Effect of omega-3 fatty acids on ventricular action potentials in a canine model of sudden cardiac death

THESIS

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Abstract

Background: Sudden cardiac death (SCD) is the most common cause of death in the United States and in most developed countries, accounting for ~ 50% of total mortality. The most common underlying cause of SCD is “ventricular fibrillation” (VF). Several clinical trials have reported conflicting results on the benefits of omega-3 fatty acids for the prevention of lethal ventricular arrhythmias following infarction. The explanation for these inconsistent results remains to be determined.

Methods: Dogs with healed left ventricular anterior wall myocardial infarctions (MI, 3-4 weeks post-MI, n = 76) were subjected to an exercise and ischemia test to stratify animals by arrhythmia risk as VF susceptible, VF+ (n = 46) and VF resistant, VF− (n = 30). The animals were then assigned to omega-3 fatty acid ethyl esters (1-4 grams/day, n = 45) or corn-oil treatment (placebo, n = 31) for 3 months. Following treatment, arrhythmia inducibility was re-evaluated with the exercise and ischemia test. The left ventricular myocytes were isolated one week following the exercise plus ischemia test. Five age matched dogs served as controls (non-infarcted). Action potentials were recorded by perforated whole cell patch clamp studies (T= 36 ± 0.5°C) to measure the resting membrane potentials (RMP) and the action potential duration at 90% repolarization (APD$_{90}$).
Results: Omega-3 treatment resulted in either sudden death or a positive exercise plus ischemia test in 5 out of 15 (33.3%) VF resistant dogs (p<0.05 vs. placebo) whereas, 4 out of 30 (13.3%) omega-3 treated VF+ dogs no longer had VF at the end of the treatment period (p>0.05 vs. placebo). In addition, 5 out of 30 (16.7%) omega-3 treated dogs VF+ had SCD (p>0.05 vs. placebo). There was no significant differences in RMP following omega-3 treatment in both VF+ and VF- animals (p>0.05 vs. placebo). The APD$_{90}$ of myocytes of dogs protected from VF following omega-3 treatment was significantly shorter (p<0.05) compared to the myocytes from placebo-treated dogs and was similar to the APD$_{90}$ from the myocytes of normal control (non-infarcted) dogs at both 0.5 Hz and 1 Hz stimulation frequency. The longest APD$_{90}$ was observed in myocytes from VF- that developed arrhythmias after omega-3 treatment [VF(-,+)] compared to the myocytes from placebo-treated VF- or normal control animals at both 0.5 Hz and 1 Hz frequency. The prolongation of APD$_{90}$ in the placebo-treated VF+ group was also associated with a significant (p<0.05 vs. control) increase in beat-to-beat variability of APD$_{90}$ (quantified as SD1 and SD2), a marker of increased risk of pro-arrhythmia which was normalized after omega-3 treatment (p<0.05). There were no significant differences in the SD1 and SD2 between the placebo-treated VF- animals and control (non-infarcted) animals (p>0.05) whereas the myocytes from VF- dogs that developed arrhythmias after treatment [VF (-,+)] exhibited increased beat-to-beat variability of APD$_{90}$ than myocytes from placebo-treated VF- or normal control dogs (p<0.05).
Conclusion: Chronic treatment with omega-3 fatty acids is not anti-arrhythmic in animals known to be at risk for sudden cardiac death. In fact, chronic treatment with omega-3 fatty acids may be pro-arrhythmic.
Dedication

This document is dedicated to my Gurudev & my family.
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Chapter 1: Introduction

1.1. Definition of Sudden cardiac death

Sudden cardiac death is defined as the unanticipated death from a cardiac cause which occurs within a short time period, usually defined as less than one hour after the onset of symptoms\textsuperscript{1,2}. In sudden cardiac death (SCD), the heart stops pumping blood and the victim abruptly collapses. Brain damage immediately sets in within just a few minutes, and death occurs within minutes after the victim collapses if there is no immediate treatment like cardiopulmonary resuscitation (CPR). When defibrillation is available, it can terminate the heart’s abnormal rhythm by delivering an electrical shock which allows normal cardiac rhythm to resume \textsuperscript{3}. These treatments, if performed in a timely manner, can enhance a patient’s chance of survival \textsuperscript{4}.

1.2. Incidence of sudden cardiac death

Sudden cardiac death (SCD) is the most widespread mode of death and accounts for \textasciitilde 50\% of the mortality in most developed countries. The total number of sudden deaths in the United States is around 300,000-400,000 deaths per year \textsuperscript{1,2,5}. Most of all sudden cardiac deaths (\textasciitilde 80\%) occur at home, and almost 60\% are witnessed \textsuperscript{6}. Due to lack of timely treatment, around 95\% of sudden cardiac arrest victims die before reaching the hospital.
1.3. Causes of sudden cardiac death

Around 80% of the victims of SCD have underlying structural cardiac defects. Holter analysis has revealed that the vast majority (>80%) of the SCDs result from tachyarrhythmias that terminate in ventricular fibrillation (VF), a condition in which there is rapid and disordered ventricular rhythm with no effective cardiac output. This chaotic ventricular rhythm causes the heart to pump little or no blood and ischemic brain injury follows. Previous myocardial ischemic injury or its sequelae has been estimated as one of the risk factors in 80% of the cases of SCD’s. In victims of SCD, there is a 50% incidence of healed scar due to a previous myocardial infarction seen in post-mortem examinations.

Despite the severity of the problem, the identification of the causes responsible for SCD and safe, effective anti-arrhythmic therapies for its management remains problematic. Indeed, many potential anti-arrhythmic drugs have been shown to promote ventricular tachyarrhythmias and sudden cardiac death. Only beta adrenergic receptor blockers; amiodarone- a class III anti-arrhythmic drug with multiple electrophysiological effects that also blocks the β-adrenergic receptor; and implantable cardioverter defibrillators (ICDs, devices that monitor and electrically treat any abnormal cardiac rhythm) have been shown to reduce sudden cardiac death. Nevertheless, even with the best currently available therapies, among survivors of acute myocardial infarction (MI) with significant left ventricular dysfunction, the one year mortality is 10% or higher, with sudden cardiac deaths accounting for nearly 1/3 of the late mortality. Also, the
adverse reactions due to long term use of amiodarone include pulmonary fibrosis, liver toxicity, and thyroid toxicity which are difficult to manage\textsuperscript{26}. Research for an effective anti-arrhythmic therapy must, therefore, continue.

1.4. Animal model of sudden cardiac death- A canine model of ventricular fibrillation

Animal models play an important role in arrhythmia research. Appropriate animal models help to understand the mechanisms responsible for the development of arrhythmias that result in sudden cardiac deaths and are also useful for the pre-clinical screening of potential anti-arrhythmic drugs. Several studies have identified important risk factors for sudden cardiac death which include the following: previous injury due to myocardial ischemia\textsuperscript{27}, patients with ischemia at a site spatially distant from previous myocardial ischemic injury\textsuperscript{28} and alterations in cardiac autonomic modulation\textsuperscript{29-30}. From post-mortem examinations it is evident that a majority of victims of SCD have underlying and often undetected coronary artery disease\textsuperscript{31-33}.

For effective assessment of anti-arrhythmic potential of compounds, development of an ideal and effective model of VF is essential. Firstly, it is necessary that the arrhythmias should be consistently and reliably induced in the animal model to mimic the clinical reality in which these sudden deaths occur. Secondly, sample size is also an important consideration in these experiments and can influence the results obtained in the pre-clinical evaluation because smaller the sample size, greater the possibility for a false positive result. Trolese-
Mongheal et al. (1985) studied the effect of sample size on sudden death rate in a large set of dogs (n=658) in which acute myocardial infarction was produced by ligation of the left anterior descending coronary artery. The sudden death varied widely (0-70%) in a small series of dogs (n=10) whereas in a large series (n=100) the sudden death rate values varied less (14-36%). An effective and reliable evaluation of preventive measures of a drug against sudden death required at least 50 animals per treatment group. However, the use of such a large number of animals is a basic limitation in the pre-clinical stage due to the long time periods in these studies that would incur a huge cost. Thus, by using the same animals in both control and in treatment groups, i.e. including an internal control group, these experiments would be a cost-effective approach and expedite the pre-clinical evaluation. In summary, an ideal animal model to study VF and SCD should imitate the disease process and also include an adequate number of animals for effective evaluation of anti-arrhythmic potential of compounds.

1.4.1. Overview of the canine model of sudden cardiac death

A canine model of sudden cardiac death was used in the present study that includes several factors thought to be responsible for the development of VF. An experimental myocardial infarction (MI) was produced in the animals by occlusion of the left anterior descending coronary artery. A vascular occluder was placed on the left circumflex coronary artery during the surgery. To determine a given animal’s arrhythmia risk, after the MI was healed, a brief and reversible left circumflex coronary artery occlusion was created for induction of acute ischemia.
during exercise \textsuperscript{35-37}. Since, alterations in cardiac autonomic regulation system are one of the risk factors for SCD, submaximal exercise was used to activate the autonomic nervous system and combined with acute ischemia to determine arrhythmia susceptibility in the animals\textsuperscript{29,38-41}. The animals were exercised on a motor-treadmill with increasing workloads until the target heart rate of 210 beats/min was achieved (70\% of the maximum heart rate). During the final minute of exercise, a reversible occlusion of the left circumflex coronary artery was carried out by inflating the vascular occluder placed on the left circumflex which was maintained for an additional minute after stopping the exercise. Thus, the total time period of occlusion was 2 minutes. The occlusion was immediately released in those animals that exhibited ventricular fibrillation followed by subsequent resuscitation. In summary, the canine model of SCD helps to identify and stratify the animals as those that are “resistant” \textit{and} those that are “susceptible” to VF \textsuperscript{35-37}. A representative example of one susceptible and one resistant dog is shown in Fig. 1.
Figure 1. A sample recording from one susceptible and one resistant animal. LVP = left ventricular pressure, CBF = coronary blood flow, HR = heart rate (beats per min)^42. (Used by permission from Elsevier Publishers Ltd: Pharmacology & Therapeutics, A comprehensive review and analysis of 25 years of data from an in vivo canine model of sudden cardiac death: Implications for future anti-arrhythmic drug development, copyright September 2006).

From fig. 1, it is evident that the exercise plus ischemia test resulted in ventricular flutter in the susceptible animal whereas in the resistant animal there is no induced tachyarrhythmia.
1.5. Omega-3 fatty acids and cardiovascular disease

1.5.1. Omega-3 fatty acids-general

Fatty acids are classified as saturated, monounsaturated, or polyunsaturated. There are two types of polyunsaturated fatty acids (PUFAs) - the omega-6 fatty acids are mainly from plant sources and the omega-3 fatty acids are mainly from marine sources. A typical Western diet mainly includes the omega-6 fatty acids present in vegetable oils which are a source of linoleic acids. Human beings do not possess the necessary enzymes to convert the precursor omega-6 fatty acid (linoleic acid) to omega-3 fatty acids and also lack the necessary metabolic pathway to synthesize the precursor omega-3 fatty acid (α-linolenic acid). Thus, omega-3 fatty acids can only be obtained from dietary intake.

Omega-3 fatty acids (ω-3 FAs) are a family of naturally occurring long chain polyunsaturated fatty acids (PUFAs) that may be obtained by dietary intake of eicosapentanoic acid (EPA) and docosahexanoic acid (DHA), that are present in oily fish or commercially available fish oil supplements. Although EPA and DHA are usually obtained from marine sources, these fatty acids are not actually produced by fish. Rather, these compounds are produced by the unicellular marine organisms which are consumed by fish.

The potential physiological benefits of omega-3 fatty acids include:

a) Anti-arrhythmic
b) Anti-inflammatory
c) Anti-atherosclerotic and plaque stabilization
d) Triglyceride lowering effect

e) Blood pressure lowering effect

f) Vasodilation

g) Anti-thrombotic

h) Regulation of autonomic function

The dose of omega-3 fatty acids has an important role in the physiological effects and the time course required to attain the clinical outcome. In humans, the anti-thrombotic, anti-inflammatory and triglyceride decreasing effects of omega-3 FA’s occur at high doses of usually 3-4 g/day whereas the anti-arrhythmic effects, regulation of autonomic function and reduction in sudden cardiac deaths can be attained at lower doses of EPA and DHA (500-1000mg/day) \(^{49}\). While the beneficial (e.g, anti-platelet) effects can be obtained within weeks, lowering of triglyceride levels, heart rate and blood pressure can be achieved only over a period of months to years\(^ {49}\).

1.5.2. Background epidemiologic evidence

The association between omega-3 fatty acids and its cardiovascular impact was recognized following the observation in the 1970s by two Danish investigators Bang and Dyerberg \(^ {50}\). The Danish investigators reported that the age-adjusted mortality from coronary artery disease for the Greenland Inuit was 10-40% lower than for the Danes, despite the fact that the Inuit diet was rich in fat and cholesterol\(^ {50-53}\). It was proposed that this protection could be due to high content of long chain omega-3 PUFA’s in the Inuit diet, mainly from fish, seal and whales
whereas typical Western diets were rich in saturated fatty acids. In agreement with these findings, several epidemiological studies were conducted which reported the cardiac benefits of fish oils. The most striking result was obtained by a study conducted by Albert et al. (2002) who reported that subjects without prior evidence of cardiovascular disease and with the highest blood omega-3 levels had an associated 70%-90% decline in the risk of SCD. On the other hand, not every epidemiological study has reported beneficial effects of fish oils in reducing cardiac mortality since the effects are limited by the type of fish: like fresh water vs. marine source, how it is consumed (fried vs. baked) or the presence of environmental toxins like mercury in fish.

1.5.3. Clinical intervention trials on omega-3 fatty acids

Several clinical trials conducted over the past few decades have examined the cardiovascular effects of fish and fish oil supplements mainly after MI. A randomized controlled trial conducted in 1989 (the Diet and Reinfarction trial [DART]) by Burr and co-workers was the first to study the effects of consuming fish on the incidence of myocardial re-infarction. In this study 2,033 men with recent MI were advised to eat at least two portions (200-400 g) of oily fish per week. The study reported a 29% decrease (p<0.05) in total mortality during a two year period in male MI survivors who received omega-3 fatty acids compared to men on a standard diet with similar re-infarction rates as the former group. The Lyon Heart study was a prospective, randomized, multicentre trial which was aimed in secondary prevention of cardiovascular deaths by diet modification in
survivors of a recent MI. In this study, 600 survivors of a recent MI were advised to adopt a Mediterranean-type diet [food with more oleic and alpha-linolenic acids (ALA), experimental group] as well as a control diet (with no dietary restrictions) for 5 years. At the end of two years, there were 16 cardiac deaths in the control group and three deaths in the experimental group. The overall mortality was 20% in the control group compared to 8% in the experimental group. It was concluded that the protective effect of the Mediterranean-type diet was due to the dietary supply of EPA and the omega-3 fatty acid precursor (ALA). The most striking results were obtained in the GISSI-Prevenzione trial, the largest intervention trial to test the efficacy of omega-3 PUFAs on secondary prevention after MI. This study randomized 11,324 post-MI patients to receive either omega-3 PUFAs (1 capsule/day, Omacor®: 850 mg of DHA+EPA) or 300 mg of vitamin E or both or neither of the treatments. The study reported a highly significant (p<0.001) 45% reduction in SCD, which was apparent after only 4 months. However, conflicting results have been obtained from several interventional studies. The DART-2 trial was a follow up study by Burr and his co-workers in 1990, where the subjects involved were the former participants of the DART trial. A total of 3,114 men with chronic angina were randomly assigned to four groups: 1) subjects advised to eat two portions of oily fish each week or to intake 3 fish oil capsules daily, 2) subjects recommended to eat more fruits, vegetables and oats, 3) subjects advised with a combination of the above and, 4) subjects not provided any dietary advice. Mortality in this study was determined after 3-9
years. The results indicated that the risk of sudden cardiac death was maximal in subjects taking fish oil capsules. Although the trial was well-designed, due to a lack of funding the trial was stopped midway and it was unclear if the participants of the trial continued to adhere to the advice. On the other hand the Nilsen study was designed to study the effect of high dose omega-3 fatty acids (4 g/day) administered to 300 Norwegian patients after an acute MI for 12-24 months, and did not observe any clinical benefit of high dose of omega-3 fatty acids. It was suggested that a high background intake of fish oil in the patient population might have masked the treatment effects. The JELIS (Japan EPA Lipid Intervention Study) trial was conducted in 18,645 hypercholesterolemic patients to determine the long-term effects of a moderate dose of EPA (1800 mg/day) for prevention of major coronary events, and observed a 19% decrease in major coronary events at the end of the 5 year study without any difference in sudden cardiac deaths compared to the control group.

In view of these conflicting findings, three double-blind randomized clinical trials were conducted in patients with implantable cardioverter defibrillators (ICDs) to determine the direct effects of omega-3 fatty acids on susceptibility to ventricular arrhythmias and sudden cardiac death. Leaf et al. conducted a double-blinded trial wherein 402 high-risk patients with ICDs were randomly assigned to a daily treatment of fish oil or olive oil supplements for 12 months. Although the time to the first ICD event for VT or VF did not yield any significant result (risk reduction of 28%, P=0.057), there was a significant risk reduction of 31% (P=0.033) for
episodes of VT or VF in individuals at high risk of ventricular arrhythmias. Thus, it was concluded that regular intake of fish oils may reduce the risk of fatal ventricular arrhythmias in high risk individuals. In contrast, a randomized, placebo-controlled trial was performed by Raitt et al. in 200 patients with an implantable cardioverter defibrillator (ICD) and a recent episode of sustained VT or VF yielded unexpected results. The patients were treated with fish oil supplements (1.8 grams/day) or placebo for a median of 718 days. It was concluded from the study that fish oil supplementation did not prevent episodes of VT or VF. Subgroup analysis further revealed that patients with VT had an earlier arrhythmia recurrence if treated with fish oil (p<0.007) and a general trend towards repeated episodes of VT/VF (p<0.001) suggesting a pro-arrhythmic potential of fish oil supplementation. Finally, the SOFA trial (Study on omega-3 fatty acids and ventricular arrhythmia), a randomized, placebo controlled trial conducted in Europe on a total of 546 patients with ICDs and prior history of VT/VF treated with 2 g/day of fish oils or a placebo (high oleic acid-sunflower oil) for a median period of 356 days found neither an anti-arrhythmic nor a pro-arrhythmic effect of treatment with omega-3 fatty acids. Thus, from the discordant results the benefits of omega-3 fatty acids for secondary prevention of lethal ventricular arrhythmias and sudden cardiac deaths remain unknown.
1.5.4. Mechanisms of Arrhythmia

Cardiac arrhythmia is defined as any disturbance in the cardiac impulse formation, its rate and regularity or an abnormality in the conduction of the impulse such that the normal activation sequence of the myocardium is altered\textsuperscript{83}. The cellular mechanisms of cardiac arrhythmias are classified into disturbances in impulse formation (triggered activity, automaticity) and disturbances in impulse propagation (reentry)\textsuperscript{83-84}. The predominant arrhythmogenic mechanisms are triggered activity and reentry. In a normal heart, an electrical impulse originates at regular intervals in the sinoatrial node and terminates after the activation of the whole heart. Reentry occurs if an impulse re-enters and re-excites an area of cardiac tissue more than once thereby resulting in abnormal impulse propagation and cardiac arrhythmias\textsuperscript{83}. The time of impulse conduction and the refractory period are important determinants of the risk for a re-entrant circuit to occur\textsuperscript{85}. Multiple reentrant circuits or continuous reactivation of ventricular electrical activity is responsible for initiation and perpetuation of ventricular fibrillation\textsuperscript{86}. In addition, reentry is initiated by a premature (triggered) beat, most often when there is an anatomic substrate (e.g. scar)\textsuperscript{83-87}.

Triggered activity results from early afterdepolarizations (EADs) and delayed afterdepolarizations (DADs)\textsuperscript{83,87}. Afterdepolarizations are oscillations of membrane potential and if the amplitude is sufficient to reach threshold may trigger a response which can result in sustained and repetitive firing of the cell\textsuperscript{83,88}. An EAD occurs when the action potential is prolonged during slow cardiac
rhythms and the are oscillations in membrane potential, interrupt phase 3 (repolarization phase) of the cardiac action potential. If the EAD is of sufficient amplitude and reaches threshold, it can result in a triggered response which can be repetitive and sustained\textsuperscript{83,88}.

1.5.5. Experimental studies of omega-3 fatty acids on arrhythmia and electrophysiology

While many scientists were investigating the actions of fish oil on the heart, two Australian investigators, Peter McLennan and John Charnock, were the first to demonstrate an anti-arrhythmic effect of omega-3 fatty acids\textsuperscript{89}. McLennan et al. (1988) reported that dietary omega-3 PUFAs provides protection from myocardial ischemia and reperfusion-induced ventricular fibrillation in anesthesized rats fed with diets rich in fish oils (tuna fish oil) for 9 months compared to the animals fed with diets rich in saturated fat or olive oil (monounsaturated fatty acid)\textsuperscript{89-93}. Also, McLennan et al.\textsuperscript{94} demonstrated that a long term diet (30 months) rich in tuna fish oil decreased the susceptibility of normal or ischemic myocardium to VF using non-human primates (marmoset monkey).

Chronic administration of a diet rich in fish oil to pigs led to omega-3 incorporation in the sarcolemma and resulted in action potential shortening in isolated ventricular myocytes\textsuperscript{95-96}. Male pigs (7 weeks old) received a diet rich in omega-3 PUFAs (EPA-0.81g/100 g feed + DHA 0.71 g/100g feed, n=11) or control diet (sunflower oil, n=8) for 8 weeks\textsuperscript{95}. This study reported that a diet rich
in fish oil shortened the action potential duration compared to the controls, inhibited L-type calcium current \( (I_{\text{ca,L}}) \), responsible for the action potential plateau and a contributor to the duration of the action potential), reduced re-opening of calcium channels at plateau potentials, which could prevent triggered activity; had no effect on transient outward current \( (I_{\text{to}}) \), responsible for early rapid repolarization phase of the action potential); but caused an increase in \( I_{K1} \) (inward rectifier current which maintains the resting membrane potential); and an increase in \( I_{Ks} \) potassium currents (slow component of delayed rectifier current responsible for repolarization of phase 3 of the action potential); and decreased the sodium-calcium exchanger\(^95\).

Not only diet, but also acute intravenous injection of a fish oil emulsion prevented ischemia induced cardiac arrhythmias. Billman et al. (1999) studied the effects of the acute intravenous infusion of a fish oil emulsion on VF induced by acute ischemia in the canine model of SCD used in the present study \(^96\). The results indicated that fish oil infusion prevented VF in the susceptible dogs compared to an emulsion prepared from soybean oil \(^96\). Similar results were obtained after dietary supplementation of omega-3 fatty acids (EPA: 100mg/kg for 8 weeks) compared to a control group (standard diet) in a canine model of acute MI\(^97\).

In contrast to the above findings, Coronel et al. (2007) reported that diets rich in omega-3 fatty acids resulted in more episodes of spontaneous ischemia-induced sustained ventricular tachycardia (sVT) and ventricular fibrillation compared to the control group \(^98\). Male piglets (7 weeks) old received a diet rich in omega-3
PUFAs (EPA 0.81g/100 g feed + DHA 0.71 g/100g feed, n=11), omega-9 FAs (sunflower oil, N=11) or a standard diet (control, n=6) for 8 weeks\textsuperscript{98}. These results indicated increased arrhythmia susceptibility (p<0.05) during acute ischemia in pigs fed with diets rich in omega-3 PUFAs compared to the control diet. The pro-arrhythmic effect of omega-3 PUFAs correlated with the decrease in excitability in the ischemic myocardium, a condition favoring reentry\textsuperscript{96,98}.

1.5.6. The Omega-3 index

The omega-3 index measures the content of the two major omega-3 fatty PUFAs (EPA and DHA) present in the red blood cell (RBC) membrane and is expressed as the EPA + DHA percent of total RBC FAs. Harris et al.(2004)\textsuperscript{99} proposed the omega-3 index as a new biomarker and risk factor for death from coronary heart disease (CHD) based on the studies by Siscovick et al.(1995)\textsuperscript{100} and Albert et al. (2002)\textsuperscript{101}, who reported a strong inverse relationship between the blood omega-3 index and the risk for primary cardiac arrest or sudden cardiac death\textsuperscript{99-102}. Subsequently, the cardioprotective target level of omega-3 index (percent of EPA+DHA in RBC) was established\textsuperscript{99}. The proposed cut point of the omega-3 index for the cardioprotective effect was estimated to be >= 8% while an omega-3 index <4% was associated with an increased risk for death due to coronary heart disease (CHD)\textsuperscript{99}.

1.5.7. Recommended intake of omega-3 FAs

The American Heart Association (AHA) recommends that patients without documented CHD consume fish at least twice a week as well as foods rich in
alpha-linoleic acids like flaxseed, canola, or soybean oils whereas patients with CHD should consume a total of 1 g/day of EPA and DHA, preferably from an oily fish. Fish oil supplements could also be consumed in consultation with a physician. Patients who need to lower their triglyceride levels can also take 2-4 g of EPA+DHA per day after consultation with a physician. Thus, the intake of omega-3 fatty acid supplements should be tailored to the need of an individual patient.

1.6. Summary

Omega-3 fatty acids have significant cellular electrophysiological effects. The safety and efficacy of omega-3 fatty acids for the prevention of lethal ventricular tachyarrhythmias and SCD remains unclear. The electrophysiological mechanisms that result in pro- or anti-arrhythmic effects after consumption of omega-3 fatty acids still remain unknown. Therefore, it was the purpose of the present study to determine whether chronic treatment with omega-3 fatty acids attenuates the electrophysiological abnormalities that contribute to ventricular fibrillation in a canine model of SCD.

1.7. Hypothesis

Chronic treatment with omega-3 fatty acids attenuates the electrophysiological abnormalities that contribute to ventricular fibrillation in a canine model of SCD.
Chapter 2: Materials and Methods

All the animal procedures were approved by the Ohio State University Institutional Animal Care and Use Committee and were in accordance with the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication N.85-23, revised 1996).

2.1. Surgical preparation

Seventy-six heartworm mixed breed dogs (male/female; 2-3 years old and 15-25kg) were used in the study. The animals were anesthetized and a surgically induced MI was created by occlusion of the left anterior descending coronary artery\(^{42,104-106}\). Briefly, 24 hours before surgery, a transdermal patch which delivers fentanyl at the rate of 100µg/hr was placed on the left side of the dog’s neck and held in place with tape to reduce post-operative pain. On the day of the surgery, the dogs were given morphine sulfate (15 mg, i.m.) and thiopental sodium (20 mg/kg, i.v.) for induction of anesthesia. The dogs were intubated and a surgical plane of anesthesia was maintained by isoflurane inhalation (1 to 1.5%). Under strict aseptic conditions, a left thoracotomy was performed. The heart was exposed and a 2-stage occlusion of the left anterior descending coronary artery was performed, approximately at one-third the distance from its origin to produce a left ventricular anterior wall MI\(^{35,36,42}\). The left anterior
descending coronary artery was then partially occluded for 20 minutes, and then, tied completely. Next the left circumflex coronary artery was isolated and a 20 MHz pulsed Doppler flow transducer and a vascular occluder were placed on the left circumflex coronary artery. Three sets of bipolar pacing electrodes (supplied by Medtronic Inc., Minneapolis, MN) were placed in the infarcted, border zone and non-ischemic regions of the left ventricle to record electrocardiograms during the exercise-ischemia test. The long-lasting local anesthetic 0.25%, bupivacaine HCl was injected in the area of incision to block the intercostal nerves and minimize post-operative discomfort. Morphine sulfate (1.0 mg/kg, s.c.) was also given as needed to reduce any post-operative pain and discomfort. The dogs also received post-operative antibiotic therapy (Amoxicillin, 500mg *per os*) twice daily for 7 days. To decrease the incidence of arrhythmias, 100 mg of lidocaine HCl was administered intra muscularily (i.m.) before surgery followed by an additional 60 mg, i.v. during the 2-stage occlusion. The dogs also received procainamide HCl (500 mg, i.m.) before the surgery. The animals were allowed to recover in a quiet place after the surgery and were monitored for the presence of any arrhythmias. They were returned to the kennel after the effects of anesthesia were gone.

2.2. Exercise plus Ischemia testing

After 3-4 weeks of recovery from surgery, the susceptibility to VF was tested by a standardized exercise plus ischemia test. The animals were trained to run on a motor driven treadmill over 3-4 days. After the training, the animals were
exercised on the treadmill for 15-18 mins until the target heart rate of 210 beats/min (70% of the maximum heart rate) was attained. During the last minute of exercise, the left circumflex artery was occluded using the vascular occluder, and the occlusion of the circumflex was maintained for another minute after termination of the exercise. Thus, the exercise-ischemia testing resulted in two reproducible populations of animals: VF susceptible, VF⁺ (n = 46) and VF resistant, VF⁻ (n = 30).

2.3. Protocol for dietary omega-3 intake

Following identification of VF susceptible and resistant animals, the dogs were randomly assigned to either placebo (1g/day, corn oil; VF⁺, n = 16 and VF⁻, n = 15), or a omega-3 PUFA supplement [docosahexaenoic acid+ eicosapentaenoic acid ethyl esters] 1g/day (VF⁺, n = 14 and VF⁻, n = 1), 2g/day (VF⁺, n = 7 and VF⁻, n = 5), or 4g/day (VF⁺, n =9 and VF⁻, n =9) groups. Each one gram capsule contained 465 mg ethyl eicosapentaenoate, EPA + ethyl docosahexaenoate, DHA, 375 mg per capsule (Lovaza®, GlaxoSmithKline Research). The capsules were administered per os every morning, 7 days per week for 3 months (90 days). Five age-matched dogs served as controls (i.e. dogs without any MI).

2.4. Isolation of Myocytes

At the end of the study period, the placebo-treated (VF⁺, n = 10 and VF⁻, n = 15); omega-3 treated dogs (VF⁺, n =30 and VF⁻, n = 15) and control dogs (n = 5) were anesthetized with pentobarbital sodium (50 mg/kg i.v.). After attaining the required plane of anesthesia, the heart was rapidly removed and flushed with
cold cardioplegic solution (containing 5% glucose, 0.1% mannitol, 22.4 mM NaHCO₃, 30 mM KCl) injected into the coronary ostia. The left circumflex coronary artery was cannulated for isolation of myocytes as previously described¹⁰⁷. Following removal of blood from heart, collagenase (Worthington type 2; 0.65 mg/ml) was added to the perfusate. After 30-45 minutes of enzyme digestion, the digested midmyocardial part of the left ventricle was separated from the epicardial and endocardial part of the left ventricle, avoiding the area of infarct and the surrounding border zone. Usually, this isolation procedure yielded 50-70% rod-shaped striated myocytes with sharp margins and staircase ends. The myocytes were suspended at room temperature in a standard incubation buffer solution containing the following (in mM): 118 NaCl, 4.8 KCl, 1.2 MgCl₂, 1.2 KH₂PO₄, 0.68 glutamine, 10 glucose, 5 pyruvate, 1 CaCl₂, along with 1 µmol/L insulin and 1% BSA until used. All myocyte experiments were completed within 10 h of isolation.

2.5. Electrophysiological studies

Myocytes were placed in a laminin coated cell chamber (Cell Microcontrols, Norfolk, VA) and superfused with bath solution containing the following: 135 mM NaCl, 5 mM MgCl₂, 5 mM KCl, 10 mM Glucose, 1.8 mM CaCl₂, 5 mM HEPES, pH adjusted to 7.40 with NaOH and the temperature maintained at 36 ± 0.5°C. Borosilicate glass micropipettes with tip resistance of 1.5 - 3 MΩ were filled with pipette solution containing the following (in mM): 130 KCl, 5 MgCl₂, 5 EGTA, 5 HEPES, pH adjusted to 7.2 with KOH. Action potentials (APs) were recorded with
the amphotericin B-perforated whole cell patch clamp technique. APs were measured as the average of the last 15 (steady state) traces obtained from 25 traces at each stimulation frequency. The resting membrane potential (RMP) action potential duration (APD) at 90 % repolarization (APD$\text{90}$) for each myocyte was calculated from the averaged trace. A standard Poincaré analysis was done to estimate the beat-to-beat instability in repolarization. Poincare plot is a geometrical representation to represent the beat-to-beat variability in a two-dimensional plane. The two standard descriptors of the plot, short term deviation (SD1) and long term deviation (SD2) is used for the quantification of the Poincaré plot geometry by fitting an ellipse to the plot geometry and is also used for the qualitative visualization of the distribution$^{108}$. The minor or short axis of the ellipse fitted to the data points is proportional to the standard deviation of the successive differences (SD1) whereas the major or long axis of the ellipse is proportional to the standard deviation of the current beat$^{108-110}$. Thus, the SD1 and SD2 together represent the total beat-to-beat variability in a data set. Similarly in our experiments, short term deviation (SD1) and the long term deviation (SD2) of the Poincaré plot was measured at each frequency as previously described$^{108-110}$. A representative Poincaré plot for our set of data points in VF resistant dogs is shown in Fig.2.

All the data was acquired using Clampex 10.2 software (Axon instruments, Sunnyvale, CA) and an Axopatch 200B patch-clamp amplifier.
Figure 2. A standard Poincare plot representing the beat-to-beat variability of APD$_{90}$ in VF resistant dogs. VF$^+$ or VF$^-$: susceptible or resistant to sustained ventricular tachyarrhythmias (VF). VF (-, +) indicates resistant to VF initially with conversion to VF$^+$ after omega-3 treatment while VF (-,-) indicates dogs that remained resistant to VF after omega-3 treatment.

2.6. Solutions and chemicals

All chemicals for buffer and stock solutions were purchased from Fisher Scientific, Sigma-Aldrich (St. Louis, MO), or Invitrogen (Carlsbad, CA). Stock solutions of amphotericin-B were prepared daily and protected from exposure to light.

2.7. Statistical analysis

All the acquired electrophysiological data were analyzed with Clampfit 10.2 (Axon instruments) and Origin 8.0 (Origin Lab). The data are presented as mean $\pm$
SEM. Statistical testing for the in vivo data on susceptibility to VF and SCD was conducted using chi-square test. Other statistical testing was conducted using one-way ANOVA, followed by post hoc testing with the Least Significant Difference (LSD) test to identify differences between groups (SAS for Windows, v. 9.1, SAS Institute, Cary, NC).
3.1. Effect of omega-3 fatty acids on left ventricular structure or function

In a previous report by Billman et al. (2010)\textsuperscript{111} using the same post-infarction canine model used in the present study, chronic omega-3 treatment resulted in significant dose-dependent increases of the omega-3 fatty acid levels in the RBC membrane and in the left ventricular tissue. Also, there was no evidence of impairment of global left ventricular structure or function after infarction in this model of SCD\textsuperscript{42,105-106}. None of the treatments altered the left ventricular fractional shortening or the end systolic and the end diastolic diameters after infarction in this model of SCD\textsuperscript{111}.

3.2. Effect of omega-3 fatty acids on susceptibility to VF and SCD

After 3 months of treatment, 6 out of 16 (37.5\%) placebo-treated dogs experienced SCD while 5 out of 30 (16.7\%) omega-3 treated dogs had SCD (Figure 3, B). All the VF\textsuperscript{+} placebo-treated dogs remained susceptible to VF at the end of the treatment period whereas, 4 out of 30 (13.3\%) omega-3 treated VF\textsuperscript{+} dogs were protected from VF (Figure 3, A). There were no significant differences in susceptibility to VF and SCD between the placebo and omega-3 treated VF\textsuperscript{+} dogs (p>0.05).
In contrast, 5 out of 15 (33.3%) omega-3 treated VF resistant dogs became susceptible to arrhythmias whereas all of the placebo treated VF resistant dogs remained resistant to arrhythmias at the end of the treatment period (Figure 3, C). Two omega-3 treated VF dogs developed spontaneous sudden death during the treatment period, however none of the placebo-treated VF resistant dogs died suddenly during the treatment. Thus, the in vivo data shows that although omega-3 treatment did not improve the outcome in dogs susceptible to VF, it significantly (p<0.05 vs. placebo) induced arrhythmias in animals that were previously resistant to it.
Figure 3. Evidence of susceptibility to VF and SCD. Panel A shows that the dogs receiving omega-3 supplement were not protected from VF compared to the placebo-treated dogs. Panel B shows the spontaneous death rate in placebo and omega-3-treated VF+ dogs. Panel C shows that omega-3 treatment increased the susceptibility to VF in the resistant population. Statistical difference is indicated above the bar. VF+ or VF−: susceptible or resistant to sustained ventricular tachyarrhythmias (VF).
3.3. Effect of omega-3 fatty acid treatment on electrophysiological studies in VF susceptible dogs

The electrophysiological studies in isolated single cardiomyocytes exhibited no differences in the resting membrane potentials between the control, placebo-treated VF\(^+\), or omega-3 treated VF\(^+\) dogs at both 0.5 Hz and 1 Hz stimulation frequencies (Figure.4.B-C). The action potential duration at 90% repolarization (APD\(_{90}\)) was significantly longer in the placebo-treated VF\(^+\) group compared to the control (692.8 ± 44.9 in placebo-treated vs. 424.7 ± 29.1 in controls at 0.5 Hz; 515.9 ± 26.0 in placebo-treated vs. 409.2 ± 35.2 in control at 1 Hz, p<0.05) while there was no significant difference in the APD\(_{90}\) between the control and omega-3 treated dogs at both 0.5 Hz and 1 Hz stimulation frequency (p>0.05) [Figure.4.D-E]. Thus, omega-3 treatment normalized the APD\(_{90}\) in the VF susceptible dogs whether the dogs converted to VF resistant (477.2 ± 32.6, 399.3 ±19.5) or still remained susceptible to VF (421.6 ± 58.9, 385.9 ± 22.8) respectively at both 0.5 Hz and 1 Hz stimulation frequencies (p<0.05) (Figure.4.D-E).
Figure 4. Electrophysiological recordings in VF susceptible dogs

Panel A shows the representative action potential tracings from control (black), placebo-treated VF+ (red), omega-3 treated VF (+,-) (blue) and omega-3 treated VF (+, +) (green) recorded at 1 Hz. Panel B & C shows the Resting membrane potential recorded at 0.5 Hz (B) and 1 Hz (C). Panel D and Panel E depict the summary of APD90 at 0.5 Hz (D) and 1 Hz (E) in all groups.
Statistical differences are indicated above the bars. VF\textsuperscript{+} or VF\textsuperscript{-} : susceptible or resistant to sustained ventricular tachyarrhythmias (VF). VF (+,-) indicates susceptible to VF initially with conversion to VF\textsuperscript{-} after omega-3 treatment while VF (+,+) indicates dogs that remained susceptible to VF after omega-3 treatment.

3.4. Effect of omega-3 fatty acid treatment on electrophysiological studies in VF resistant dogs

The electrophysiological studies in isolated single cardiomyocytes showed no differences in the resting membrane potentials in the control, placebo-treated VF\textsuperscript{-} or omega-3 treated VF\textsuperscript{-} dogs (Figure.5.B-C). The omega-3 treated VF(-,+) group exhibited a significant increase in action potential duration at 90\% repolarization (APD\textsubscript{90}) compared to the control and the placebo-treated VF\textsuperscript{-} groups (Figure.5.D-E). There was no significant difference in the APD\textsubscript{90} between the control, placebo-treated VF\textsuperscript{-} and omega-3 treated VF (-,-) dogs (p>0.05) (Figure.5.D-E).
Figure 5. Electrophysiological recordings in VF resistant dogs

Panel A shows the representative action potential tracings from control (black), placebo-treated VF (red), omega-3 treated VF (-,-) (blue) and omega-3 treated VF (-,+) (green) recorded at 1 Hz. Panels B and C shows the resting membrane potential 0.5 Hz (B) and 1 Hz (C) which was similar in all groups. Panels D and Panel E depicts the summary of APD$_{90}$ at 0.5 Hz (D), 1 Hz (E) in all groups.
Statistical differences are indicated above the bars. VF$^+$ or VF$^-$: susceptible or resistant to sustained ventricular tachyarrhythmias (VF). VF (-,+) indicates resistant to VF initially with conversion to VF$^+$ after omega-3 treatment while VF (-,-) indicates dogs that remained resistant to VF after omega-3 treatment.

3.5. Effect of omega-3 fatty acid treatment on beat-to-beat variability of APD$_{90}$ in VF susceptible dogs

The prolongation in APD$_{90}$ in placebo-treated VF$^+$ group was also associated with increase in beat-to-beat variability of APD$_{90}$. Increased beat-to-beat variability is a marker of increased risk of pro-arrhythmia\textsuperscript{112-114}. Omega-3 fatty acid treatment subsequently normalized the beat-to-beat variability of APD$_{90}$ at 1 Hz (Figure.6, C-D). The short-term deviation, SD1 was significantly greater in the placebo-treated VF$^+$ group compared to normal controls (28.3 ± 3.9 in placebo treated VF$^+$ vs. 16.4 ± 2.5 in control at 1 Hz, p<0.05) and was normalized following omega-3 treatment [15.7 ± 2 in O-3 VF(+,-); 16.0 ± 2.8 in O-3 VF(+,+)] vs. 28.3 ± 3.9 in placebo-treated VF$^+$, respectively at 1 Hz, (Figure.6,C). A similar trend was observed in the long-term deviation, SD2 of APD$_{90}$ [19.2 ± 2.5 in control, 28.9 ± 3.9 in placebo-treated VF$^+$, 18.0 ± 2.6 in O-3 VF (+,-) and 16.2 ± 2.8 in O-3 VF (+,+), respectively at 1 Hz stimulation frequency, p<0.05] (Figure.6,D). There was a significant increase in beat-to-beat variability in the APD$_{90}$ from the placebo-treated VF$^+$ dogs, quantified as SD2 of APD$_{90}$ [54 ± 13.1 in placebo-treated VF$^+$ vs. 18.2 ± 2.1 in control at 0.5 Hz stimulation frequency, p<0.05] (Figure.6,B). However, there was no significant difference in the beat-to-
beat variability of APD$_{90}$ between the control and omega-3 treated VF$^*$ dogs at 0.5Hz stimulation frequency (p>0.05) (Figure.6,A-B). Thus at a faster frequency of stimulation, omega-3 fatty acid treatment resulted in normalization of beat-to-beat variability of APD$_{90}$ in the VF susceptible group.
Figure 6. Beat-to-beat variability of APD$_{90}$ in VF susceptible dogs.

Panel A & C indicates the averaged short-term variability (SD1) of APD$_{90}$ measured from each myocyte in all groups plotted as a function of the stimulation frequency at 0.5 Hz (A) and 1 Hz (C) respectively. Panel B & D indicates the averaged long-term variability (SD2) of APD$_{90}$ measured from each myocyte in all groups plotted as a function of the stimulation frequency at 0.5 Hz (B) and 1 Hz (D) respectively. Statistical differences are indicated above the bars. VF$^+$ or VF$^-$: susceptible or resistant to sustained ventricular tachyarrhythmias (VF). VF (+,-)
indicates susceptible to VF initially with conversion to VF after omega-3 treatment while VF (+, +) indicates dogs that remained susceptible to VF after omega-3 fatty acid treatment.

3.6. Effect of omega-3 fatty acid treatment on beat-to-beat variability of APD$_{90}$ in VF resistant dogs
The prolongation in APD$_{90}$ in the omega-3 treated VF (-, +) group was also associated with an increase in beat-to-beat variability of APD$_{90}$, a known marker of arrhythmia susceptibility$^{112-114}$ (Figure 7, A-B). The short-term variability, SD1 was significantly greater in the omega-3 treated VF (-,+) group compared to either the control, placebo-treated VF$^- -$ or omega-3 fatty acid treated VF (-,-) dogs [43.5 ± 5.0 in O-3 treated VF(-,+)$ vs. 18.2 ± 2.0 in control, 27.4 ± 6.8 in placebo-treated VF$^- -$ and 11.2 ± 1.9 in omega-3 treated VF(-,-)$ respectively at 0.5 Hz, 27.1 ± 3.4 in O-3 treated VF(-,+)$ vs. 16.4 +/-2.5 in control, 14.1 ± 1.7 in O-3 treated VF(-,-)$ respectively at 1 Hz, p<0.05] (Figure 7, A). The long-term variability, SD2 of APD$_{90}$ was also significantly greater in VF (-,+) group compared either the control or the omega-3 fatty acid treated VF (-,-) group [38.1 ± 5.5 in O-3 treated VF (-,+) vs. 18.2 ± 2.0 in control & 14.7 ± 3.2 in omega-3 treated VF(-,-)$ respectively at 0.5 Hz, p<0.05] (Figure 7,B). However, at 1 Hz there were no significant differences in the long-term variability, SD2 between the control, placebo-treated VF$^- -$ and omega-3 treated VF$^- -$ dogs (p>0.05) (Figure 7, C-D). There were no significant differences in the averaged SD1, SD2 between the control, placebo-treated VF$^- -$ and omega-3 treated VF (-,-) dogs (p>0.05). Thus
the increased beat-to-beat variability in the APD$_{90}$ following omega-3 treatment in VF resistant group is associated with the observed risk of pro-arrhythmia.

Figure 7. Beat-to-beat variability of APD$_{90}$ in VF resistant dogs. Panel A & C indicates the averaged short-term deviation (SD1) of APD$_{90}$ measured from each myocyte in all groups plotted as a function of the stimulation frequency at 0.5 Hz (A) and 1 Hz (C) respectively. Panel B & D indicates the averaged long-term deviation (SD2) of APD$_{90}$ measured from each myocyte in all groups plotted as a function of the stimulation frequency at 0.5 Hz (B) and 1 Hz (D) respectively. Statistical differences are indicated above the bars. VF$^+$ or VF$^-$: susceptible or resistant to sustained ventricular tachyarrhythmias (VF). VF (-,+)...
indicates resistant to VF initially with conversion to VF+ after omega-3 treatment while VF (-,-) indicates dogs that remained resistant to VF after omega-3 fatty acid treatment.
Chapter 4: Discussion and Summary

4.1. Discussion
Sudden cardiac death is the principle cause of cardiovascular mortality in patients with previous myocardial ischemia, and most often results from sustained ventricular tachyarrhythmias that terminate in ventricular fibrillation. Multiple clinical trials\textsuperscript{73-82} have reported conflicting results on the benefits of omega-3 fatty acids for the secondary prevention of lethal ventricular arrhythmias and sudden cardiac arrest. In the present study, omega-3 fatty acid treatment not only failed to elicit significant protection against ventricular fibrillation or sudden cardiac death (i.e., spontaneous death rates were similar in treated and placebo groups) but actually induced arrhythmias in some post-MI dogs previously shown to be resistant to sudden death. While in animals susceptible to VF, there was no evidence of anti-arrhythmic benefit with omega-3 fatty acid supplements, cellular electrophysiological data suggest that omega-3 fatty acid supplements improved the action potential duration at 90\% repolarization. Additionally, omega-3 treatment normalized the increased beat-to-beat variability of \( \text{APD}_{90} \), which may underlie the substrate of susceptibility to VF in myocytes from previously VF-susceptible dogs. Previous studies by Sridhar et al. (2008)\textsuperscript{115} have shown that multiple repolarizing currents must be inhibited leading to impaired “repolarization reserve” which leads to APD prolongation and beat-to-
beat variability in the animal model used in this study. The APD prolongation we observed in the VF susceptible myocytes could be due to abnormal repolarizing K⁺ currents or due to dysregulated intracellular calcium handling processes. Thus, treatment with omega-3 PUFAs in the VF susceptible dogs may induce changes in the repolarizing K⁺ currents and normalize the abnormal calcium regulation leading to improved APD characteristics.

In contrast, in animals initially resistant to provoked VF, omega-3 fatty acid treatment resulted in a significant increase in VF. Further, the electrophysiological studies suggest that omega-3 fatty acid treatment significantly prolonged the action potential duration at 90% repolarization (APD₉₀) and increased the beat-to-beat variability of APD₉₀ in myocytes from previously VF-resistant dogs. The omega-3 fatty acid treatment resulted in increased arrhythmia susceptibility in a subset of animals that were previously resistant to it. The omega-3 treatment in the VF resistant myocytes may have caused reductions in potassium currents like I_K1, I_Kr and I_Ks which could have prolonged the APD, increased the APD variability and induced afterdepolarizations, thereby providing a substrate for arrhythmogenesis and conversion of VF-resistant myocytes to VF-susceptible phenotype.

Previous experiments by Sridhar et al. (2008)¹¹⁵ have reported that in addition to action potential (AP) prolongation and increased beat-to-beat variability in repolarization, frequent early after depolarizations (EADs) at low stimulation rates were observed in myocytes from VF-susceptible dogs. EADs are inverse rate-
dependent membrane potential oscillations that interrupt the repolarization phase (Phase 2) of the AP and occur during AP prolongation at low heart rates. Frequent ventricular premature depolarization and non sustained VT were evident in VF susceptible dogs, especially in those which exhibit EADs at the cellular level.

However, in this canine model of SCD, when the heart was stressed with myocardial ischemia at high heart rates were occurring during exercise. This suggest that EADs are unlikely to contribute to the in vivo arrhythmias, since EADs occur at lower heart rates.

In contrast, our data on myocytes from the VF resistant demonstrated that a pro-arrhythmic response to omega-3 fatty acid supplements was associated with increased beat-to-beat variability of APD$_{90}$, suggesting that omega-3 fatty acid treatment may have predisposed to EADs, a mechanism that could trigger reentrant ventricular arrhythmias in animals that were previously resistant to it. Given the rate dependence, this is more likely to have contributed to sudden death, rather than arrhythmias provoked by the exercise plus ischemia test.

Inhibition of multiple repolarizing currents on the background of reduced “repolarization reserve” may cause prolongation of the APD, increase in the beat-to-beat variability of APD and induce EADs at low heart rates which may ultimately have contributed to the spontaneous pro-arrhythmic effects of omega-3 fatty acid treatment$^{115}$. 

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4.2. Summary

In the present study, chronic treatment with omega-3 fatty acids had no protective anti-arrhythmic efficacy in animals susceptible to ischemically induced VF. In contrast, chronic treatment with omega-3 fatty acids prolonged myocyte repolarization and induced VF in a subset of dogs with healed infarctions that were previously resistant to VF. In conclusion, omega-3 treatment has some cellular anti-arrhythmic effects which do not translate into in vivo anti-arrhythmic efficacy in VF susceptible animals, while having pro-arrhythmic effects in VF resistant animals. Thus, evidence supporting the safety and efficacy of fish oil supplements for the prevention of lethal ventricular arrhythmias and sudden cardiac death remains elusive. Future studies should be directed toward studying the electrophysiogical changes that result from specific repolarizing K⁺ currents or myocyte calcium handling after chronic treatment with omega-3 fatty acids.
References


