Oral Administration of Eicosapentaenoic Acid or Docosahexaenoic Acid Modifies Cardiac Function and Ameliorates Congestive Heart Failure in Male Rats1–3

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Abstract

This study assessed the effects of eicosapentaenoic acid (EPA) or docosahexaenoic acid (DHA) on normal cardiac function (part 1) and congestive heart failure (CHF) (part 2) through electrocardiogram analysis and determination of EPA, DHA, and arachidonic acid (AA) concentrations in rat hearts. In part 2, pathologic assessments were also performed. For part 1 of this study, 4-wk-old male rats were divided into a control group and 2 experimental groups. The rats daily were orally administered (1 g/kg body weight) saline, EPA–ethyl ester (EPA–Et; E group), or DHA–ethyl ester (DHA–Et; D group), respectively, for 28 d. ECGs revealed that QT intervals were significantly shorter for groups E and D compared with the control group (P < 0.05). Relative to the control group, the concentration of EPA was higher in the E group and concentrations of EPA and DHA were higher in the D group, although AA concentrations were lower (P < 0.05). In part 2, CHF was produced by subcutaneous injection of monocrotaline into 5-wk-old rats. At 3 d before monocrotaline injection, rats were administered either saline, EPA–Et, or DHA–Et as mentioned above and then killed at 21 d. The study groups were as follows: normal + saline (control), CHF + saline (H group), CHF + EPA–Et (HE group), and CHF + DHA–Et (HD group). QT intervals were significantly shorter (P < 0.05) in the control and HD groups compared with the H and HE groups. Relative to the H group, concentrations of EPA were higher in the HE group and those of DHA were higher in the control and HD groups (P < 0.05). There was less mononuclear cell infiltration in the myocytes of the HD group than in the H group (P = 0.06). The right ventricles in the H, HE, and HD groups showed significantly increased weights (P < 0.05) compared with controls. The administration of EPA–Et or DHA–Et may affect cardiac function by modification of heart fatty acid composition, and the administration of DHA–Et may ameliorate CHF. J. Nutr. 144: 467–474, 2014.

Introduction

Cardiovascular disease is the leading cause of death worldwide. As such, preventive measures, particularly measures involving food intake, have been proposed (1). Since the late 1960s, when Bang et al. (2) reported a low prevalence of coronary heart disease among the Inuit in Greenland, many epidemiologic studies, clinical trials, animal studies, and in vitro experiments have supported the cardiovascular protective effects of n–3 PUFAs (1,3–5). On the basis of this evidence, cardiac societies in America, Europe, and Japan now recommend a daily intake of 1 g of both EPA (20:5n–3) and DHA (22:6n–3) for cardiovascular disease prevention, treatment after a myocardial infarction, prevention of sudden death, and secondary prevention of cardiovascular disease (5,6). To support human trial results, experimental studies using animal models have been performed. Animal models allow investigations that are not possible in human trials and can provide a better understanding of the roles of EPA and DHA in coronary heart disease from the results of their effects on the heart (3). In several models, n–3 PUFAs improved endothelial function and diminished the incidence of atheroma, although such results were inconsistent, presumably because of the differences between the animal species used and the study designs (7). Rats fed n–3 PUFAs from fish or plant sources and subjected to surgically induced myocardial infarction, reversible surgical ischemia, or ischemia/reperfusion showed survival benefits and decreased arrhythmias (8–13). Similarly, isolated papillary muscles and myocytes in the hearts of rats fed

1 Supported in part by Grants-in-Aid (No. 21500788) for Scientific Research from Japan Society for the Promotion of Science and by the Ohtsuka award from the Japan Society for Lipid Nutrition.

2 Author disclosures: T. T. Yamanushi, H. Kabuto, E. Hirakawa, N. Janjua, F. Takayama, and M. Mankura, no conflicts of interest.

3 Supplemental Figures 1 and 2 are available from the “Online Supporting Material” link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

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n–3 PUFAs showed improved recovery from ischemic insults and increased resistance to arrhythmias (14–17). These findings appear to support the benefits of n–3 PUFAs and their role in cardiac protection.

Purified n–3 PUFA samples are necessary to determine the role of each n–3 PUFA component. However, n–3 PUFAs are highly unstable when exposed to heat and oxidation, and effective purification methods have not been developed (18). The n–3 PUFA EPA has been purified industrially and used as medicine since 1990 (18). For DHA purification, enzymatic purification and reversed-phase HPLC methods have been successful in obtaining DHA with >90% purity (18,19). In the present study, highly purified (97% purity) EPA–ethyl ester (EPA-Et) or DHA–ethyl ester (DHA-Et) was administered to rats to investigate the effects of these FAs on normal cardiac function (part 1) and heart failure (HF) (part 2). We assessed cardiac function via analysis of electrocardiogram. The QT intervals were our primary focus because interval prolongation is associated with ventricular arrhythmias (20).

For HF assessment, we used a well-established rat model of right-sided congestive HF (CHF). CHF was induced by injection of monocrotaline, a pyrrolizidine alkaloid. Monocrotaline undergoes hepatic metabolism by the cytochrome P450 monoxygenase system to form a reactive metabolite, monocrotaline pyrrolo. This metabolite, in turn, circulates to the pulmonary vascular bed where it induces lung injury. This initial lung injury causes vasculitis of the lung arterioles, leading to pulmonary hypertension, which further impairs blood flow to the lungs (21). The chronically elevated right ventricle (RV) afterload causes RV hypertrophy, reducing the ability of the heart to pump blood through the lungs and ultimately causing right HF. In addition, monocrotaline-treated rats demonstrate marked neutrophil migration, widespread leukocyte infiltration, and multiple inflammatory cell foci in the RV (22–24). These reports suggest that monocrotaline induces lymphocytic myocarditis, n–3 PUFAs are also potentially potent anti-inflammatory agents (reviewed in reference 1). They perform anti-inflammatory tasks, such as replacing arachidonic acid (AA; 20:4n–6) as an eicosanoid substrate, inhibiting AA metabolism, and altering the expression of inflammatory genes through their effects on transcription factor activation. Furthermore, n–3 PUFAs are metabolized to a family of anti-inflammatory mediators called resolvins (1,25). Thus, we based our adoption of this model on the assumption that the anti-inflammatory actions of EPA or DHA would improve CHF.

Materials and Methods

Materials. All chemicals were purchased from Wako Pure Chemical Industries or Sigma-Aldrich unless mentioned otherwise. Highly purified (97% purity) EPA-Et and DHA-Et were kindly donated by Ikeda Tohka Industries and Bizen Chemical Co. The TaqMan assay reagent and primer/probe sets were purchased from Applied Biosystems.

Rats and housing. Three-week-old Sprague-Dawley male rats, initially weighing 35–55 g, were purchased from Clea Japan and housed under constant temperature and humidity conditions on a 12-h light/dark cycle. Rats were provided with a feed mixture containing −23% crude protein, 5% crude fat, and 3% fiber (catalog name MF; Oriental Yeast). Food and tap water were consumed ad libitum. Body weights of rats were measured once per week.

All experimental procedures were performed in accordance with the Japanese Act on Welfare and Management of Animals (Act 105 of October 1, 1973). The ethics and animal committee of Kagawa Prefectural University for Health Sciences approved our protocol.

Part 1. Rats were divided into the following 3 groups: control (n = 8), EPA-Et (E group; n = 6), and DHA-Et (D group; n = 6). Four-week-old rats in the E and D groups were orally administered 1 g/d of EPA-Et or DHA-Et per kilogram of body weight for 28 d. In the case of the control group, saline was administered in place of EPA-Et and DHA-Et. To prevent EPA-Et and DHA-Et from oxidative damage, daily aliquots were stored under nitrogen at −20°C.

Part 2. To induce CHF in rats, we used a previously described method (26) with modifications. Rats were kept under the conditions described above. At the age of 5 wk, rats were subcutaneously injected in the interscapular region with a single dose of monocrotaline (solution of 60 mg/kg body weight). Monocrotaline solution was prepared by dissolving monocrotaline in distilled water and then adjusted to a neutral pH. Saline was used for the control group rats. Beginning 3 d before monocrotaline injection, rats were administered saline, EPA-Et, or DHA-Et daily. All rats were killed on day 21. The part 2 group names were as follows: normal rats administered saline (control group; n = 4–5); CHF rats administered saline (H group; n = 10–11); CHF rats administered EPA-Et (HE group; n = 9–10), and CHF rats administered DHA-Et (HD group; n = 10). Because CHF rats die −3 wk after monocrotaline injection, the number of rats killed was less than the initial number of rats. Therefore, the sample numbers used for each measurement were not the same as the initial rat numbers.

Sample collection. Rats were killed by an overdose of pentobarbital. Blood was drawn from the RV to obtain serum. To record the leukocyte count, blood was drawn in the presence of EDTA and kept at 4°C until measurement. The heart was rapidly removed, placed in saline, and then dissected into the right atrium (RA), the left atrium (LA), the RV, and the left ventricle (LV), including the interventricular septum. Each part was then weighed. Serum and RAs were frozen and kept at −80°C for further analysis. For histopathology, after the blood was drawn, the heart was removed and fixed by immersion in 4% paraformaldehyde solution for 2–4 wk at room temperature.

Blood pressure measurement. Indirect blood pressure (BP) measurements were performed 1 d before rats were killed. A tail-cuff apparatus (BP-98; Softron) was used for the conscious rats (27). The rat was placed in a temperature-controlled cylinder, and the temperature of the environment was increased to 38°C. The cuff was then placed on the base of the tail. Regression curves were calculated from the peak values of each pulse volume oscillation by the least-squares method. Systolic BP (SBP) and mean BP were determined at the points at which this pressure curve began, and where it attained a peak. Diastolic BP was calculated from the SBP and mean BP values. The BP-98 apparatus acquired the heart rate (HR) from the pulse. Each measurement was performed in triplicate, and the mean values were calculated.

Electrocardiogram recording. Electrocardiogram recordings were made immediately before rats were killed. Rats were anesthetized as described above and underwent electrocardiogram recording with an electrocardiogram amplifier (SAP-2; Softron) connected to an electrocardiogram processor interface (EP95U-2; Softron). Limb electrocardiogram electrodes were attached and surface electrocardiogram recordings were obtained. The electrocardiogram plots were saved and processed by using electrocardiogram processor software (SP-2000; Softron). The electrocardiogram variables analyzed were as follows: P wave duration, QRS interval, RR interval, PR interval, and the QT interval. The QT interval was corrected by using Bazett’s method (28). Electrocardiogram
measurements were derived from heartbeats over a 4-s interval. The measurements were repeated at least 6 times for each rat. In part 1, electrocardiograms were recorded in 1 experiment. In part 2, electrocardiograms were recorded in 2 experiments, and all the data were then combined.

**FA quantification.** FAs were extracted following a modified method originally reported by Folch et al. (29). Briefly, frozen RA tissue was weighed and homogenized with a 2:1 (v:v) chloroform:methanol mixture containing 1 μg of heptadecanoic acid as an internal standard. Total lipids were extracted from the chloroform layer and dissolved in hexane. Subsequently, transmethylation was performed by using an FA methylation kit (Nacalai Tesque). FA analysis was carried out by GC-MS (Shimadzu GCMS-QP5050A; Shimadzu). Methylated FAs were separated with a DB-5 column (J and W Scientific) by using helium as the carrier gas. Methylated FAs were identified by comparison of retention times with authentic standards and from fragmentation patterns in the mass spectra. In part 1, FAs were quantified in 2 experiments, and all the data were then combined. In part 2, FAs were quantified in 1 experiment.

**mRNA extraction and qPCR.** We performed mRNA quantification following the kit manufacturer’s instructions. Total RNA was isolated from frozen RA tissue by using an RNA purification kit (Life Technologies). The isolated RNA concentration was measured by spectrophotometry. Then, 1000 ng of total RNA from each sample was reverse-transcribed by random priming with a high-capacity cDNA reverse transcription kit (Life Technologies). Real-time qPCR was performed by using the TaqMan system (Applied Biosystems). Gene expression levels of target products and internal reference Gapdh were measured by using TaqMan probes. Real-time PCR amplification and product detection were performed by using an ABI StepOnePlus System (Applied Biosystems), as recommended by the manufacturer, at the Division of Research Instrument and Equipment in the Life Science Research Center at Kagawa University. Relative expression was determined by using the standard curve method according to the manufacturer’s protocol. Each assay was performed in duplicate and included a standard curve sample, a no-template control, and a cDNA sample from the RA. All samples with a CV >10% were retested.

**Histopathology.** We dissected the rat hearts (as described above) and performed standard histologic procedures (30). Slices (3–4 mm thick) were removed from the middle of the ventricles in the short axis plane and embedded in paraffin. Next, 2–4 μm sections were cut and stained with hematoxylin and eosin. Standard light microscopy was used to obtain images and to assess the histopathologic features. The thickness of the RV free wall and the RV myocyte diameters were determined (31, 32). Mononuclear cell infiltration in RV tissue was assessed as described by Akhavein et al. (33). Pathologic observations were scored 1 for negative, 2 for weakly positive, 3 for positive, and 4 for strongly positive.

**Leukocyte counting and brain natriuretic peptide quantification.** One-day-old blood samples were diluted 1:10 with Turk’s solution (Merck). Leukocytes in blood were stained and counted with a hemocytometer under light microscopy. Brain natriuretic peptide (BNP), a 32-amino acid polypeptide secreted by the ventricles of the heart in response to excessive stretching of myocytes, was detected by using an Assay Max rat BNP-32ELISA kit (Assaypro).

**Survival rate.** To calculate the survival rate, the number of rats that survived until being killed at day 21 was divided by the number of rats at the start of each experiment. The survival rates from 3 experiments were calculated, and the mean and SEM values were obtained.

**Statistical analyses.** Statistical analyses were performed by using the Origin Software package (Microcal Software), SPSS (IBM), and Excel (Microsoft) software. Unequal variances were tested by the F-test. Data with equal variances were tested by 1-factor ANOVA, and when significant effects were present, post hoc comparisons were carried out by using Tukey’s test. For data with unequal variances, the Welch test was conducted. A Kruskal-Wallis test was performed to test for significant differences between scored pathologic observations. A chi-square test was performed to test for significant differences between survival rates. Data are represented as means ± SEMs. Differences were considered to be significant when P ≤ 0.05, and in cases in which 0.05 < P ≤ 0.1, the result, although not reaching significance, was considered to indicate a tendency.

**Results**

**Part 1**

**Growth.** There were no significant differences between the body weights of the experimental and control groups (Supplemental Fig. 1). The weights of whole heart, RA, LA, RV, and LV showed no significant differences between groups (data not shown).

**Heart function.** There were no significant differences in SBP and diastolic BP between the experimental and control groups (Table 1). HR tended to be lower in group E than in the control group (P = 0.07; Table 1). The average HR in group D was significantly lower than in the control group (P ≤ 0.05; Table 1). QT intervals for groups E and D were significantly shorter than those observed in the control group (P ≤ 0.05; Table 1). There were no significant differences between groups with respect to other electrocardiogram variables.

**Heart FAs.** Compared with the control group and group D, EPA concentrations in the RA in group E were significantly higher (P ≤ 0.05; Fig. 1A). EPA concentrations were significantly higher in group D than in the control group (P ≤ 0.05; Fig. 1A). DHA concentrations in the RA were significantly higher in group D than in the control group and group E (P ≤ 0.05; Fig. 1B), and AA concentrations in the RA were significantly lower in group D than in the control group and group E (P ≤ 0.05; Fig. 1C). The EPA:AA ratio, an indicator of inflammation, was significantly greater in groups E and D than in the control group (P ≤ 0.05; Fig. 1D). Furthermore, the EPA:AA ratio in group E was significantly greater than in group D (P ≤ 0.05; Fig. 1D). The concentrations of linoleic acid (18:2n-6) did not differ between groups (data not shown).

**Heart gene expression.** The endothelin 1 (End1) mRNA levels tended to be lower in group E than in the control group (P = 0.09; Supplemental Fig. 2). mRNA levels for Tnf, transforming growth factor β1 (Tgfβ1), cyclooxygenase 2 (Cox2), and angiotensin-converting enzyme 2 (Ace2) did not differ significantly between groups (data not shown).

**TABLE 1**

<table>
<thead>
<tr>
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<th>Control (n = 8)</th>
<th>E (n = 6)</th>
<th>D (n = 6)</th>
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<tr>
<td>SBP, mm Hg</td>
<td>116 ± 5</td>
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<td>DBP, mm Hg</td>
<td>95.9 ± 4.3</td>
<td>95.6 ± 5.2</td>
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<td>HR, beats/min</td>
<td>421 ± 9</td>
<td>379 ± 13b</td>
<td>358 ± 17b</td>
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<td>P wave duration, ms</td>
<td>19.5 ± 0.7</td>
<td>17.2 ± 1.6</td>
<td>18.5 ± 0.8</td>
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<td>ORS interval, ms</td>
<td>14.8 ± 0.4</td>
<td>14.7 ± 0.5</td>
<td>15.3 ± 0.3</td>
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<tr>
<td>RR interval, ms</td>
<td>146 ± 5</td>
<td>160 ± 7</td>
<td>153 ± 6</td>
</tr>
<tr>
<td>PR interval, ms</td>
<td>50.5 ± 13</td>
<td>49.6 ± 1.1</td>
<td>50.7 ± 1.5</td>
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<tr>
<td>QT interval, ms</td>
<td>74.3 ± 14a</td>
<td>66.3 ± 1.0b</td>
<td>67.7 ± 2.9b</td>
</tr>
</tbody>
</table>

1 Values are means ± SEMs. Labeled means in a row without a common letter differ, P ≤ 0.05. BP, blood pressure; Control, normal rats administered saline; D, normal rats administered DHA–ethyl ester; DBP, diastolic blood pressure; E, normal rats administered EPA–ethyl ester; HR, heart rate; SBP, systolic blood pressure.

Modification of cardiac function by EPA or DHA
Part 2

Growth. From 7 d after monocrotaline injection, body weights for groups H, HE, and HD were significantly lower than those observed in the control group ($P < 0.05$; Table 2). The QT interval was significantly shorter in the control group and group HD than in groups H and HE ($P < 0.05$; Table 2). There were no significant differences in the PR interval between groups. We attempted to measure BP and HR for CHF rats; however, in a pilot experiment, we found that the CHF rats needed to remain within the temperature-controlled cylinder at 38°C for a longer time than the control rats because their pulse was weak and took longer to measure. This resulted in aggravating their symptoms. Thus, we did not measure BP or HR in part 2.

Heart function. P wave duration, QRS interval, RR interval, and QT interval were significantly greater in group H than in the control group ($P < 0.05$; Table 2). The QRS interval was significantly longer in group HD than in the control group and groups H and HE ($P < 0.05$; Table 2). The QT interval was significantly shorter in the control group and group HD than in groups H and HE ($P < 0.05$; Table 2). There were no significant differences in the PR interval between groups. We attempted to measure BP and HR for CHF rats; however, in a pilot experiment, we found that the CHF rats needed to remain within the temperature-controlled cylinder at 38°C for a longer time than the control rats because their pulse was weak and took longer to measure. This resulted in aggravating their symptoms. Thus, we did not measure BP or HR in part 2.

Heart FAs. The concentrations of EPA were significantly higher in group HE than in the control group and groups H and HD ($P < 0.05$; Fig. 3A). EPA concentrations were significantly higher in group HD than in the control group and group H ($P < 0.05$; Fig. 3A). The concentrations of DHA were significantly lower in group H than in the control group and group HD ($P < 0.05$; Fig. 3B). DHA concentrations in group HE were generally lower compared with the control group ($P = 0.08$) and group HD ($P = 0.10$) (Fig. 3B). The concentrations of AA in groups H, HE, and HD were significantly lower than in the control group ($P < 0.05$; Fig. 3C). The EPA:AA ratio was significantly greater in group HE than in the control group and groups H and HD ($P < 0.05$; Fig. 3D). Linoleic acid concentrations did not show significant differences between groups (data not shown).

Heart gene expression. Cox2 mRNA levels were significantly higher in groups H and HE than in the control group ($P < 0.05$; Fig. 4A). End1 mRNA levels were significantly higher in groups H and HE than in the control group ($P < 0.05$; Fig. 4B). End1 mRNA levels in the HD group tended to be greater than those in the control group ($P = 0.08$; Fig. 4B). The mRNA expression levels for Tnf, Tgfb1, and Ace2 did not exhibit significant differences between groups (data not shown).

FIGURE 1 Concentrations of EPA (A), DHA (B), and AA (C) and the EPA:AA ratio (D) in the RA of rats orally administered EPA–ethyl ester or DHA–ethyl ester daily for 28 d (study part 1). Values are means ± SEMs; $n = 11$ (C group), $n = 9$ (E group), or $n = 9$ (D group). Within each graph, labeled means without a common letter differ, $P < 0.05$. AA, arachidonic acid; C, normal rats administered saline; D, normal rats administered DHA–ethyl ester; E, normal rats administered EPA–ethyl ester; RA, right atrium.

FIGURE 2 Body weights of CHF rats orally administered EPA–ethyl ester or DHA–ethyl ester daily for 24 d (study part 2). Values are means ± SEMs; $n = 5$ (group C), $n = 11$ (group H), $n = 9$ (group HE), or $n = 10$ (group HD) at day 0 and $n = 5$ (group C) or $n = 7$ (groups H, HE, and HD) at day 21. Labeled means without a common letter differ, $P < 0.05$. The solid arrow shows the start of EPA–ethyl ester or DHA–ethyl ester administration. The dashed arrow shows the start of MCT injection. C, normal rats administered saline; CHF, congestive heart failure; H, CHF rats administered saline; HD, CHF rats administered DHA–ethyl ester; HE, CHF rats administered EPA–ethyl ester; MCT, monocrotaline.
TABLE 2 P wave duration or intervals between waves in electrocardiogram recordings of CHF rats administered EPA–ethyl ester or DHA–ethyl ester daily for 24 d (study part 2)1

<table>
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<th>Group</th>
<th>Control (n = 9)</th>
<th>H (n = 15)</th>
<th>HE (n = 11)</th>
<th>HD (n = 12)</th>
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<tr>
<td>P wave duration, ms</td>
<td>17.6 ± 0.6a</td>
<td>20.2 ± 1.1b</td>
<td>17.6 ± 0.8b</td>
<td>16.4 ± 1.0b</td>
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<td>QR interval, ms</td>
<td>143 ± 0.4c</td>
<td>19.7 ± 1.5b</td>
<td>16.9 ± 0.6c</td>
<td>23.6 ± 1.7a</td>
</tr>
<tr>
<td>RR interval, ms</td>
<td>142 ± 5a</td>
<td>167 ± 10a</td>
<td>153 ± 9b</td>
<td>164 ± 11b</td>
</tr>
<tr>
<td>PR interval, ms</td>
<td>49.6 ± 1.4</td>
<td>50.7 ± 1.7</td>
<td>48.0 ± 1.6</td>
<td>46.8 ± 1.8</td>
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<tr>
<td>QT interval, ms</td>
<td>69.0 ± 4.1b</td>
<td>95.7 ± 3.1a</td>
<td>90.2 ± 2.3a</td>
<td>77.1 ± 3.1b</td>
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</table>

1 Values are means ± SEMs. Labeled means in a row without a common letter differ, P ≤ 0.05; Table 3.

Morbidity. Pathologic observations revealed that the average thickness of the RV free wall was significantly greater in groups H, HE, and HD than in the control group (P ≤ 0.05; Table 3). The volumes of the RV myocytes (cell width) in groups H, HE, and HD were also significantly greater than in the control group (P ≤ 0.05; Table 3). RV sections obtained from CHF rats showed that infiltration of mononuclear cells by myocytes was significantly greater in groups H and HE than in the control group (P ≤ 0.05; Table 3). Finally, the score of mononuclear cell infiltration between myocytes in the HD group tended to be lower than in the H group (P = 0.06; Table 3). The BNP concentrations in the H, HE, and HD groups were significantly higher than in the control group (P ≤ 0.05; Table 3).

At 21 d after monocrotaline injection, rat body weights in groups H, HE, and HD were significantly lower than in the control group (P ≤ 0.05; Table 3). Although the whole-heart weight did not differ between groups, compared with the control group, RV weights significantly increased in groups H, HE, and HD (P ≤ 0.05; Table 3), whereas LV weights significantly decreased (P ≤ 0.05; Table 3). Compared with the control group, the number of leukocytes significantly increased (P ≤ 0.05) and the survival rates significantly decreased (P ≤ 0.05) among rats in groups H, HE, and HD (Table 3).

Discussion

For normal rats that did not receive CHF (part 1), the administration of EPA-Et and DHA-Et resulted in significantly shortened QT intervals (Table 1). Furthermore, the administration of EPA-Et increased EPA concentrations in the RA (Fig.1A), whereas treatment with DHA-Et resulted in increased EPA and DHA concentrations (Fig.1A, B) and reduced AA concentrations (Fig.1C). It has been proposed that n–3 PUFAs are metabolized through both “orthodox” and “new” pathways to signaling molecules (reviewed in reference 1). In orthodox pathways, COX1 or COX2 mediates the conversion of EPA into 3-series prostanoids, such as prostaglandin I3 and thromboxane A3, while also mediating the conversion of AA into 2-series prostanoids, such as prostacyclin (PGI2) and thromboxane A2. In the new pathways, during the resolution of inflammation, EPA can metabolize to resolvin E1 by the reaction with acetylated COX2 (25), and DHA can give rise to the following novel eicosanoid derivatives: protectin D1, 17R resolvin D1, and 17R resolvin D1 (25). Furthermore, direct effects of n–3 PUFAs that do not involve their metabolism have been proposed. Some of these effects, including antiarrhythmic effects, occur rapidly without incorporation of n–3 PUFAs into the cell membranes (34). Free n–3 PUFAs can sterically interfere with sodium and calcium channels. Among other direct effects of n–3 PUFA, their incorporation into cell membranes may alter the properties of lipid rafts and caveolae, thereby contributing to membrane fluidity (35,36). Consequently, the functions of membrane-associated proteins, including ion channels (3), may be affected. Such remodeling of myocardial membrane and proteins may

FIGURE 3 Concentrations of EPA (A), DHA (B), and AA (C) and the EPA:AA ratio (D) in the RA of CHF rats orally administered EPA–ethyl ester or DHA–ethyl ester daily for 24 d (study part 2). Values are means ± SEMs; n = 4 (group C) or n = 6 (groups H, HE, and HD). Within each graph, labeled means without a common letter differ, P ≤ 0.05. AA, arachidonic acid; C, normal rats administered saline; CHF, congestive heart failure; H, CHF rats administered saline; HD, CHF rats administered DHA–ethyl ester; HE, CHF rats administered EPA–ethyl ester; RA, right atrium.
alter their functionality and subsequent downstream signaling (36,37). Because cardiac membrane action potential, which is the origin of cardiac function, is controlled by ion channel currents (38), remodeling of ion channels may thus alter cardiac function. Therefore, the administration of EPA-Et and DHA-Et in the present study may have altered downstream events, including signal transduction and ionic movement, through at least one of the mechanisms described above. Such alterations may have modified normal cardiac function.

In part 2, EPA concentrations were greater in CHF rats administered EPA-Et (Fig. 3A) and the RA EPA:AA ratio increased (Fig. 3D). The EPA:AA ratio provides information about the effects of proinflammatory eicosanoids and cytokines (39,40). A prospective randomized trial investigating combination treatments with statins and EPA found that greater EPA:AA ratios are indicative of reduced risk of a major coronary event, such as sudden cardiac death, fatal myocardial infarction, or unstable angina (41). In the present study, the heart function in the HE group did not differ significantly from that in group H (Table 2). The administration of EPA-Et did not improve the morbidity rates that occurred in group H (Table 3). Thus, in the present study, the effect of supplementing EPA concentrations and the EPA:AA ratio may have been too weak to offset the damaging effects caused by CHF.

In CHF rats administered DHA-Et (part 2), the prolongation of the QT interval observed in group H did not occur (Table 2). Furthermore, unlike in group H, there was no reduction in RA DHA concentrations in group HD (Fig. 3B). These observations suggest that DHA protects the heart from the effects of CHF by normalizing the downstream signaling of n-3 PUFA metabolism and/or remodeling the cardiac membrane (as discussed above in relation to part 1). In CHF rats administered DHA-Et, the upregulation of Cox2 expression observed in CHF-treated rats was suppressed (Fig. 4A), and the mononuclear cell infiltration observed in CHF was reduced (Table 3). A study of human saphenous vein endothelial cells challenged with proinflammatory stimuli while exposed to DHA reported reductions in COX2 expression (42). PGI2 production was reduced in human endothelial cells briefly exposed to DHA (43). From these reports, in response to proinflammatory stimuli, DHA reduces PGI2 production by the reduction of COX2. Accordingly, the results of the present study suggest that DHA prevents

**TABLE 3** Heart pathology, BNP concentrations, weights of body and heart, number of leukocytes, and survival rate of CHF rats administered EPA–ethyl ester or DHA–ethyl ester daily for 24 d (study part 2)1

<table>
<thead>
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<th>Group</th>
<th>Control</th>
<th>H</th>
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<th>HD</th>
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<tbody>
<tr>
<td></td>
<td>Heart pathology, fold of Control</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Thickness of RV wall</td>
<td>1.0 ± 0.0a</td>
<td>1.7 ± 0.2b</td>
<td>1.8 ± 0.2b</td>
<td>1.6 ± 0.2b</td>
</tr>
<tr>
<td>Cell width</td>
<td>1.0 ± 0.0a</td>
<td>2.3 ± 0.3b</td>
<td>2.0 ± 0.4b</td>
<td>2.0 ± 0.4b</td>
</tr>
<tr>
<td>Mononuclear cell infiltration</td>
<td>1.0 ± 0.0a</td>
<td>2.1 ± 0.3b</td>
<td>2.0 ± 0.4b</td>
<td>1.2 ± 0.2b</td>
</tr>
<tr>
<td>BNP, μg/L</td>
<td>0.11 ± 0.02a</td>
<td>0.71 ± 0.17b</td>
<td>0.74 ± 0.12b</td>
<td></td>
</tr>
<tr>
<td>Weight, g</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Body</td>
<td>332 ± 14a</td>
<td>254 ± 10b</td>
<td>250 ± 7b</td>
<td>256 ± 7b</td>
</tr>
<tr>
<td>Heart</td>
<td>0.99 ± 0.03</td>
<td>1.04 ± 0.05</td>
<td>0.93 ± 0.04</td>
<td>1.03 ± 0.03</td>
</tr>
<tr>
<td>RV</td>
<td>0.17 ± 0.01b</td>
<td>0.32 ± 0.02b</td>
<td>0.32 ± 0.02b</td>
<td>0.34 ± 0.02b</td>
</tr>
<tr>
<td>LV</td>
<td>0.76 ± 0.02a</td>
<td>0.60 ± 0.04b</td>
<td>0.54 ± 0.03b</td>
<td>0.61 ± 0.02b</td>
</tr>
<tr>
<td>Number of leukocytes, ×106 cells/mL</td>
<td>5.7 ± 1.3b</td>
<td>11.7 ± 1.3b</td>
<td>12.7 ± 1.4b</td>
<td>11.3 ± 0.6b</td>
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<tr>
<td>Survival rate, %</td>
<td>100 ± 0a</td>
<td>74 ± 6b</td>
<td>65 ± 5b</td>
<td>70 ± 12b</td>
</tr>
</tbody>
</table>

1 Values are means ± SEMs; n = 5 (control group), n = 7 (group H), n = 5 (group HE), or n = 5 (group HD) for heart pathology, BNP, and leukocytes and n = 4 (control group CI), n = 8 (group H), n = 6 (group HE), or n = 9 (group HD) for weight. Labeled means without a common letter differ, P ≤ 0.05. BNP, brain natriuretic peptide; CHF, congestive heart failure; Control, normal rats administered saline; H, CHF rats administered saline; HD, CHF rats administered DHA–ethyl ester; HE, CHF rats administered EPA–ethyl ester; LV, left ventricle; RV, right ventricle.

2 Pathologic observations were measured as follows: 1, negative; 2, weakly positive; 3, positive; 4, strongly positive. Data were analyzed by using the Kruskal-Wallis test.

3 Data were collected from 3 experiments and were analyzed by using the chi-square test.
myocarditis through COX2 reduction, thereby protecting the heart from CHF abnormalities. However, because BNP concentrations and the numbers of leukocytes were unchanged (Table 3), the preventive effects of DHA on myocarditis may be limited. Furthermore, hypertrophy of the RV in CHF rats was not suppressed by administration of DHA-Et (weight of RV in Table 3). Therefore, DHA may have little effect on hypertrophy. Moreover, because these limited effects on myocarditis and hypertrophy, although DHA may ameliorate the symptoms of CHF, it does not fully cure them. Thus, the administration of DHA may not reduce mortality rates. The HD group exhibited a prolonged QRS interval (Table 2). We attributed this to either a delay in the conduction of impulses in the ventricle (e.g., fascicular block) or a ventricular premature contraction (20). Further studies that use software for arrhythmia analysis are needed to understand this QRS prolongation.

The vasoconstrictive system may be affected by the administration of EPA and DHA. END induces the constriction of blood vessels to increase blood pressure, which induces hypertrophy of cardiomyocytes (44–47). Shimojo et al. (47) reported that EPA may suppress END1-induced hypertrophy of cardiomyocytes (44–47). According to the results of their study, EPA may suppress END1-induced cardiomyocyte hypertrophy by inhibition of c-Jun NH2-terminal kinase signaling. The DHA monoacylglyceride decreases END1-induced Ca2+ sensitivity and proliferation in human pulmonary arteries (48). In the present study, in the normal-heart rat group, EPA tended to suppress End1 gene expression (Supplemental Fig. 2). However, the upregulation of End1 expression in the H group was not suppressed in the HE and HD groups (Fig. 4B). Furthermore, treatment with EPA-Et or DHA-Et did not prevent hypertrophy of the heart (Table 3). Unlike other reports (47,48), our study found the effect of EPA and DHA to be too weak to prevent the vasoconstrictive effect caused by END1. The expression of Ace2, which is involved in the renin-angiotensin-aldosterone vasoconstrictive system, was unaffected by EPA-Et or DHA-Et administration in the present study (data not shown). Therefore, the effects of EPA and DHA are also weak in relation to the renin-angiotensin-aldosterone system.

In conclusion, the present study found that the administration of EPA-Et or DHA-Et affects normal cardiac function by altering the FA composition in rat heart. The study also found that although DHA-Et might contribute to reducing the effects of CHF exposure, the effect of EPA-Et might be too weak to prevent such effects. Because the QT interval is prolonged concomitantly with the emergence of coronary heart disease accompanied by arrhythmia, long QT syndrome, and hypocalcemia (20), the administration of EPA or DHA is considered to be beneficial for the prevention of coronary heart disease. Further research regarding the alteration of downstream signaling of n–3 PUFA metabolism and ion channel alteration in order to elucidate the detailed mechanism of the effects of EPA or DHA on cardiac function is warranted.

Acknowledgments

T.T.Y. was the main author of the manuscript and contributed to designing the study, preparing protocols, performing the experiments, interpreting the data, and preparing the manuscript; H.K. carried out quantification of FAs, interpretation of data, and preparation of the manuscript; E.H. observed pathologic samples and diagnosed and interpreted data; N.J. contributed to writing, editing, and proofreading the manuscript; and F.T. and M.M. contributed to interpretation of data and preparation of the manuscript. All authors read and approved the final manuscript.

Literature Cited


