Dietary Omega-3 Fatty Acids and Susceptibility to Ventricular Fibrillation
Lack of Protection and a Proarrhythmic Effect

George E. Billman, PhD; Cynthia A. Carnes, PharmD, PhD; Philip B. Adamson, MD; Emilio Vanoli, MD; Peter J. Schwartz, MD

The cardiovascular benefits of dietary omega-3 polyunsaturated fatty acids (n-3 PUFAs) have been actively investigated for nearly 40 years. Epidemiological data provide strong evidence for an inverse relationship between fatty fish consumption and cardiac mortality. In contrast to these observational studies, interventional studies using n-3 PUFAs for the secondary prevention of adverse cardiovascular events in patients recovering from myocardial infarction (MI) have yielded conflicting results (Table 1) and are a current area of active debate. Nevertheless, the American Heart Association first recommended fish oils for secondary prevention in post-MI patients in 2003. Based in part on these recommendations, consumer demand for n-3 PUFAs has exploded. It has been estimated that 5–10% of the adult US population use a fish oil supplement, and sales are projected to exceed 7 billion dollars by the end of 2011 (www.marketresearch.com).

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Background—Recent clinical studies that evaluated the effects of supplemental omega-3 polyunsaturated fatty acids (n-3 PUFAs) on sudden cardiac death have yielded conflicting results. Our aim was to clarify this issue using an established and clinical relevant canine model of sudden cardiac death.

Methods and Results—Susceptibility to ventricular fibrillation (VF) was evaluated using a 2-minute left circumflex artery occlusion during the last minute of an exercise test in 76 dogs (from 2 independent studies) with healed myocardial infarctions (MI); 44 developed VF (susceptible, VF+), whereas 32 did not (resistant, VF−). These dogs were then randomly assigned to either placebo (1 g/d, corn oil; 15 VF+, 11 VF−) or n-3 PUFAs (1–4 g/d, docosahexaenoic acid+eicosapentaenoic acid ethyl esters, 29 VF+, 21 VF−) groups. Seven sham (no-MI) dogs were also treated with n-3 PUFAs (4 g/d). After treatment (3 months), the exercise+ischemia test was repeated. Dietary n-3 PUFAs produced significant (P<0.01) increases in red blood cell and left ventricular n-3 PUFAs levels. Nine post-MI (5 placebo versus 4 n-3 PUFAs) and 2 sham dogs died suddenly during the 3-month treatment period. The n-3 PUFAs treatment failed to prevent arrhythmias in VF+ dogs (decreased in 27% placebo versus 24% n-3 PUFAs, P=0.5646) but induced VT/VF in VF− animals (n-3 PUFAs 33% versus placebo 0%, P=0.0442).

Conclusions—Despite large increases in cardiac tissue n-3 PUFA content, dietary n-3 PUFAs did not prevent ischemia-induced VF and actually increased arrhythmia susceptibility in both noninfarcted and low-risk post-MI dogs. (Circ Arrhythm Electrophysiol. 2012;5:553-560.)

Key Words: omega-3 polyunsaturated fatty acids ■ fish oil ■ ventricular fibrillation ■ myocardial ischemia ■ myocardial infarction
Methods

This report includes the results obtained from 2 independent laboratories that coincidentally explored the same issue using the identical canine model. All the animal procedures were approved by the University of Oklahoma Health Sciences Center (study 1) or the Ohio State University (study 2) Institutional Animal Care and Use Committee and conformed to the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health.

Study 1 (the University of Oklahoma Protocol)

Surgical Preparation and Sudden Death Testing
Thirty-eight animals (heartworm-free, mixed-breed male dogs; weight, 20–25 kg) were instrumented and had an MI induced by the 2-stage ligation of the left anterior descending artery as previously described22,23; 11 (29%) dogs died within 48–72 hours and 3 could not be classified because of malfunction of the left circumflex coronary artery occluder.

The susceptibility to VF was evaluated 3–4 weeks after MI in the remaining 24 dogs, using an exercise plus ischemia test as previously described.22,23 This test induced VF in 10 dogs (VF+) but not in the remaining 14 (VF–). Two of the VF+ dogs were not successfully resuscitated. The VF– dogs were not used to evaluate n-3 PUFA treatment in study 1. The remaining VF+ dogs (n=8) were then treated with n-3 PUFAs (1 g/d for 8 weeks). The dogs were given supplements similar to those used in the GISSI-Prevenzione study.5,8 Each 1-g capsule contained 465 mg ethyl eicosapentaenoate (EPA) and 375 mg ethyl docosahexaenoate (DHA) (GlaxoSmithKline, Research Triangle Park, NC). The exercise plus ischemia test was repeated at the end of the 8-week n-3 PUFA treatment period. Blood samples were collected at baseline and at the end of the treatment period to assess n-3 PUFA plasma levels.

Study 2 (The Ohio State University Protocol)

The surgical preparation and the classification as to the susceptibility to VF for the animals in study 2 were identical to that used in study 1. Heartworm-free, mixed-breed dogs (n=115, 19 males, 76 females, 2–3 years old) weighing 19.4±0.2 kg were used in this study (Figure 1). Of the 115 animals that underwent surgery, 24 animals could not be tested either due to death within 72-hours of the MI (n=17, 15%) or occluder failure (n=7). Thus, the exercise plus ischemia test was performed on 91 of the original 115 post-MI dogs. Fifty-five dogs developed VF (VF+, susceptible), whereas the remaining 36 did not (VF–, resistant). Eight VF+ animals were not successfully defibrillated. Seven sham-operated (ie, left anterior descending artery was isolated but not ligated) dogs were also classified through the use of this exercise plus ischemia test (2 VF+, 5 VF–).

The n-3 PUFA treatment protocol used in study 2 lasted 3 months and included doses of 1, 2, and 4 g/d. The dogs were maintained on a diet that did not contain any n-3 PUFAs (Harlan Teklad, Harlan Laboratories, Inc, Indianapolis, IN), beginning 1 week before the
Myocardial Infarction
(n = 115, 17 died within 72 hr)

Exercise + Ischemia Test
(n = 98, occluder failure n = 7)

Susceptible
(VF+, n = 55)
Unable to defibrillate (n = 8)

Placebo
(n = 20)
(*n = 5)

Placebo
(n = 12)
(*n = 2)

n-3 PUFA
(n = 27)
(*n = 6)

n-3 PUFA
(n = 24)
(*n = 3)

Resistant
(VF−, n = 36)

Red Blood Cell and Cardiac Tissue Fatty Acid Analysis

Fasting blood samples (5 mL) were drawn into EDTA tubes from a cephalic vein between 8:00 and 9:00 am at the following time points: 1 day before treatment and when tissue was harvested at the end of the study (~14 weeks of treatment). Right atrial and left ventricular tissues were obtained when the hearts were harvested; the tissue and red blood cells (RBC) were flash-frozen in liquid nitrogen and stored at –80°C for subsequent analysis.

RBC and phospholipids from cardiac tissue were analyzed for fatty acid composition24,25 by gas chromatography, using a GC2010-FID instrument (Shimadzu Corporation, Columbia, MD) equipped with a 100-mm capillary column (SP-2560, Supelco, Bellefonte, PA). Fatty acids of interest were identified by comparison with known standards and expressed as a percentage of total fatty acids. The coefficient of variation for the RBC EPA+DHA assays was <5%.

Data Analysis

The lipid analysis data are reported as mean±SEM. The ECG data were digitized (1 kHz) and recorded, using a Biopac MP-100 data acquisition system (Biopac Systems, Inc, Goleta, CA). RBC and cardiac tissue lipid compositions were compared, using a 2-factor (dose, pre-post) ANOVA with repeated measures on 1-factor (pre-post) or a 1-factor ANOVA, respectively (NCSS statistical software, Kaysville, UT). Post hoc comparisons were made by using the Tukey-Kramer multiple comparison test. The effects of the interventions (placebo versus n-3 PUFA) on arrhythmias/mortality were evaluated using the Fisher exact test (one-tailed test). Arrhythmias severity was quantified by using the Lambeth convention26 criteria for arrhythmia score: 0=no arrhythmias, 1=presetim ventricular complexes; 2=ventricular tachycardia (of at least 4 beats duration); 3=ventricular fibrillation; and 4=spontaneous death.

Results

Study 1

Dietary n-3 PUFA treatment (8 weeks) significantly (P=0.0340) increased plasma omega-3 index (EPA+DHA, from 2.5±0.4% to 4.1±0.4%) levels. No animals died spontaneously during the course of this study. At the end of the treatment with 1 g/d, the exercise plus ischemia test was repeated and VF recurred in 7 of the 8 animals tested (87.4%, pretreatment versus posttreatment, P=0.5000).

Study 2

Dietary n-3 PUFA treatment dose-dependently increased RBC membrane and cardiac tissue n-3 PUFA content, whereas the lipid composition did not change in the placebo-treated animals (online-only Data Supplement Tables I and II). Because all 3 n-3 PUFA doses had similar effects on the susceptibility to VF, the results for all the post-MI dogs (study 2) treated with n-3 PUFA were combined for all subsequent analyses and are displayed in Figure 2 (VF+) and 3 (VF−).

VF+ Dogs

Nine dogs died spontaneously during the 3-month feeding study: 5 of 20 placebo (10, 32, 65, 72, and 79 days after treatment onset) and 4 of 27 [1 (84 days, witnessed VF) with 1 g/d; 1 (52 days) with 2 g/d and 2 (40 and 59 days after treatment onset) with 4 g/d] n-3 PUFA (P=0.3055). With 1 notable exception (witnessed collapse and subsequent VF confirmation), the cause for the spontaneous deaths could not be determined but, consistent with clinical practice, were assumed to result from arrhythmias. The arrhythmia score was not affected by either the placebo (pretreatment: 2.5±0.1, versus posttreatment: 2.8±0.3) or n-3 PUFA treatment (pretreatment: 2.8±0.1, versus posttreatment: 2.5±0.3). Similar reductions (placebo: 4 of 15, 26.7%, versus n-3 PUFA: 6 of 21, 28.6%; P=0.6023) and increases (placebo: 5 of 15, versus n-3 PUFA: 6 of 21, P=0.5209) in the occurrence of ventricular tachyarrhythmias were noted after treatment in both groups.

VF− Dogs

No VF− dogs died spontaneously in either the placebo-treated (Figure 3A) or the n-3 PUFA–treated groups (Figure 3B). However, n-3 PUFA treatment significantly (P=0.0442)
increased the incidence of ventricular tachyarrhythmias. The exercise plus ischemia test did not alter arrhythmias in the 10 placebo-treated dogs (Figure 3A) but induced arrhythmias in 7 of 21 (2 of 5 with 2 g/d and 4 of 15 with 4 g/d) n-3 PUFA–treated animals (Figure 3B), including the induction of VF in 3 dogs. The arrhythmia score doubled after n-3 PUFA treatment (pretreatment: 0.4±0.1, versus posttreatment: 0.8±0.2) but did not change in the placebo group (pretreatment: 0.4±0.1, versus posttreatment: 0.3±0.3).

**Sham (No MI) Dogs**

Seven sham dogs (2 VF+, 5 VF–) were also tested with 4 g/d n-3 PUFA; 2 of these dogs (1 VF+, 35 days, 1 VF–, 74 days after treatment, 28.6%) died spontaneously during the 3-month n-3 PUFA treatment, whereas there was no change in the arrhythmias in the remaining 5 dogs. There were no placebo-treated sham dogs. However, none of 195 noninfarcted dogs died spontaneously after thoracic surgery in previous studies (n-3 PUFA–treated versus historic data, \(P=0.0010\)).

**Discussion**

There were 2 major findings from this study that have important clinical implications. First, despite large increases in cardiac tissue n-3 PUFA concentration, n-3 PUFA treatment did not reduce life-threatening ventricular arrhythmias in post-MI dogs at high risk for ventricular fibrillation. Second, and contrary to both current views and to our initial expectations, n-3 PUFA treatment significantly increased the susceptibility to malignant arrhythmias in low-risk dogs (both dogs with and without MI). Long-term dietary n-3 PUFA treatment induced ventricular tachyarrhythmias in one-third of the VF– post MI dogs, whereas 2 noninfarcted dogs died spontaneously during the 3-month n-3 PUFA treatment. This latter observation is particularly noteworthy, as these noninfarcted dogs would normally exhibit a negligible risk for sudden death. In fact, these are the only noninfarcted dogs that have died suddenly after thoracic surgery in our laboratories during the last 35 years (n=195). Furthermore, it should also be emphasized that it is difficult to induce ventricular tachyarrhythmias in VF– dogs; repetition of the exercise plus ischemia test never induced ventricular
tachyarrhythmias in our combined experience with over 220 post-MI dogs initially classified as VF–. When considered together, these data strongly suggest that dietary n-3 PUFAs not only lack significant antiarrhythmic benefits in this model but also actually enhance the risk for severe ventricular tachyarrhythmias in some settings.

Interventional studies using n-3 PUFAs for the secondary prevention of adverse cardiovascular events in patients recovering from MI have yielded conflicting results (Table 1). The DART3 and GISSI-Prevenzione5 trials reported 10–20% reductions in all-cause mortality with up to a 45% reduction in sudden death.5 In marked contrast, Burr et al6 reported that n-3 PUFAs increased rather than decreased all-cause mortality (15% increase over 9-year follow-up period, with a 54% increase in sudden death), whereas the JELIS trial found that EPA supplements did not alter either sudden death or fatal MI despite decreasing nonfatal coronary events.7 Most recently, both the OMEGA9 and the Alpha Omega10 trials reported that n-3 PUFA supplements failed to alter either total mortality or sudden death rates in post-MI patients. Similar inconsistent findings have been obtained from meta-analysis of these trials.28-31 Two analyses failed to find a relationship between fish or fish oil consumption and a reduction in cardiac mortality28,29; a third analysis found an inverse relationship between the incidence of sudden death in MI patients and n-3 PUFA consumption, but an increased risk for adverse cardiac events in patients with angina30; and a fourth study reported a significant dose-independent reduction in cardiac mortality but not in sudden death or in all-cause mortality.31

Omega-3 PUFA supplements have also been given to patients with a demonstrated risk for ventricular arrhythmias (ie, those with implanted cardioverter-defibrillators [ICDs]) yielding similar mixed results (Table 2). Leaf et al11 reported that fish oil supplements did not reduce death rates, but they found a trend toward benefit in the combined end point of time to ICD discharge and all-cause mortality. In contrast, Raitt et al12 reported that fish oil supplements not only did not reduce ICD events or mortality but also increased arrhythmic events in the subgroup of patients (67%) who received an ICD with ventricular tachycardia as an indication. Heart failure patients (New York Heart Association class II and class III) with the highest RBC n-3 PUFA levels also exhibited an increased risk for ventricular arrhythmias that required antitachycardic therapy.32 However, the largest ICD study to date found that n-3 PUFA treatment had no effect on adverse cardiac events.13 Accordingly, meta-analysis of these ICD trials found that n-3 PUFA treatment was neither antiarrhythmic nor proarrhythmic in this patient population.33,34

Variable results have also been reported in animal studies.21,35-37 For example, Coronel et al21 found that dietary n-3 PUFA increased the incidence of VF during regional ischemia in isolated pig heart preparations. Conversely, tuna oil supplements prevented ventricular tachyarrhythmias during ischemia and reperfusion in isolated rat hearts35 and increased the current necessary to induce VF in nonhuman primate hearts.36 A meta-analysis of 27 (23 feeding, 4 acute intravenous

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**Table 2. Effect of Omega-3 Fatty Acids on Cardiac Events in ICD Patients**

<table>
<thead>
<tr>
<th>Study</th>
<th>Patients</th>
<th>Treatment</th>
<th>Time to First Event or Death</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Leaf et al,11 2005</td>
<td>50% EF ≤30% 78% CHD</td>
<td>EPA 18.2 mg+DHA 2.4 g/d</td>
<td>↑</td>
<td></td>
</tr>
<tr>
<td>Raitt et al,12 2005</td>
<td>46% EF ≤30% 73% CHD</td>
<td>756 mg EPA+540 mg DHA per day</td>
<td>No change</td>
<td>Increased VT in subgroup analysis</td>
</tr>
<tr>
<td>Brouwer et al,13 2006</td>
<td>33% EF ≤30% 76% CHD</td>
<td>464 mg EPA+335 mg DHA per day</td>
<td>No change</td>
<td></td>
</tr>
</tbody>
</table>

ICD indicates implantable cardioverter-defibrillator; EF, ejection fraction; CHD, coronary heart disease; VT, ventricular tachycardia; MI, myocardial infarction; n-3 PUFA, omega-3 polyunsaturated fatty acid; EPA, ethyl eicosapentaenoate; and DHA, ethyl docosahexaenoate.
infusion) animal studies found that n-3 PUFA (fish oil, EPA, or DHA but not alpha linolenic acid [ALA] treatment) attenuated ischemia-induced ventricular arrhythmias but was ineffective in ischemia-reperfusion models. Thus, as emphasized in a recent review, the effects of n-3 PUFA on sudden death—whether harmful or beneficial—have yet to be convincingly demonstrated.

Strengths and Limitations of the Study

A major strength of our findings lies in the very high translational value of our experimental model. To provide an accurate assessment of the factors responsible for sudden cardiac death, a model must mimic, as closely as possible, the underlying pathological conditions associated with a high risk of sudden death in patients. Clinical studies indicate that among the most important factors associated with a high risk of sudden death are the following: acute myocardial ischemia, previous myocardial ischemic injury, and alterations in cardiac autonomic regulation. Nearly 30 years ago, we developed a canine model of sudden cardiac death that fulfills these criteria. More than 50 peer-reviewed publications have resulted from these studies including a recent comprehensive review. Most importantly, the results first obtained in our model have been subsequently validated in large human studies. For example, autonomic markers for sudden death (both baroreflex sensitivity and HR variability) first identified in our model were confirmed in large, prospective clinical studies in post-MI patients. Furthermore, the efficacy of antiarrhythmic interventions as determined in our model was subsequently confirmed in major clinical trials. Specifically, azimilide (10%, 1 of 10), d-sotalol (11.1%, 2 of 18), and dofedilide (14.3%, 1 of 7) (used without success in the clinical trials ALIVE, SWORD, and DIAMOND) each failed to provide protection from VF, whereas clinically effective agents, β-adrenoceptor blockers (68.4%, 63 of 92) and amiodarone (94.2%, 33 of 35), successfully reduced VF in our model. Although great care is always necessary when attempting to draw clinical implications from experimental studies, we believe that our preparation for sudden death has proven to be reliable over time.

Defining human-equivalent doses of n-3 PUFA for animal studies is challenging. In study 2, the average n-3 PUFA dose (adjusted for body surface area) was equivalent to about 4 g/d in human subjects. As such, this dose is higher than those used in the most of the interventional studies in Table 1. However, it is equivalent to the dose of prescription n-3 PUFA (Lovaza, GlaxoSmithKline) used to treat hypertriglyceridemia, and this dose was ineffective in the treatment of paroxysmal atrial fibrillation. Furthermore, the doses used in the present study yielded RBC membrane EPA+DHA levels that were associated with a significant reduction the risk for sudden death in epidemiological studies. A mean RBC n-3 PUFA concentration of 6.9% was associated with a 90% reduction in the risk for sudden death, a value that compares favorably with that obtained the present study (after 3 months at 4 g/d, mean RBC concentration was 6.8%; range, 4.3–10.7%).

There were no placebo-treated noninfarcted dogs, and, as such, it is difficult to access the role that n-3 PUFA treatment played in the deaths noted in this group. However, historically, no noninfarcted dog (n=195) has died spontaneously after thoracic surgery. It therefore seems likely that the deaths in these dogs could be attributed to the n-3 PUFA treatment. With 1 notable exception (witnessed collapse and confirmed VF), the cause of the spontaneous death was not determined but, consistent with clinical studies, was assumed to result from arrhythmias.

Finally, although the potential proarrhythmic effects of n-3 PUFA ingestion were assessed by the inclusion of low-risk animals, the present study was not designed to investigate arrhythmogenic mechanisms if this hypothesis were to be confirmed. As a consequence, it was not possible to determine the electrophysiological basis for the apparent proarrhythmic action of the n-3 PUFA treatment. However, there are at least 2 mechanisms by which n-3 PUFA might enhance the risk for arrhythmias in our model: alterations in repolarization and/or myocardial calcium dysregulation. We previously demonstrated that MI provokes reductions in repolarization reserve in both VF- and VF+ dogs, with the largest reductions noted in the VF+ animals. As dietary n-3 PUFA treatment has been shown to prolong myocyte action potential duration through inhibition of outward potassium currents, these fatty acids could provoke further reductions in repolarization reserve, that lead to marked regional differences in repolarization during myocardial ischemia. Indeed, dietary n-3 PUFA enhanced arrhythmia formation in isolated porcine hearts during ischemia but not during normoxic conditions. These regional differences in repolarization would be difficult to detect with a body surface ECG but should be more obvious with multi-electrode mapping and refractory period studies. Thus, changes in repolarizing currents could explain both the lack of beneficial actions in the VF+ dogs and the induction of tachyarrhythmias in some of the VF- dogs (by “exhausting” the repolarization reserve, tipping the balance in favor arrhythmia formation). In a similar manner, cardiomyocyte calcium dysregulation also appears to contribute to arrhythmia formation in VF+ animals. Myocytes from these animals exhibit greater spontaneous calcium release and calcium alternans, phenomena that could be eliminated by reducing agents and replicated in control myocytes by oxidant stress. It is possible that n-3 PUFA treatment could further disrupt the regulation of sarcoplasmic reticular calcium release particularly during the oxidant stress associated with ischemia and/or in response to sympathetic nerve activation (β-adrenoceptor stimulation) in dogs previously shown to be resistant to VF. The relative contribution, if any, of n-3 PUFA mediated changes in calcium regulation and repolarization reserve to the proarrhythmia noted in the present study remain to be determined.

Conclusions

In the present study, despite large increases in cardiac tissue n-3 PUFA content, dietary n-3 PUFAs not only did not prevent ischemia-induced VF but actually induced arrhythmias in about one-third of the dogs that were previously resistant to malignant arrhythmias. Given the inconsistent benefits reported in clinical and experimental studies and the potential adverse actions on cardiac rhythm noted in the
present study, recommendations to use n-3 PUFA in the post-MI patient should be reconsidered.

Acknowledgments

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Disclosures

None.

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**CLINICAL PERSPECTIVE**

Although epidemiological data provide strong evidence for an inverse relationship between fatty fish consumption and cardiac mortality, interventional studies using omega-3 polyunsaturated fatty acids (n-3 PUFAs) for the secondary prevention of adverse cardiovascular events in patients recovering from myocardial infarction (MI) have yielded conflicting results. Despite an exploding consumer demand (as much as 10% of American adults use a fish oil supplement, with annual sales exceeding 7 billion dollars), the safety and efficacy of n-3 PUFAs supplements have not been determined. The present study evaluated the actions of the long-term ingestion of n-3 PUFAs on cardiac rhythm in animals at high risk and a low risk for arrhythmia development. There were 2 major findings that have important clinical implications. First, despite large increases in cardiac tissue n-3 PUFA concentration, n-3 PUFA treatment did not reduce life-threatening ventricular arrhythmias in post-MI dogs at high risk for ventricular fibrillation. Second, and contrary to current views, n-3 PUFA treatment significantly increased the susceptibility to malignant arrhythmias in low-risk dogs, inducing ventricular tachyarrhythmias in one-third of these post-MI animals and provoking spontaneous death in 2 of 7 noninfarcted dogs. This latter observation is particularly noteworthy, as these are the only noninfarcted dogs that have died suddenly after thoracic surgery in our laboratories during the last 35 years (none of 195). Thus, given the lack of benefit and the potential adverse actions on cardiac rhythm noted in the present study, recommendations to use n-3 PUFA in the post-MI patient should be reconsidered.
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**SUPPLEMENTAL MATERIAL**

**Table 1.** Red blood cell omega-3 polyunsaturated fatty acid content

<table>
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<th>EPA</th>
<th></th>
<th>EPA</th>
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<th>Omega-3 Index</th>
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<tbody>
<tr>
<td></td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
<td>Pre</td>
<td>Post</td>
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<tr>
<td><strong>Placebo</strong></td>
<td>0.16 ± 0.03</td>
<td>0.20 ± 0.03</td>
<td>0.25 ± 0.04</td>
<td>0.23 ± 0.04</td>
<td>0.40 ± 0.05</td>
<td>0.44 ± 0.06</td>
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<tr>
<td><strong>1 g/day</strong></td>
<td>0.23 ± 0.05</td>
<td>1.22 ± 0.14*#</td>
<td>0.17 ± 0.02</td>
<td>1.86 ± 0.17*#</td>
<td>0.40 ± 0.04</td>
<td>3.02 ± 0.23*#</td>
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<td>(n = 7)</td>
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<tr>
<td><strong>2 g/day</strong></td>
<td>0.16 ± 0.02</td>
<td>1.66 ± 0.12*#</td>
<td>0.27 ± 0.08</td>
<td>2.01 ± 0.13*#</td>
<td>0.55 ± 0.12</td>
<td>3.72 ± 0.22*#</td>
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<tr>
<td>(n = 12)</td>
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<tr>
<td><strong>4 g/day</strong></td>
<td>0.20 ± 0.01</td>
<td>3.91 ± 0.19*#</td>
<td>0.23 ± 0.02</td>
<td>2.83 ± 0.15*#</td>
<td>0.42 ± 0.03</td>
<td>6.76 ± 0.28*#</td>
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<tr>
<td>(n = 29)</td>
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</table>

All values as are mean ± SE and are expressed as % total lipid content; EPA = eicosapentaenoic acid; DHA = docosahexaenoic acid; omega-3 index = EPA + DHA * P<0.01 Pre vs. Post; # = P<0.01 omega-3 dose vs. placebo.

**ANOVA results**

EPA: Dose $F_{3/57} = 84.79$, $P<10^{-6}$; Pre-post, $F_{1/57} = 196.67$, $P<10^{-6}$; Dose x Pre-post interaction, $F_{3/57} = 81.07$, $P<10^{-6}$

DHA: Dose $F_{3/57} = 52.64$, $P<10^{-6}$; Pre-post, $F_{1/57} = 228.30$, $P<10^{-6}$; Dose x Pre-post interaction, $F_{3/57} = 42.25$, $P<10^{-6}$

Omega-3 Index: Dose $F_{3/57} = 101.74$, $P<10^{-6}$; Pre-post, $F_{1/57} = 322.22$, $P<10^{-6}$; Dose x Pre-post interaction, $F_{3/57} = 91.27$, $P<10^{-6}$
**Table 2.** Cardiac tissue omega-3 polyunsaturated fatty acid content

<table>
<thead>
<tr>
<th></th>
<th>EPA</th>
<th>DHA</th>
<th>Omega-3 Index</th>
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<tbody>
<tr>
<td><strong>Right Atrium</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (n = 12)</td>
<td>0.22 ± 0.08</td>
<td>0.38 ± 0.11</td>
<td>0.58 ± 0.16</td>
</tr>
<tr>
<td>1 g/day (n = 7)</td>
<td>1.20 ± 0.10*</td>
<td>3.26 ± 0.21*</td>
<td>4.46 ± 0.22*</td>
</tr>
<tr>
<td>2 g/day (n = 12)</td>
<td>1.45 ± 0.23*</td>
<td>2.89 ± 0.41*</td>
<td>4.34 ± 0.64*</td>
</tr>
<tr>
<td>4 g/day (n = 29)</td>
<td>2.10 ± 0.28*</td>
<td>2.71 ± 0.33*</td>
<td>4.80 ± 0.59*</td>
</tr>
<tr>
<td><strong>Left Ventricle</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Placebo (n = 12)</td>
<td>0.23 ± 0.04</td>
<td>0.36 ± 0.07</td>
<td>0.59 ± 0.09</td>
</tr>
<tr>
<td>1 g/day (n = 7)</td>
<td>1.22 ± 0.08*</td>
<td>2.92 ± 0.21*</td>
<td>4.14 ± 0.25*</td>
</tr>
<tr>
<td>2 g/day (n = 12)</td>
<td>1.69 ± 0.20*</td>
<td>2.67 ± 0.21*</td>
<td>4.35 ± 0.39*</td>
</tr>
<tr>
<td>4 g/day (n = 29)</td>
<td>3.25 ± 0.24*</td>
<td>2.99 ± 0.17*</td>
<td>6.24 ± 0.39*</td>
</tr>
</tbody>
</table>

All values are as mean ± SE and are expressed as % total lipid content; EPA = eicosapentaenoic acid; DHA = docasahexaenoic acid; omega-3 index = EPA + DHA

* P<0.01 omega-3 dose vs. placebo.

**ANOVA results**
Right Atrium:
EPA, \( F_{3/56} = 7.83, P = 0.000188 \); DHA \( F_{3/56} = 9.51, P = 0.000036 \); Omega-3 Index: \( F_{3/56} = 8.58, P = 0.000089 \)

Left Ventricle:
EPA, \( F_{3/56} = 30.80, P<0.10^{-6} \); DHA, \( F_{3/56} = 36.46, P<10^{-6} \); Omega-3 Index, \( F_{3/56} = 34.52, P<10^{-6} \)