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# Omega-3 Fatty Acids in Boxer Dogs with Arrhythmogenic Right Ventricular Cardiomyopathy

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**Background:** Omega-3 fatty acids have been shown to reduce arrhythmia in animal models and people. These effects have not been studied in dogs with spontaneously occurring arrhythmia.

**Hypothesis:** Fish oil will reduce the frequency of ventricular arrhythmia in Boxer dogs with arrhythmogenic right ventricular cardiomyopathy (ARVC).

**Animals:** Twenty-four Boxers with ARVC were included in this study.

**Methods:** Asymptomatic Boxers not receiving antiarrhythmic medications were evaluated with echocardiogram and electrocardiogram. Dogs with at least 1 ventricular premature contraction (VPC) received 24-hour ambulatory electrocardiography (AECG) recordings. Dogs with >95 VPCs in 24 hours were randomized to 1 of 3 treatments: (1) Fish oil, 2 g; (2) Flax oil, 2 g; or (3) sunflower oil, 2 g (Control group), for 6 weeks. Investigators and owners were blinded to the treatment groups. All baseline measurements were repeated after the 6-week supplementation.

**Results:** There were no differences at baseline for age, sex, blood pressure, weight, echocardiographic measurements, or VPCs. Median number of VPCs in 24 hours for all dogs was 543 (range, 96–40,063) at baseline and 193 (range, 6–14,825) after 6 weeks of supplementation. VPCs/24 h were reduced for the Fish oil group (baseline median = 397 [range, 249–10,587]; 6-week median = 162 [range, 16–3,781]; $P = .02$), but not for the Flax oil ($P = .58$) or Control ($P = .48$) groups.

**Conclusions and Clinical Importance:** These data suggest that fish oil, but not flax oil, supplementation for 6 weeks reduces arrhythmia in Boxers with ARVC and that it could be useful in treating this common disease. Further studies are needed to determine optimal dose and duration of treatment.

**Key words:** Arrhythmia; Cardiac disease; Dietary supplements; Nutrition; Ventricular premature contraction.

Ventricular arrhythmias are a common and often fatal complication in dogs with cardiomyopathy, including the form called arrhythmogenic right ventricular cardiomyopathy (ARVC), which is common in Boxers and also occurs in people. The exact incidence of ARVC in Boxers is unknown, but arrhythmias caused by this form of cardiomyopathy have been observed in >36% of healthy Boxers over 9 months old.[1] In 1 study of 23 Boxers with ARVC, mortality due to sudden cardiac death was 39%.[2] Routine electrocardiography is insensitive for the detection of ventricular arrhythmias in Boxers compared with 24-hour ambulatory electrocardiography (AECG), which provides a more sensitive means of identifying affected dogs.[1] While the frequency of ventricular premature contractions (VPC) which establishes a definitive diagnosis of ARVC is not absolutely defined, Boxers with ARVC generally have >50 VPCs in 24 hours.[3,4]

Histopathologic analysis of the hearts of Boxers that died as a result of ARVC consistently demonstrates cardiomyocyte loss and replacement with adipose cells (fatty form) or adipose and fibrous tissue (fibro-fatty form).[2] The relationship between these myocardial changes and metabolic changes in Boxers with ARVC is unknown, although dogs with dilated cardiomyopathy have low plasma concentrations of the 2 major omega-3 fatty acids, eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA).[5]

Cardiomyopathies appear to have a heritable basis, with an autosomal dominant mode of inheritance likely for ARVC in Boxer dogs,[3] but few studies have investigated the manner and extent to which environmental factors might influence the expression or progression of cardiomyopathies or ventricular arrhythmias in dogs. In people it is clear that dietary and other environmental factors play a very large role in the development and progression of cardiac disease. Early observational studies reporting an inverse relationship between dietary factors such as fish intake and coronary heart disease mortality in people led to investigations of a possible cardioprotective role of long chain omega-3 fatty acids (EPA and DHA), the predominant fatty acids in fish.[6] A prospective, case-control analysis of 94 healthy men demonstrated a significant, inverse relationship between whole blood omega-3 fatty acid concentrations and sudden death from cardiac causes.[7]

Clinical trials in people further support the hypothesis that while the cardioprotective effects of omega-3 fatty acids are probably multi-factorial, their antiarrhythmic effects are likely to be primary. In the GISSI Prevenzione trial, supplementation with EPA and DHA of 11,324 survivors of recent myocardial infarction reduced total mortality and sudden cardiac death.[8] Time-course analysis of the GISSI mortality data revealed an early protective effect that was attributed by investigators to antiarrhythmic rather than to anti-atherosclerotic effects of omega-3 fatty acids.[9] Omega-3 fatty acid effects on ventricular arrhythmias were measured directly in

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a double-blind, placebo-controlled trial of 68 human patients with moderate to low grade spontaneous VPC.[10] Dietary supplementation with fish oil was associated with reductions of >70% of VPCs occurring in a 24-hour period in 44% of these patients.[10]

In addition to the antiarrhythmic effects associated with dietary intake of omega-3 fatty acids, intravenous infusions of omega-3 fatty acids have also been associated with reduced arrhythmias in 2 species.[11,12] In dogs with ischemia-induced arrhythmia, the infusion of 3 individual omega-3 fatty acids, DHA, EPA, or alpha-linolenic acid (ALA; a plant-based omega-3 fatty acid), prevented arrhythmias and sudden death compared with placebo infusions.[11] In a pilot study designed to test the safety of omega-3 fatty acid infusion in people, infusions of marine-derived omega-3 fatty acids prevented the induction of sustained ventricular tachycardia in 5 of 7 individuals with documented spontaneously occurring ventricular tachycardia.[12] While this study is limited because of its lack of controls and very small sample size, it suggests that omega-3 fatty acid infusions may be antiarrhythmic in people with preexisting arrhythmias.

In spite of the large body of evidence supporting antiarrhythmic activity of omega-3 fatty acids in experimental animals, human patients, and individual cardiomyocytes, the effects of omega-3 fatty acids in dogs with naturally occurring ventricular arrhythmia are unknown. The goal of this study was to determine whether supplements containing ALA or a DHA/EPA combination could reduce the number of VPCs in asymptomatic Boxers with documented ventricular arrhythmia.

## Materials and Methods

### *Subjects*

Boxer dogs recruited from area breed clubs and from hospital clients were evaluated for possible enrollment in the study. Dogs with symptomatic heart disease, concurrent major diseases, left ventricular outflow tract velocity >3 m/s, and those receiving antiarrhythmic medication were excluded. Dogs with dietary intakes of total omega-3 fatty acids exceeding 1.5 g per day were excluded unless they were switched to a diet providing less than 1.5 g per day for a washout period of at least 7 weeks. Dogs taking supplements containing fatty acids were excluded unless the owner discontinued the supplement for at least 7 weeks before undergoing cardiac evaluation. The study was approved by the Tufts University Animal Care and Use Committee, and owners signed a consent form before enrolling their dogs in the study.

### *Study Design*

Dogs were fasted for 8 hours before the first visit. Detailed diet and clinical histories were obtained for each dog. A cardiac examination and baseline echocardiogram (2D, M-mode, and color-flow Doppler) were performed to measure chamber dimensions and cardiac function. Continuous-wave and pulsed-wave Doppler echocardiography was used to measure aortic and pulmonary outflow tract velocities. All echocardiograms were performed by the same cardiologist (JER). A 6-lead electrocardiogram (ECG) of 2 minutes duration was performed. For dogs demonstrating at least 1 VPC during the echocardiogram or during the ECG, further testing was performed. Dogs without arrhythmia were not studied further unless they had documentation of prior arrhythmia or a close relative diagnosed with ARVC. Further testing consisted of blood pressure measured by Doppler technique and the average of 3 systolic measurements recorded, blood collection for a biochemistry profile and fatty acid analysis, and 24-hour AECG recording. Serum for fatty acid analysis was centrifuged, separated, and frozen at −80°C until analysis. Dogs returned 24 hours later for 24-hour AECG recorder[a] removal, and recordings were analyzed by software[b] to obtain VPC frequency and average heart rate. AECG recordings included at least 20 hours of recording, and baseline and postintervention analyses for each subject were performed by the same AECG technician. Arrhythmia severity was categorized into 4 grades based on the modified Lown criteria: 1 (single uniform VPC), 2 (bigeminy, trigeminy, or both), 3 (ventricular couplets, triplets, or both), or 4 (R on T, ventricular tachycardia, or both).[13] Dogs with ≥95VPCs/24 h were eligible for enrollment in the dietary intervention trial.

**Table 1.** Composition of the 3 fatty acid supplements administered to Boxers for 6 weeks (in milligrams/capsule). Each dog received 2 capsules daily.

| Fatty Acid | Sunflower Oil (Control) | Flax Oil | Fish Oil |
|---|---|---|---|
| c18:1, n-9 | 251.3 | 180.9 | 0 |
| c18:2, n-6 | 666.8 | 156.6 | 0 |
| c18:3, n-3 | 0 | 562.1 | 0 |
| c20:5, n-3 | 0 | 0 | 390.0 |
| c22:6, n-3 | 0 | 0 | 248.5 |

After baseline measurements, eligible dogs were randomized to either the Fish oil (EPA/DHA), Flax oil (ALA), or Control group (Table 1). Randomization was performed by means of a computer-generated randomization table with stratification of dogs having >10,000 VPCs/24 h into each of the fatty acid groups. Dogs each were administered two 1-g capsules per day. Dogs in the Fish oil group received 780 mg EPA and 497 mg DHA per day. Dogs in the Flax oil group received 1124 mg of ALA/day, and dogs in the Control group received 2 g of sunflower oil/day. The dose of fish oil was based on a previous study of omega-3 fatty acids in dogs with dilated cardiomyopathy.[5] An identical number of capsules was provided for the Flax oil and Control groups; this provided a similar intake of omega-3 fatty acids to the Flax oil group compared with the Fish oil group. Investigators and owners were blinded to the capsule formulation until the entire study was completed. Owners were instructed to maintain the baseline diet for the duration of the study and were contacted by phone after 3 weeks for follow-up.

After 6 weeks of fatty acid administration, dogs returned after an 8-hour fast for repeat measurements, including a serum biochemistry profile and fatty acid analysis, echocardiogram, 2-minute ECG, blood pressure, and 24-hour AECG. Compliance was assessed by capsule counts and by serum fatty acid changes. Ambulatory ECG recordings were analyzed and postintervention VPC frequency, average heart rate, and arrhythmia severity grade were compared with baseline values.

### *Lipid Extraction*

Total phospholipid fatty acid assessments were analyzed at Lipid Technologies (Austin, MN). Plasma lipids were extracted according to the method of Bligh and Dyer,[14] whereby mixtures of plasma, methanol, chloroform, and water are prepared such that lipid is recovered in a chloroform layer. Trace amounts of butylated hydroxyl toluene (0.05%) were added to the chloroform to prevent oxidation of polyunsaturated fatty acids. The resulting

lipid extracts were maintained under an atmosphere of nitrogen after extraction and kept frozen before additional processing.

### *Separation of Lipid Classes*

Immediately before lipid class separation, lipid samples were dried under a gentle stream of nitrogen and rediluted in 50 μL of chloroform and prepared for lipid class separation. Lipid classes including total phospholipid, nonesterified fatty acids, triglyceride, and cholesterol esters were separated on commercial silica gel G plates.[c] The chromatographic plates were developed in a solvent system consisting of distilled petroleum-ether (30–60°C):diethyl-ether:acetic acid (80:20:1, by volume). After development, the silica gel plates were sprayed with a methanolic solution containing 0.5% 2,7-dichlorofluorescein, which was then used to visualize lipid classes under ultraviolet light. Desired corresponding lipid bands were then scraped into screw-cap tubes. The samples were then transesterified with boron trifluoride (12%) in methanol[d] in an 80°C water bath for 90 minutes. Resulting fatty acid methyl esters were extracted with water and petroleum ether and stored frozen until gas chromatographic analysis was performed.

### *Fatty Acid Analysis*

Lipid class fatty acid methyl ester composition was determined by capillary gas chromatography. Methyl ester samples were blown to dryness under nitrogen and resuspended in heptane containing methyl-tridecanoic acid[e] as an internal standard. Resulting fatty acid methyl esters were separated and quantified with a gas chromatograph[f] with a 30-m Restek free fatty acid phase (FFAP) coating and commercial software.[g] The instrument temperature was programmed from 190–240°C at 7°C/min with a final hold of 10 minutes, separating and measuring fatty acid methyl esters ranging from 12:0 to 24:1. The detector temperature was 250°C. Helium carrier gas was used at a flow rate of 1.4 mL/min and a split ratio of 1:25. Chromatographic data were collected and processed with commercial software.[g] Individual peaks, representing as little as 0.05% of the fatty acid methyl esters, were distinguished.

Fatty acids were identified by comparison to authentic fatty acid standards and quantitated with peak area and internal standard. Resulting data are expressed as percentage composition.

### *Statistical Analysis*

Data are reported as median (range). Data were examined graphically; data that were not normally distributed were transformed before analysis for baseline comparisons among the groups. Baseline comparisons between the Fish oil, Flax oil, and Control groups were made by analysis of variance (ANOVA) with Tukey's post hoc test. The changes in variables (eg, blood pressure, mean heart rate, fatty acids) from baseline to follow-up within each of the Fish oil, Flax oil, and Control groups were compared with Wilcoxon Signed Ranks test. Because of the large variation in VPC frequency, the percentage change in VPC frequency was also compared. Correlations between fatty acid concentrations and VPC frequency were calculated by the Spearman correlation coefficient. Commercial statistical software was used for analysis.[h] $P$ values $<.05$ were considered significant.

## Results

A total of 91 Boxers were screened for enrollment in the study. Of these 91 dogs, 42 received a 24-hour AECG based on at least 1 VPC observed during the echocardiogram or ECG or a history of previously documented arrhythmia. Of these 42, 15 did not qualify for enrollment in the fatty acid trial because of an insufficient number of VPCs over 24 hours (<95). Of the 27 dogs that did qualify for enrollment, 24 completed the study. One dog was not enrolled in the fatty acid trial because the owner decided to initiate antiarrhythmic medication, which disqualified the dog from enrollment. A second dog began fatty acid supplementation but was withdrawn from the study and replaced after the owner elected to initiate antiarrhythmic medication during week 5 of the supplementation phase of the study, although the dog remained asymptomatic. A third dog was withdrawn from the study after enrollment and replaced because of preputial hemorrhage and subsequent anesthesia and surgical removal of a penile plasmacytoma during the final week of the supplementation phase.

At baseline, dogs were not different in weight, body condition score, blood pressure, heart rate, VPC number, or Lown grade (Table 2). Total cholesterol was higher in the Flax oil group than in the Control ($P$ = .04) or Fish oil ($P$ = .01) groups. Palmitic acid was higher in the Control group than in the Flax or Fish oil groups ($P < .001$). Arachidonic acid was higher in the Flax oil group than in the Control group ($P$ = .005). The arachidonic acid:EPA ratio was higher in the Fish oil group than in the Control group ($P$ = .04). Echocardiographic measurements at baseline were not different among the groups.

Compliance with fatty acid supplementation was based on capsule count (median = 98%; range = 84–100%) and fatty acid changes for the 24 dogs that completed the study. Comparisons of the fatty acid changes from the baseline to follow-up visit within each of the 3 groups revealed changes consistent with the supplement administered (Table 3). The Fish oil group had significant increases in EPA ($P$ = .01), docosapentaenoic acid ($P$ = .02), DHA ($P$ = .02), and total omega-3 fatty acids ($P$ = .01) and significant decreases in arachidonic acid ($P$ = .02), docosatetraenoic acid ($P$ = .01), total omega-6 fatty acids ($P$ = .01), omega-6:omega-3 ratio ($P$ = .01), and the arachidonic acid:EPA ratio ($P$ = .01; Table 3). The Flax oil group had a significant increase in EPA ($P$ = .046) and a significant decrease in docosatetraenoic acid ($P$ = .02; Table 3). The Control group had only a significant decrease in docosapentaenoic acid ($P$ = .01; Table 3). Within-group comparison did not show significant changes in weight, blood pressure, heart rate, number of dogs with a VPC reduction or increase of >85%, or Lown grade (Table 3). Although the number of dogs that had a decrease in VPC number was not different between groups (6/8 in the Flax oil and Control groups versus 7/8 in the Fish oil group), only the Fish oil group had a significant reduction in VPC number ($P$ = .02; Fig 1; Table 3). The number of VPCs that occurred during the 2-minute ECG was also significantly reduced in the Fish oil group ($P$ = .04; Table 3), but not in the Flax oil or Control groups. There were no within-group changes in echocardiographic measurements in any of the groups (data not shown).

Significant correlations between plasma fatty acid concentrations after supplementation and percentage

**Table 2.** Baseline characteristics of dogs receiving Control, Flax oil, or Fish oil supplements. All data are presented as median (range).

| | Control (n = 8) | Flax Oil (n = 8) | Fish Oil (n = 8) | *P* Value |
|---|---|---|---|---|
| Age | 6.6 (4.5–9.3) | 6.6 (2.1–9.8) | 6.1 (4.2–9.3) | .95 |
| Weight (kg) | 30.6 (23.6–40.2) | 29.9 (21.8–31.8) | 28.0 (23.6–35.6) | .60 |
| Body condition score (1–9) | 6.0 (5.0–7.5) | 5.8 (5.0–6.0) | 5.75 (5.0–7.0) | .82 |
| Blood pressure (systolic) | 142 (135–167) | 146 (120–157) | 135 (118–148) | .17 |
| Heart rate (per min)[a] | 80 (69–85) | 82 (64–94) | 81 (60–93) | .99 |
| VPC number during 2-minute ECG | 2 (0–11) | 9 (0–144) | 4 (0–396) | .58 |
| VPC number in 24 hours | 397 (249–10,587) | 794 (96–8,439) | 1400 (131–40,063) | .72 |
| Lown grade | 3 (1–4) | 3 (1–4) | 3 (1–4) | .70 |
| **Lipids** | | | | |
| Total cholesterol (mg/dL) | 246 (178–288)[b] | 281 (232–387)[c] | 224 (193–281)[b] | .009 |
| Triglycerides (mg/dL) | 30 (27–48) | 36 (28–54) | 36 (28–61) | .23 |
| Palmitic acid (C16:0,%) | 15.13 (13.55–16.56)[b] | 12.96 (12.38–14.21)[c] | 13.44 (12.62–14.25)[c] | <.001 |
| Stearic acid (C18:0,%) | 25.52 (23.64–26.97) | 27.21 (19.65–27.87) | 26.37 (24.58–28.83) | .56 |
| Oleic acid (C18:1 n-9,%) | 6.79 (6.02–8.18) | 6.26 (5.27–6.63) | 7.09 (5.05–8.74) | .21 |
| Linoleic acid (C18:2 n-6,%) | 15.8 (14.4–20.8) | 13.3 (12.4–17.3) | 15.4 (11.7–17.3) | .10 |
| Alpha-linolenic acid (C18:3 n-3,%) | 0.15 (0.11–0.26) | 0.20 (0.05–0.24) | 0.17 (0.05–0.34) | .90 |
| Gamma-linolenic acid (C18:3 n-6,%) | 0.19 (0.14–0.24) | 0.24 (0.15–0.28) | 0.20 (0.15–0.25) | .10 |
| Arachidonic acid (C20:4 n-6,%) | 20.9 (19.7–24.5)[b] | 24.5 (22.1–26.1)[c] | 23.9 (19.2–25.9)[b,c] | .006 |
| Eicosapentaenoic acid (C20:5 n-3,%) | 0.82 (0.23–1.26) | 0.52 (0.18–0.88) | 0.25 (0.17–0.80) | .06 |
| Docosatetraenoic acid (C22:4 n-6,%) | 0.89 (0.66–2.42) | 0.99 (0.71–2.80) | 1.37 (0.65–2.41) | .56 |
| Docosapentaenoic acid (C22:5 n-3,%) | 2.03 (1.52–3.21) | 2.70 (1.85–2.94) | 2.05 (1.05–3.74) | .39 |
| Docosahexaenoic acid (C22:6 n-3,%) | 2.15 (0.49–3.99) | 2.20 (0.72–3.88) | 0.96 (0.32–3.28) | .35 |
| Total n-3 fatty acids (%) | 5.45 (3.24–8.09) | 5.21 (4.00–8.01) | 4.35 (1.96–6.84) | .24 |
| Total n-6 fatty acids (%) | 39.7 (38.9–44.7) | 41.4 (37.9–45.3) | 41.9 (38.8–44.5) | .42 |
| Omega-6:omega-3 fatty acid ratio | 7.33 (4.84–13.23) | 7.82 (4.73–11.33) | 9.94 (5.88–22.65) | .17 |
| Arachidonic acid:EPA ratio | 25.3 (16.5–107)[b] | 48.2 (25.0–132)[b,c] | 94.6 (25.3–153)[c] | .04 |
| **Echocardiographic measurements** | | | | |
| Left atrium-standard (cm) | 2.70 (2.07–3.22) | 2.44 (2.29–2.75) | 2.47 (2.06–2.94) | .34 |
| Left atrium-2 dimensional (cm) | 3.45 (3.03–3.86) | 3.61 (3.30–3.96) | 3.49 (2.93–3.89) | .31 |
| Aorta (cm) | 2.12 (1.95–2.44) | 2.16 (1.82–2.50) | 2.17 (1.95–2.42) | .84 |
| Right ventricle-diastole (cm) | 0.42 (0.23–1.01) | 0.54 (0.21–1.21) | 0.46 (0.30–0.94) | .93 |
| Interventricular septum-systole (cm) | 1.48 (1.17–2.36) | 1.46 (1.24–2.13) | 1.65 (0.81–1.82) | .94 |
| Interventricular septum-diastole (cm) | 1.17 (0.90–1.52) | 1.16 (0.93–1.40) | 1.27 (0.83–1.51) | .79 |
| LVIDd (cm) | 4.01 (3.60–4.30) | 3.99 (3.53–4.24) | 3.91 (3.66–4.04) | .88 |
| LVIDs (cm) | 2.49 (2.27–2.93) | 2.75 (2.25–3.17) | 2.56 (2.09–3.14) | .62 |
| Left ventricular free wall-diastole (cm) | 1.23 (1.07–1.40) | 1.18 (0.95–1.30) | 1.26 (0.96–1.31) | .59 |
| Left ventricular free wall-systole (cm) | 1.74 (1.53–2.00) | 1.63 (1.29–1.82) | 1.71 (1.22–1.79) | .28 |
| Fractional shortening (%) | 38 (24–43) | 32 (23–36) | 33 (21–44) | .58 |

VPC, ventricular premature contraction; ECG, electrocardiogram; LVIDd, left ventricular internal dimension in diastole; LVIDs, left ventricular internal dimension in systole.

[a] Heart rate is the average heart rate obtained from the 24-hour ambulatory electrocardiography recording.

[b] Values with different superscripts are significantly different from one another ($P < .05$).

change in VPC frequency were present only in the Fish oil group. EPA and total omega-3 concentrations after supplementation were negatively correlated with percentage change in VPC frequency ($r = -.881$; $P = .004$ and $r = -.833$; $P = .01$, respectively; Fig 2). Conversely, the ratio of the omega-6 fatty acid arachidonic acid to EPA and the omega-6:omega-3 ratio after supplementation in the Fish oil group were positively correlated with percentage change in VPC frequency ($r = .905$; $P = .002$ and $r = .786$; $P = .02$, respectively).

## Discussion

This study demonstrates that administration of fish oil providing 780 mg/d EPA and 497 mg/d DHA per day is associated with decreased ventricular arrhythmia in Boxers with ARVC. Two human trials, with similar design to the current study, investigated the effects of EPA/DHA combinations on ventricular arrhythmia.[10,15] In the first study, a double-blinded, placebo-controlled trial of 68 people with a minimum of 2000 VPCs/24 h, fish oil supplementation reduced VPC number by >70% in 44% of subjects but the reduction compared with placebo did not reach significance ($P = .052$).[10] A second trial evaluating the effect of fish oil on 402 people with implantable cardioverter/defibrillators gave similar results ($P = .057$) for reduction in ventricular tachycardia or ventricular fibrillation events that triggered device discharges.[15] Poor compliance and technical complications may have affected the primary endpoints.[15] In this same study, a significant reduction ($P = .006$) in the risk of sudden cardiac death was observed in patients supplemented with fish oil who completed the 11-month treatment.[15] The current study did not evaluate mortal-

**Table 3.** Comparison of changes from baseline visit to follow-up visit in dogs receiving Control, Flax oil, or Fish oil supplement. All data are presented as median (range).

| | Control (n = 8) | Flax Oil (n = 8) | Fish Oil (n = 8) |
|---|---|---|---|
| Weight (kg) | −0.3 (−2.5 to 1.2) | 0.1 (−0.5 to 1.3) | 0.2 (−2.2 to 1.0) |
| Blood pressure (systolic) | −5 (−37 to 10) | 1 (−14 to 15) | 7 (−16 to 13) |
| Heart rate (per min)[a] | −3 (−18 to 4) | 1 (−10 to 12) | −3 (−10 to 3) |
| VPC number during 2-minute ECG | 1 (−6 to 19) | −4 (−16 to 12) | −4 (−371 to 1)[b] |
| VPC number in 24 hours | −181 (−7,352 to 1527) | −146 (−4,901 to 14,266) | −787 (−36,963 to 75)[b] |
| No. of dogs with VPC reductions of >85% | 1/8 | 4/8 | 3/8 |
| No. of dogs with VPC increases of >85% | 1/8 | 1/8 | 0/8 |
| VPC percentage change | −53 (−97 to 141) | −84 (−99 to 2552) | −68 (−98 to 55) |
| Lown grade | 0 (−2 to 2) | 0 (−2 to 1) | 0 (−2 to 0) |
| **Lipids** | | | |
| Total cholesterol (mg/dL) | 12 (−43 to 42) | 1 (−48 to 20) | 6 (−7 to 34) |
| Triglycerides (mg/dL) | 2 (−2 to 29) | 3 (−12 to 20) | −2 (−6 to 10) |
| Palmitic acid (C16:0,%) | −0.095 (−3.02 to 0.85) | 0.28 (−0.99 to 8.17) | 0.00 (−0.98 to 2.07) |
| Stearic acid (C18:0,%) | −0.01 (−1.07 to 4.34) | 0.08 (−7.43 to 4.77) | 0.18 (−3.69 to 1.09) |
| Oleic acid (C18:1,%) | −0.01 (−0.46 to 1.23) | 0.13 (−0.72 to 1.58) | −1.04 (−1.85 to 1.51) |
| Linoleic acid (C18:2 n-6,%) | 1.04 (−5.87 to 3.54) | 1.92 (−1.83 to 11.45) | −1.23 (−2.33 to 4.19) |
| Alpha-linolenic acid (C18:3 n-3,%) | 0.005 (−0.16 to 0.05) | 0.08 (−0.05 to 0.48) | 0.00 (−0.15 to 0.31) |
| Gamma-linolenic acid (C18:3 n-6,%) | 0.02 (−0.10 to 0.09) | −0.03 (−0.09 to 0.03) | 0.05 (−0.05 to 0.17) |
| Arachidonic acid (C20:4 n-6,%) | −0.84 (−2.50 to 4.09) | −2.1 (−14.24 to 1.34) | −3.42 (−5.82 to 0.95)[b] |
| Eicosapentaenoic acid (C20:5 n-3,%) | −0.12 (−0.37 to 0.30) | 0.04 (−0.01 to 3.01)[b] | 1.3 (0.71 to 3.05)[b] |
| Docosatetraenoic acid (C22:4 n-6,%) | −0.04 (−1.82 to 0.09) | −0.18 (−0.79 to 0.00)[b] | −0.93 (−1.44 to −0.27)[b] |
| Docosapentaenoic acid (C22:5 n-3,%) | −0.15 (−1.2 to −0.09)[b] | 0.01 (−0.41 to 0.21) | 0.87 (−0.50 to 1.96)[b] |
| Docosahexaenoic acid (C22:6 n-3,%) | −0.12 (−1.14 to 2.35) | −0.19 (−1.13 to −0.48) | 2.59 (−0.23 to 3.25)[b] |
| Total omega-3 fatty acids (%) | −0.44 (−2.70 to 1.76) | 0.14 (−0.59 to 2.47) | 5.09 (1.44 to 6.73)[b] |
| Total omega-6 fatty acids (%) | 0.55 (−4.13 to 3.03) | −0.34 (−5.64 to 1.85) | −4.42 (−7.34 to −1.89)[b] |
| Omega-6:omega-3 fatty acid ratio | 0.58 (−4.74 to 4.07) | −0.41 (−2.42 to 1.93) | −5.52 (−18.22 to −1.25)[b] |
| Arachidonic acid:EPA ratio | 2.84 (−11.9 to 27.9) | −7.71 (−51.8 to 6.69) | −87.2 (−139 to −13.2)[b] |

VPC, ventricular premature contraction; ECG, electrocardiogram; EPA, eicosapentaenoic acid.

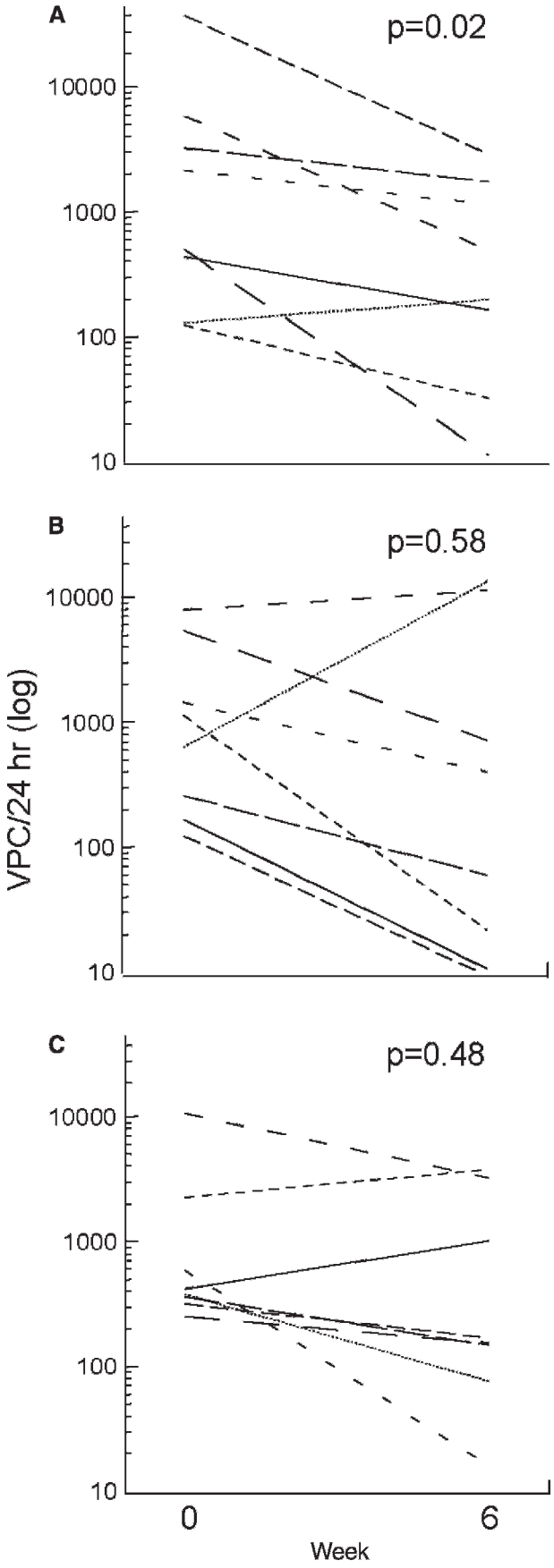
[a] Heart rate is the average heart rate obtained from the 24-hour ambulatory electrocardiography recording.

[b] Indicates a within-group change from baseline visit to follow-up visit.

ity, and reduction in VPC number may not necessarily equate to reduced mortality in Boxers with ARVC.

The correlations between EPA and total omega-3 fatty acid plasma concentrations and changes in VPC frequency in the Fish oil group, but not in the Control or Flax oil groups, suggest that a particular threshold of omega-3 fatty acids may need to be reached in order for changes in arrhythmia frequency to become detectable, although the spontaneous day-to-day variability in VPC number also may contribute to the finding that only 7 of 8 dogs in the Fish oil group had a reduction in VPC number. Further, these correlations support the existence of a dose-response relationship. In people, a supplement providing 0.9 g/d EPA and 1.5 g/d DHA significantly reduced VPCs.[10] Another trial that reported reduced atrial fibrillation in people used 2 g/d of EPA/DHA in a ratio of 1:2.[16] The large-scale GISSI trial, which attributed reduced cardiac mortality to antiarrhythmic effects, used supplements containing only 1 g/d of EPA/DHA in a ratio of 1:2, but baseline concentrations of dietary omega-3 fatty acids were not evaluated or controlled in this Mediterranean population.[9] The current study used capsules providing 2 g/d of fish oil in a 3:2 ratio providing a mean combined EPA/DHA dose of 45 mg/kg/d with a range of 36–54 mg/kg/d. Further studies are needed to evaluate the optimal dose and optimal ratio of EPA to DHA with respect to antiarrhythmic effects.

In addition to their antiarrhythmic properties, omega-3 fatty acids are associated with changes in other physiologic variables that were not observed in the current study. Changes in triglycerides, heart rate, blood pressure, and cholesterol are among the effects reported. In people, the hypotriglyceridemic effects are sufficiently established so that pharmaceutical forms of EPA/DHA (3.4 g/d) are used therapeutically as adjuncts to other lipid-lowering drugs.[17,18] Fish oil is also associated with reductions in heart rate in several species. In people, resting heart rate is decreased and heart rate recovery after exercise is improved,[19,20] and in horses heart rates during exercise are lower in those supplemented with fish oil.[21] Lower heart rates are cardioprotective and could reduce the risk of arrhythmia, although we did not observe this effect in our study. The effects on blood pressure and cholesterol in people are more variable and appear to be somewhat dependent on dosing. While significant reductions in blood pressure have been reported at a dose of 6 g/d EPA/DHA with no changes seen at doses as high as 3.6 g/d,[22] effects are generally more commonly observed in hypertensive subjects rather than in normal subjects.[23] Results on the effects on cholesterol concentrations in people are similarly




mixed. While many studies have reported negligible effects of EPA/DHA on cholesterol concentrations at doses <1 g/d in people,[24] a few have reported increases in low-density lipoprotein (LDL) or total cholesterol.[25,26] In horses, a dose of fish oil providing 60 mg/kg/d total omega-3 fatty acids was associated with decreased concentrations of cholesterol.[21] The extent to which differences in physiologic responses can be attributed to species differences, abnormalities in baseline parameters, and differences in dose or ratio of omega-3 fatty acids is unclear. While we did not observe additional effects of omega-3 fatty acids beyond VPC reduction, which might be beneficial to Boxers with ARVC, we did not see any changes that might be considered harmful to Boxers with this disease.

Mechanisms for the variety of effects attributed to omega-3 fatty acids are not completely understood, but their antiarrhythmic effects have been investigated with ventricular myocytes. The addition of micromolar concentrations of EPA or DHA to the media of spontaneously beating cardiomyocytes has been shown to reduce excitability by inhibiting voltage-activated $Na^+$ currents and voltage-gated L-type $Ca^{++}$ currents.[27,28] ALA reduced the spontaneous beating of myocytes as did DHA and EPA, but to a lesser degree.[29] Additional cellular studies have demonstrated that omega-3 fatty acids exert antiarrhythmic effects only while in their free, unesterified form and via partitioning into the plasma membrane, without covalent bonding to membrane phospholipids.[30]

The results of cellular studies have been extended by research with experimental animals in which evidence for a relationship between different types of dietary fatty acids (eg, saturated, monounsaturated, polyunsaturated) and arrhythmia has also been shown. In rats supplemented with varying types of fatty acids, the omega-3 fatty acids, ALA, DHA, and EPA reduced ischemia-induced and reperfusion-induced ventricular arrhythmias and associated mortality.[31,32] While both fish oil-derived DHA and EPA and plant-derived ALA were shown to have antiarrhythmic effects in the rats, a greater antiarrhythmic effect was seen with DHA and EPA than with ALA.[31,32] Marmoset monkeys supplemented with DHA and EPA were shown to have higher thresholds for current-induced ventricular fibrillation under both ischemic and nonischemic conditions.[33]

In the present study, supplementation with ALA was not associated with a statistically significant change in VPC number. Dietary ALA supplementation has been associated with antiarrhythmic effects[34] and reduced cardiac deaths[35] in people, and intravenous ALA has been associated with reduced fatal arrhythmia in dogs with ischemia-induced ventricular fibrillation.[11] People

←

**Fig 1.** Number of ventricular premature contractions (VPC) at baseline and after 6 weeks of supplementation of **(A)** fish oil, **(B)** flax oil, and **(C)** sunflower oil (Controls). Changes for individual dogs (n = 8) are shown for each of the groups. VPC number was significantly reduced only in the Fish oil group.

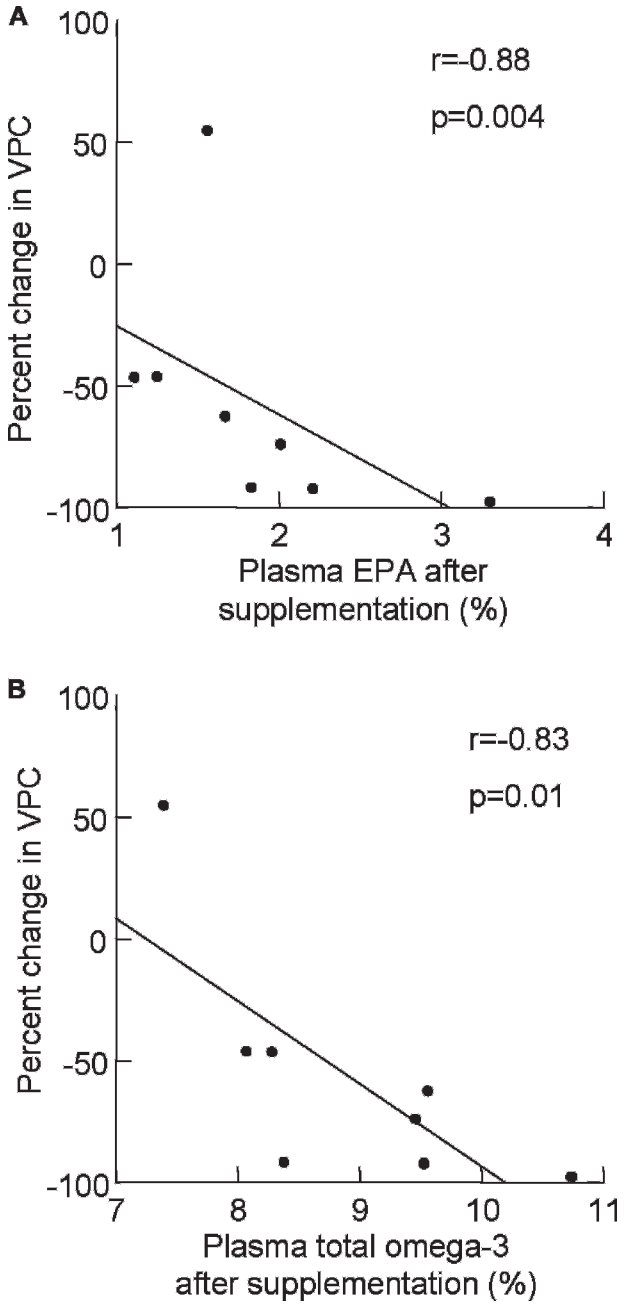



**Fig 2.** Spearman correlations in the Fish oil group (n = 8) between the percentage change in **(A)** plasma eicosapentaenoic acid (EPA) or **(B)** total omega-3 fatty acids after 6 weeks of supplementation and the percentage change in ventricular premature contraction (VPC) number.

surpass dogs in their capacity to convert ALA to the longer chain fatty acids EPA and DHA,[36,37] and the antiarrhythmic effects of ALA in people may be attributed to this conversion rather than to ALA directly. In the current study, dogs that received ALA demonstrated a significant increase in EPA concentrations (median presupplementation concentration of 0.52% versus median postsupplementation concentration of 0.72%, $P = .046$), but these postsupplementation concentrations were much lower than those in dogs that received fish oil supplementation (median presupplementation EPA concentration of 0.25% versus median postsupplementation concentration of 3.30%, $P = .01$). Our results are similar to those from an earlier study in which dogs supplemented with ALA demonstrated increased plasma cholesterol ester concentrations of EPA (from 0.1% ± 0.1% to 0.7% ± 0.3%) but did not show increased concentrations of DHA.[36]

In spite of the lack of statistical significance of VPC reduction after ALA supplementation, 4 of the 8 dogs (50%) in this group demonstrated a >85% reduction in VPC number and 2 of the 8 dogs had smaller reductions. Earlier studies have shown that in Boxers with arrhythmia, as in people, the rate of spontaneous day-to-day variability in ventricular arrhythmia frequency can be as high as 80–100%.[38] One of the criteria for judging an antiarrhythmic agent's effectiveness is its ability to reduce arrhythmia frequency by >85%.[13] Whether ALA could be directly antiarrhythmic, as intravenous ALA appears to be in dogs with induced arrhythmias,[11] or whether the relatively small amount of increase in EPA concentrations resulting from conversion of ALA may be responsible for some degree of antiarrhythmic action, is unknown. Of interest, similar to dogs in the Flax oil group, 6 of 8 dogs in the Control group had a numerical reduction in VPC number. However, only 1 dog in this group had an 85% reduction in VPCs (from 586 to 16 VPC/24 h) over the 6-week study.

One of the study's limitations is its small sample size, although its restriction to a single breed of dog reduced the number of animals needed in each group. Related to the small number of dogs is a relatively high variability in plasma fatty acid concentrations and potential variability in antiarrhythmic responses. Some of this variability could be genetically based; different lipid responses to fish oils have been documented among people with different ApoE polymorphisms.[39] Further, while dogs with diets containing >1.5 g of total omega-3 fatty acids were excluded from the study and owners were instructed to maintain a constant diet, many commercial diets contain some level of omega-3 supplementation, so baseline plasma fatty acid concentrations were variable. Repeating the study with a larger group of dogs eating a single type of dog food could produce less variable results. In addition, although significant changes in VPC frequency were seen in the Fish oil group, further study will be needed to determine optimal dose and whether a higher dose of ALA also might have a significant effect. There also is large day-to-day variability in VPC number, so additional studies with a larger number of dogs and multiple AECGs also would be useful. Most studies on AECGs, to date, have focused on day-to-day variation and whether the changes observed in VPC number in the current study over 6 weeks are to be expected (ie, an 85% reduction in VPC number in 1/8 dogs in the Control group) is not known. Although further research is needed in this area, the results of this study suggest that supplementation with omega-3 fatty acids from fish oil resulted in a significant reduction in VPC number in Boxers with ARVC.

## Footnotes

[a] Ambulatory electrocardiography (AECG) Aria recorder, Del Mar Reynolds Medical Inc, Irvine, CA
[b] Del Mar Holter Analysis System, Del Mar Reynolds Medical Inc, Irvine, CA
[c] Silica gel G plates, AnalTech, Newark, DE
[d] Methanol, Supelco, Bellefonte, PA
[e] Heptane containing methyl-tridecanoic acid, NuCheck Prep, Elysian, MN
[f] Gas chromatograph, model GC17, Shimadzu, Tokyo, Japan
[g] EZChrom software, Scientific Software, Pleasanton, CA
[h] Systat Version 10.0, SPSS, Chicago, IL

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