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Beneficial Effects of ω-3 Fatty Acid Treatment on the Recovery of Cardiac Function After Cold Storage of Hyperlipidemic Rats

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Cardiac effects of ω-3 polyunsaturated fatty acids (PUFAs) were studied in female Wister rats fed a standard diet (control [C] diet) or a high-cholesterol (HC) diet. Subgroups of rats from these groups were treated with eicosapentaenoic acid-E (EPA) or docosahexaenoic acid-95E (DHA) for 5 weeks. Although plasma total cholesterol (TC) and triglyceride (TG) levels were higher in each group fed the HC diet versus each group fed the C diet, EPA administration with the HC diet (HC + EPA) significantly ($P < .05$) reduced these levels. An isolated working-heart preparation was used to determine cardiac function. Cardiac output (CO) was lower in rats fed the HC diet and HC + DHA versus any of the groups fed the C diet ($P < .05$). In addition, left ventricular (LV) maximum differentiation of pressure-time curve (dp/dt) was lower in the rats fed the HC diet versus any of the C diet groups ($P < .05$). After evaluation of cardiac function in each rat, the heart was stored in a histidine-tryptophan-ketoglutarate solution for 8 hours at 4°C. The heart was then reperfused, and recovery of cardiac function was evaluated. No significant differences were observed for post-preservative cardiac function within the C diet groups. However, within the HC diet groups, HC + EPA significantly ($P < .05$) improved the recovery of cardiac function. In addition, HC + DHA also significantly ($P < .05$) improved the recovery of coronary flow (CF) and LV dp/dt. No significant differences were observed for plasma TC and TG concentrations in the C diet groups. EPA administration significantly decreased cardiac levels of palmitic, oleic, and linoleic acids in the HC diet groups. No significant differences were observed for cardiac levels of free fatty acids (FFAs) within the C diet groups. Cardiac EPA and DHA levels were significantly ($P < .05$) elevated in EPA- or DHA-treated rats compared with the other diet-fed rats. Cardiac EPA levels were also elevated in DHA-treated rats compared with untreated rats ($P < .05$). These results suggest that EPA attenuates coronary and myocardial preservation injuries through an increase in serum lipids and an accumulation of myocardial FFAs resulting from a HC diet.

HIGH SERUM CHOLESTEROL levels that impair coronary endothelium and smooth muscle lead to impaired cardiac function. Hypercholesterolemia itself injures endothelial cells and reduces their ability to release endothelium-derived relaxing factor (EDRF), which then influences vascular tone and thus causes arterial stiffness.[1,2] In contrast, elevated free fatty acid (FFA) levels during ischemia result in an increased incidence of arrhythmia and impaired cardiac-pump performance.[3-6] FFAs, which are the most common metabolic substrates of the myocardium under aerobic conditions, have been shown to be harmful under ischemic conditions.[7] The proposed mechanism for the detrimental effects of FFAs include an accumulation of toxic intermediates of fatty acid metabolism, inhibition of glucose utilization, particularly glycolysis during ischemia and/or reperfusion, and uncoupling of oxidative metabolism from electron transfer. FFAs accumulate in the myocardium during ischemia and reperfusion, and thus may be an indicator of myocardial damage.[8-11]

Epidemiologic studies indicate that the intake of large amounts of fish oil–derived ω-3 polyunsaturated fatty acids (PUFAs), of which eicosapentaenoic acid (20:5 [EPA]) and docosahexaenoic acid (22:6 [DHA]) are the major components, prevents coronary artery disease.[12,13] Cardiac function is improved, susceptibility to arrhythmia is reduced, and infarct size is decreased in animals that receive a fish oil diet compared with those that receive a high-cholesterol diet.[14] Although the mechanisms underlying this protection have not been fully elucidated, alterations in the lipid composition of cellular membranes in myocardial cells, leukocytes, and platelets may represent the most plausible explanation.[12] Studies have shown that dietary supplementation with ω-3 PUFAs has direct protec-

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tive effects on the myocardium after exposure to warm ischemia and reperfusion.[15,16] Diet-induced alterations in myocardial membrane phospholipids are associated with alterations in the cardiac function and response to stress.[17-20] Supplementation with ω-3 PUFAs, which changes the fatty acid composition of myocardial phospholipids, may affect cellular transport processes or enzyme activities and thus lead to alterations in cardiac function and in the response of the heart to ischemic stress.[1]

As the need for donor organs becomes greater, a number of centers have decided to increase the age of potential donors, pending the results of a careful examination of the donor's medical history.[21-23] Recently, there has been an increase in the number of individuals with hyperlipidemia due to a high-cholesterol diet and/or a hereditary predisposition for the condition. These hyperlipidemic individuals, in most cases, are considered for cardiac surgery or heart transplantation. In the present study, we investigated the influence of a high-cholesterol diet on myocardial FFAs, serum lipid, and baseline and post-preservative hemodynamics. In addition, we also examined the effects on these parameters of supplementing a high-cholesterol diet with ω-3 PUFAs.

MATERIALS AND METHODS

Animals and Diets

The present study conformed to the guidelines specified in *The Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (Publication No. 85-23, revised 1985). A total of 35 female 30-week-old Wistar rats were used. The experimental groups were as follows: (1) standard diet (control [C]), ie, a laboratory commercial chow pellet that does not contain any fish products (F1R; Funabashi Farm, Chiba, Japan) (n = 5), (2) C diet with EPA-E (EPA) supplement 300 mg/kg/d (n = 6), (3) C diet with DHA-95E (DHA) supplement 300 mg/kg/d (n = 6), (4) high-cholesterol (HC) diet (F1R diet containing 1% cholesterol and 1% cholic acid; Funabashi Farm) (n = 6), (5) HC diet with EPA supplement 300 mg/kg/d (n = 6), and (6) HC diet with DHA supplement 300 mg/kg/d (n = 6). The composition of the C diet and HC diet is shown in Table 1. The rats were housed under constant temperature on a 12-hour light/dark cycle and fed their respective diets for 5 weeks. EPA or DHA diets were orally administered via a stomach tube for 5 weeks. Food and water were given ad libitum. Body weight and food intake were measured before and after the experiment. The rats were anesthetized using sodium pentobarbital (65 mg/kg intraperitoneally), and 1 mL blood was collected from the inferior vena cava using heparinized syringes. EDTA (1 mmol/L) was added to the blood, which was then centrifuged at 3,000 rpm for 20 minutes at 4°C. The plasma was collected and used to measure plasma lipid levels.

Table 1. Composition of the C Diet and the HC Diet (%)

Component	C Diet (F1R)	HC Diet
Water	8.0	6.0
Protein	21.3	21.3
Fat	5.1	5.1
Fiber	3.1	3.1
Carbohydrate	5.0	5.0
Non-nitrogen	57.5	57.5
Cholesterol	—	1.0
Cholic acid	—	1.0
Total energy (kcal/g)	4.2	4.2

Heart Perfusion

Parallel experiments were performed in vitro using isolated hearts from each group. Following collection of 1 mL blood for plasma lipid analysis, the hearts were excised and immediately immersed in Krebs-Henseleit bicarbonate buffer (KHB) solution consisting of NaCl 118 mmol/L, KCl 4.7 mmol/L, $MgSO_4$ 1.2 mmol/L, KH_2PO_4 1.2 mmol/L, $CaCl_2$ 2.5 mmol/L, $NaHCO_3$ 25.0 mmol/L, and glucose (11.0 mmol/L at 37°C. The heart was then immediately mounted on a Langendorff (L) apparatus (IPH-W; Labo Support, Osaka, Japan) via the aorta and perfused at a constant pressure of 60 mm Hg in nonrecirculating L-mode for 3 minutes. Perfusion was performed with filtered (0.22 μm) KHB solution that was previously equilibrated with 95% O_2 and 5% CO_2, and maintained at 37°C. The KHB perfusate in this circuit was not recycled. During preparation, the excised hearts were cannulated rapidly to minimize the ischemic time. Following cannulation of the left atrium via the pulmonary vein, the apparatus was switched to working mode (W-mode) with a left atrial filling pressure of 10 mm Hg and an afterload of 60 mm Hg. The baseline cardiac function was determined following 30 minutes of W-mode. The following parameters were measured: aortic flow ([AF] milliliters per minute), coronary flow ([CF] milliliters per minute), cardiac output ([CO] milliliters per minute), heart rate ([HR] beats per minutes), systolic pressure ([SP] mm Hg), aortic mean pressure (mm Hg), rate pressure product ([RPP] HR × SP), and left ventricular maximum dp/dt (LV dp/dt). Following evaluation of the pre-preservative cardiac function, the hearts were arrested by administration of a histidine-buffered Bretschneider (HTK) solution (60 mL/kg at 4°C) via an aortic cannula at a pressure of 60 mm Hg. The HTK solution was used because of its suitability for cardiac preservation.[24] Each heart was then stored in 30 mL HTK preservation solution at 4°C for 8 hours. Next, the hearts were mounted on the L-apparatus and reperfused for 15 minutes on L-mode. Reperfusion was also performed with filtered (0.22 μm) KHB solution, that was previously equilibrated with 95% O_2 and 5% CO_2, and maintained at 37°C. The KHB perfusate in this circuit was not recycled. The apparatus was then switched to W-mode. Recovery of cardiac function in the stored hearts after 45 minutes of W-mode reperfusion was evaluated and calculated as a percentage of the pre-preservative baseline function.

After calculating the ability of the stored hearts to recover cardiac function, a part of the LV specimen was frozen in liquid nitrogen and stored at −80°C until needed for further analysis.

Total Fatty Acid Profiles for Cardiac Lipids

A modification of the one-step reaction reported by Lepage and Roy[25] was used for preparation and analysis of fatty acids in heart tissue. Gas chromatographic separation was performed on a model 5890 II gas chromatograph (Hewlett-Packard, Avondale, PA) equipped with a flame ionization detector and an automatic sampler (model 7673). Peak identities were established by spiking with reference compounds and in part by gas chromatography–mass spectrometry (JMS-D 300; JEOL, Tokyo, Japan).

Chemicals and Reagents

EPA-E (EPA), an ethyl-ester derivative of all-*cis*-5,8,11,14,17-eicosapentaenoic acid (C20:5, n-3), and DHA-95E (DHA), an ethyl all-*cis*-4,7,10,13,16,19-docosahexaenoic acid (C22:6, n-3), were prepared by Mochida Pharmaceutical (Tokyo, Japan) and Harima Chemicals (Hyoga, Japan), respectively. EPA and DHA were emulsified gently in a 5% gum arabic solution in ice-cold water with an ultrasonic cell homogenizer and administered orally via a stomach tube. The vehicle (5% gum arabic solution) was administered to the C groups with no EPA or DHA supplement. Tricosanoic acid, arachidonic acid, EPA, and DHA as fatty acid standards were obtained from Sigma Chemical (St. Louis, MO).

Statistical Analysis

All results are presented as the mean ± SEM. Statistical evaluation of the data was performed by one-way ANOVA and Scheffe's F test within each dietary group. Significance was established at a P level of less than .05.

RESULTS

Food Intake and Body Weight

The mean food intake in groups 1 to 6 was 18.7 ± 0.7, 18.4 ± 2.1, 17.9 ± 1.3, 18.6 ± 0.7, 19.8 ± 3.4, and 19.5 ± 0.5 g/d per rat, respectively. The mean body weight in groups 1 to 6 was 203 ± 3, 200 ± 5, 208 ± 11, 207 ± 3, 205 ± 19, and 204 ± 4 g, respectively. There were no significant differences in food intake or body and heart weight between groups.

Fatty Acid and Lipid Profiles

Figure 1 shows the fatty acid profiles for cardiac lipids. Administration of EPA or DHA increased cardiac EPA and DHA levels compared with the other dietary groups ($P < .05$). DHA supplementation also significantly increased cardiac EPA levels compared with the untreated groups. No significant differences

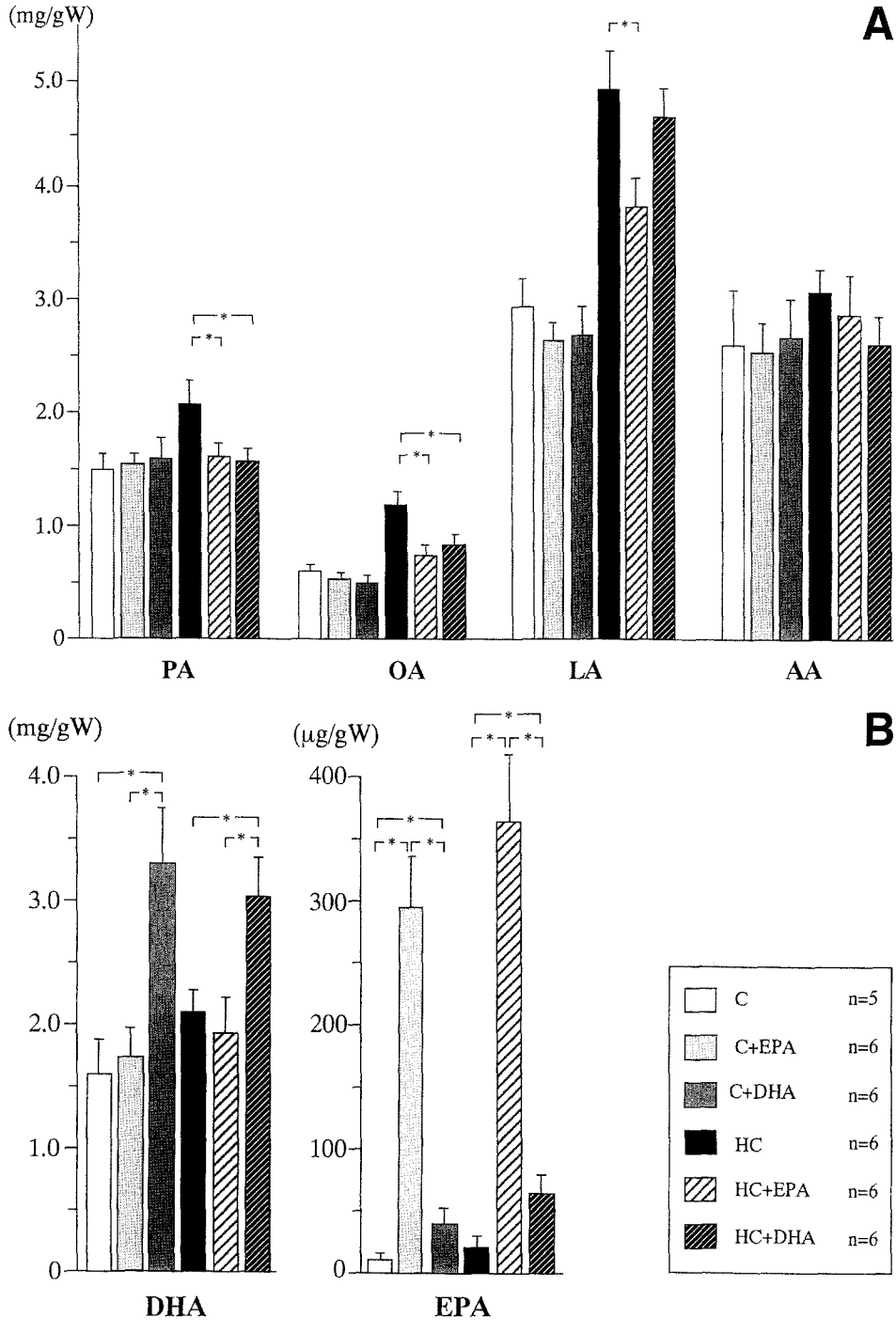

Fig 1. Fatty acid profiles of cardiac lipids. (A) PA, palmitic acid; OA, oleic acid; LA, linoleic acid; AA, arachidonic acid. (B) DHA and EPA. C, group 1; C + EPA, group 2; C + DHA, group 3; HC, group 4; HC + EPA, group 5; HC + DHA, group 6. *Significant difference between groups.

were observed for cardiac arachidonic acid levels among any of the diet-fed rats, but EPA administration significantly decreased cardiac levels of palmitic, oleic, and linoleic acids in HC diet groups. No significant differences were observed for cardiac FFA levels in C diet rats.

Figure 2 shows the plasma concentration of total cholesterol (TC) and triglyceride (TG). Although plasma TC and TG levels were higher in HC diet groups versus C diet groups, EPA administration with the HC diet significantly reduced these levels. No significant differences were observed for plasma TC and TG concentrations in C diet rats.

Basal Cardiac Function

Figure 3 shows the effects of ω-3 PUFAs on basal cardiac function. Although there were no significant differences in basal cardiac function within the C diet groups or within the HC diet groups, CO was lower in groups 4 and 6 than in any of the C diet groups. In addition, LV dp/dt was lower in group 4 than in any of the C diet groups.

Recovery of Cardiac Function After Preservation

Figure 4 shows the recovery of cardiac function after preservation. No significant differences were observed for post-preservative cardiac function in the C diet groups. However, in the HC diet groups, the recovery of AF and CO was significantly higher in group 5 versus groups 4 and 6. In addition, the recovery of CF, RPP, and LV dp/dt in group 5 and recovery of CF and LV dp/dt in group 6 were significantly higher than the corresponding values in group 4.

DISCUSSION

In the present study, the HC diet caused an impaired recovery of cardiac function, and the resulting cardiac levels of palmitic, oleic, and linoleic acids were lower and the recovery of cardiac function was improved in the EPA-treated group compared with the HC diet group.

Plasma FFAs are the main source of fatty acids that are esterified and incorporated into membrane phospholipids, and thus a change in plasma FFAs should be reflected in the fatty acid composition of membrane phospholipids in the myocardium.[1] FFAs that accumulate in the myocardium during ischemia and reperfusion reportedly cause myocardial damage.[8-11] Indeed, many studies have shown that elevated FFA levels during ischemia result in an increased incidence of arrhythmia and impaired cardiac-pump performance.[3-6] In addition, high plasma lipid concentrations have a direct detrimental effect on myocardial performance.[3] Inhibitors of FFA metabolism have been shown to decrease the size of myocardial infarction and to lessen postischemic cardiac dysfunction during global ischemia.[7] The mechanism through which dietary ω-3 PUFAs are responsible for limiting myocardial ischemia and reperfusion damage may thus be related to alterations in the fatty acid composition of myocardial phospholipid and an effect produced by serum lipid and myocardial FFA levels.[12] Other potential hypolipidemic actions of EPA are as follows: an inhibitory effect on intestinal lipid absorption, an inhibitory effect on lipid biosynthesis or promotion of lipid secretion in the liver, and a stimulatory effect on lipid catabolism in the blood.[26]

Significant modulation of myocardial eicosanoid production induced by dietary supplementation with ω-3 PUFAs causes increased synthesis of prostaglandin I_2 and reduced production of thromboxane A_2 in myocytes.[27,28] Enrichment of membrane phospholipids by ω-3 PUFAs increases the postischemic CF, possibly through the receptor-mediated release of EDRF and nitric oxide–mediated mechanisms.[29,30] Hypercholesterolemia itself injures endothelial cells, and the resultant reduced ability of regenerated endothelial cells to release EDRF could partly explain the endothelial dysfunction associated with hypercholesterolemia.[1] In the present study, whereas post-preservative CF in HC diet groups was significantly decreased, post-preservative CF in rats that received ω-3 PUFAs was significantly increased. Dietary ω-3 PUFA supplementation, which amelio-

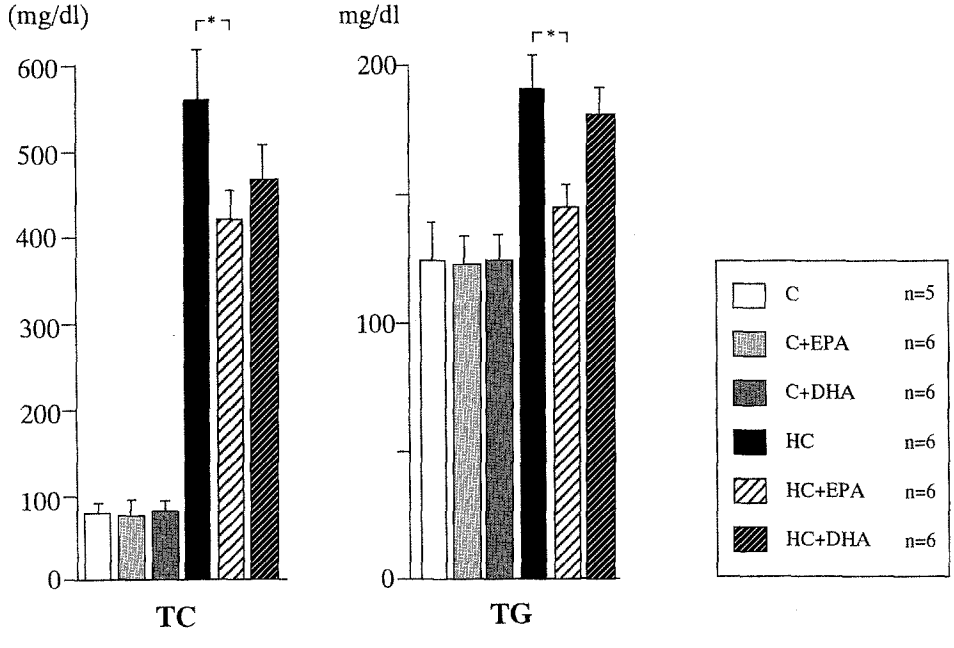

Fig 2. Plasma concentration of TC and TG. C, group 1; C + EPA, group 2; C + DHA, group 3; HC, group 4; HC + EPA, group 5; HC + DHA, group 6. *Significant difference between groups.

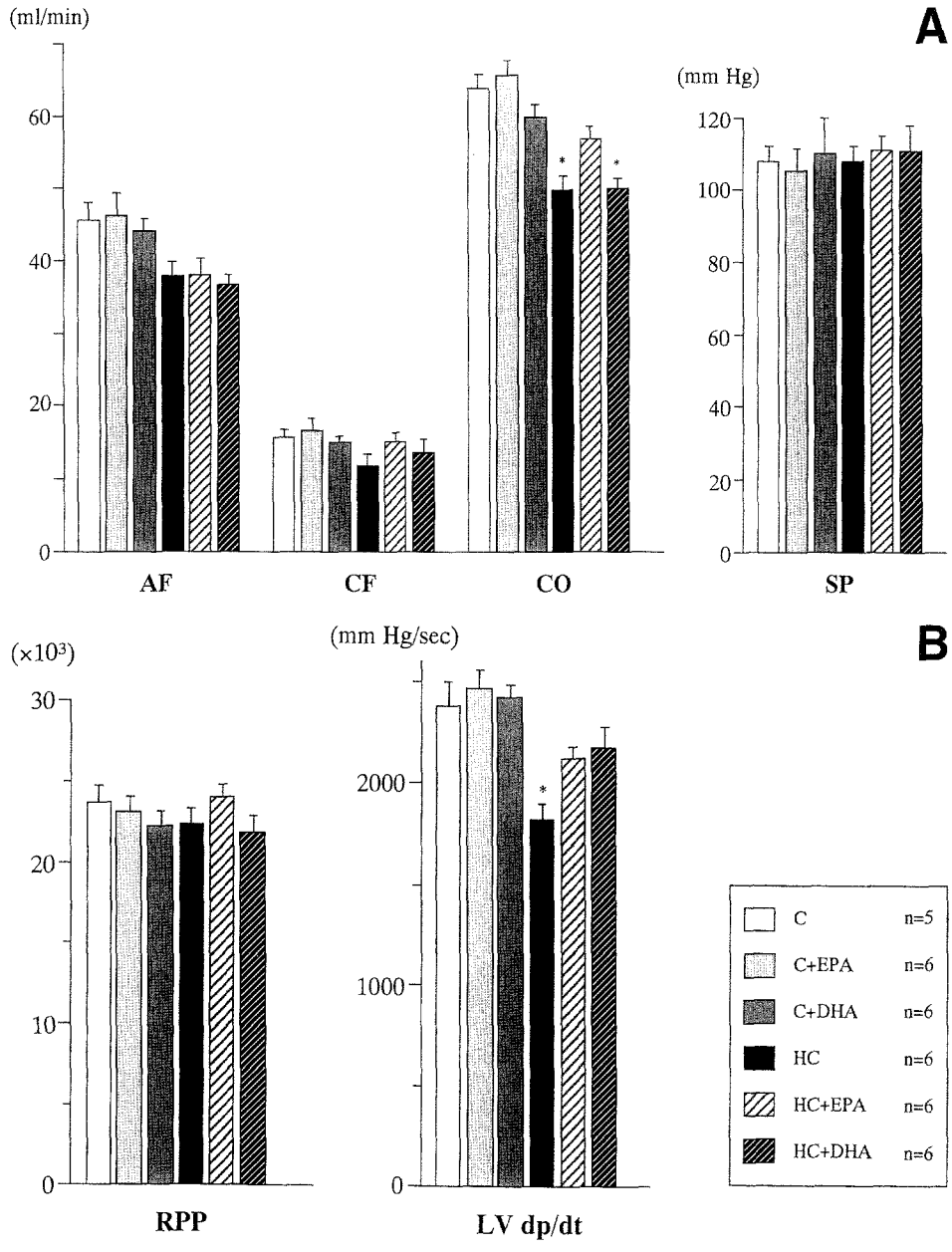

Fig 3. Basal cardiac function. (A) AF, CF, CO, and SP. (B) RPP and LV dp/dt. No significant differences were observed in basal cardiac function within the C diet groups (groups 1-3) or within the HC diet groups (groups 4-6). CO was lower in groups 4 and 6 *v* any of the C diet groups (groups 1-3). LV dp/dt was lower in group 4 *v* any of the C diet groups (groups 1-3). C, group 1; C + EPA, group 2; C + DHA, group 3; HC, group 4; HC + EPA, group 5; HC + EPA, group 5; HC + DHA, group 6. *Significant difference *v* C diet groups (groups 1-3).

rates the development of coronary artery disease and restores coronary endothelium-dependent relaxation in impaired coronary endothelium in hypercholesterolemia, may produce an improved recovery of cardiac function after preservation in rats fed a HC diet.

Few studies have described the effects of dietary supplementation with DHA on cardiac function after preservation. In the present study, dietary supplementation with DHA also significantly increased cardiac EPA compared with the levels in untreated HC-fed rats. In addition, the recovery of CF and LV dp/dt was higher in DHA-treated versus HC-fed rats. In contrast, no significant differences were observed for basal and post-preservative CO between untreated HC-fed and DHA-treated rats. The feeding duration in the present study was shorter than that of several other studies, and thus a longer period may be required to increase the retroconverted quality of DHA to EPA leading to the improved CO values in DHA-treated rats in the present study. DHA supplementation, which causes some retroconversion of DHA to EPA, may alter myocardial membrane phospholipids and lead to an improved recovery of cardiac function after preservation.[31]

In the present study, among the C diet groups, the cardiac EPA concentration was also significantly higher in the EPA-treated group, but there were no significant differences for plasma TC and TG or cardiac FFA levels. EPA administration to rats fed the C diet that does not affect the level of plasma lipids and cardiac FFAs beyond a normal range may not cause any significant difference in cardiac function within the C diet groups. Some studies have shown that dietary fish oil does not alter the parameters of basal cardiac function.[32,33] Although in the present study neither EPA nor DHA supplementation changed the baseline cardiac function in C diet groups, EPA

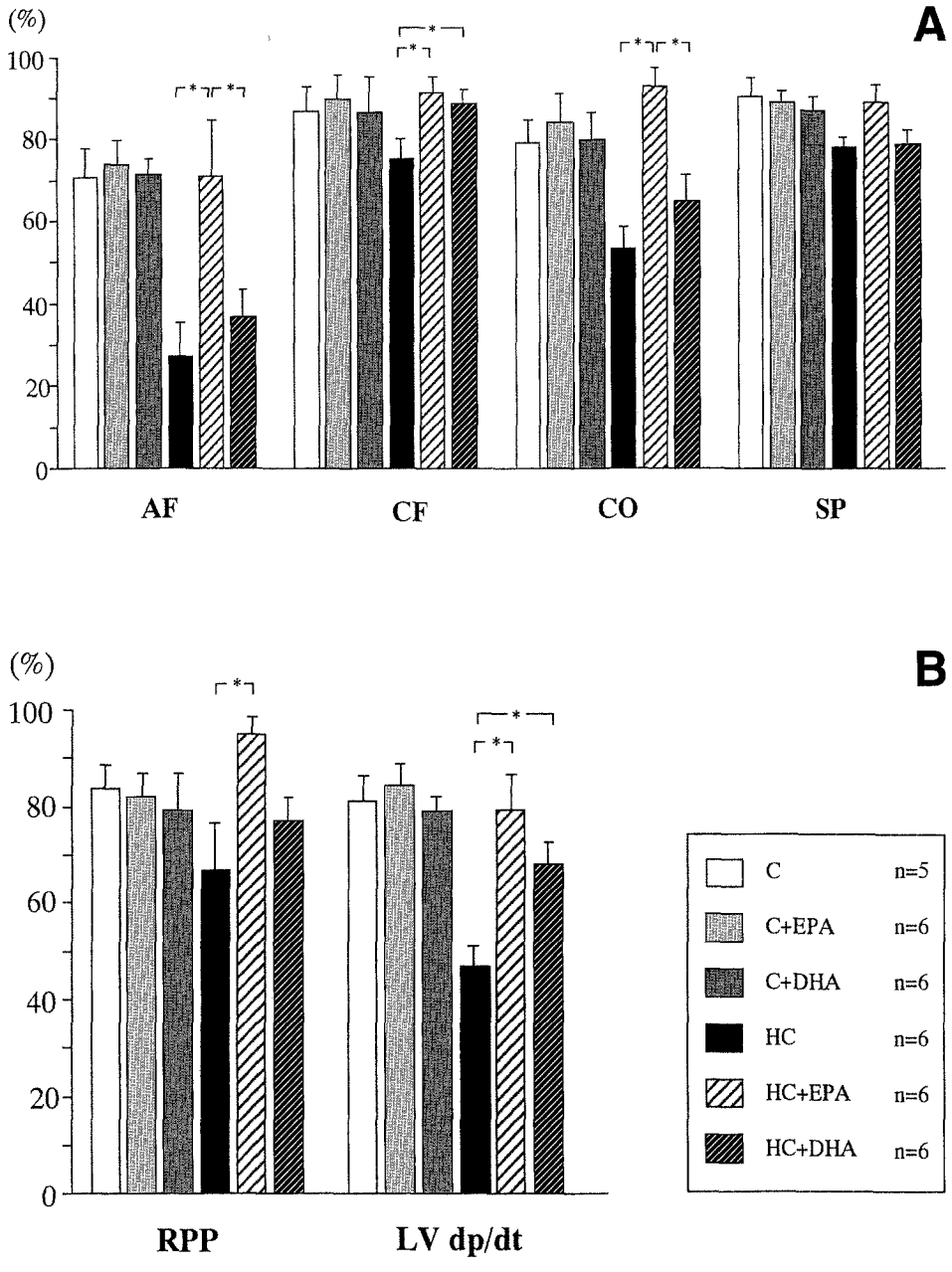

Fig 4. Recovery of cardiac function after preservation. (A) AF, CF, CO, and SP. (B) RPP and LV dp/dt. C, group 1; C + EPA, group 2; C + DHA, group 3; HC, group 4; HC + EPA, group 5; HC + DHA, group 6. *Significant difference between groups.

administration led to improved baseline CO and LV dp/dt in HC-fed rats. Thus, the decreased cardiac function induced by a HC diet may be attenuated by EPA supplementation. Kool et al[34] have reported that a short-term decrease of plasma cholesterol does not alter the hemodynamics and vessel-wall properties. As already mentioned, the feeding duration in the present study was short compared with that of other studies,[32,33] and thus a longer period of supplementation with ω-3 PUFAs may be required to increase myocardial EPA levels, which exert a beneficial effect on basal cardiac function. In addition, in the present study, only female rats were used, and as a result, gender differences may be somewhat responsible for the difference between the results of the present study for plasma and cardiac lipid levels and those of other studies.

Fish oils reduce whole-blood viscosity and increase red blood cell deformability, and fish oil supplementation may also produce fewer oxygen free radicals from neutrophils.[35,36] In the isolated heart model used in the present study, neutrophils and platelets were absent from the perfusion solution. In addition, high serum cholesterol levels in rats fed a HC diet are never found clinically. Therefore, the findings of the present study cannot be immediately extrapolated to other clinical conditions such as the blood-perfused transplanted heart. However, the present study demonstrates that dietary supplementation with EPA improves the recovery of cardiac function after preservation in hyperlipidemic rats. In addition, DHA administration, wherein DHA was retroconverted to EPA, also produced some beneficial effects on the recovery of cardiac

function. Hence, the alterations in myocardial phospholipid composition induced by EPA supplementation may be responsible for attenuating myocardial preservation injuries. Further studies are needed to determine whether EPA perfusion during reperfusion bestows a similar protective effect on the human cardiovascular system. If this is indeed the case, then widespread EPA treatment of patients with severe hypercholesterolemia may prove beneficial in clinical practice for coronary intervention, cardiac surgery, and/or heart transplantation.

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