol.

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The evidence of H_2O_2 inactivation is represented as decreased hepatic malondialdehyde (Kamanli *et al.* 2004), which is the best available index to measure global reactive oxygen species by presenting the extent of lipid peroxidation (Hsiang *et al.* 1997; Qujeq *et al.* 2004).

There have been several reports on the effect of various PUFA:SFA (P:S) ratios of dietary fatty acids on lipid metabolism (Buckingham, 1985; Lee *et al.* 1989; Kang *et al.* 2003, 2004) and enzymic antioxidant system (Kang *et al.* 2003, 2004). In a previous study (Kang *et al.* 2004), we compared only the effects of two P:S ratios (0·38 and 4·81) on lipid parameters and antioxidant enzyme activities when PI value was controlled at the same level. Because it was not easy to find an appropriate PI value in the diet, the dietary PI value was settled at 81·22 by calculation from results of several studies (Nam & Park, 1993; Kang *et al.* 2003). When PI value is at the ideal level, lipid metabolism or antioxidant enzyme activities may by influenced by the dietary P:S ratio (Kang *et al.* 2003, 2004). Alternatively, it is possible that effects of the P:S ratio may be modified by the PI value. However, there are few studies investigating the interrelated effects of dietary PI values and P:S ratios of dietary fatty acids on lipid metabolism or hepatic enzymes.

In the present study, therefore, we investigated the effects of dietary P:S ratios with a fixed PI value on serum lipid parameters and hepatic enzyme activities in rats fed different combinations of fats in order to establish a basis for recommending a desirable P:S ratio in the diet. In addition, we analysed the effects of dietary PI values with a constant P:S ratio in order to explain whether those phenomena were only due to the P:S ratio and find an adequate PI value in the diet.

Materials and methods

Animals and diet

All animals were cared for in accordance with the National Research Council's *Guide for the Care and Use of Laboratory Animals* (National Research Council, 1996). Two experiments (experiment 1 and experiment 2) were carried out on female Sprague–Dawley rats weighing 240–280 g. The rats were adapted to their surroundings for 1 week before being fed experimental diets. The rats were divided into three groups in experiment 1 and four groups in experiment 2 (n 9) by using a randomised complete block design (Table 1). Thus, rats were fed seven different experimental diets with various P:S ratios (experiment 1) and PI values (experiment 2) for 4 weeks. Water and food were provided *ad libitum*. The rats were housed in suspended stainless steel mesh cages and kept in an environmentally controlled room at 22 ± 2°C, relative humidity of 50 ± 10 %, with automatic lighting from 06.00 to 18.00 hours.

Fatty acid analysis in fat sources

The fatty acid composition of fat sources was analysed by GC (HP 6890; Hewlett Packard Co., Wilmington, DE, USA). A HP-1 (crosslinked methyl siloxane) capillary column (50 m × 0·32 mm × 0·52 μm) (Morrison & Smith, 1964) and a flame ionisation detector were used. Injector and detector temperatures were 250 and 300°C, respectively. N_2 was employed as the carrier gas. The fatty acid composition was determined by area percentage and confirmation of each fatty acid was performed by comparison of methyl ester with retention time (Nam & Park, 1993). Table 2 shows the fatty acid composition of seven fat sources used in the two experimental diets.

Experimental diets

The experimental diets were prepared by using a modified American Institute of Nutrition-93 growth (AIN-93G) diet (Reeves, 1997). The diets contained the following (% by weight): casein, 15; maize starch, 50; sucrose, 10; fat, 15; DL-methionine, 0·3; choline chloride, 0·2; α-cellulose powder, 5; vitamin mixture (AIN-93), 1·0; mineral mixture (AIN-93), 3·5. The dietary fat was mixed with soyabean oil (CJ Co., Seoul, South Korea), maize oil (CJ Co.), palm oil (Lotte Samkang Co., Seoul, South Korea), perilla oil (directly extracted from perilla), sesame oil (Ottogi Co., Seoul, South Korea), fish oil (Dongwon Co., Seoul, South Korea) and beef tallow (Lottesamkang Co.).

The experimental diet of each group in experiments 1 and 2 is shown in Table 1. In experiment 1, we prepared the lowest and highest P:S ratio of 0·4 and 4·8 under the same PI level of 81 according to our previous study (Kang *et al.* 2003). The middle P:S ratio was prepared on a reference level of 1·0 (Lands *et al.* 1990). In experiment 2, we calculated PI values from seven fats when the P:S ratio was maintained at 1·0. The lowest PI value was 36 and the highest PI value was 217 when the P:S ratio was maintained at 1·0. The average of the lowest PI value and the highest PI value was 126. Then, the average of 36 and 126 was 81, chosen to be the desirable range in our previous study (Kang *et al.* 2003). Table 3 shows the dietary fatty acid composition in experiments 1 and 2. These fatty acid compositions were calculated by using Table 2.

Sample collection

After being deprived of food for 9 h, the rats were weighed and lightly anaesthetised with diethyl ether. Blood samples were collected from heart puncture and the serum was separated by centrifugation at 3000 ***g*** for 15 min at 4°C in a centrifuge (MF 550; Hanil Science Industrial Co., Ltd, Incheon, South Korea).

Table 1. Grouping of experimental rats according to diet conditions in the experiments

	Group	Peroxidisability index*	P:S ratio
Expt 1	LP	81	0·4
	MP	81	1·0
	HP	81	4·8
Expt 2	LPI	36	1·0
	MLPI	81	1·0
	MHPI	126	1·0
	HPI	217	1·0

P:S, polyunsaturated:saturated fatty acid; LP, low polyunsaturated:saturated fatty acid ratio; MP, middle polyunsaturated:saturated fatty acid ratio; HP, high polyunsaturated:saturated fatty acid ratio; LPI, low peroxidisability index; MLPI, mid–low peroxidisability index; MHPI, mid–high peroxidisability index; HPI, high peroxidisability index.

* Peroxidisability index (Du *et al.* 2003) is calculated as follows: peroxidisability index = (% monoenoic acid × 0·025) + (% dienoic acid × 1) + (% trienoic acid × 2) + (% tetraenoic acid × 4) + (% pentaenoic acid × 6) + (% hexaenoic acid × 8).

For details of diets and procedures, see p. 527.

Table 2. Fatty acid composition of fat sources used in the experimental diets (% of total fatty acid methyl esters)

	Fat						
Fatty acid	Soyabean oil	Maize oil	Palm oil	Perilla oil	Sesame oil	Fish oil	Beef tallow
12 : 0	–	–	0·80	–	0·03	–	–
14 : 0	0·06	0·11	1·21	0·01	0·03	3·91	3·00
14 : 1	–	–	–	–	–	–	0·67
16 : 0	10·49	11·25	44·34	6·04	9·33	18·90	26·50
16 : 1	–	0·09	0·18	–	0·14	17·50	2·90
18 : 0	3·51	2·17	4·31	1·89	5·09	5·42	17·00
18 : 1*n*-9	22·21	24·90	39·10	18·09	40·44	16·20	43·40
18 : 2*n*-6	55·23	56·67	9·12	12·37	43·88	1·51	3·40
18 : 3*n*-3	7·49	0·53	0·18	61·00	0·26	0·72	0·30
20 : 0	0·23	0·43	0·27	0·11	0·52	–	0·29
20 : 1	0·78	–	–	–	0·28	1·01	0·38
20 : 4*n*-6	–	–	–	–	–	1·83	–
20 : 5*n*-3	–	–	–	–	–	5·41	–
22 : 6*n*-3	–	–	–	–	–	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
Σ*n*-3	7·49	0·53	0·18	61·00	0·26	33·44	0·30
Σ*n*-6	55·23	56·67	9·12	12·37	43·88	3·34	3·40
Σ*n*-6:Σ*n*-3 Ratio	7·73	106·92	50·67	0·20	168·77	0·10	11·33
Peroxidisability index*	70·78	58·35	10·46	134·82	45·42	262·08	5·18

SFA, saturated fatty acids; P:S, PUFA:SFA.

* Peroxidisability index (Du *et al.* 2003) is calculated as follows: peroxidisability index = (% monoenoic acid × 0·025) + (% dienoic acid × 1) + (% trienoic acid × 2) + (% tetraenoic acid × 4) + (% pentaenoic acid × 6) + (% hexaenoic acid × 8).

For details of diets and procedures, see p. 527.

The livers were promptly excised and washed with ice-cold isotonic saline. Both serum and liver tissue samples were stored at −70°C until needed for analysis.

Measurement of serum lipid concentration

The concentrations of triacylglycerol (TG), total cholesterol (T-C), and HDL-cholesterol (HDL-C) in serum were measured using a commercial diagnostic kit (Shinyang Chemical Co., Seoul, South Korea) at 505, 500, and 555 nm with a spectrophotometer (DU 600; Beckman Coulter, Inc., Fullerton, CA, USA), respectively. The concentration of LDL-cholesterol (LDL-C) was calculated by Friedewald's formula (Friedewald *et al.* 1972). LDL-C:HDL-C ratio was calculated to compare the degrees of cardiovascular risk among groups (Bermingham *et al.* 1995).

Liver tissue preparation for assay

Liver samples were weighed, mixed to 1:10 (w/v) with 10 mM-phosphate buffer (pH 7·4) and homogenised under cold conditions. This

Table 3. Fatty acid composition of the experimental diets in the two experiments (% of total fatty acid methyl esters)

	P:S ratio (Expt 1)			PI value (Expt 2)			
Fatty acid	0·4	1·0	4·8	36	81	126	217
14 : 0	2·96	1·80	0·06	1·16	1·80	2·43	3·70
14 : 1	0·38	0·17	–	0·19	0·17	0·15	0·11
16 : 0	25·71	21·36	9·78	21·72	21·36	21·00	20·28
16 : 1	6·66	4·45	0·02	1·00	4·45	7·91	14·82
18 : 0	11·83	7·59	3·28	7·71	7·59	7·47	7·24
18 : 1*n*-9	34·62	31·60	21·83	35·17	31·60	28·03	20·90
18 : 2*n*-6	4·99	21·54	47·71	27·95	21·54	15·13	2·31
18 : 3*n*-3	0·78	2·43	16·34	2·98	2·43	1·88	0·78
20 : 4*n*-6	0·52	0·38	–	–	0·38	0·75	1·50
20 : 5*n*-3	1·54	1·13	0·01	0·03	1·13	2·23	4·44
22 : 6*n*-3	7·78	5·70	0·03	0·14	5·70	11·27	22·39
P:S ratio	0·38	1·00	4·81	1·00	1·00	1·00	1·00
n-6:*n*-3 Ratio	0·55	2·37	2·91	8·88	2·37	1·03	0·14
PI*	81·22	81·22	81·22	36·12	81·22	126·34	216·56

P:S, PUFA:saturated fatty acids; PI, peroxidisability index.

* PI (Du *et al.* 2003) is calculated as follows: PI = (% monoenoic acid × 0·025) + (% dienoic acid × 1) + (% trienoic acid × 2) + (% tetraenoic acid × 4) + (% pentaenoic acid × 6) + (% hexaenoic acid × 8).

For details of diets and procedures, see p. 527.

homogenate was sonicated for 30 s with a sonicator (GE 50; Sonics & Materials Inc., Danbury, CT, USA) in an ice bath and used for thiobarbituric acid reactive substances (TBARS). The homogenate was centrifuged at 20 000 ***g*** and 4°C for 30 min in an ultracentrifuge (Optima™ TL; Beckman Coulter, Inc.) in order to use as samples for assaying SOD and CAT. Liver samples were mixed to 1:20 (w/v) with 50 mM-phosphate buffer (0·25 mM-sucrose–0·5 mM-EDTA; pH 7·4) and homogenised under cold conditions. This homogenate was sonicated in an ice bath and subjected to two centrifugation steps before being used for GSH-Px and GST samples. The homogenate was centrifuged at 10 000 ***g*** and 4°C for 20 min in an ultracentrifuge (Optima™ TL; Beckman Coulter, Inc.). The supernatant fraction was then recentrifuged at 100 000 ***g*** and 4°C for 1 h (Optima™ TL; Beckman Coulter, Inc.). The final supernatant fraction was used as a sample for enzyme assay.

Hepatic enzyme activities assay

SOD (*EC* 1.15.1.1) activity was measured by using pyrogallol (Marklund, 1984). The reaction was started by the addition of 24 mM-pyrogallol (in 10 mM-degasing acetic acid) and read at 420 nm. CAT (*EC* 1.11.1.6) activity was measured by the disappearance rate of H_2O_2 monitored spectrophotometrically at 240 nm according to the method of Aebi (1984) and Claiborne (1984). GSH-Px (*EC* 1.4.1.9) activity was assayed according to the method of Flohe & Gunzler (1984). The GSH-Px activity was expressed as unit per mg protein as measured by the method of Bradford (1976). An enzyme unit was defined as the amount of enzyme that catalyses the release of 1 μmol NADPH/min at 37°C (Qujeq *et al.* 2004). The activity of GST (*EC* 2.5.1.18) was measured by the methods of Warholm *et al.* (1985) using 1-chloro-2, 4-dinitrobenzene (Sigma Aldrich Korea Ltd, Seoul, South Korea) as a substrate.

Hepatic thiobarbituric acid reactive substance assay

The concentration of the lipid peroxide products of hepatic TBARS, mainly malondialdehyde (Saito & Kubo, 2003), was measured by a modification of the method of Fraga *et al.* (1988) using thiobarbituric acid, 1,1,3,3-tetraethoxy-propane (Aldrich Chemical Co., Seoul, South Korea) as the standard. TBARS values were expressed as ng/mg protein. Protein determination was performed by the method of Bradford (1976) using bovine serum albumin (Sigma Aldrich Korea Ltd) as standard.

Statistical analysis

For statistical analysis, Statistical Package for Social Science version 11.0 (SPSS Inc., Chicago, IL, USA) was used. Data were expressed as means with their standard errors. The significance of differences among mean values was assessed by one-way ANOVA coupled with Duncan's multiple range test at $P<0·05$.

Results

When the dietary P:S ratio was changed while the dietary PI value was constant (81; experiment 1; Table 4), the serum TG and LDL-C concentrations were not significantly different among groups. The serum T-C and HDL-C concentrations increased significantly with increasing dietary P:S ratio. The serum LDL-C:HDL-C ratio was not influenced by dietary P:S ratio. When dietary PI value was changed while the dietary P:S ratio was controlled at the same level (1·0; experiment 2; Table 4), the serum TG and HDL-C concentrations, and LDL-C:HDL-C ratio were significantly different by the PI value. The serum T-C and LDL-C concentrations were not affected by dietary PI value.

When the dietary P:S ratio was changed while the dietary PI value was controlled at the same level (81; experiment 1; Table 5), the hepatic SOD, CAT, and GSH-Px activities were significantly different by dietary P:S ratio. The hepatic SOD, CAT, and GSH-Px had a tendency to decrease with increasing dietary P:S ratio when PI value was maintained at the same level. The hepatic GST activities were not significantly different by dietary P:S ratio when dietary PI value was maintained at the same level. When PI value was changed while the P:S ratio was constant (1·0; experiment 2; Table 5), hepatic SOD, GSH-Px, and GST activities were significantly different among four groups. The hepatic GSH-Px and GST activities were the lowest in the mid–low PI condition.

The hepatic TBARS concentration decreased significantly with increasing dietary P:S ratio while the PI value was fixed

Table 4. Serum lipid profiles of rats fed diets with different polyunsaturated:saturated fatty acids (P:S) ratios and peroxidisability index (PI) values of dietary fats (nine rats per group)

(Mean values with their standard errors)

	P:S ratio (Expt 1)						PI value (Expt 2)							
	LP (0·4)		MP (1·0)		HP (4·8)		LPI (36)		MLPI (81)		MHPI (126)		HPI (216)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
TG (mg/l)	589·0	64·0	588·0	60·9	438·0	32·9	502·3[b]	12·6	491·4[b]	31·3	547·9[b]	21·3	400·3[a]	33·7
T-C (mg/l)	489·0[a]	19·5	678·9[b]	52·5	720·9[b]	64·8	590·4	37·4	615·7	45·2	534·7	53·7	502·7	41·0
HDL-C (mg/l)	196·0[a]	19·1	298·7[b]	33·1	336·4[b]	33·0	339·8[b]	12·2	260·0[a]	17·2	261·9[a]	22·8	272·7[a]	16·4
LDL-C(mg/l)	153·3	35·2	266·1	43·1	252·5	52·2	151·8	43·7	256·5	29·7	164·0	39·7	149·9	30·7
LDL-C: HDL-C ratio	0·93	0·34	0·98	0·22	0·81	0·18	0·46[a]	0·13	0·99[b]	0·12	0·60[a]	0·12	0·54[a]	0·10

LP, low polyunsaturated:saturated fatty acid ratio; MP, middle polyunsaturated:saturated fatty acid ratio; HP, high polyunsaturated:saturated fatty acid ratio; LPI, low peroxidisability index; MLPI, mid–low peroxidisability index; MHPI, mid–high peroxidisability index; HPI, high peroxidisability index; TG, triacylglycerol; T-C, total cholesterol; HDL-C, HDL-cholesterol; LDL-C, LDL-cholesterol.

[a,b] Mean values within a row with unlike superscript letters were significantly different by ANOVA coupled with Duncan's multiple range tests ($P<0·05$).

For details of diets and procedures, see p. 527.

Table 5. Hepatic enzyme activities of rats fed diets with different polyunsaturated:saturated fatty acids (P:S) ratios and peroxidisability index (PI) values of dietary fats (nine rats per group)

(Mean values with their standard errors)

	P:S ratio (Expt 1)						PI value (Expt 2)							
	LP (0·4)		MP (1·0)		HP (4·8)		LPI (36)		MLPI (81)		MHPI (126)		HPI (217)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
SOD	11·55^{b}	0·96	9·91^{a}	0·59	9·18^{a}	0·68	10·94^{b}	0·50	9·39^{a,b}	0·99	7·60^{a}	0·44	7·70^{a}	0·69
CAT	30·53^{b}	1·86	25·44^{a,b}	2·98	21·35^{a}	1·34	23·15	1·13	24·53	2·25	25·40	2·62	27·25	1·90
GSH-Px	2·74^{b}	0·25	2·46^{a,b}	0·35	1·81^{a}	0·07	3·20^{b}	0·16	2·55^{a}	0·11	3·20^{b}	0·24	2·81^{a,b}	0·19
GST	12·21	0·55	13·09	2·77	12·51	0·91	20·45^{a,b}	1·36	15·99^{a}	1·45	24·26^{b}	1·71	23·58^{b}	2·18

LP, low polyunsaturated:saturated fatty acid ratio; MP, middle polyunsaturated:saturated fatty acid ratio; HP, high polyunsaturated:saturated fatty acid ratio; LPI, low peroxidisability index; MLPI, mid–low peroxidisability index; MHPI, mid–high peroxidisability index; HPI, high peroxidisability index; SOD, superoxide dismutase; CAT, catalase; GSH-Px, glutathione peroxidase; GST, glutathione-*S*-transferase.

[a,b,c] Mean values within a row with unlike superscript letters were significantly different by ANOVA coupled with Duncan's multiple range tests ($P<0·05$).

For details of diets and procedures, see p. 527.

(81; experiment 1; Table 6). In experiment 2, the hepatic TBARS concentration was significantly increased with increasing dietary PI value above 81 when the P:S ratio was maintained at the same level (1·0; experiment 2; Table 6).

Discussion

In a previous study, the serum TG concentration was not affected by the dietary P:S ratio when the dietary PI value was constant (Kang *et al.* 2003). In the present study, also, the serum TG concentration was not significantly different although we changed the dietary P:S ratio of three diets in experiment 1. Through results in experiment 2, we were able to ascertain whether this phenomenon is due to the effect of fixed PI value or not. In experiment 2, when we changed dietary PI value while the dietary P:S ratio was maintained at the same level, the serum TG concentration was significantly different by dietary PI value. Hence, these results suggest that the serum TG concentration is more influenced by the dietary PI value than by the dietary P:S ratio.

It has been shown that the serum T-C is increased by the consumption of SFA and decreased by the intake of PUFA (Friedewald *et al.* 1972; Knezevic *et al.* 2000). Lee *et al.* (1989) reported that the P:S ratio of approximately 2 is the point of maximum influence in regulating serum cholesterol. However, in the present study, the serum T-C concentration increased with increasing P:S ratio up to 4·8 (experiment 1), but it was not significantly affected by change of dietary PI value under the fixed P:S ratio (experiment 2). Because of these results, it seems that the change of serum T-C concentration is dependent on the unique effect of the dietary P:S ratio.

Although the dietary P:S ratio is associated with the serum HDL-C concentration, the relationship has not been equally observed in all investigations (Ehnholm *et al.* 1982). Ehnholm *et al.* (1982) reported that the serum HDL-C concentration decreased as the ratio of the P:S increased in the diet. In addition, in contrast to the *n*-6 PUFA-rich vegetable oils that lower HDL-C concentration, *n*-3 PUFA-rich fish oil did not decrease HDL-C concentration (Conner, 2000). In the present study, the serum HDL-C concentration was affected by dietary PI value (experiment 2) as well as by dietary P:S ratio (experiment 1). Perhaps the confounding interrelated effects of dietary P:S ratio and PI value implicate the roles as regulator of serum HDL-C concentration. Thus, serum HDL-C concentration was the highest in the low PI group, P:S ratio 1·0 and PI value 36, among the groups.

Muller *et al.* (2003) reported that the lowering of dietary total SFA without a change in the P:S ratio does not lower LDL-C. But, in their study, replacing dietary SFA by PUFA reduced the serum LDL-C concentration and to some extent reduced the serum HDL-C concentration. In the present study, the serum LDL-C concentration was significantly different by dietary PI value (experiment 2) as well as by dietary P:S ratio (experiment 1). In many studies, serum LDL-C, HDL-C, and LDL-C:HDL-C ratio has been known as a risk marker for CVD (Ehnholm *et al.* 1982; Green *et al.* 1985; Muller *et al.* 2003). The serum LDL-C concentration is positively associated with the incidence of CVD (Ehnholm *et al.* 1982). However, the relationship between HDL-C level and CVD is more complex (Ehnholm *et al.* 1982). LDL-C:HDL-C ratio has been known as an important factor to predict the evidence of CVD (Green *et al.* 1985). When this

Table 6. Hepatic thiobarbituric acid reactive substances (TBARS) of rats fed diets with different polyunsaturated:saturated fatty acids (P:S) ratios and peroxidisability index (PI) values of dietary fats (nine rats per group)

(Mean values with their standard errors)

	P:S ratio (Expt 1)						PI value (Expt 2)							
	LP (0·4)		MP (1·0)		HP (4·8)		LPI (36)		MLPI (81)		MHPI (126)		HPI (217)	
	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM	Mean	SEM
TBARS	20·33^{b}	0·70	14·59^{b}	2·73	5·21^{a}	0·54	16·79^{a}	1·24	16·01^{a}	2·30	28·19^{b}	1·93	37·54^{c}	3·17

LP, low polyunsaturated:saturated fatty acid ratio; MP, middle polyunsaturated:saturated fatty acid ratio; HP, high polyunsaturated:saturated fatty acid ratio; LPI, low peroxidisability index; MLPI, mid–low peroxidisability index; MHPI, mid–high peroxidisability index; HPI, high peroxidisability index.

[a,b,c] Mean values within a row with unlike superscript letters were significantly different by ANOVA coupled with Duncan's multiple range tests ($P<0·05$).

For details of diets and procedures, see p. 527.

ratio is higher than 5, it is a serious risk sign in CVD (Meyer *et al.* 2004). In the present study, the serum LDL-C:HDL-C ratio was in the range of 0–2 when dietary PI value (experiment 1) was maintained at 81 (experiment 1) and when P:S ratio was kept at 1·0 (experiment 2). Accordingly, if it is possible to control a desirable P:S ratio (approximately 1·0–1·5) (Oliver, 1987; Lee *et al.* 1989; Kang *et al.* 2004) or an ideal PI value (approximately 80–90) (Nam & Park, 1993; Kang *et al.* 2004) in the diet, the level of serum LDL-C:HDL-C ratio may be maintained in the range not exceeding 5.

In experiment 1, the activities of hepatic SOD, CAT, and GSH-Px as antioxidant defence were the highest in rats fed the lowest P:S ratio-containing diet. Also, the increasing and decreasing patterns of these enzyme activities by dietary P:S ratio were similar to hepatic TBARS concentration in respect of relationship with dietary P:S ratio. In experiment 1, we controlled PI value equally through three experimental diets. In the preparation of experimental diets, fish oil, especially DHA (22 : 6), was added in the low P:S diets more than in the high P:S diets. Because of this reason, it seems that hepatic TBARS decreased with increasing P:S ratio in the diet. Accordingly, the hepatic TBARS was affected by specific fatty acid such as DHA more than by P:S ratio when dietary PI value was fixed at the same time. SOD was positively correlated with CAT (r 0·623; P=0·001), GSH-Px (r 0·426; P=0·038), and TBARS (r 0·407; P=0·041) (data not shown). In the present study, we concluded that the activities of SOD, CAT, and GSH-Px may be directly dependent on hepatic TBARS concentration. TBARS concentration of the low P:S group was elevated by abundant DHA content when dietary PI value was maintained at the same level, and SOD activity was increased with this increase of TBARS. It seems that the elevation of SOD activity caused the continuous increase of CAT and GSH-Px activities. Accordingly, we reconfirmed that SOD (Lutoslawska *et al.* 2003), CAT, and GSH-Px (Qujeq *et al.* 2004) protect liver tissue from oxidative damage such as TBARS. However, in experiment 2, GSH-Px and GST activities, and TBARS were significantly different by dietary PI value, and were not consistent according to PI value. As a result, it seems that the activities of GSH-Px may be dependent on both the dietary P:S ratio and PI value. On the other hand, glutathione-related enzyme activities such as GSH-Px and GST are more influenced by dietary PI value than by dietary P:S ratio. In addition, GSH-Px was correlated with GST (r 0·619; P=0·001), Hence, the dietary PI value of 81 may be the optimal PI level for GSH-Px and GST activities when dietary P:S ratio was fixed at 1·0.

In conclusion, if a desirable dietary P:S ratio and PI value in the diet is maintained, the LDL-C:HDL-C ratio may be controlled within an adequate range in order to decrease the risk of CVD. However, it is difficult to make a clear recommendation for the dietary PI value based on the results of the present study. We may conclude, however, that it is desirable to maintain a P:S ratio (approximately 1·0–1·5) with an ideal PI value (approximately 80–90) in the diet in order to reduce the risk of CVD and oxidative stress.

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