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Effects of dietary fish oil and trans fat on rat aorta histopathology and cardiovascular risk markers*

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Abstract

Fish oil and shortening have been suggested to have opposite effects on cardiovascular disease (CVD). This study investigated the effect of shortening and fish oil on CVD risk factors and aorta histopathology, and the association between risk factors and aorta histopathology. Male Wister rats (n=30) were fed an AIN-93G diet containing 20% fat in the form of fish oil, shortening, or soybean oil for 4 weeks. Total cholesterol (TC), triacylglyceride (TG), and C-reactive protein levels were significantly ($P<0.001$) lower in the fish oil than in soybean oil and shortening groups. HDL-cholesterol concentrations were significantly different ($P<0.001$) between groups. In addition, LDL-cholesterol levels were significantly ($P<0.001$) lower in the fish oil and shortening groups than in the soybean oil group. Insulin and glucose concentrations did not differ among groups. Effect of dietary fat on tissue fatty acid composition significantly differed in abdominal fat and brain compared with RBC, heart, kidney and liver. The aortic wall was significantly ($P=0.02$) thinner in the fish oil group than in the soybean oil and shortening groups. The aortic wall thickness was positively correlated with TG and TC, but negatively with EPA + DHA levels of all tissues. These results suggested that fish oil had protective effects on aorta histopathology by hypolipidemic action in this rat model.

Key Words: Aorta histopathology, C-reactive protein, fish oil, lipid profile, trans fat

Introduction

Diet has been known as an important risk factor for cardiovascular disease (CVD). Studies suggest that fish oil such as eicosapentaenoic acid (EPA; C20:5n-3), docosahexaenoic acid (DHA; C22:6n-3), and trans fatty acid alter lipoprotein metabolism (Colandré *et al.*, 2003; Idris & Sundram, 2002; Morgado *et al.*, 2005; Othman *et al.*, 2008; Qi *et al.*, 2008) amd insulin resistance (Holness *et al.*, 2003; Kavanagh *et al.*, 2007; Mahmud *et al.*, 2004; Natarajan *et al.*, 2005). Previous studies reported that fish oil lowered total cholesterol (TC), low-density lipoprotein (LDL)-cholesterol, high-density lipoprotein (HDL)-cholesterol, and triacylglycerol (TG) concentrations (Morgado *et al.*, 2005; Othman *et al.*, 2008; Qi *et al.*, 2008;). On the other hand, trans fatty acids increased LDL-cholesterol, TC, and TG (Colandré *et al.*, 2003; Idris & Sundram, 2002; Natarajan *et al.*, 2005) and decreased HDL-cholesterol (Idris & Sundram, 2002). There were several studies to show the effects of fish oil and trans fatty acids on insulin and glucose, but the findings were inconclusive. Holness *et al.* (2003) reported that n-3 fatty acids decreased insulin and increased glucose levels, but Mahmud *et al.* (2004) did not. Trans fatty acids decreased insulin levels in some studies (Ibrahim *et al.*, 2005; Natarajan *et al.*, 2005), but not in others (Bernal *et al.*, 2006; Lichtenstein *et al.*, 2003).

Inflammation is another risk factor for CVD and a few human studies have shown that n-3 fatty acids decrease (Ciubotaru *et al.*, 2003; Niu *et al.*, 2006) and trans fatty acids increase C-reactive protein (CRP; Baer *et al.*, 2004; Lopez-Garcia *et al.*, 2005). Unfortunately, there is no animal study showing the effects of fish oil and trans fatty acids on CRP. Unlikely with lipid profile, insulin, or CRP, vascular morphology is an intermediate marker of CVD (Cao *et al.*, 2007). There has been only one study reporting that vascular wall thickness and coronary artery diameter were decreased in DHA-fed rats. Therefore, we investigated the effect of shortening and fish oil on CVD risk factors and aorta histopathology, and the association between risk factors and aorta histopathology.

Materials and Methods

Animals and diet

Four-week-old male Wistar rats (Japan SLC. Inc., Japan) weighing 100-120 g were fed a commercial chow diet for one week and randomly assigned to one of three groups (n=10 each) for four weeks. Throughout the experiment, rats were housed in standard stainless cages and maintained in a temperature-

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Table 1. Composition of experimental diets[1]

(g/kg)	Soybean oil	Fish oil	Shortening
Starch	399.3	399.3	399.3
Casein	200	200	200
Sucrose	100	100	100
Rapeseed oil	30	30	30
Soybean oil	170		
Fish oil			170
Shortening		170	
Fiber	50	50	50
Mineral mix[2]	35	35	35
Vitamin mix[2]	10	10	10
L-systein-L-metionine	3	3	3
Choline	2.5	2.5	2.5
Butylated hydroxytuoluen	0.2	0.2	0.2

[1] Animals were fed an isocaloric semi-synthetic diet containing identical dietary constituents differing only in the composition of dietary oil for 4 weeks.
[2] Mineral mix and Vitamin mix were prepared according to the AIN-93 (23).

controlled animal facility at 22 ± 1℃ with a 12-h light/dark cycle and humidity-controlled at 60 ± 10%. Daily intake was calculated by subtracting residual food from the amount given. Body weight was measured twice a week. Rats were housed two per cage and fed with one of three isocaloric purified AIN-93G diets *ad libitum*, which were stored at -5℃ temperature and replaced with fresh diets every day (Reeves *et al.*, 1993). The experimental diet contained 20% fat from soybean oil (Back-Seol, CJ, Korea), fish oil (Carlson the very finest fish oil, Carlson lab, Norway), or shortening (Crisco, JM Smuckers Company, USA; Table 1). The fatty acid composition of fish oil was 27.2% n-3 fatty acids, 17.0% saturated fat (SFA), 34.0% polyunsaturated fat (PUFA), and 17.0% monounsaturated fat (MUFA). The fatty acid composition of shortening was 10.6% trans fatty acids, 21.2% SFA, 21.2% PUFA, and 28.33 MUFA. After four weeks, the rats were deprived of food overnight and carbon dioxide asphyxiated. The experimental protocols were approved by the Animal Care Committee of Hanyang University.

Procedures

Blood samples were collected in EDTA and SST tubes by cardiac puncture. Plasma and serum were separated by centrifugation at 3,000 g for 15 min (HA1000-3, Hanil Sciences Industrial Co. Ltd., Korea) and stored at <-70℃ for later analysis. Serum TG, TC, and plasma HDL-cholesterol levels were measured by enzymatic methods (Ultrospec 2100 pro, Amersham Pharmacia Biotech, England). Serum LDL-cholesterol concentrations were calculated using the Friedewald equation (Friedewald *et al.*, 1972). Plasma insulin (EIA kit, SPI bio, France), CRP (BD™ ELISA, BD Biosciences, USA) and serum glucose (Glucose Assay Kit, Biovision, USA) levels were measured by enzyme immunoassay at 415, 450, and 570 nm, respectively with microplate reader (ELx 800 uv, BIO-TEK Instruments. INC, USA).

Liver, brain, kidney, heart, and abdominal fat (100 mg) were mixed with 5ml of chloroform: methanol: distilled water, 2:2:1 (v/v/v). Tissue phospholipids were separated by thin layer chromatography (TLC; Silica gel G, Analtech, USA) and re-extracted by hexane: ether: acetic acid, 40:10:1 (v/v/v). Red blood cells (RBC) and tissue phospholipids were methylated by adding boron trifluoride methanol-benzene (B1252; Sigma-Aldrich, MO, USA), and heated at 100℃ for 10 min. Fatty acid methyl esters were analyzed by Gas Chromatography (GC; Shimadzu 2010AF; Shimadzu Scientific Instrument, Japan) with a 100-m SP2560 capillary column (Supelco; Bellefonte, PA, USA). Fatty acids were identified by comparison with known standards (GLC-727; Nu-Check Prep, Elysian, MN, USA). The C18:1t standard were the mixture of C18:1n-12t, C18:1n-9t, and C18:1n-7t, and the C18:2n-6t standard contained 18:2n-6tt. The control sample was made from pooled RBC and the CV was 4.6%.

Portions of aortic tissue were fixed in 10% formalin in pH 7.4 (Kim *et al.*, 1995). The washed tissue was dehydrated in descending isopropanol grades, cleared in xylene, and embedded in paraffin. Sections were cut to be 5 μm thick and stained with hematoxylin, eosin, Venhoeff, and Van Gieson. Haematoxylin stains cell nuclei blue, while eosin stains cytoplasm, connective tissue and other extracellular substances pink or red. Eosin is strongly absorbed by red blood cells, coloring them bright red. Van Gieson's and Venhoeff stain is a mixture of picric acid and acid fuchsin. The sections were viewed under a light microscope (DM RXE, Leica, Germany) for histopathological changes. Intima wall thickness, media, and lumen area were examined at 0°, 90°, 180° and 270° in every section and the average measure was calculated (Analysis v.3.2, SIS Gmbh, Germany).

Statistical analysis

All data were expressed as the mean ± SEM, and differences among the three groups were compared using one-way ANOVA with post-hoc Turkey's test. A p-value of <0.05 was considered statistically significant. Statistical analysis was performed using SPSS software version 12 (SPSS Inc., Chicago, IL, USA).

Results

Rat weight, diet, and weight of organs

Average daily weight, initial and final body weight, weight change, and heart and brain weights did not significantly differ among groups (Table 2). Rats fed fish oil had significantly lower abdominal fat weight, but significantly higher liver and kidney weights, as compared with those fed soybean oil or shortening.

Lipid profile, insulin, glucose, and CRP

TC, TG, and CRP concentrations were significantly lower in the fish oil than the soybean oil and shortening groups (Table

Table 2. Dietary intake, body weight, and organ weights

	Soybean oil	Shortening	Fish oil
Dietary intake (g/day)	16.6 ± 0.7[1]	16.8 ± 0.4	16.0 ± 0.4
Initial body weight (g)	111.6 ± 2.0	111.9 ± 2.1	111.5 ± 1.9
Final body weight (g)	290.7 ± 7.6	291.1 ± 6.4	286.5 ± 8.3
Weight gain (g)	179.1 ± 6.7	179.2 ± 5.4	175.0 ± 7.1
Liver weight (g)	10.5 ± 0.4[a2]	9.9 ± 0.4[a]	12.5 ± 0.5[b]
Kidney weight (g)	2.3 ± 0.1[a]	2.3 ± 0.1[a]	2.6 ± 0.1[b]
Heart weight (g)	1.2 ± 0.0	1.2 ± 0.0	1.2 ± 0.1
Brain weight (g)	1.7 ± 0.1	1.8 ± 0.1	0.9 ± 0.0
Abdominal fat weight (g)	4.3 ± 0.3[a]	3.6 ± 0.2[a]	1.8 ± 0.2[b]

[1] Data were expressed as mean ± SEM of 10 rats per group.
[2] Values in a row with different letters were significantly different, P<0.05 (ANOVA with post-hoc Tukey's test).

Table 3. Lipid profile, insulin, glucose, and C-reactive protein

	Soybean oil	Shortening	Fish oil
TC[1] (mg/dL)	133.10 ± 7.98[a2,3]	117.80 ± 6.12[a]	52.00 ± 3.55[b]
TG (mg/dL)	206.90 ± 17.58[a]	194.10 ± 21.14[a]	94.60 ± 10.49[b]
HDL-cholesterol (mg/dL)	38.65 ± 2.69[a]	58.28 ± 1.76[c]	13.98 ± 2.46[b]
LDL-cholesterol (mg/dL)	53.07 ± 7.22[a]	20.70 ± 3.71[b]	19.10 ± 2.69[b]
Insulin (ng/mL)	2.26 ± 0.19	2.54 ± 0.22	2.32 ± 0.21
Glucose (nmol/μL)	6.43 ± 0.75	7.37 ± 0.90	7.63 ± 0.82
CRP (mg/dL)	38.13 ± 1.14[a]	35.76 ± 0.58[a]	29.52 ± 0.81[b]
Aorta thickness (μm)	86.38 ± 2.07[a]	87.93 ± 3.67[a]	72.98 ± 2.74[b]

[1] TC, total cholesterol; TG, triacylglyceride; HDL-cholesterol, high-density lipoprotein-cholesterol; LDL-cholesterol, low-density lipoprotein-cholesterol; CRP, C-reactive protein
[2] Data were expressed as mean ± SEM of 10 rats per group.
[3] Values in a row with different letters were significantly different, P<0.01 (ANOVA with post-hoc Tukey's test).

3). HDL-cholesterol level was the lowest in the fish oil group and the highest in the shortening group. LDL-cholesterol level was significantly lower in the fish oil and shortening groups than the soybean oil group. There was no significant difference in insulin or glucose levels among groups.

Fatty acids composition of tissues

Total n-3 fatty acids, EPA, DPA, DHA and EPA+DHA in RBC, liver, kidney, heart, brain, and abdominal fat were significantly higher in the fish oil than soybean oil and shortening

Table 4. Fatty acid composition of red blood cell and other tissues

(%)	Soybean oil	Shortening	Fish oil
Red blood cell			
C18:3n3	5.57 ± 0.65[1]	4.77 ± 0.70	4.81 ± 0.61
C20:5n3 (EPA[2])	0.35 ± 0.01[a3]	0.25 ± 0.01[a]	11.86 ± 0.44[b]
C22:5n3	1.80 ± 0.04[a]	1.75 ± 0.05[a]	3.02 ± 0.08[b]
C22:6n3 (DHA)	3.26 ± 0.06[a]	3.71 ± 0.10[b]	8.98 ± 0.49[c]
EPA + DHA	3.61 ± 0.05[a]	3.96 ± 20.84[a]	20.84 ± 0.52[b]
Total n-3 FAs	10.97 ± 0.63[a]	10.48 ± 0.71[a]	28.67 ± 0.36[b]
C16:1n7t	0.03 ± 0.00[a]	0.11 ± 0.01[b]	0.21 ± 0.01[c]
C18:1t	0.40 ± 0.01[a]	2.15 ± 0.09[b]	0.21 ± 0.01[a]
C18:2n6t	0.27 ± 0.02[a]	0.20 ± 0.02[b]	0.31 ± 0.01[a]
Total trans FAs	0.69 ± 0.05[a]	2.46 ± 0.21[b]	0.74 ± 0.07[a]
Liver			
C18:3n3	0.17 ± 0.02[a]	0.08 ± 0.01[b]	0.08 ± 0.00[b]
C20:5n3	0.09 ± 0.01[a]	0.05 ± 0.00[a]	4.60 ± 0.41[b]
C22:5n3	0.81 ± 0.03[a]	0.72 ± 0.04[a]	1.16 ± 0.10[b]
C22:6n3	9.31 ± 0.26[a]	8.28 ± 0.36[a]	21.28 ± 0.70[b]
EPA + DHA	9.40 ± 0.25[a]	8.34 ± 0.35[a]	25.88 ± 0.77[b]
Total n-3 FAs	10.38 ± 0.22[a]	9.13 ± 0.36[a]	27.13 ± 0.73[b]
C16:1n7t	0.03 ± 0.00[a]	0.11 ± 0.01[c]	0.08 ± 0.04[b]
C18:1t	0.13 ± 0.01[a]	1.90 ± 0.03[b]	0.13 ± 0.00[a]
C18:2n6t	0.00 ± 0.00[a]	0.01 ± 0.00[b]	0.00 ± 0.00[a]
Total trans FAs	0.16 ± 0.02[a]	2.02 ± 0.09[b]	0.21 ± 0.02[a]
Kidney			
C18:3n3	0.58 ± 0.04[a]	0.21 ± 0.02[b]	0.19 ± 0.02[b]
C20:5n3	0.37 ± 0.02[a]	0.18 ± 0.01[a]	12.17 ± 0.89[b]
C22:5n3	0.42 ± 0.02[a]	0.38 ± 0.02[a]	1.03 ± 0.06[b]
C22:6n3	2.62 ± 0.05[a]	2.43 ± 0.09[a]	10.38 ± 0.51[b]
EPA + DHA	2.99 ± 0.06[a]	2.61 ± 0.10[a]	22.55 ± 1.30[b]
Total n-3 FAs	4.00 ± 0.08[a]	3.20 ± 0.10[a]	23.77 ± 1.28[b]
C16:1n7t	0.05 ± 0.01[a]	0.39 ± 0.04[c]	0.17 ± 0.01[b]
C18:1t	0.36 ± 0.03[a]	1.89 ± 0.26[b]	0.17 ± 0.02[a]
C18:2n6t	0.22 ± 0.02[a]	0.10 ± 0.01[b]	0.08 ± 0.01[b]
Total trans FAs	0.63 ± 0.12[a]	2.39 ± 0.94[b]	0.40 ± 0.09[a]
Heart			
C18:3n3	0.20 ± 0.00[a]	0.11 ± 0.01[b]	0.13 ± 0.01[b]
C20:5n3	0.06 ± 0.00[a]	0.04 ± 0.00[a]	2.40 ± 0.06[b]
C22:5n3	2.74 ± 0.11[a]	2.54 ± 0.08[a]	2.02 ± 0.03[b]
C22:6n3	14.30 ± 0.22[a]	14.29 ± 0.37[a]	28.56 ± 0.42[b]
EPA + DHA	14.36 ± 0.22[a]	14.33 ± 0.37[a]	30.95 ± 0.39[b]
Total n-3 FAs	17.30 ± 0.23[a]	16.98 ± 0.36[a]	33.10 ± 0.38[b]
C16:1n7t	0.04 ± 0.01[a]	0.09 ± 0.01[b]	0.13 ± 0.01[c]
C18:1t	0.29 ± 0.01[a]	1.96 ± 0.04[c]	0.15 ± 0.01[b]
C18:2n6t	0.28 ± 0.02[a]	0.10 ± 0.01[b]	0.08 ± 0.01[c]
Total trans FAs	0.61 ± 0.07[a]	2.15 ± 0.14[b]	0.36 ± 0.06[c]
Brain			
C18:3n3	0.02 ± 0.01	0.00 ± 0.00	0.02 ± 0.01
C20:5n3	0.00 ± 0.00[a]	0.00 ± 0.00[a]	0.37 ± 0.02[b]
C22:5n3	0.10 ± 0.03[a]	0.19 ± 0.01[a]	0.87 ± 0.03[b]
C22:6n3	17.18 ± 0.61[a]	17.68 ± 0.33[a]	20.97 ± 0.39[b]
EPA + DHA	17.18 ± 0.61[a]	17.69 ± 14.33[a]	21.33 ± 30.95[b]
Total n-3 FAs	17.31 ± 0.59[a]	17.88 ± 0.33[a]	22.22 ± 0.37[b]
C16:1n7t	0.04 ± 0.00[a]	0.15 ± 0.02[b]	0.10 ± 0.02[ab]
C18:1t	0.06 ± 0.02[a]	0.03 ± 0.02[b]	0.14 ± 0.02[c]
C18:2n6t	0.23 ± 0.03[a]	0.30 ± 0.04[a]	0.05 ± 0.01[b]
Total trans FAs	0.33 ± 0.13[a]	0.75 ± 0.10[b]	0.29 ± 0.08[a]
Abdominal fat			
C18:3n3	0.68 ± 0.07[a]	0.46 ± 0.09[ab]	0.42 ± 0.05[b]
C20:5n3	0.05 ± 0.01[a]	0.00 ± 0.00[a]	4.69 ± 0.25[b]
C22:5n3	0.10 ± 0.02[a]	0.00 ± 0.00[a]	1.71 ± 0.16[b]
C22:6n3	1.52 ± 0.14[a]	0.00 ± 0.00[b]	6.96 ± 0.29[c]
EPA + DHA	1.57 ± 0.13[a]	0.00 ± 0.00[b]	11.65 ± 0.42[c]
Total n-3 FAs	2.35 ± 0.13[a]	0.46 ± 0.09[b]	13.77 ± 0.48[c]
C16:1n7t	0.13 ± 0.02	0.19 ± 0.03	0.18 ± 0.01
C18:1t	1.24 ± 0.14[a]	5.77 ± 0.37[b]	0.52 ± 0.16[a]
C18:2n6t	0.24 ± 0.03[a]	0.25 ± 0.02[a]	0.06 ± 0.02[b]
Total trans FAs	1.61 ± 0.50[a]	6.22 ± 1.20[b]	0.77 ± 0.55[a]

[1] Data were expressed as mean ± SEM of ten rats per group.
[2] EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid; FA, fatty acid
[3] Values in a row with different letters were significantly different, P<0.05 (ANOVA with post-hoc Turkey's test).

groups (Table 4). However, alpha-linolenic acids (ALA; C18:3n3) in the liver, kidney, heart, and abdominal fat were significantly higher in the soybean oil than fish oil and shortening groups. Total trans fatty acids, C16:1n7t and C18:1t in RBC, liver, kidney, brain, and abdominal fat, and C18:1t in abdominal fat were significantly higher in the shortening than fish oil and soybean oil groups. Interestingly, there was no consistent pattern in the distribution of C18:2n6t. Dietary effect on fatty acid composition of RBC was similar to those of liver, kidney and heart, but to those of abdominal and brain, suggesting that dietary fatty acid composition highly influenced abdominal fatty acid composition but less influenced brain fatty acid composition.

Aorta histopathology

Aortic wall thickness was significantly lower in the fish oil than soybean oil and shortening groups (Table 3). The density of tunica media smooth muscle nuclei was significantly higher in the fish oil than soybean oil and shortening groups (Fig. 1). In addition, tunica media smooth muscle nuclei in the fish oil group were longer and finer than those in the other groups. The aortic wall lumen area and number of elastin bands did not differ significantly among groups (Fig. 2). The aortic wall thickness was positively correlated with TG and TC, and negatively correlated with EPA+DHA in RBC, liver, heart, kidney, brain, and abdominal fat (Table 5). There was no significant association between aortic wall thickness and trans fatty acids (data not shown).

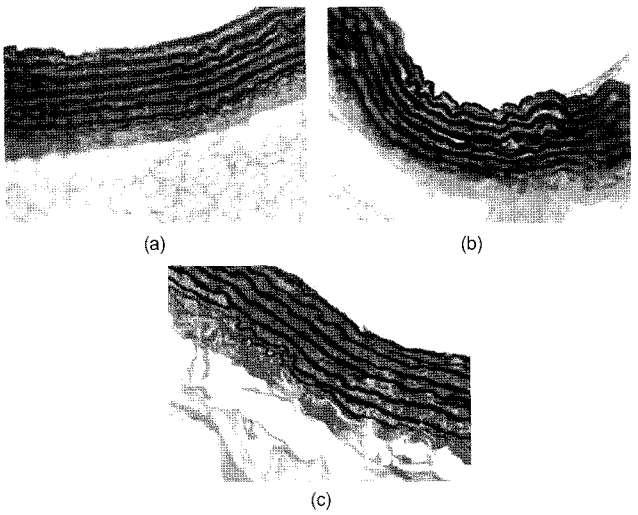

Fig. 1. Aorta wall stained by Venhoeff & van Gieson in rats; (a) soybean oil; (b) fish oil; (c) shortening

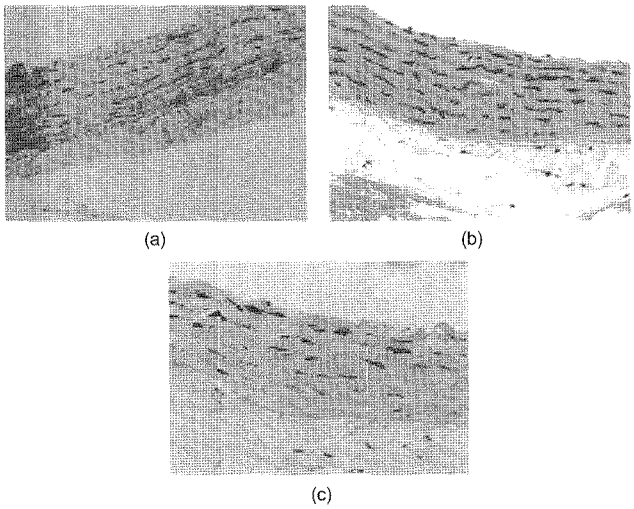

Fig. 2. Aorta wall stained by Hematoxylin-eosin staining in rats; (a) soybean oil; (b) fish oil; (c) shortening

Table 5. Cardiovascular risk factors according to tertile of aortic wall thickness

	Aortic wall thickness tertile (μm)		
	1	2	3
Aortic lumen area (mm^2)	1.13 ± 0.07[1]	1.06 ± 0.06	1.11 ± 0.04
TC[2] (mg/dL)	69.00 ± 11.72[a3]	109.75 ± 14.50[ab]	123.75 ± 9.08[b]
TG (mg/dL)	117.86 ± 19.14[a]	183.13 ± 20.56[ab]	196.13 ± 20.10[b]
HDL-cholesterol (mg/dL)	25.02 ± 7.01	37.77 ± 8.11	46.33 ± 4.68
LDL-cholesterol (mg/dL)	20.40 ± 2.21	35.35 ± 8.81	38.19 ± 9.13
Insulin (ng/mL)	2.00 ± 0.22	2.67 ± 0.10	0.52 ± 0.30
Glucose (nmol/μL)	7.61 ± 1.03	6.40 ± 0.68	7.17 ± 0.52
CRP (mg/dL)	31.92 ± 1.20	35.19 ± 2.16	35.47 ± 0.97
EPA + DHA (%)			
RBC	16.04 ± 2.59[a]	8.56 ± 3.19[ab]	3.71 ± 0.06[b]
Liver	22.09 ± 3.09[a]	13.14 ± 2.64[b]	9.05 ± 0.49[b]
Brain	20.50 ± 0.74[a]	18.56 ± 0.78[ab]	17.73 ± 0.61[b]
Heart	27.28 ± 2.64[a]	18.07 ± 2.57[b]	14.52 ± 0.35[b]
Kidney	18.64 ± 3.82[a]	7.10 ± 2.80[b]	2.95 ± 0.06[b]
Abdominal fat	9.19 ± 2.03[a]	3.52 ± 2.03[b]	0.79 ± 0.32[b]

[1] Data were expressed as mean ± SEM.
[2] RBC, red blood cell; TC, total cholesterol; TG, triacylglyceride; HDL-cholesterol, high density lipoprotein-cholesterol; LDL-cholesterol, low density lipoprotein-cholesterol; CRP, C-reactive protein; RBC, red blood cell; EPA, eicosapentaenoic acid; DHA, docosahexaenoic acid
[3] Values in a row with different letters were significantly different, $P<0.05$ (ANOVA with post-hoc Turkey's test).

Discussion

Aortic wall was significantly thinner, and TC, HDL-cholesterol, TG, and CRP levels were significantly lower in the fish oil group than the soybean oil and shortening groups in this study. Aortic wall thickness was negatively associated with n-3 fatty acids of all tissues, but positively associated with TC and TG concentrations. Interestingly, HDL-cholesterol level was higher and LDL-cholesterol was lower in the shortening group than the soybean oil group, but there was no association between trans fatty acids and aortic wall thickness.

Increased wall thickness is a common structural feature of hypertensive resistant vessels (Folkow, 1990) and conduit arteries such as the aorta (Chamiot-Clerc *et al.*, 2001). Hypertensive structural alterations of the aortic wall may affect arterial mechanics. Fish intake has been shown to reduce coronary artery atherosclerosis progression (Erkkilä *et al.*, 2004), while trans fat

accelerated it (Merchant *et al.*, 2008). Engler *et al.* (2003) also showed previously that supplementation of DHA decreased vascular wall thickness, but there was no other study to investigate the effect of trans fatty acids or fish oil on aorta pathophysiology.

Previous studies consistently found that fish oil consumption decreased TG, TC, LDL-cholesterol, and HDL-cholesterol (Lu *et al.*, 1996; Morgado *et al.*, 2005). On the other hands, trans fatty acids has been shown to increase TG, TC, and LDL-cholesterol concentration and decrease HDL-cholesterol (Colandré *et al.*, 2003; Ibrahim *et al.*, 2005; Idris & Sundram, 2002; Natarajan *et al.*, 2005). However, we observed that shortening increased HDL-cholesterol, but decreased LDL-cholesterol as compared to those fed soybean oil (Table 3). This inconsistency may be partly caused by either that we used young rats and shortening made from soybean oil, or fed diet not long enough or that we did not separated HDL 2 and 3 .

Anti-inflammatory effect of n-3 fatty acids have been studied in human models and CRP levels are believed to reflect a chronic, low-grade inflammatory process, and associated with increased CVD (Ciubotaru *et al.*, 2003; Niu *et al.*, 2006; Zampelas *et al.*, 2005). Trans fatty acids, however, have been known as pro-inflammatory (Mozaffarian *et al.*, 2004). A few clinical studies (Baer *et al.*, 2004; Mozaffarian *et al.*, 2004) demonstrated that CRP was positively associated with trans fat intake. There was no animal study to show the effects of fish oil and shortening on CRP, but we found that fish oil consistently reduced CRP in this rat model but shortening did not.

It is well documented that membrane fatty acid composition is modified by diet (Hulbert *et al.*, 2005). In the present study, dietary effect on fatty acid composition of RBC was similar to those of liver, kidney and heart, but to those of abdominal and brain, suggesting that dietary fatty acid composition highly influenced abdominal fatty acid composition but less influenced brain fatty acid composition (Table 4). Baylin and Campos (2006) previously reported that the fatty acid composition of adipose tissue was considered to be the best choice for the assessment of long-term dietary intake of fatty acids due to a slow turnover rate, with blood fractions reflecting shorter-term intake. Porsgaard *et al.* (2007) found that the fatty acid composition of the brain and adipose tissue was not highly affected by dietary fats, because of slow fatty acid turnover. Once DHA is synthesized in the brain, it is very efficiently retained, and thus is not easily affected by dietary fatty acids (Bazan *et al.*, 1993). Additionally, Edmond *et al.* (1998) found that the activities of Δ5 and Δ6-desaturase in the brain, the rate-limiting step in n-3 fatty acid synthesis, did not significantly differ between rats fed n-3 PUFA adequate and deficient diets.

Interestingly, abdominal fat was significantly lower in rats fed fish oil as compared with the rats fed shortening or soybean oil in the present study (Table 2). It was consistent with lower CRP levels in rats fed fish oil. Previous studies have also suggested that fish oil decreased abdominal, epididymal, and lumbar fat by altering hepatic lipogenic genes and fatty acid oxidation (Halvorsen *et al.*, 2001; Jang *et al.*, 2003; Rustan *et al.*, 1998; Ruzickova *et al.*, 2004). Although our study did not significantly decrease body weight, rats fed fish oil were weighed less.

Unfortunately, we did not find significant changes in insulin and glucose concentrations after feeding fish oil or shortening (Table 3). This finding, however, was not surprising since the effects of fish oil and trans fatty acids on insulin and glucose levels have been inconsistent. Mahmud *et al.* (2004) reported that fish oil increased insulin levels in non-diabetic rats but decreased in diabetic rats, with no effect on glucose levels. Holness *et al.* (2003) demonstrated that n-3 fatty acids decreased insulin and increased glucose concentration in Wister rats. Trans fats also have been shown to decrease (Ibrahim *et al.*, 2005; Natarajan *et al.*, 2005) and increase (Alstrup *et al.*, 1999; Lichtenstein *et al.*, 2003) insulin levels.

In conclusion, aortic wall thickness was positively correlated with TG and TC, but negatively with EPA + DHA levels of all tissues, suggesting that fish oil had cardio-protective effects on aorta histopathology by hypolipidemic action in this rat model. Shortening had some beneficial effects on lipid profile, but no effect on aorta histopathology.

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