Dietary n-3 fatty acids promote arrhythmias during acute regional myocardial ischemia in isolated pig hearts☆

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Abstract

Objective: Dietary supplementation with fish oil-derived n-3 fatty acids reduces mortality in patients with myocardial infarction, but may have adverse effects in angina patients. The underlying electrophysiologic mechanisms are poorly understood. We studied the arrhythmias and the electrophysiologic changes during regional ischemia in hearts from pigs fed a diet rich in fish oil.

Methods: Pigs received diets rich in fish oil, in sunflower oil, or a control diet for 8 weeks. Hearts were isolated and perfused. Ischemia was created by occluding the left anterior descending artery. Diastolic stimulation threshold, refractory period, conduction velocity, activation recovery intervals and the maximum downstroke velocity of 176 electrograms were measured in the ischemic zone. Spontaneous arrhythmias during 75 min of regional ischemia were counted.

Results: More episodes of spontaneous ischemia-induced sustained ventricular tachycardia and ventricular fibrillation occurred in the fish oil and sunflower oil group than in the control group. More inexcitable myocardium was present in the ischemic zone in the group fed fish oil or sunflower oil than in the control group after 20 min of ischemia. After 40 min of ischemia, more block occurred in the control group than in the other groups. The downstroke velocity of the electrograms in the ischemic border zone was lower in the fish oil group and sunflower oil group than in the control after 20 min.

Conclusions: A diet rich in fish oil results in proarrhythmia compared to a control diet during regional ischemia in pigs. Myocardial excitability is reduced in the fish oil and sunflower oil group during the early phase of arrhythmogenesis. In the late phase of arrhythmogenesis, excitability is more reduced in the control group than in the fish oil and sunflower oil group.

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Keywords: Arrhythmias (mechanisms); Lipid metabolism; Diet; Nutrition; Fish oil; Excitability

1. Introduction

Clinical studies have demonstrated that a diet rich in n-3 fatty acids from fish oil reduces the risk of cardiac death [1]. In a large trial in patients with prior myocardial infarction, dietary n-3 fatty acids decreased the incidence of fatal heart disease relative to placebo [2]. Risk reduction of sudden cardiac death was larger than that of total cardiovascular mortality suggesting an
antiarrhythmic effect [2]. Indeed, there is an inverse correlation between serum phospholipid n-3 fatty acids and the occurrence of ventricular arrhythmias [3]. However, an advice to eat fish or to take fish oil capsules in 3114 men with angina pectoris (without myocardial infarction) tended to increase the risk of sudden cardiac death [4]. Two trials on patients with an automatic cardioverter defibrillator (AICD) did not show differences in mortality between patients with and without fish oil supplementation [5,6]. Another trial in patients with an AICD demonstrated a significant increase of recurrent ventricular tachycardia (VT) or ventricular fibrillation (VF) during a 2-year treatment with n-3 fatty acids [7]. Patients elected for AICD implantation, however, constitute a diverse population with respect to cardiac pathologies. It may well be that a diet rich in fish oil is antiarrhythmic in some patients and proarrhythmic in others.

So far, the electrophysiological mechanisms of n-3 fatty acids were investigated following acute administration in dogs [8], single myocytes or expression systems [9–12]. Superfusion of n-3 fatty acids directly influence sarcolemmal sodium- [13,14] and calcium-channels [15,16]. However, the electrophysiological mechanism(s) of dietary, incorporated n-3 fatty acids on arrhythmogenesis have not been elucidated.

We hypothesize that dietary n-3 fatty acids are proarrhythmic during acute regional myocardial ischemia. We therefore documented arrhythmogenesis during acute regional ischemia in hearts of pigs fed a diet rich in fish oil, sunflower oil or a control diet, and studied the underlying electrophysiological changes.

2. Methods

The experimental protocol complied with the Guide for the Care and Use of Laboratory Animals published by the US National Institutes of Health (NIH publication 85-23, revised 1996) and was approved by the institutional animal experiments committee. Male piglets (7 week old) entered the study and were assigned to diets rich in fish oil, sunflower oil or a control diet, and studied the underlying electrophysiological changes.

Table 1 Composition of food

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>N-9</th>
<th>N-3</th>
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<tr>
<td>g/100g feed (%)</td>
<td></td>
<td></td>
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<tr>
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<td>6.22(14.58)</td>
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<td>Total</td>
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<td>0.72(1.69)</td>
<td>1.09(2.57)</td>
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<td>Mono-unsaturated fatty acids</td>
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<tr>
<td>Total</td>
<td>1.60(3.76)</td>
<td>3.82(9.02)</td>
<td>1.08(2.54)</td>
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<td>C18:1n-9</td>
<td>1.41(3.34)</td>
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<tr>
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<td>1.40(3.31)</td>
<td>3.02(7.12)</td>
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<tr>
<td>C18:2n-6</td>
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<td>1.39(3.28)</td>
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<tr>
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<td>0.03(0.07)</td>
<td>0.64(1.51)</td>
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docosahexaenoic acid (DHA, C22:6n-3), and oleic acid (C18:1n-9) content. The control diet did not contain EPA or DHA. Oleic acid (sunflower oil) was used to detect a potential specific effect of multiple double bonds and/or a double bond at the n-3 position.

After 8 weeks of feeding, pigs received ketamine (Nimetek, Animal Health BV, NL) 350 mg, azaperone (Stresnil, Janssen, NL) 80 mg and atropine (Centrafarm) 0.5 mg (intramuscularly) and were anesthetized with 20 mg/kg pentobarbital (Nembutal, CevaSate Animale) intravenously. They were intubated and ventilated with room air and isoflurane (Forene, Abbott). Expiratory CO2 was monitored. Heparin (Leo Pharmaceuticals, 5000 IU) was injected intravenously. Blood was collected and the heart was isolated after a midsternal thoracotomy, and perfused with blood—Tyrode’s mixture [17]. A short occlusion of the left anterior descending artery (LAD) showed the extent of cyanosis. A ligature was left around the LAD. AV-block was made. The perfusate [K+] was 4.2+−0.2 mM (m+−sem, n=8).

The occlusion was made just below the first diagonal branch. The relative size of the ischemic area was 25.6+−1.3, 23.9+−1.4 and 26.4+−1.3% (m+−sem, N-3, N-9 and control, respectively) of ventricular weight (NS).

2.1. Data acquisition

A rectangular 176 electrode matrix (interelectrode distance 2 mm) was sutured over the prospective ischemic border. One electrode at about 1 cm into the prospective ischemic border was selected for pacing (exact distance was measured later). A bipolar stimulating electrode was placed in the normal myocardium near the multi-electrode. A virtual ground electrode was connected to the aortic root and served as a reference for the unipolar recordings.

2.2. Electrophysiologic studies

After equilibration (30 min), the heart was paced (cycle length 450 ms) from a site within the prospective ischemic zone or from the normal zone. There, the diastolic stimulation threshold was determined (using cathodal rectangular current pulses) with 1 μA accuracy and was adjusted during ischemia. A diastolic stimulation threshold exceeding 1500 μA was considered a sign of inexcitability. The effective refractory period (ERP) was measured by programmed stimulation (8 basic stimuli at diastolic stimulation threshold, one premature stimulus at twice diastolic stimulation threshold) in the ischemic tissue. Ischemia was produced after ERP and diastolic stimulation threshold measurements had stabilized.

During ischemia, stimulation was performed from the normal myocardium. Electrograms were acquired every minute (sample interval 0.5 ms, duration 3.5 s). Every 5 min diastolic stimulation threshold and ERP were determined. If VF occurred it was terminated by a DC shock. When more than 6 consecutive shocks were necessary to defibrillate the heart the protocol was discontinued. This occurred in 2/11, 3/11 and 1/6 heart in the N-3, N-9 and control group respectively.

Data analysis was performed with a custom made program [18]. Maximum downstroke velocity (dV/dtmin) of the initial
Deflection was used as an index of local excitability [19]. Conduction velocity (longitudinally and transversely to fiber direction) was calculated from isochronal maps of local activation times recorded following stimulation in the center of the electrode grid. Conduction block was defined as a site with a monophasic electrogram and no Laplacian activity [20]. Activation recovery intervals were calculated as a measure of local action potential duration, as the time difference between the moment of activation and repolarization. The latter was defined as the time of maximal \( \frac{dV}{dt} \) during the upstroke of the T-wave (in any configuration).

After the ischemic period (75 min), the circumflex artery was cannulated and flushed with Tyrode’s solution (composition see [17]). A biopsy was taken from the perfused tissue for the determination of the lipid profile.

A VT was defined as a rapid ventricular rhythm of more than 3 subsequent premature complexes. Sustained VT (sVT) was defined as a VT of more than 30 s duration. In accordance with earlier studies, the early phase (1a) of arrhythmogenesis was defined as the period between 0–30 min of ischemia, the delayed phase (1b) period between 30 and 75 min [19].

2.3. Lipid analyses

Phospholipids from plasma and heart were isolated with aminopropyl bonded phase columns (Varian Bond Elut) [21]. Phospholipids were saponified and methylated with boron trifluoride (Pierce, IL, USA). Formed fatty acid methyl esters were subjected to capillary gas chromatography using a Chrompack column (Fused Silica, Chrompack), a flame ionisation detector and H2 as carrier gas.

2.4. Statistics

Data are means±SEM. Data were statistically analyzed using one-way ANOVA (if appropriate on ranked data) on the three experimental groups. Post-hoc testing for multiple comparisons was done with the Holm–Sidak or Dunn’s test. A \( p \leq 0.05 \) was considered statistically significant.

3. Results

3.1. Feeding experiments

The diet rich in fish oil increased EPA and DHA in plasma phospholipids at the expense of both n-9 and n-6 fatty acids.

![Fig. 1. Arrhythmias during ischemia quantified in 5 min bins in the three experimental groups. Ventricular extrasystoles, doublets and non-sustained ventricular tachycardias are included in the category ‘other arrhythmias’. Note that number of arrhythmias in the latter category is divided by 10 for clarity. In the three groups the arrhythmias occur in two phases (1a and 1b).]
Table 3

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<tr>
<td>1a+1b</td>
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*Indicates significant differences compared to the control group.

VTs were monomorphic in all three groups. The percentage of sVTs of all VTs in each group was not significantly different (ANOVA).

Fig. 2 shows examples of activation maps of a basic beat at 0, 20 and 40 min of ischemia and of the first beat of VF (40 min) in the three groups. VF did not occur in the control group and the onset of a VT is shown. Note that more crowding of isochrones and areas of activation block occurs in the N-3 and N-9 groups than the control group at 20 min. After 40 min of ischemia there is more block and slowed conduction in the control group. This is also evident from the appearance of monophasic electrograms in the normal, the border and the central ischemic zone (N, B, and C respectively). In the examples the first activation from VF originated from outside the electrophysiological border (dotted line) leading to increased conduction slowing. Overall, the origin of VF was not different between the three groups.

3.4. Excitability and activation block

Because sVT/VF was more prevalent in the N-3 and N-9 groups than in control group, whereas the sum of all arrhythmias was not significantly different in these groups, we...
argued that the difference is caused by a different substrate for reentry rather than by a difference in the number of triggers. We therefore studied excitability, the prime determinant of the substrate of ischemia-induced reentrant arrhythmias in both the early and delayed phase of arrhythmogenesis [19,22].

3.5. Activation block

Monophasic electrograms are indicative of the absence of local activation. In Fig. 3 more monophasic electrograms occur in the N-3 and N-9 heart than in the control heart after 20 min of ischemia. The reverse is true after 40 min. Fig. 4 shows the time course of development of sites of activation block (within the ischemic area) in the three groups. The area under the curve (up to the secondary rise) was significantly larger in the N-3 than in the control group (p < 0.05 ANOVA). The N-9 group did not differ from the other groups. At 20 min of ischemia the N-3 and N-9 groups differed significantly from the control group (ANOVA on ranks, all p < 0.01). At 40 min of ischemia no differences in the percentage of activation block was detected.

The onset of the second rise in block-electrograms was later in the N-3 and N-9 groups than in the control group (N-3: 39.0 +/- 1.6 (n=8), N-9: 39.3 +/- 1.7 (n=8) and control: 25.8 +/- 1.5 (n=6) min of ischemia, p < 0.001 ANOVA).

3.6. Local electrograms and diastolic stimulation threshold

The site of stimulation was on the average 13.4 ± 0.67 mm distant from the electrophysiological border (defined by the region separating tissue with ST-elevation from that with ST-depression in subsequent mapping experiments) and was not significantly different between the three groups (ANOVA).

Fig. 5 shows the averaged change of the diastolic stimulation threshold (DST) in the three groups. The area under the curve (up to the moment of the secondary rise in diastolic stimulation threshold) was larger in the N-3 group than in both the N-9 and control group (ANOVA). Analysis of variance of
the values at the time points 20 and 40 min of ischemia yielded a statistically significant difference at 20 min between N-3 and both the N-9 and control groups. At 40 min the DST was not significantly different.

To obtain a more generalized measure of excitability in the entire ischemic zone we analyzed the downstroke velocity of the initial deflection \( \frac{dV}{dt_{\text{min}}} \) of all electrograms recorded from the ischemic zone (stimulation from the normal zone) at 20 min and 40 min of ischemia. Sites with monophasic electrograms were exempted.

Fig. 6 shows the averaged distribution of \( \frac{dV}{dt_{\text{min}}} \) in the ischemic tissue from the three groups at 20 min of ischemia. The averaged \( \frac{dV}{dt_{\text{min}}} \) was significantly lower in the N-3 (\( n = 10 \)) and the N-9 (\( n = 10 \)) groups compared to the control (\( n = 6 \)) group (\( -2.1^{+/-} 0.4, -3.1^{+/-} 1.6 \) V/s and \( -5.6^{+/-} 1.0 \) V/s, respectively, \( p = 0.005 \), ANOVA). The lower panel shows the percentage of sites within the central ischemic zone (sites with activation block excepted) with a \( \frac{dV}{dt_{\text{min}}} \) less than 4 V/s: it was higher in the N-3 and N-9 groups than in control.

After 40 min of ischemia, \( \frac{dV}{dt_{\text{min}}} \) was not different between the groups, either in the border zone, the central zone or in the entire ischemic zone. The \( \frac{dV}{dt_{\text{min}}} \) of the electrograms recorded from the non-ischemic myocardium after 20 min of ischemia was not significantly different between the groups (data not shown).

### 3.7. Refractory period

Measurements of the refractory period in the ischemic zone (at the same sites where diastolic stimulation threshold was measured) and conduction velocity were hampered by the development of inexcitability and arrhythmias. Effective refractory period data from the first 40 min of ischemia were obtained. No differences in effective refractory period were detected between the groups (ANOVA, \( p = 0.08 \)).
4. Discussion

Our study shows that a diet rich in n-3 fatty acids results in a larger incidence of life-threatening arrhythmias than a control diet. In the 1b phase of arrhythmias VF constitutes a larger fraction of these arrhythmias in the fish oil fed group than the group fed sunflower oil. The proarrhythmia observed during ischemia in the N-3 group, in combination with the unchanged number of triggers, supports the notion that the arrhythmogenic substrate was altered by n-3 fatty acids. The proarrhythmic potential of a diet rich in fish oil is correlated with the development of a larger mass of inexcitable tissue in the ischemic zone, particularly in the central part of the ischemic myocardium, and with a later secondary decrease in excitability.

Prior to the initiation of regional ischemia, measures of refractoriness, conduction or excitability did not differ between the three groups. In contrast, n-3 fatty acids resulted in action potential shortening in isolated myocytes of pigs fed a diet rich in fish oil [23]. The action potentials were recorded under highly controlled experimental conditions whereas larger variabilities may be displayed in perfused hearts. Also, it cannot be excluded that circulating n-3 fatty acids present in the perfusion experiments but absent during action potential recordings may have modified the electrophysiologic changes. The individual contribution of circulating versus incorporated n-3 fatty acids is yet to be determined.

In our study, dietary n-3 and n-9 fatty acids resulted in a moderate increase in inexcitable tissue and thereby favored the onset of reentrant arrhythmias. A larger increase in the amount of inexcitable tissue is antiarrhythmic after administration of lidocaine or after preconditioning with low-flow ischemia [19,24].

N-3 fatty acids are not antiarrhythmic in every clinical trial. In trials involving patients with prior myocardial infarction fish oil reduced the risk in sudden death up to 45% [25,26]. Many other studies support this beneficial role of increased fish intake [1,2,26–28]. However, in a trial in patients with angina pectoris without prior myocardial infarction, the incidence of sudden death tended to be increased [4]. A recent trial in patients with an AICD demonstrated a significant increase of recurrent VT/VF during a 2-year treatment with n-3 fatty acids [7], whereas other AICD trials did not show a difference between the patients with and without n-3 fatty acids supplementation [5,6].

Our data may help to explain these discrepant findings of fish oil supplementation in clinical trials. In patients with heart-failure associated arrhythmias based on triggered activity [29] decreased myocardial excitability is antiarrhythmic. However, a decrease in myocardial excitability is proarrhythmic under conditions of prolonged myocardial ischemia. A diet rich in fish oil may therefore be proarrhythmic in patients with angina.

We have compared three groups of pigs in which the fish oil and the sunflower oil diets were equal in fat content, energy content but differed in the amount of n-3 fatty acids. The observation that VF constituted a larger fraction of life-threatening arrhythmias during the 1b phase in the N-3 group than in the N-9 group suggests that at least some of the observed differences are explained by the n-3 position and/or the number of double bonds of the fatty acid. However, the large differences in excitability between the N-3 and N-9 groups on one hand versus the control group on the other hand indicates that other dietary factors play a role as well. Because the control diet in our experiments was different in more than a single aspect from both the fish oil and sunflower oil rich diets it is difficult to assign a cause for the observed differences between the groups. Sunflower oil therefore appears to be a poor control fatty acid in the setting of ischemia.

The absence of an antiarrhythmic effect in our study is at variance with previous experimental work on n-3 fatty acids [8,30,31]. One explanation may be that some of these studies used acute administration of free n-3 fatty acids rather than feeding. Another explanation may be that we have selected a pig model with a moderate amount of arrhythmias, allowing proarrhythmia to be uncovered. In a highly arrhythmogenic model n-3 fatty acids may be antiarrhythmic while in a less arrhythmogenic model n-3 fatty acids may facilitate arrhythmias. For example, the study by Billman et al. involved a maximally arrhythmogenic model by selecting dogs with reproducible VF for inclusion in the study [8]. Furthermore, species differences may explain these contrasting findings. Pigs and dogs display two phases of arrhythmogenesis during regional myocardial ischemia, whereas small rodents do not [32]. In studies on long term dietary intake of n-3 fatty acids in rats the severity and amount of arrhythmias induced by ischemia/reperfusion were reduced [30,31].

Acutely administered n-3 fatty acids block the cardiac sodium channel, possibly by a direct interaction [13]. Alterations in myocardial excitability during regional ischemia in our study are in concordance with this idea and are possibly regulated through circulating n-3 fatty acids or incorporated n-3 fatty acids. Incorporated n-3 fatty acids alone do not affect sodium current density [23]. However, it is possible that release of incorporated n-3 fatty acids during regional ischemia may have caused alterations in excitability [5].

The 1b phase of ischemia-induced arrhythmogenesis is related to the closure of gap junctions. The high incidence of VF in this phase together with the delayed development of inexcitability in the fish oil group supports the idea that n-3 fatty acids alter gap junctions. The latter may be mediated by a decrease of the intracellular Ca²⁺-concentration induced by fish oil [33].

4.1. Strengths and limitations

Mechanistic information has so far been derived from experimental studies in which n-3 fatty acids were administered acutely [8,12]. Some feeding studies have been performed in pigs but these have focused on recovery of hemodynamic function [34]. Our study is the first to demonstrate changes in electrophysiology underlying arrhythmogenesis of n-3 fatty acids in a chronic feeding model.
Although the occurrence of VF is larger in the N-3 group compared to the N-9 group we cannot explain this difference in terms of excitability.

We did not evaluate the influence of other arrhythmogenic factors (myocardial stretch, the autonomous nervous system [35]), but the results indicate that at least part of the electrophysiological effects of dietary n-3 fatty acids are conveyed in the absence of these factors.

In conclusion, our study demonstrates that dietary n-3 and n-9 fatty acids reduce excitability and cause arrhythmias during regional ischemia.

Acknowledgements

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References

