Review
Pro- and antiarrhythmic properties of a diet rich in fish oil

Hester M. Den Ruijter a,⁎, Géza Berecki a, b, Tobias Ophof a, Arie O. Verkerk a, b, Peter L. Zock c, Ruben Coronel a

Abstract
Increased consumption of fish rich in omega-3 polyunsaturated fatty acids (ω3-PUFAs) is associated with decreased incidence of sudden cardiac death in post-myocardial infarction patients, but is also related to increased incidence of sudden death and arrhythmias in patients with acute myocardial ischemia. This review discusses the possible pro- and antiarrhythmic mechanisms of ω3-PUFAs in relation to various cardiac pathologies. Differences between circulating and incorporated ω3-PUFAs with respect to electrophysiology are emphasized.

We conclude that ω3-PUFAs alter cardiac electrophysiology and thereby may be pro- or antiarrhythmic, dependent on the mechanism of arrhythmia. As ω3-PUFAs may be antiarrhythmic under conditions that favour triggered activity, they may facilitate re-entrant arrhythmias. This may explain the contradictory outcomes of increased intake of fish oil on sudden death and arrhythmias in clinical trials.

Advice to increase intake of ω3-PUFA supplements or fatty fish should be tailored to individual patients with respect to the arrhythmogenic mechanisms associated with the underlying pathology.

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1. Introduction
Epidemiological studies support the protective effect of omega-3 polyunsaturated fatty acids (ω3-PUFAs) on cardiac death and, more specifically, on sudden cardiac death [1]. The Physicians Health Study reported that eating fish at least once a week was associated with a reduced risk of sudden cardiac death, but not of myocardial infarction in men [1]. In particular men with high blood levels of ω3-PUFAs had risk reductions of sudden death up to 90% [2]. As sudden cardiac death is in the majority of cases preceded by ventricular arrhythmias, it has been suggested that ω3-PUFAs are antiarrhythmic.

The risk reductions in sudden death by ω3-PUFAs were confirmed in several randomized controlled trials [3,4]. In DART, men with a prior MI were advised to increase their fatty fish intake. 2 years later, a 29% reduction in all-cause mortality was observed in the fish advised group compared to the control group [3]. The GISSI-Prevenzione Trial, a randomized clinical trial, investigated the effects of ω3-PUFAs supplements in the secondary prevention of myocardial infarction [4]. Treatment with ω3-PUFAs lowered mortality and sudden cardiac death was reduced by 45% [4].

Following publication of these data, the American Heart Association issued an advise to eat (fatty) fish at least two times a week [5]. Patients with documented coronary heart disease are recommended by the American Heart Association to consume about 1 g of fish oil fatty acids eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) per day. Fig. 1 displays the structural formulas of EPA and DHA.

Contrary to reports on the beneficial effects of fish oil, DART-2 reported that fish oil supplementation resulted in an increase in cardiac death in patients with angina pectoris [6]. Moreover, in patients with implanted cardioverter defibrillators...
Patients while an increased risk of cardiac death was reduced sudden cardiac death in post myocardial infarction observed in patients with angina pectoris[3,6]. This may be between circulating and incorporated parameters of life-threatening arrhythmias. Differences ω study the effects of fish oil supplementation on arrhythmias. Differences between circulating and incorporated ω-3-PUFAs may be limited to a population subgroup (e.g. patients with myocardial infarction and with heart failure) [10]. Indeed, fish oil supplementation reduced sudden cardiac death in post myocardial infarction patients [4] while an increased risk of cardiac death was observed in patients with angina pectoris [3,6]. This may be related to different mechanisms of the prevailing arrhythmia in these population subgroups. Arrhythmias in heart failure are induced by triggered activity [11,12], while those in acute myocardial ischemia are caused by reentry [13].

Increased mortality has also been observed in several antiarrhythmic drug trials [14–17]. The main reason for the disappointing outcomes in these trials is that drugs that suppress one type of arrhythmia may facilitate another. As a consequence, the Sicilian Gambit Working Group initiated a rational approach to study antiarrhythmic drugs against the background of various arrhythmia mechanisms and their vulnerable parameters [18]. A similar approach is required to study the effects of fish oil supplementation on arrhythmias.

In this paper we review the electrophysiological effects of ω-3-PUFAs in fish oil, EPA and DHA, against the vulnerable parameters of life-threatening arrhythmias. Differences between circulating and incorporated ω-3-PUFAs on electrophysiology are emphasized. Modulating factors of the autonomic nervous system and intracellular pathways are outside the scope of this review.

2. Arrhythmogenic mechanisms

Arrhythmia mechanisms are traditionally divided into abnormalities in impulse initiation (triggered activity, automaticity) and abnormalities in impulse conduction (reentry) [19]. Life-threatening arrhythmias are predominantly caused by triggered activity and reentry. This review limits itself to these mechanisms. Abnormal automaticity only plays a minor role in the sub-acute phase of ischemia and does not lead to life-threatening arrhythmias [13] and thus has minor relevance on the effects of fish oil on sudden cardiac death in the population.

2.1. Triggered activity and reentry mechanisms

Triggered activity may arise from early and delayed afterdepolarizations [20]. An early afterdepolarization (EAD, Fig. 3, panel A (top)) occurs when the action potential is prolonged during slow cardiac rhythms [21]. They may give rise to a particular polymorphic ventricular tachycardia named Torsade des Pointes after its typical appearance of the electrocardiogram. The congenital or acquired long-QT syndrome is a predisposing disorder which can lead to ventricular tachycardia and ultimately sudden death from these arrhythmias. One of the antiarrhythmic interventions for EADs is action potential shortening.

A delayed afterdepolarization (DAD, Fig. 3, panel A (bottom)) can occur during rapid rhythms when intracellular Ca2+ is elevated e.g. in heart failure. Antiarrhythmic interventions for DADs include action potential shortening, slowing heart rate, lowering intracellular Ca2+ and reducing cardiac excitability. Both types of afterdepolarizations may form the trigger for a re-entrant arrhythmia.

In normal heart, an impulse is generated by the sinus node and dies out when the whole heart has been activated. Reentry occurs if the same impulse continues to re-excite tissue. It is a mechanism for the maintenance of arrhythmias (Fig. 3, panel B). Conditions that favour a re-entrant circuit to arise are slow conduction and short refractory periods (the mathematical product of conduction velocity and refractory period — the wavelength — determines the length of the circle) [13]. In addition, the presence of a unidirectional block is prerequisite for a re-entrant circuit to arise [22].

2.2. Action potential duration, excitability and conduction velocity

Interventions aimed to alter action potential duration, excitability and conduction velocity can have both pro- and antiarrhythmic actions, depending on the mechanism of arrhythmia. Action potential shortening is antiarrhythmic when the underlying arrhythmia is caused by triggered activity initiated by EADs or DADs. However, action potential shortening promotes reentry by shortening the refractory period and, therefore, the wavelength. Conversely, action potential prolongation is an antiarrhythmic strategy for reentry, but may induce EADs and thereby Torsade de Pointes.

Reducing cardiac excitability is an antiarrhythmic intervention when the underlying arrhythmia is based on triggered activity. However, it may also slow conduction, one of the conditions that favour reentry.

In the following section we review the effects of fish oil on arrhythmogenesis, vulnerable parameters of arrhythmias [18], sarcolemmal ionic currents and intracellular Ca2+ handling. We will make a distinction between acute administrations of ω-3-PUFAs to ventricular myocytes and ω-3-PUFAs incorporated into the sarcolemma of ventricular myocytes caused by a dietary intervention with fish oil.
These treatments have dissimilar electrophysiological consequences although they are often presented as counterparts.

3. Experimental studies of fish oil on arrhythmias and electrophysiology

3.1. Fish oil and experimental arrhythmias

McLennan et al. [23] studied arrhythmias, induced by myocardial ischemia, in rats fed a 3-month diet rich in tuna fish oil, sunflower oil, or sheep fat. Only rats fed the tuna fish oil rich diet were protected from ischemia- and reperfusion-induced ventricular fibrillation [23]. Identical experiments in marmoset monkeys showed that a long term diet (30 months) rich in tuna fish oil decreased ventricular fibrillation threshold and resulted in a lower incidence of sustained ventricular fibrillation [24]. A diet rich in fish oil protected rabbits against dofetilide-induced Torsade de Pointes [25].

Not only a diet, but also intravenous administration of a fish oil emulsion reduces cardiac arrhythmias. Intravenous administration of EPA or DHA was studied in a dog model of cardiac sudden death where anterior myocardial infarction was created surgically. A hydraulic inflatable cuff was placed around the left circumflex artery so it could be compressed at will [26]. After recovery from surgery, the dogs were trained to run on a treadmill. During the final minute of a 20-minute episode of exercise, the circumflex artery was occluded. Dogs with reproducible ventricular fibrillation were selected for the experiments. Intravenous administration of EPA or DHA prevented fatal ischemia-induced arrhythmies [26]. An uncontrolled pilot study in patients with ICDs who had repeated episodes of ventricular tachycardia showed that infusion of ω-3-PUFAs during electrophysiological testing reduced the inducibility of sustained ventricular tachycardia in 5 out of 7 patients [27]. However, an important drawback of these two infusion studies is that pro-arrhythmia cannot be revealed as a direct consequence of the study designs. In cellular experiments, acute administration of EPA reduced the incidence of DADs in isolated myocytes from rabbits with heart failure [28].

A meta-analysis evaluating the effects of fish oil on arrhythmia outcome in various animal studies concluded that EPA and DHA can prevent arrhythmias in ischemia- but not reperfusion-induced arrhythmia models [29]. However, this study did not discriminate between dietary administration of fish oil and direct infusion of fish oil in the different animal models.

3.2. Action potential duration, excitability and conduction velocity

Acute administration of 5–25 μM ω-3-PUFAs resulted in action potential shortening in neonatal rat cardiomyocytes, rabbit ventricular myocytes and guinea pig ventricular myocytes, respectively [30–32]. Action potential shortening caused by superfusion of 10 μM EPA was accompanied by a threefold increase in relative refractory period in neonatal rat cardiomyocytes [31]. Normally, this phenomenon is referred to as post-repolarization refractoriness and is only present in depolarized cells during myocardial ischemia [13]. However, the increase in relative refractory period occurred at normal resting membrane potentials and can, therefore, only be explained by reduced membrane excitability [31].

Incorporated ω-3-PUFAs, resulting from a diet rich in fish oil in pigs, also lead to action potential shortening in isolated ventricular myocytes [33]. Rabbits fed a diet rich in α-linolenic acid (ALA), an ω-6-PUFA from flaxseed, had shorter QT intervals compared to the control [30].

On the contrary, direct infusion of (1–20 μM) ALA and EPA prolonged QTc in isolated rabbit hearts [34]. Also, in adult rat ventricular myocytes, an increase in action potential duration was observed with low (<10 μM) ω-3-PUFA concentrations, whereas action potential duration was decreased with higher (>10 μM) ω-3-PUFA concentrations [32, 35]. Therefore, ω-3-PUFAs may shorten or prolong action potential duration, depending on differences in species and on whether they are circulating (through acute administration) or incorporated into the sarcolemma (dietary administration). Fig. 2 shows typical examples of APD

Fig. 2. A; Action potentials recorded from isolated ventricular myocytes from pigs fed a control diet (control) or a diet rich in fish oil (ω3). Incorporation of ω3-PUFAs results in action potential shortening. B; Action potentials recorded after acute administration of different concentrations of ω3-PUFAs in guinea pig ventricular myocytes. Acute administration of ω3-PUFAs results in action potential shortening. C; Action potentials recorded after acute administration of different concentrations of ω3-PUFAs in adult rat ventricular myocytes. Acute administration of ω3-PUFAs results in both prolongation and shortening of the action potential. Adapted from Ref. [32], with the permission of Elsevier.
changes in response to either incorporated or acutely superfused ω3-PUFAs.

Membrane excitability in single cardiomyocytes was reduced following acute administration of fish oil fatty acids EPA and DHA (5–10 μM) [31]. EPA stabilized the resting membrane potential and increased diastolic stimulation threshold [31]. This finding is in line with observations in Langendorff-perfused rabbit hearts, where direct infusion of (1–20 μM) EPA and DHA increased the threshold for induction of a ventricular extrasystole in a concentration-dependent manner [34]. Contrarily, excitability of ventricular myocytes isolated from pigs fed a diet rich in fish oil was unchanged by incorporated ω3-PUFAs [33]. In the same model, maximal upstroke velocity remained the same, suggesting that a diet rich in fish oil does not modify conduction velocity.

Direct addition of DHA (1–20 μM) to the perfusate of spontaneously beating isolated rabbit hearts resulted in a reduction of longitudinal and transversal conduction velocities, although the latter to a lesser extent [34]. This may point to reduced sodium channel availability [34] and/or reduced gap junction conductance. At present, the effect of fish oil on gap junction intracellular communication is unknown. Results of fish oil on sodium channels are reviewed in the following section.

In conclusion, fish oil alters vulnerable parameters important in arrhythmogenesis, dependent on the manner of administration. Alterations in transmembrane currents underlie these changes.

3.3. Ion channels, exchangers and intracellular calcium handling

Fig. 3, panel C illustrates the cardiac ventricular action potential (top). The numbers 0–4 define the various phases of the action potential. They respectively represent: The upstroke of the action potential (phase 0); Initial rapid repolarization, also denominated as the notch of the action potential (phase 1); The action potential plateau, or persistent depolarization of the action potential, (phase 2); Rapid repolarization (phase 3) and diastole (phase 4) [36]. The time courses of the most relevant transmembrane currents that underlie the ventricular action potential are shown in the bottom panel. Inward, depolarizing currents are schematically represented by a downward deflection. Outward, repolarizing currents are represented by an upward deflection.

3.3.1. Sodium current

The cardiac sodium current (INa) is responsible for the upstroke of the action potential and plays an important role in impulse conduction (Fig. 3, panel C). Peak INa was reduced by 51% after acute administration of EPA and DHA (5–10 μM) in neonatal rat cardiomyocytes [37]. In addition, these concentrations of EPA and DHA shifted steady-state inactivation by ∼20 mV towards more negative potentials, without modifying activation properties. Similar results were obtained in human embryonic kidney (HEK293t) cells upon expression of the pore-forming subunit cDNA of the human cardiac sodium channel, hH1α, in HEK293t cells [38]. INa was suppressed in a concentration and voltage-dependent manner by extracellular application of EPA. Furthermore, steady state inactivation of INa was shifted by ∼27 mV towards more negative potentials. EPA enhanced slow inactivation and markedly prolonged recovery from inactivation of hH1α [38]. Several other ω3-PUFAs had similar effects on hH1α as EPA [38]. Generally, ω3-PUFAs showed higher affinity to block channels that were in the inactivated state compared to channels in closed or resting state [38].

Fig. 3. A; Early afterdepolarization (EAD, top panel) and delayed afterdepolarization (DAD, bottom panel). B; Schematic representation of normal conduction and a reentrant circuit. C; Normal ventricular action potential (top panel). Numbers denote the different phases of the ventricular action potential. The underlying ionic membrane currents and their schematic time course (bottom panel). See text for identification of the currents.
Interestingly, substitution of a single amino acid, asparagine with lysine at site 406 (N406K) in the D1-S6 region of hH1α reduced the potency of EPA to inhibit I_{Na} suggesting a direct interaction between EPA and the ion channel protein [39]. Activation properties of the sodium channel were unaltered by acute administration of EPA and DHA in both neonatal rat cardiomyocytes and HEK293t cells expressing hH1α cDNA, respectively [37,38]. However, in adult rat ventricular myocytes, acute administration of α3-PUFAs (25 μM) shifted the voltage dependence of activation of the cardiac sodium channel to more positive potentials [40]. In this study, the effects of EPA and DHA on the cardiac sodium channel correlated with their ability to increase membrane fluidity [40]. Mutations in the α-subunit of human cardiac Na⁺ channel cause an inactivation-deficient Na⁺ channel with a long-lasting persistent I_{Na}. This late I_{Na} was more sensitive to suppression by α3-PUFAs (IC_{50} < 1 μM) compared to the corresponding peak I_{Na} (IC_{50} < 5 μM) in HEK-293t cells [41].

In contrast with the acute effects, peak I_{Na} was unaffected by incorporated α3-PUFAs in ventricular myocytes isolated from pigs and rats that were fed a diet rich in fish oil [33,42]. In both studies voltage dependence of activation remained unaltered, whereas a shift (< 8 mV) in inactivation towards more negative potentials was observed [33,42].

In conclusion, acutely administered α3-PUFAs reduce I_{Na} [37,38,40]. This contributes to the observed reduction in excitability and to the slowing of ventricular conduction [31,34]. However, incorporated α3-PUFAs do not alter I_{Na} [33,42].

3.3.2. Calcium current (L- and T-type)

The L-type calcium current (I_{Ca,l}) is responsible for the plateau and contributes to the duration of the ventricular action potential (Fig. 3, panel C). I_{Ca,l} was suppressed by acute administration of α3-PUFAs in a concentration-dependent manner (IC_{50} < 10 μM) in ventricular myocytes (neonatal rat, adult rat and guinea pig) [32,43,44]. Activation properties of I_{Ca,l} remained unchanged, and a negative shift of steady-state inactivation was observed following acute administration of α3-PUFAs [43]. Block of I_{Ca,l} by acutely administered DHA (10 μM) was also demonstrated by Ferrier et al. [44] in guinea pig ventricular myocytes. Remarkably, α3-PUFAs reduced I_{Ca,l}, while preserving myocardial function [44].

Incorporation of α3-PUFAs into the sarcolemma also reduced I_{Ca,l} (∼20%), leaving activation properties unaltered [33]. At plateau potentials, ‘reopening’ of the L-type calcium channel was reduced in ventricular myocytes with incorporated α3-PUFAs compared to the control VMs [33]. This may prevent EAD formation and thereby triggered activity [33].

The effects of acute and incorporated α3-PUFAs on T-type calcium currents of the heart have not yet been described. However, it appears that the effects of acutely administered EPA and DHA on adrenal T-type calcium channels are similar to those described for L-type calcium channels [45]. In conclusion, acute administration of α3-PUFAs to ventricular myocytes reduces I_{Ca,l} [43,44] and thereby lowers the plateau of the action potential. Incorporated α3-PUFAs also reduce I_{Ca,l} and, more importantly, inhibit ‘reopening’ of the calcium channel at plateau potentials. This may prevent EADs and Torsade de Pointes [33].

3.3.3. Repolarizing currents

Early rapid repolarization or the notch of the action potential (phase 1) is caused by the transient outward current, carried by potassium ions (I_{to1}) and/or chloride ions (I_{Cl(cA) or I_{to2}}) (Fig. 3, panel C). I_{to} is larger in subepicardial ventricular myocytes compared to mid- and endomyocardial ventricular myocytes and thereby contributes to transmural dispersion in repolarization [46]. The slow and rapid components of the delayed rectifier current (I_{Kr} and I_{Ks}, respectively) are responsible for rapid repolarization (phase 3) of the action potential. The inward rectifier current (I_{Kr}) contributes to the terminal phase of repolarization and to the maintenance of the resting membrane potential.

The Kv4.3 gene encodes a large proportion of the ion channel responsible for I_{to1}. DHA blocked the Kv4.3 current in a concentration dependent manner with an IC_{50} of ~4 μM in a stable transfected mammalian cell line [47]. I_{to1} was inhibited following acute administration of EPA and DHA (IC_{50} < 10 μM) in rat and ferret ventricular myocytes [32,35,48]. In the presence of the antioxidant alphatocopherol, the inhibiting effect of DHA on I_{to1} is less pronounced, but still significant in rat ventricular myocytes [49]. However, incorporated α3-PUFAs did not alter I_{to1} in ventricular myocytes isolated from rats fed a diet rich in fish oil [33].

Block of I_{to1} would be expected to attenuate transmural dispersion of repolarization and thereby prevent reentrant tachyarrhythmias [46] but so far the effects of fish oil on transmural dispersion of repolarization have not been investigated.

I_{Kc} decreased following acute administration of EPA and DHA with an IC_{50} of 20 μM in ferret cardiomyocytes [48]. I_{Kc} consists of two components I_{Kr} and I_{Ks}. Acutely administered DHA (10 μM) blocked the human ether-a-go-go-related gene (HERG) channel which encodes the pore-forming subunit of the ion channel carrying I_{Kr}, in a time-, voltage- and use-dependent manner [50]. These data suggested that DHA preferentially binds to the open state of the channel [50]. However, incorporated α3-PUFAs did not cause any change in I_{Kr} of pig ventricular myocytes [33].

I_{Ks} has been studied in Xenopus oocytes upon expression of the channel pore-forming subunit, KvLQT1, in the presence or absence of the auxiliary subunit, hminK. I_{Ks} was enhanced (∼32%) by acute administration of DHA (20 μM), but not by EPA [51]. Upon incorporation of α3-
PUFAs, $I_{KS}$ was increased (∼70%) in pig ventricular myocytes [33].

A decrease of $I_{KS}$ following acute administration of ω3-PUFAs may, at least in part, explain why ω3-PUFAs prolong the action potential as less repolarizing current is present during the repolarization phase of the action potential. On the other hand, augmentation of $I_{KS}$ by acutely administered ω3-PUFAs leads to increased repolarizing current during the repolarization phase of the action potential. Whether the observed changes in repolarizing potassium currents caused by ω3-PUFAs lead to action potential prolongation or shortening will largely depend on the delicate balance between these and other depolarizing and repolarizing currents, species-differences regarding channel protein expression and the concentration of EPA and DHA in the superfusate [32].

$I_{K1}$ did not change upon acute administration of ω3-PUFAs in ferret cardiomyocytes [48]. Incorporation of ω3-PUFAs resulted in an increase of $I_{K1}$ by ∼50% in ventricular myocytes isolated from pig fed a diet rich fish oil [33]. Increased $I_{K1}$ by incorporated ω3-PUFAs contributes to the observed action potential shortening [33]. In addition, an increase in $I_{K1}$ may decrease excitability and thereby reduce DADs and triggered activity.

The transient outward current is not always carried by potassium ions but is, in several species, carried by chloride ions ($I_{Cl(Ca)}$) [33]. Data on $I_{Cl(Ca)}$ following acute administration of ω3-PUFAs are lacking. Incorporation of ω3-PUFAs into ventricular myocytes left $I_{Cl(Ca)}$ unaltered [33].

Other currents that affect action potential shape and duration, e.g. ultra-rapid delayed rectifier K+ current, have not yet been investigated in the presence of (circulating or incorporated) ω3-PUFAs.

3.3.4. Na+/Ca2+ exchanger

The Na+/Ca2+ exchanger (NCX) exchanges 3 Na+ ions for 1 Ca2+ ion and is therefore electrogenic. It can generate inward or outward current and contributes to the shape and duration of the action potential (Fig. 3, panel C).

Outward and inward Na+/Ca2+ exchanger current ($I_{NCX}$) was inhibited by acute administration of ω3-PUFAs (IC50<1 μM) in HEK293t cells [52].

Leifert et al. showed that a diet rich in fish oil increased the time constant of decay of Ca2+ transients in response to caffeine in isolated rat cardiomyocytes and suggested that NCX was involved [53]. This finding was supported by experiments in ventricular myocytes, where both outward and inward $I_{NCX}$ were reduced by ∼60% in the presence of incorporated ω3-PUFAs [33].

Inhibition of $I_{NCX}$ results in action potential shortening because less depolarizing current is available during the final repolarizing phase of the action potential (Fig. 3) [33,52]. Furthermore, $I_{NCX}$ contributes to DAD formation after spontaneous sarcoplasmic reticulum Ca2+-release. Possibly, reduced $I_{NCX}$ by both acute administration and incorporated ω3-PUFAs reduce DADs and triggered activity. Iso- and lusitropic effects of fish oil are outside the scope of this review. An antiarrhythmic action of fish oil may occur together with negative lusitropy.

3.3.5. Calcium homeostasis and sarcoplasmic reticulum function

Intracellular Ca2+ handling plays an important role in the genesis of triggered activity [54]. In experiments on rat ventricular myocytes, acute administration of EPA (1.5–15 μM) reduced the amplitude of Ca2+ transients and Ca2+ sparks without modifying Ca2+ spark kinetics [43]. The frequency of spontaneous waves of Ca2+-release was also diminished in the presence of EPA (10 μM). This indicates that sarcoplasmic reticulum function is affected by acute administration of EPA. Additionally, EPA decreased diastolic Ca2+ concentrations and imaging of Ca2+ waves showed that EPA also increased Ca2+ wave amplitudes and propagation [55]. Increased Ca2+ wave amplitudes by EPA correlated with enhanced sarcoplasmic reticulum load in rat ventricular myocytes [55–57]. It was concluded that ω3-PUFAs reduce sarcoplasmic reticulum Ca2+ uptake and also inhibit Ca2+-release (ryanodine receptors (RyR)). The potency of ω3-PUFAs (10–100 μM) to reduce open probability ($P_o$) of RyR was demonstrated in isolated sarcoplasmic reticulum vesicles [56,58].

Spontaneous Ca2+-release from the sarcoplasmic reticulum underlies DAD-related arrhythmias in heart failure [12,59]. Increased diastolic Ca2+ levels have been shown to induce spontaneous Ca2+-releases from the sarcoplasmic reticulum in a rabbit model of heart failure [12]. In that model, diastolic and systolic [Ca2+]i levels were reduced after acute administration of EPA (20 μM) to isolated ventricular myocytes [28]. Furthermore, spontaneous Ca2+-releases and DADs were reduced by EPA after burst-pacing in the presence of noradrenalin [28].

In contrast, incorporated ω3-PUFAs did not cause any alterations in diastolic Ca2+ or Ca2+ transient amplitude in pig ventricular myocytes. In these animals, the duration of the Ca2+ transient was shortened probably secondary to the shorter action potential recorded in these myocytes [33]. Similar results were obtained in ventricular myocytes isolated from rats fed a diet rich in fish oil where Ca2+ transients and diastolic Ca2+ values remained unaltered in the presence of incorporated ω3-PUFAs. In this study, sarcoplasmic reticulum Ca2+ content was also unaffected by incorporated ω3-PUFAs [53].

In summary, the effects of acute administration of ω3-PUFAs on intracellular Ca2+ handling are different from those with incorporated ω3-PUFAs. Acute administration of ω3-PUFAs leads to a decrease in diastolic Ca2+ concentration in rat ventricular myocytes and in ventricular myocytes isolated from rabbits with heart failure [28,55,57]. Furthermore, EPA reduces spontaneous waves of Ca2+-release from the sarcoplasmic reticulum that underlie DAD-related arrhythmias [28,12]. Therefore, acute administration of...
ω3-PUFAs may reduce triggered activity based on spontaneous Ca^{2+}-releases and DADs.

ω3-PUFAs have profound but various effects on cardiac electrophysiology, dependent on whether they circulate in the bloodstream (acute administration) or are incorporated into the sarcolemma of the myocyte (dietary intervention). Table 1 schematically summarizes ω3-PUFAs-induced changes in ion current densities and intracellular Ca^{2+} handling.

### 4. Pro- or antiarrhythmic effects in humans

Fish oil fatty acids EPA and DHA may be considered as electrophysiological drugs as they alter parameters important for the generation and maintenance of arrhythmias. Earlier antiarrhythmic drug trials were unsuccessful in reducing mortality in patients and in some cases, even increased mortality [14–17]. The negative outcomes in the antiarrhythmic drug trials were probably due to different mechanisms of arrhythmias of inhomogeneous patient populations. For example, the Cardiac Arrhythmia Suppression Trial (CAST) was designed to reduce ventricular ectopy in post myocardial infarction patients [16]. However, the initial effective suppression of ventricular ectopy by sodium channel blockers was followed by an increase in arrhythmic death in the following months [16]. Sodium channel blockade probably facilitates reentry by slowing conduction.

In line with the observations in CAST, ω3-PUFAs may also cause arrhythmias through sodium channels blockade and action potential shortening, two conditions that favour reentry-based arrhythmias [22]. Incorporated ω3-PUFAs shorten action potentials and thereby refractory periods [33] and acute administration of ω3-PUFAs slows ventricular conduction [34]. Therefore, arrhythmias based on reentry may occur more often in the presence of either circulating or incorporated ω3-PUFAs.

The conflicting outcomes of clinical trials with increased intake of fish oil regarding propensity to cardiac arrhythmias may be explained by inhomogeneous patient populations. The various patient subpopulations represent different arrhythmogenic mechanisms. Increased consumption of fish oil was antiarrhythmic in patients with a prior myocardial infarction. Likely, in this patient population, arrhythmias may have been based on triggered activity due to spontaneous Ca^{2+}-releases and prolonged action potentials. Harmful effects of fish oil were seen in patients with acute ischemia and in patients with a history of sustained ventricular tachycardia/ventricular fibrillation. The arrhythmogenic mechanism in these patients may have been based on reentry.

### 5. Acute administration versus incorporation of ω3-PUFAs

Increased consumption of fish oil leads to increased (circulating) blood-levels of ω3-PUFAs [2] and ω3-PUFAs incorporation into various tissues [60]. The relative contributions of circulating and incorporated ω3-PUFAs to electrophysiology have not yet been discriminated.

Data on plasma levels of ω3-PUFAs after dietary interventions are scarce. In post menopausal women, fish oil supplements (2 g EPA and 1.4 g DHA) for 5 weeks increased plasma concentrations of EPA and DHA up to 0.5–0.7 mM [61]. In dogs, a fish oil rich diet for 8 weeks increased EPA and DHA serum levels to 1.0–1.3 mM [62]. To which extent these plasma levels are actually ‘free’ EPA and DHA, is unknown. Negretti et al. estimated that maximum levels of free circulating ω3-PUFAs are ∼8–32 μM [55].

Dietary administration of fish oil leads to the incorporation of ω3-PUFAs into all the membranes including myocardial membranes of the heart. Several weeks after the start of the dietary intervention with fish oil, ω3-PUFAs account for ∼25% of total lipids in the sarcolemma [33,53].

What are the primary sites of action of circulating and incorporated ω3-PUFAs? Leaf and coworkers showed that substitution of a single amino acid in the hH1α diminished the inhibitory effect of the acutely administered EPA on I_{Na} [39], supporting a hypothesis of direct interaction between the fatty acid and ion channel. However, this explanation alone was unsatisfactory because other ligand gated ion channels that lack amino acid homology with voltage gated ion channels are also inhibited by an acute administration of ω3-PUFAs [63]. Therefore, it has been suggested that fatty acids primarily alter membrane properties close to ion channels rather than a direct interaction with the ion channel protein. Andersen and coworkers hypothesized that incorporation of the long acyl chain of the fatty acid...
compresses the phospholipid bilayer resulting in a mismatch with the hydrophobic length of the transmembrane channel [64]. The resulting compression or stretch by the long chain fatty acids will, therefore, alter the conformational state and conductance of the ion channel [65].

Another hypothesis is that acutely administered ω3-PUFAs alter membrane fluidity. Gating properties of INa were altered by acute administration of ω3-PUFAs, but also by benzyl alcohol [40]. The latter compound is known to increase membrane fluidity [40]. In this study, block of INa by ω3-PUFAs correlated with an increased membrane fluidity [40]. Nevertheless, another study debated the potency of PUFAs to alter membrane fluidity, since the molar ratio’s of PUFAs to membrane phospholipids was found to be very low (<1%) [66].

Dietary administration of fish oil alters lipid rafts and specific microdomains of the plasma membrane [67]. There is an increasing body of evidence for significant ion channel localization in lipid rafts [68]. Incorporated ω3-PUFAs enhance the propensity to form PUFA-rich microdomains and would thereby modify the function of proteins [69].

Changes in intracellular pathways, alterations in cellular redox status, gene expression and metabolism of phospholipids have also been reported by ω3-PUFAs and are reviewed by Judé and coworkers [70].

6. Conclusions

1. Circulating ω3-PUFAs have different electrophysiological effects from ω3-PUFAs incorporated into the sarcolemma.

2. Fish oil ω3-PUFAs are cardiac electrophysiological drugs that may result in pro- or antiarrhythmia depending on the underlying arrhythmogenic mechanism.

3. An advice to increase intake of ω3-PUFA supplements or fatty fish should be tailored to individual patients regarding the arrhythmogenic mechanisms associated with their pathology.

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