Intravenous infusion of n-3 polyunsaturated fatty acids and inducibility of ventricular tachycardia in patients with implantable cardioverter defibrillator

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Aims Marine n-3 polyunsaturated fatty acids (PUFA) may have antiarrhythmic effects. The aim of this study was to investigate the effect of intravenously administered n-3 PUFA on the inducibility of ventricular tachycardia (VT) in patients with an implantable cardioverter defibrillator (ICD).

Methods and results In a randomized, placebo-controlled cross-over study, patients with an ICD underwent two electrophysiological studies using the stimulation possibilities in the ICD preceded by intravenous infusion of either a lipid emulsion delivering 3.9 g n-3 PUFA or placebo (0.9% saline). The level of stimulation required to induce sustained monomorphic VT was ranked in order from least to most aggressive, and non-inducibility was ranked highest. The content of n-3 PUFA as free fatty acids (FFA), plasma phospholipids, and platelet phospholipids was measured by gas chromatography. Eight patients were included, and six of these completed the study. The content of n-3 PUFA as FFA and in platelet phospholipids increased more after n-3 PUFA infusion than after placebo ($P < 0.001$). Of the five patients who were inducible after placebo, two were no longer inducible after n-3 PUFA infusion and another two required stronger stimulation to induce VT. The difference in the stimulation required after placebo and after n-3 PUFA was borderline significant ($P = 0.063$, Wilcoxon signed-rank test).

Conclusion Intravenous n-3 PUFA tended to decrease VT inducibility, but a larger study is warranted.

Keywords Fish oil • n-3 Polyunsaturated fatty acids • Implantable cardioverter defibrillator • Ventricular tachycardia • Inducibility • Electrophysiological study

Background

Sudden cardiac death (SCD), often the first manifestation of ischaemic heart disease (IHD), is primarily caused by ventricular arrhythmias.1 Despite advances in the management of IHD, SCD continues to account for a large proportion of cardiovascular mortality.2 Marine n-3 polyunsaturated fatty acids (PUFA), in particular eicosapentaenoic acid (EPA; 20:5 n-3) and docosahexaenoic acid (DHA; 22:6 n-3), have been associated with a reduced risk of SCD in observational studies3,4 and in intervention trials.5 Patients with an implantable cardioverter defibrillator (ICD) provide a unique possibility to study a possible direct antiarrhythmic effect of n-3 PUFA. The incidence of ventricular arrhythmias is high and arrhythmic episodes are recorded by the device and can be documented accurately. Furthermore, ventricular arrhythmias can be induced through the ICD during an electrophysiological (EP) study, which is useful in testing antiarrhythmic drug effects. Schrepf et al.6 performed an EP study before and after intravenous n-3 PUFA infusion, and in five of seven patients who were initially inducible, ventricular tachycardia (VT) could...
no longer be induced after n-3 PUFA, suggesting an immediate anti-arrhythmic effect of n-3 PUFA. These interesting findings prompted us to conduct a similar study, to see if the results could be confirmed in a randomized, placebo-controlled design.

The aim of this study was to investigate the effect of intravenously administered n-3 PUFA on inducibility of VT in patients with an ICD. Furthermore, the effect of intravenous n-3 PUFA infusion on levels of n-3 PUFA in plasma free fatty acids (FFA), plasma phospholipids, and platelet phospholipids was measured.

**Methods**

We conducted a randomized, placebo-controlled, double-blind cross-over study. Informed consent was obtained from all participants. The study was approved by the Regional Ethics Committee of Northern Jutland, Denmark, (VN-20050030) and the Danish Medicine Agency (EudraCT 2005-002386-37). The study complied with the Helsinki Declaration and the principles of Good Clinical Practice and was registered in clinicaltrials.gov (NCT00259025).

**Participants**

All patients with an ICD who were scheduled for regular follow-up visits at the Department of Cardiology, Aalborg Hospital, Aarhus University Hospital, Denmark were evaluated by reviewing their medical records. The study included clinically stable patients with an ICD and in addition the following was required.

1. The ICD had been implanted at least 3 months before inclusion.
2. Sustained monomorphic VT had been induced before implantation (primary EP study)
3. The last VT episode prior to inclusion had been terminated by anti-tachycardia pacing (ATP) or (if no VT episodes had occurred since ICD implantation) monomorphic VT induced at the primary EP study had been terminated by ATP.

Patients were excluded for the following reasons: (a) Age <18 years or >80 years, (b) premenopausal status in women, (c) allergy to fish or egg protein, (d) haemoglobin A1c >10%, (e) creatinine clearance <30 mL/min, (f) alanine aminotransferase >150 U/L, (g) international normalized ratio >3.5, (h) fasting plasma triglycerides >3 mmol/L, (i) serum potassium <3.5 mmol/L, (j) blood pressure >160/90 mmHg, (k) myocardial infarction (MI) or coronary revascularization within 6 months, (l) other serious illness, (m) inability to give informed consent. Exclusion criteria (b) through (h) were related to the safety of infusing a lipid emulsion, whereas (i) through (k) were related to EP study issues. Patients who normally used fish oil supplements were asked to refrain from fish oil in the 8 weeks preceding and during the investigations. Medications were not changed during the study.

**Procedure**

On two occasions 4 weeks apart, the patients underwent an EP study via the ICD using a standardized stimulation protocol (see below for details). Each EP study was preceded by intravenous infusion with n-3 PUFA or placebo, and performed within 30 min after the end of the infusion. Four weeks later, the EP study was repeated and patients who received n-3 PUFA in relation to the first EP study now received placebo and vice versa. The n-3 PUFA infusion consisted of 100 mL of a lipid emulsion with a high content of n-3 PUFA (Omegaven® 10%, Fresenius Kabi, Uppsala, Sweden). The same batch of Omegaven® was used throughout the study, and 100 mL of the lipid emulsion contained 3.9 g n-3 PUFA (2.07 g EPA and 1.87 g DHA). One hundred millilitre of 0.9% saline was used as placebo. The infusions were given during the 4 h immediately preceding EP study at a constant infusion rate of 25 mL/h. The tubing and the bottles containing lipid emulsion or placebo were covered to maintain the blinding to all other than the study nurse who administered the infusions. The EP studies were performed by an experienced electrophysiologist, and an anaesthesiologist was present during the EP procedure. All EP studies were performed between noon and 1 PM, and the patients took their usual medication 5–6 h prior to inducibility testing.

**Electrophysiological study protocol**

Inducibility was evaluated using eight-beat drive trains at a pacing cycle length of 500 ms. Up to three extra stimuli were delivered, and the coupling interval of the last extra stimulus was decreased in 10 ms decrements until ventricular refractoriness was reached. When extra stimuli were necessary, the previous extra stimulus was set at 20 ms longer than refractoriness. If monomorphic VT was not induced using this protocol, the stimulation was repeated with a pacing cycle length of 400 ms and up to three extra stimuli. If sustained VT was induced, it was first attempted to terminate the arrhythmias with ATP. During EP study, the shock modality of the ICD was disabled, and any potentially fatal arrhythmia not terminated by ATP was to be treated with external defibrillation. The level of stimulation required to induce sustained monomorphic VT was ranked in order from least aggressive (500 ms pacing cycle length with one extra stimulus) to most aggressive (400 ms pacing cycle length with three extra stimuli), and non-inducibility was ranked highest (Figure 1).

**Fatty acid analyses**

Fatty acid composition of plasma FFA and platelet phospholipids was determined in blood drawn after an overnight fast before the infusions started and 15 min before the EP study while the patients were still fasting. Plasma was separated from ethylenediaminetetraacetic acid (EDTA) blood, flushed with nitrogen to avoid oxidation, and stored at −80°C until analysis. Total lipids were extracted from plasma with chloroform:methanol (CHCl3:MeOH) containing butyraldehydelhydroxytoluene as antioxidant. Lipid extracts were dissolved in CHCl3 and transferred to a Bond Elut NH2 column (Varian, Middleburg, The Netherlands) pre-conditioned with hexane. The column was washed with CHCl3 to remove triglycerides and cholesterol esters. Phospholipids were eluted with CHCl3–MeOH, and additional MeOH, and FFAs were eluted with CHCl3–MeOH–acetic acid. The FFA fraction was methylated with sulphuric acid in MeOH, and the phospholipid fraction was methylated with potassium hydroxide (KOH) in MeOH.

Platelets were isolated from EDTA blood, washed twice with 0.9% saline, flushed with nitrogen, and stored at −80°C until analysis. Platelet phospholipids were extracted with dichloromethane (CH2Cl2): MeOH containing butyraldehydelhydroxytoluene and methylated with KOH in MeOH.

The fatty acid composition was analysed by gas chromatography using a Varian 3900 GC equipped with a CP-8400 auto sampler, a flame ionization detector, and a CP-sil 88 60 m × 0.25 mm ID capillary column (Varian, Middleburg, The Netherlands). Split injection mode, temperature programming from 90 to 210°C, and constant flow were used. Helium was used as carrier gas. Commercially available standards (Nu-chek-Prep, Inc., Elysian, MN, USA) were used to identify individual fatty acids. FFA concentrations were expressed as µg/mL, and the fatty acid composition in phospholipids was expressed as percent of total fatty acids.
Statistics
The study by Schrepf et al. included 10 patients. A double-blinded, randomized, placebo-controlled cross-over study with a similar number of patients would have larger statistical power and, therefore, we aimed at including 12 ICD patients. Wilcoxon signed-rank test was used to test for difference in VT inducibility after \( n \)-3 PUFA infusion and placebo. Paired t-test was used to test for differences in fatty acid levels before and after infusions and differences in the changes seen after placebo and \( n \)-3 PUFA infusion. A \( P \)-value less than 0.05 was considered statistically significant.

Results
Of 228 patients assessed, 198 were not eligible for inclusion, and 22 refused to participate. The most common reason for non-eligibility was that inducibility testing had not been performed before ICD implantation or that VT was not induced during the primary EP study. Eight patients gave written informed consent, but two patients had to be withdrawn from the study before randomization due to worsening of their clinical condition. Thus, only six patients were randomized and completed both investigations. Tables 1 and 2 show the characteristics of the participants. Five of the patients had a prior MI. Patient B had a biventricular ICD. There were no complications to the infusions or to the EP studies.

Ventricular tachycardia inducibility
The individual results of the EP studies are shown in Figure 1. Patient C was non-inducible during both EP studies. Of the five patients inducible after placebo, two patients were no longer inducible after \( n \)-3 PUFA infusion, whereas two patients required a stronger stimulation and one patient required the same stimulation for VT induction. The difference in the stimulation required after placebo and after \( n \)-3 PUFA was borderline significant (\( P = 0.063, \) Wilcoxon’s signed-ranks test).

Discussion
We found a trend towards a decreased VT inducibility after \( n \)-3 PUFA. This effect was seen in spite of all patients being treated with beta-blockers. Also, Patient B who was treated with amiodarone required a considerably stronger stimulation after \( n \)-3 PUFA. The patient who was non-inducible during both EP studies differed from the other five patients in having a normal LVEF and no IHD.
To our knowledge, only two other studies have investigated the antiarrhythmic effect of n-3 PUFA with VT inducibility as outcome measure, and only one of these studies used intravenous n-3 PUFA administration. The study by Schrepf et al.6 was small, non-blinded, and without a placebo group but, nevertheless, the results were intriguing. They performed baseline EP studies in 10 ICD patients (nine with IHD). Seven patients who were inducible at baseline were given intravenous n-3 PUFA infusion after which the EP study was repeated, and five of these patients were no longer inducible after n-3 PUFA. Schrepf et al.6 used the same fish oil preparation and dose as in the present study and found an increase in n-3 PUFA as FFA after n-3 PUFA infusion, whereas n-3 PUFA in plasma phospholipids were only measured before the infusions. The EP study protocol was different, and the patients underwent EP study twice during the same day (before and immediately after n-3 PUFA infusion). Schrepf et al.6 used the same fish oil preparation and dose as in the present study and found an increase in n-3 PUFA as FFA after n-3 PUFA infusion, whereas n-3 PUFA in plasma phospholipids were only measured before the infusions. The EP study protocol was different, and the patients underwent EP study twice during the same day (before and immediately after n-3 PUFA infusion). We performed two EP studies 4 weeks apart, because of concern that performing an EP study twice during the same day might affect inducibility during the second testing and also because a wash-out period was mandatory in our cross-over design.

Recently, Metcalf et al.7 reported a study in patients with IHD undergoing an EP study through the ICD immediately following ICD implantation. Twelve patients who were inducible subsequently consumed 900 mg/day n-3 PUFA as oral supplements for 6 weeks followed by repeated testing. In five of the 12 patients treated with n-3 PUFA, VT was no longer inducible at the second testing, and in another five patients, VT was more difficult to induce. Another 14 inducible patients served as controls with repeated testing after 6 weeks, and in this group, there was no difference in the stimulation required to induce VT between baseline and the repeated EP study. There was no statistically significant effect of fish oil vs. no fish oil on the single endpoint of inducibility ($P = 0.062$). In the fish oil-treated groups, EPA and DHA in erythrocyte phospholipids increased, but n-3 FFA concentrations were not determined. Thus, both acute administration of n-3 PUFA and longer-term n-3 PUFA supplementation may reduce VT inducibility.

The antiarrhythmic effect of marine n-3 PUFA has also been studied in ICD patients with VT/VF occurrence as an endpoint. In an observational study, ICD patients with a low content of n-3 PUFA in serum phospholipids had a higher incidence of ventricular arrhythmias during a 12-month follow-up period compared with patients with high n-3 PUFA levels.8 Three intervention studies in ICD patients have investigated the effect of oral supplements (doses between 0.9 and 2.6 g/day n-3 PUFA) with conflicting results.9–11 The first of these studies showed no reduction of VT/VF occurrence. In fact, recurrent VT/VF events

<table>
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<tr>
<th>Table 1</th>
<th>Demographics and medical history of each participant</th>
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<td>Patient</td>
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</tr>
<tr>
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</tr>
<tr>
<td>B</td>
<td>58</td>
</tr>
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<td>C</td>
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<td>74</td>
</tr>
<tr>
<td>F</td>
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IHD, ischaemic heart disease; MI, myocardial infarction; LVEF, left ventricular ejection fraction; NYHA, New York Heart Association.

<table>
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<tr>
<th>Table 2</th>
<th>Time since implantable cardioverter defibrillator implantation, indication for implantable cardioverter defibrillator, and antiarrhythmic treatment in each participant</th>
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<tr>
<td>Patient</td>
<td>Years with an ICD</td>
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ICD, implantable cardioverter defibrillator; IHD, ischaemic heart disease; VT, ventricular tachycardia.
were more common in the $n$-3 group, and the authors raised concerns of a proarrrhythmic effect of $n$-3 PUFA. The second study showed a non-significant risk reduction ($P = 0.06$) in the primary endpoint of time to first confirmed ICD event (VT or VF) or death, whereas secondary analyses pointed at a reduced risk of fatal ventricular arrhythmias in the $n$-3 PUFA group. The third study showed no effect of $n$-3 PUFA on time to first confirmed ICD event (VT or VF) or death from any cause. In a meta-analysis, it was concluded that these three studies showed no overall effect of $n$-3 PUFA.

When $n$-3 marine PUFA are consumed through the diet or as oral supplements, the fatty acids are gradually incorporated into phospholipids of plasma and cell membranes. Intravenous $n$-3 PUFA infusion may be a way to immediately increase PUFA

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**Figure 2** Changes in $n$-3 PUFA levels in platelet phospholipids. *P*, 0.001, after vs. before; †P, 0.001, change after $n$-3 PUFA vs. change after placebo.

**Figure 3** Changes in $n$-3 PUFA levels in platelet phospholipids. *P*, 0.001, after vs. before; †P, 0.001, change after $n$-3 PUFA vs. change after placebo.
status. Rapid n-3 PUFA incorporation into blood cells has also been reported in a few other small studies, but it is unknown whether n-3 PUFA are just as rapidly incorporated into, e.g. myocardial cells. Furthermore, there is some evidence that n-3 PUFA may be antiarrhythmic in the form of n-3 FFA rather than as membrane incorporated fatty acids. Under normal conditions, the concentration of n-3 FFA in plasma and tissues is low, but it is possible that n-3 PUFA incorporated into cell membranes are released during, for instance, acute myocardial ischaemia to exert their effect as FFA. In a dog model of SCD, infusion of purified EPA and DHA resulted in a significant reduction of ischaemia-induced tachyarrhythmias. The infusions were accompanied by an increase in n-3 FFA whereas no change was seen in plasma phospholipids, and the authors concluded that infusions were effective through an increase in n-3 FFA. In the present study, the n-3 PUFA infusion resulted in a large increase in n-3 FFA as well as some incorporation into platelet phospholipids. Of note, Patient E who required the highest stimulation after placebo and became non-inducible after n-3 PUFA had the highest content of n-3 PUFA in plasma and platelet phospholipids.

Strengths and limitations

A strength of the study is the randomized, placebo-controlled cross-over design with participants serving as their own controls. However, the small size of the study is an obvious limitation. It was more difficult than anticipated to find eligible patients mainly because, in recent years, a large proportion of patients in our department had received an ICD without prior testing for VT inducibility according to changes in clinical guidelines. Thus, we were only able to include eight patients of which two had to be withdrawn before the EP studies.

The trend towards a reduced VT inducibility after n-3 PUFA infusion may indicate an immediate antiarrhythmic effect under the highly controlled conditions of an EP study and supports the hypothesis that n-3 PUFA infusion has immediate antiarrhythmic action most likely mediated by n-3 FFA. However, an effect of n-3 PUFA during EP studies does not necessarily translate into an immediate effect of intravenous n-3 PUFA on spontaneously occurring ischaemia-induced ventricular arrhythmias.

Conclusion and perspectives

The present study showed that two of the five patients who were inducible after placebo were no longer inducible after intravenous n-3 PUFA infusion and another two required stronger stimulation to induce VT. Although the number of patients was limited and does not allow for any firm conclusions to be made, the results are in line with a previous uncontrolled study in ICD patients that indicated an immediate antiarrhythmic effect of intravenous n-3 PUFA infusion. It would be of interest to perform a larger randomized study with a design similar to the present study.

From a clinical perspective, it is not the effect of intravenous n-3 PUFA on VT/VF inducibility during an EP study in ICD patients per se that is interesting, but rather the possibility to use intravenous n-3 PUFA as an antiarrhythmic drug in acute conditions. A randomized trial in critically ill patients with recurrent VT would be of interest.

Conflict of interest: none declared.

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