

Acute Administration of Fish Oil Inhibits Triggered Activity in Isolated Myocytes From Rabbits and Patients With Heart Failure

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Background—Fish oil reduces sudden death in patients with prior myocardial infarction. Sudden death in heart failure may be due to triggered activity based on disturbed calcium handling. We hypothesized that superfusion with ω 3-polyunsaturated fatty acids (ω 3-PUFAs) from fish inhibits triggered activity in heart failure.

Methods and Results—Ventricular myocytes were isolated from explanted hearts of rabbits with volume- and pressure-overload-induced heart failure and of patients with end-stage heart failure. Membrane potentials (patch-clamp technique) and intracellular calcium (indo-1 fluorescence) were recorded after 5 minutes of superfusion with Tyrode's solution (control), ω -9 monounsaturated fatty acid oleic acid (20 μ mol/L), or ω 3-PUFAs (docosahexaenoic acid or eicosapentaenoic acid 20 μ mol/L). ω 3-PUFAs shortened the action potential at low stimulation frequencies and caused an \approx 25% decrease in diastolic and systolic calcium (all $P < 0.05$). Subsequently, noradrenalin and rapid pacing were used to evoke triggered activity, delayed afterdepolarizations, and calcium aftertransients. ω 3-PUFAs abolished triggered activity and reduced the number of delayed afterdepolarizations and calcium aftertransients compared with control and oleic acid. ω 3-PUFAs reduced action potential shortening and intracellular calcium elevation in response to noradrenalin. Results from human myocytes were in accordance with the findings obtained in rabbit myocytes.

Conclusion—Superfusion with ω 3-PUFAs from fish inhibits triggered arrhythmias in myocytes from rabbits and patients with heart failure by lowering intracellular calcium and reducing the response to noradrenalin. (*Circulation*. 2008;117:536-544.)

Key Words: arrhythmia ■ calcium ■ electrophysiology ■ fatty acids ■ heart failure

Increased consumption of ω 3-polyunsaturated fatty acids (ω 3-PUFAs) from fish reduces sudden death in patients with prior myocardial infarction.^{1,2} Because sudden death often is preceded by ventricular arrhythmias, these findings suggest that fish oil is antiarrhythmic.

Clinical Perspective p 544

Arrhythmias in patients with heart failure (HF) often are initiated by membrane potential oscillations related to disturbances in calcium handling, also referred to as triggered activity.^{3,4} Isolated myocytes from rabbits with HF show elevated levels of diastolic calcium, disturbed sarcoplasmic reticulum (SR) calcium handling, and prolonged action potentials leading to spontaneous SR calcium release (calcium aftertransient).^{5,6} This sudden rise in calcium can, in turn,

cause membrane depolarizations because the excess calcium is removed by the electrogenic sodium-calcium exchanger (NCX).^{4,7} These membrane depolarizations, called delayed afterdepolarizations (DADs), can initiate action potentials.⁴

The mechanisms underlying the antiarrhythmic action of fish oil are poorly understood. Cellular experiments show that acute administration of ω 3-PUFAs leads to action potential shortening resulting from ionic remodeling of several ionic currents such as sodium current (I_{Na}) and L-type calcium current ($I_{Ca,L}$).⁸⁻¹⁰ A shorter action potential prevents calcium overload and DADs¹¹ as a result of the longer diastolic interval and increased time for calcium removal.⁴ Furthermore, acute application of ω 3-PUFAs to normal myocytes also decreases calcium transients, inhibits calcium sparks, and reduces spontaneous calcium release from the SR.^{10,12,13}

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Table 1. Patient Data

	Patient 1	Patient 2	Patient 3	Patient 4
Age, y	60	66	48	54
Sex	M	M	M	M
Diagnosis	ICM	ICM	DCM	ICM
NYHA class	IV	IV	IV	IV
EF, %	14	22	21	14
Previous medications	Bumetanide, digoxin, acenocoumarol, isosorbide mononitrate, simvastatin, carvedilol, spironolactone	Eplerenone, furosemide, potassium chloride, captopril, acenocoumarol	Spironolactone, bisoprolol, digoxin, amiodarone, candesartan cilexetil, bumetanide	Amiodarone, bumetanide, spironolactone

ICM indicates ischemic cardiomyopathy; DCM, dilated cardiomyopathy; NYHA, New York Heart Association; and EF, ejection fraction.

We hypothesized that fish oil ω 3-PUFAs docosahexaenoic acid (DHA) and eicosapentaenoic acid (EPA) inhibit DAD-induced triggered activity in HF and normalize calcium handling. We used an established arrhythmogenic rabbit model of HF^{6,14} to study the effect of acute administration of fish oil on the occurrence of triggered activity, DADs, and calcium aftertransients. Superfusion with monounsaturated ω 9 fatty acid oleic acid (OA) was used as a control. In addition, we confirmed the hypothesis in human myocytes isolated from explanted hearts of patients undergoing cardiac transplantation for end-stage HF.

Methods

Rabbit Model of Pressure- and Volume-Overload-Induced HF

The investigation in rabbits was approved by the local ethics committee and conformed to the *Guide for the Care and Use of Laboratory Animals* published by the US National Institutes of Health (NIH publication No. 85-23, revised 1996). HF was induced in 20 rabbits (male; New Zealand White; specific pathogen free; weight, 3 to 3.5 kg) by a 12-week period of combined volume and pressure overload as described in detail previously.^{6,14} Briefly, volume overload was induced by rupture of the aortic valve until pulse pressure increased by 100%. Three weeks later, pressure overload was created by a suprarenal stenosis of \approx 50% of the abdominal aorta. The rabbits received a normal diet that did not contain fish oil or EPA and DHA.

The severity of HF was qualified on the basis of the following parameters: left ventricular end-diastolic pressure $>$ 5 mm Hg, relative heart weight $>$ 5 g/kg, relative lung weight $>$ 3.5 g/kg, and the presence of ascites.⁶ Rabbits were anesthetized by a combination of ketamine (100 mg IM) and xylazine (20 mg IM) and heparinized (5000 IU heparin, LEO Pharma, Buckinghamshire, UK). Then, left ventricular end-diastolic pressure was measured with a fluid-filled catheter (Portex fine-bore polythene tubing; internal diameter, 0.76 mm; outer diameter, 1.22 mm). Subsequently, the animal was killed by an injection of pentobarbital (240 mg), and body weight, heart weight, and lung weight were measured, as well as the presence of ascites.⁶

Human Hearts

Human explanted hearts were obtained from 4 patients at the time of cardiac transplantation. Table 1 lists the clinical characteristics of the 4 patients. The patients were in New York Heart Association class IV and received standard therapy for chronic HF. Informed consent was obtained before heart transplantation. The protocol complied with institutional guidelines and the Declaration of Helsinki.

Myocyte Preparation and Test Perfusates

For cellular isolation of rabbit ventricular myocytes, both coronary arteries were cannulated; for isolation of human myocytes, the circumflex artery was cannulated.

A part of the ventricular wall was mounted on a Langendorff-perfusion apparatus and subjected to Tyrode's solution for 15 minutes, low- Ca^{2+} Tyrode's solution for 15 minutes, and low- Ca^{2+} Tyrode's solution containing a mixture of enzymes (collagenase type B 0.15 mg/mL, collagenase type P 0.05 mg/mL, trypsin inhibitor 0.1 mg/mL, and hyaluronidase 0.15 mg/mL). After about 30 minutes of recirculation with the Tyrode's solution containing the enzyme mixture at a flow rate of 30 to 50 mL/min, the left ventricular wall was cut into small pieces and further fractionated using a shaking protocol. For a more detailed description, see elsewhere.^{15,16}

Before the experiments, myocytes were kept at room temperature in separate vials, each containing $\approx 10^5$ myocytes in HEPES-buffered solution (pH 7.3) containing (mmol/L) Na^+ 156, K^+ 4.7, Ca^{2+} 1.3, Mg^{2+} 2.0, Cl^- 150.6, HCO_3^- 4.3, HPO_4^{2-} 1.4, HEPES 17, and glucose 11 supplied with 1% fatty acid free albumin. Small aliquots of cell suspension were placed in a recording chamber on the stage of an inverted microscope and allowed to adhere for 5 minutes. Quiescent rod-shaped myocytes with cross-striations and smooth surface were selected for measurements and superfused with Tyrode's solution containing (mmol/L) NaCl 140, KCl 5.4, CaCl_2 1.0, glucose 5.5, and HEPES 5.0, pH 7.4 (NaOH). The microscope stage and perfusion chamber were temperature controlled at 37°C.

The concentrations of EPA or DHA (20 $\mu\text{mol/L}$) used in this study were based on free fatty acid levels in the plasma of 6 patients included in the Study on Omega-3 Fatty Acids and Ventricular Arrhythmia (SOFA) trial¹⁷ who were taking 2 g/d fish oil. Plasma free fatty acids were measured by gas-liquid chromatography.¹⁸ Results showed that the averaged concentration of free EPA and DHA was ≈ 10 $\mu\text{mol/L}$ (range, 5.0 to 16.4 $\mu\text{mol/L}$). The test perfusates contained Tyrode's solution (control calcium; 1.8 mmol/L for action potential recordings and 2.6 mmol/L for calcium measurements), OA (20 $\mu\text{mol/L}$), ω 3-PUFAs DHA (20 $\mu\text{mol/L}$), or EPA (20 $\mu\text{mol/L}$). OA, an ω 9 monounsaturated fatty acid, was used as a control fatty acid. We superfused the human ventricular myocytes with fish oil fatty acid EPA or control fatty acid OA because of the limited number of cells. Separate aliquots of myocytes were used for electrophysiology and calcium measurements. The same cells were used for the experiment before and after application of noradrenalin.

Action Potentials and DADs

Transmembrane potentials were recorded with the use of the amphotericin-B perforated patch-clamp technique. Patch pipettes (1 to 3 mol/L Ω) were pulled from borosilicate glass and filled with pipette solution containing (mmol/L) K-gluconate 125, KCl 20, NaCl 5, HEPES 10, and amphotericin-B 2.2, pH 7.2 (KOH). Data acquisition and analysis were accomplished with custom software. Membrane potentials were low-pass filtered with a cutoff frequency of 5 kHz, digitized 10 kHz, and were corrected for liquid junction

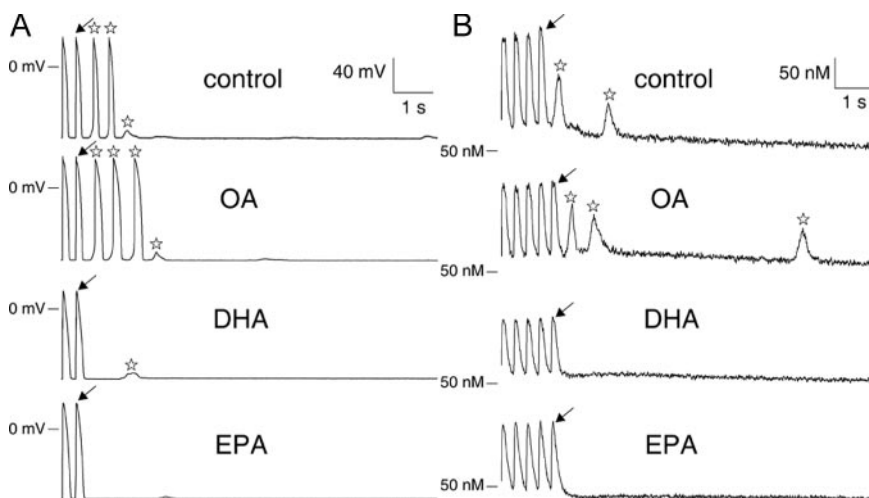


Figure 1. ω 3-PUFAs inhibit triggered action potentials and reduce the number of DADs and calcium aftertransients. A, Representative examples of the last stimulated action potentials (arrow) and the tracing periods in control, OA, DHA, and EPA. B, Representative examples of the last stimulated calcium transient (arrow) and the tracing period in control, OA, DHA, and EPA. Stars point to spontaneous action potentials, DADs, and calcium aftertransients. The action potential and calcium measurements were performed in different myocytes.

potentials. Action potentials were elicited at 0.5 to 4 Hz by 3-ms, $1.5\times$ threshold current pulses through the patch pipette, except for human action potentials, which were stimulated at 0.5 and 1 Hz.¹⁹

In each experiment, parameters from 6 consecutive action potentials were averaged. After the 5-minute intervention with one of the test perfusates, myocytes were challenged with noradrenalin for 1 minute ($1\ \mu\text{mol/L}$). Subsequently, spontaneous action potentials and DADs were evoked by rapid pacing (10 seconds at 3 Hz) and recorded during an 8-second pause. In human ventricular myocytes, we used the highest possible pacing frequency to evoke triggered action potentials and DADs. In a separate set of experiments, the rapid pacing protocol was repeated 5 times in the same cell to ensure reproducibility in the occurrence of triggered action potentials and DADs in the same cell.

Calcium Transients and Aftertransients

Myocytes were loaded with indo-1. Dual-wavelength emission of indo-1 was recorded (405/505 nm; excitation, 340 nm), and cellular-free intracellular Ca^{2+} concentration was calculated and calibrated as described previously.²⁰ Signals were corrected for background recorded from indo-1-free myocytes ($\approx 10\%$ of raw signals).

Each experiment started with a conditioning 5-minute episode of stimulation at 2 Hz (1.5 Hz for human ventricular myocytes) in the presence of Tyrode's solution. After the 5-minute intervention with one of the test perfusates, myocytes were challenged with noradrenalin ($100\ \text{nmol/L}$) for 1 minute. Subsequently, calcium aftertransients were evoked by rapid pacing (10 seconds at 3 Hz) and recorded during an 8-second pause. For human ventricular myocytes, we used the highest possible pacing frequency to evoke calcium aftertransients. In a separate set of experiments, the rapid pacing protocol was repeated 5 times in the same cell to ensure reproducibility in the occurrence of calcium aftertransients in the same cell.

Drugs

OA, DHA, and EPA were prepared as 10-mmol/L stock solutions in dimethyl sulfoxide and stored under nitrogen at -20°C . The fatty acids were diluted in Tyrode's solution to $20\ \mu\text{mol/L}$ and freshly prepared before each single recording (used within 20 minutes). Amphotericin-B was prepared as a 52-mmol/L stock solution in dimethyl sulfoxide (kept at 4°C in dark for a maximum of 2 days) and freshly diluted to $2.2\ \text{mmol/L}$ daily.

Statistical Analysis

Data are mean \pm SEM (n is number of cells; N is number of hearts). Data were statistically analyzed with the *t* test, 1- or 2-way ANOVA on ranks, or repeated-measures ANOVA if appropriate. Statistical analysis was performed using the number of myocytes (n) assuming independence between myocytes within hearts. The use of myocytes as the unit for the statistical analysis was validated by calculating the

intraclass correlation coefficient. The variation between rabbits (N=9) was less than the variation within rabbits as shown by the negative intraclass correlation coefficient (-0.104) for the action potential duration at 90% repolarization (APD_{90}). Multiple comparisons of groups after ANOVA were based on the Holm-Sidak or Dunn test, depending on which was the most appropriate for the group size and variance differences. If the main effects were significant, means were compared by use of the Holm-Sidak method (parametric ANOVA), or medians were compared through the use of Dunn's test (ANOVA on ranks). A value of $P \leq 0.05$ was considered statistically significant. On the basis of the rabbit data on DADs, we calculated that we required 10 myocytes per group (a minimum detectable difference of means of 85% and an expected SD of 65% with a desired power of 0.8 and an α of 0.05) to confirm our hypothesis in human myocytes.

The authors had full access to and take responsibility for the integrity of the data. All authors have read and agree to the manuscript as written.

Results

Rabbit Data

The rabbit was included in the HF study group if 3 of the following 4 criteria were met: (1) left ventricular end-diastolic pressure $>5\ \text{mm Hg}$, (2) relative heart weight $>5\ \text{g/kg}$, (3) relative lung weight $>3.5\ \text{g/kg}$, or (4) the presence of ascites. Of the 20 rabbits, 16 were included. In 6 animals, the severity of HF precluded the measurement of left ventricular end-diastolic pressure (the anesthesia caused cardiac arrest). These rabbits, however, met the 3 remaining criteria. Similar to previous studies,^{5,6} the left ventricular end-diastolic pressure of these failing animals was high at $21.8 \pm 4.3\ \text{mm Hg}$ (N=10); relative heart weight was $6.0 \pm 0.2\ \text{g/kg}$; relative lung weight was $5.3 \pm 0.5\ \text{g/kg}$; and ascites was present in 87.5% of the HF rabbits.

ω 3-PUFAs Inhibit Triggered Activity in Isolated Myocytes From Rabbits With HF

Figure 1 shows representative examples of the effects of ω 3-PUFAs on transmembrane potentials (Figure 1A) and intracellular calcium concentration (Figure 1B) in individual rabbit myocytes in the presence of noradrenalin during and after rapid pacing. The last stimulated action potential (Figure 1A) and last stimulated calcium transient (Figure 1B) of the rapid pacing protocol in the presence of noradrenalin are

Table 2. Triggered Activity in Control and in OA-, DHA-, and EPA-Superfused Rabbit Myocytes

Rabbit	Control	OA	DHA	EPA
Triggered action potentials, n (n, N)	0.8±0.13 (6, 3)	0.9±0.20 (6, 3)	0±0*† (7, 3)	0±0*† (7, 3)
DADs, n (n, N)	1.8±0.11 (6, 3)	2.1±0.13 (6, 3)	1.0±0.15† (7, 3)	0.3±0.08*† (7, 3)
Calcium aftertransients, n (n, N)	2.7±0.65 (41, 15)	2.5±0.28 (43, 16)	1.5±0.37† (22, 9)	1.4±0.26*† (35, 11)

n indicates number of myocytes; N, number of hearts. Values are expressed as mean±SEM.

* $P<0.05$, significant difference vs control (ANOVA); † $P<0.05$, significant differences vs OA (ANOVA).

indicated with arrows. Under control conditions (normal Tyrode's solution) and in the presence of OA, the last stimulated action potential (Figure 1A) is followed by multiple triggered action potentials and a single DAD. In the presence of DHA or EPA, triggered action potentials do not occur (Figure 1A). Similarly, the last stimulated calcium transient (Figure 1B) is followed by multiple calcium aftertransients under control conditions and in the presence of OA. In the presence of DHA or EPA, calcium aftertransients do not arise (Figure 1B).

Table 2 summarizes the averaged number of triggered action potentials and DADs in control and in the presence of OA, DHA or EPA. The number of triggered action potentials and DADs is not significantly different between control and OA or between DHA and EPA. Post hoc testing shows that both EPA and DHA significantly reduce the number of triggered action potentials and DADs compared with OA and/or control (Table 2). The amount of calcium aftertransients also is reduced in the presence of DHA and EPA (Table 2). The number of calcium aftertransients between control and OA or between DHA and EPA is not different.

DHA or EPA Reduces the Sensitivity to Noradrenalin

Because noradrenalin was used to evoke triggered activity, DADs, and calcium aftertransients, we studied the effect of noradrenalin on action potential duration and on diastolic and systolic calcium concentrations in all groups. Figure 2A shows typical examples of action potentials in the absence (–) and presence (+) of noradrenalin in control, OA, DHA, and EPA at a pacing frequency of 3 Hz. Superfusion with noradrenalin for 1 minute shortens the APD₉₀ in control, OA-, or DHA-superfused but not in the EPA-superfused myocytes (Figure 2B).

Figure 2C shows typical examples of calcium transient recordings after 5 minutes of superfusion with control, OA, DHA, or EPA in the absence (–) and presence (+) of noradrenalin. The noradrenalin-induced increase in diastolic and systolic calcium is less in the DHA- and EPA-superfused myocytes compared with control and OA-superfused myocytes. Figures 2D shows that the average response of diastolic calcium to noradrenalin is significantly lower in the EPA-superfused myocytes compared with both control and

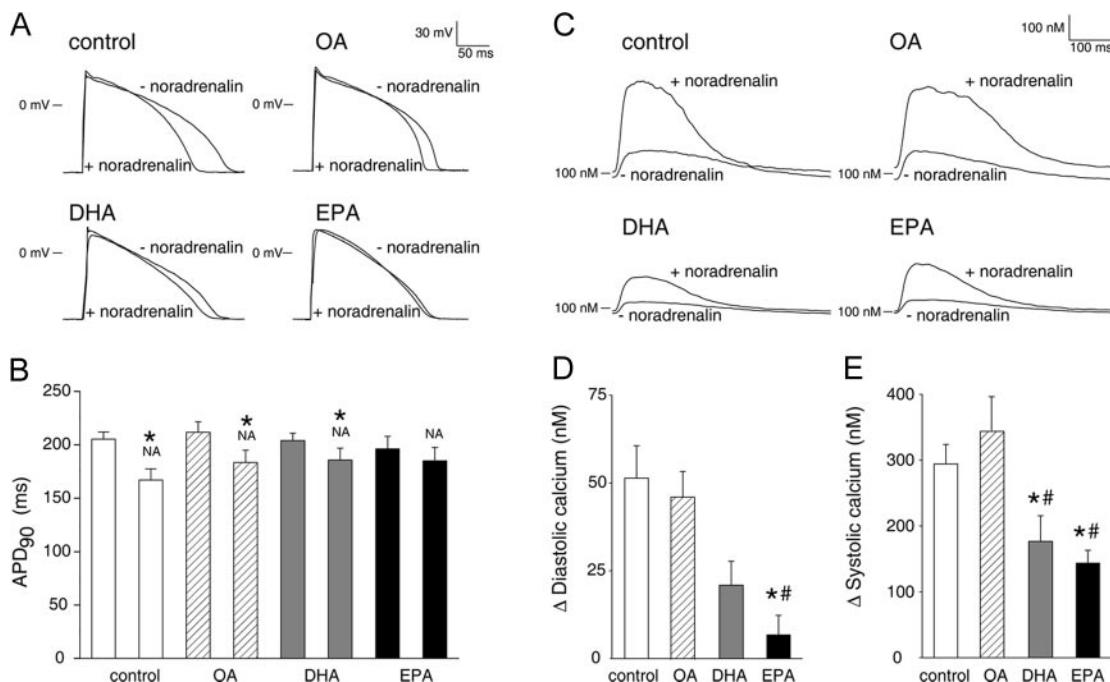


Figure 2. ω 3-PUFAs reduce the sensitivity to noradrenalin. A, Representative examples of action potential recordings in control, OA, DHA, and EPA in the absence and presence of noradrenalin. B, Changes in APD₉₀ in response to noradrenalin (NA) in control (n=6; N=3), OA (n=6; N=3), DHA (n=7; N=3), and EPA (n=7; N=3). * $P<0.05$ indicates statistical differences with or without noradrenalin (repeated-measures ANOVA). C, Representative examples of the intracellular calcium in control, OA, DHA, and EPA in the absence and presence of noradrenalin. D and E, Diastolic (D) and systolic (E) calcium elevation in response to noradrenalin in control (n=40; N=15), OA (n=35; N=16), DHA (n=20; N=9), and EPA (n=27; N=11). * $P<0.05$ indicates statistical differences between DHA or EPA and control (ANOVA on ranks). # $P<0.05$ indicates statistical differences between DHA or EPA and OA (ANOVA on ranks).

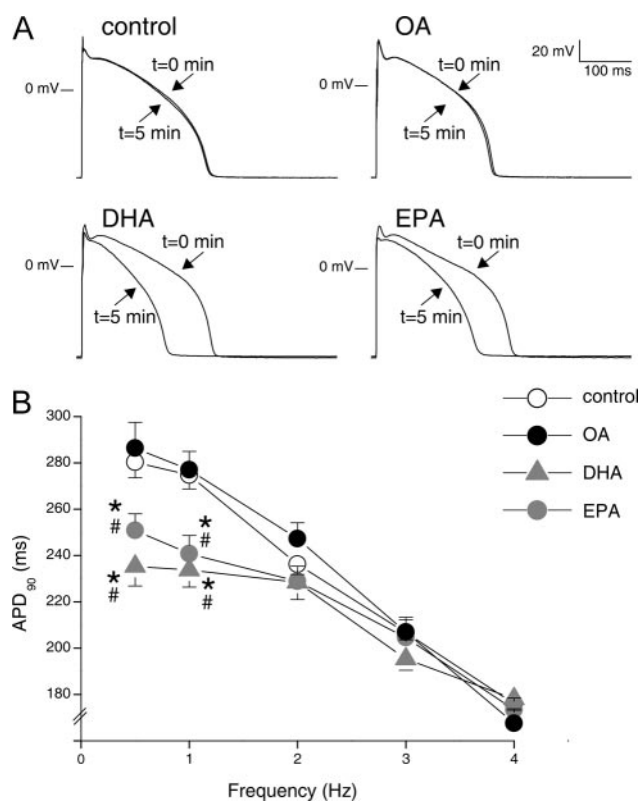


Figure 3. ω 3-PUFAs shorten the action potential at low stimulation frequencies but not at higher pacing frequencies. A, Representative examples of action potential recordings at 1 Hz before ($t=0$ minutes) and after ($t=5$ minutes) superfusion in control ($n=21$; $N=3$), OA ($n=7$; $N=3$), DHA ($n=7$; $N=3$), and EPA ($n=7$; $N=3$). B, Dependence of APD_{90} on stimulation frequency. * $P<0.05$ indicates statistical differences between DHA or EPA and control (ANOVA). # $P<0.05$ indicates statistical differences between DHA or EPA and OA (ANOVA).

OA-superfused myocytes. Figure 2E shows that the average response of systolic calcium to noradrenalin is significantly lower in both the DHA- and EPA-superfused myocytes compared with control and OA-superfused myocytes. In accordance, before the onset of rapid pacing, diastolic and systolic calcium levels are significantly lower in the DHA- (102 ± 9 and 345 ± 44 nmol/L; $n=20$; $N=9$) and EPA- (100 ± 8 and 333 ± 27 nmol/L; $n=27$; $N=11$) superfused myocytes compared with control (152 ± 11 and 517 ± 36 nmol/L; $n=40$; $N=15$) and OA-superfused myocytes (149 ± 9 and 575 ± 56 nmol/L; $n=35$; $N=16$).

Effects of ω -3 PUFAs on Action Potential Duration

Figure 3A shows typical examples of action potential recordings before ($t=0$ minutes) and after 5 minutes ($t=5$ minutes) of superfusion in control and with OA, DHA, and EPA at 1 Hz. On average, the APD_{90} of the myocytes in control and after superfusion with OA was 275 ± 6 ms ($n=21$; $N=6$) and 277 ± 8 ms ($n=7$; $N=3$), respectively. Superfusion with DHA or EPA resulted in a significantly shorter APD_{90} , 234 ± 7 ms ($n=7$; $N=3$) and 241 ± 8 ms ($n=7$; $N=3$), compared with either control or OA. Figure 3B displays the frequency dependence of APD_{90} in the 4 groups. In all groups, APD_{90}

shortened with increasing stimulation frequencies. Note that at stimulation frequencies of ≥ 2 Hz, no significant differences exist between groups. At all stimulation frequencies, resting membrane potentials and action potential amplitude were not different between groups (not shown).

Effects of ω -3 PUFAs on Cytoplasmic and SR Calcium

Typical examples of calcium transients before ($t=0$ minutes) and 5 minutes after ($t=5$ minutes) application of OA, DHA, and EPA are shown in Figure 4A. Five minutes of superfusion with DHA and EPA decreases diastolic and systolic calcium levels more than control and OA. The decrease in diastolic calcium and systolic calcium by DHA and EPA is $\approx 25\%$. At $t=0$ minutes, diastolic calcium and systolic calcium are 118 ± 4.0 and 268 ± 5.5 nmol/L, respectively. After 5 minutes of superfusion, the diastolic and systolic calcium levels are significantly more reduced by DHA and EPA than in control conditions (Figure 4B and 4C). EPA causes a larger reduction in diastolic and systolic calcium levels compared with OA (Figure 4B and 4C).

At 5 minutes of superfusion, intracellular calcium levels reached a steady state in all groups, and subsequent superfusion with Tyrode's solution did not change diastolic or systolic calcium concentrations (not shown).

Human Data

ω 3-PUFAs Inhibit Triggered Activity in Isolated Myocytes From Patients With End-Stage HF

Figure 5A and 5B shows representative examples of transmembrane potentials and intracellular calcium concentrations during and after rapid pacing (1 to 2 Hz for human myocytes) in the presence of noradrenalin. Similar to our findings in rabbit myocytes, the last stimulated action potential and the last stimulated calcium transient (Figure 5A) are followed by multiple triggered action potentials and a single DAD in the OA-superfused human myocyte. None are present in the EPA-superfused myocyte. Contrary to the rabbit myocytes, human myocytes superfused with OA displayed early afterdepolarizations (EADs) (Figure 5A).

Table 3 summarizes the average number of triggered action potentials, DADs, and calcium aftertransients and the incidence of EADs in the presence of OA or EPA. The number of DADs is significantly reduced in EPA-superfused myocytes compared with OA. In addition, the incidence of EADs was significantly reduced in the EPA-superfused myocyte compared with OA. The difference in triggered action potentials and calcium aftertransients between OA- and EPA-superfused myocytes did not reach statistical significance.

Figure 5C and 5D shows typical examples of the effect of noradrenalin on APD_{90} and intracellular calcium in OA- and EPA-superfused human myocytes. In contrast to rabbit myocytes, noradrenalin prolongs the APD_{90} , as has been documented previously,¹⁹ and therefore was accompanied by EADs in the OA-superfused human myocyte. The noradrenalin-induced prolongation of the action potential is less in the EPA-superfused myocyte and did not result in EAD formation. Similar to our rabbit data, noradrenalin causes an increase in diastolic and systolic calcium. However, this

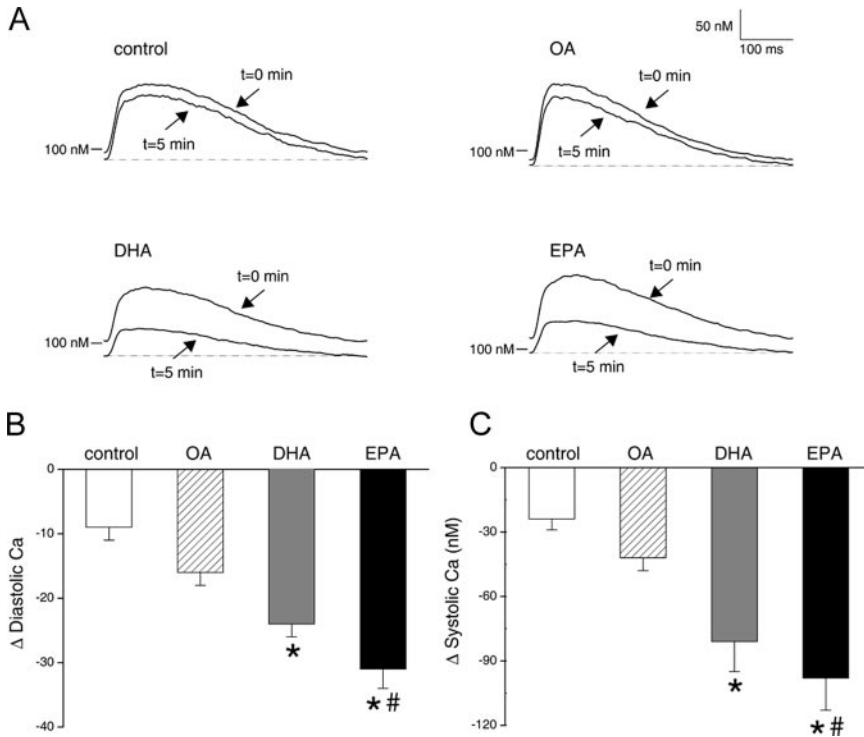


Figure 4. ω 3-PUFAs decrease diastolic and systolic calcium concentrations. A, Representative examples of calcium transient recordings at 2 Hz before (t=0 minutes) and after (t=5 minutes) superfusion in control, OA, DHA, or EPA. B and C, Reduction in diastolic and systolic calcium levels after 5 minutes of superfusion with control (n=42; N=15), OA (n=30; N=16), DHA (n=20; N=9), or EPA (n=27; N=11). * P <0.05 indicates statistical differences vs control (repeated-measures ANOVA). # P <0.05 indicates statistical differences vs OA (repeated-measures ANOVA).

increase is similar in the EPA-superfused myocyte and the OA-superfused myocyte (Figure 5D). The EAD formation complicated accurate measurements of APD₉₀ in the presence of noradrenalin in OA-superfused myocytes. The absolute increase in diastolic and systolic calcium in response to noradrenalin was 5 ± 5.7 and 73 ± 13.3 nmol/L in OA-superfused myocytes (n=9; N=4) and 9 ± 3.4 and 67 ± 13.1 nmol/L in EPA-superfused myocytes (n=8; N=4, P =NS).

Figure 5E and 5F shows typical examples of action potentials and calcium transients after 5 minutes of superfu-

sion with either EPA or OA at a stimulation frequency of 1 Hz (action potential recordings) and 1.5 Hz (calcium transient recordings) in the absence of noradrenalin. EPA causes a pronounced shortening of the action potential and decreases diastolic and systolic calcium levels compared with OA.

Before (t=0 minutes) the application of OA or EPA, APD₉₀ was 520 ± 24.3 ms (n=23; N=3) at 1 Hz. After 5 minutes of superfusion with EPA, the APD₉₀ was shortened to 412 ± 22.6 ms (n=12; N=3; P <0.05, ANOVA) in human myocytes. After 5 minutes of superfusion with OA, the APD₉₀ was

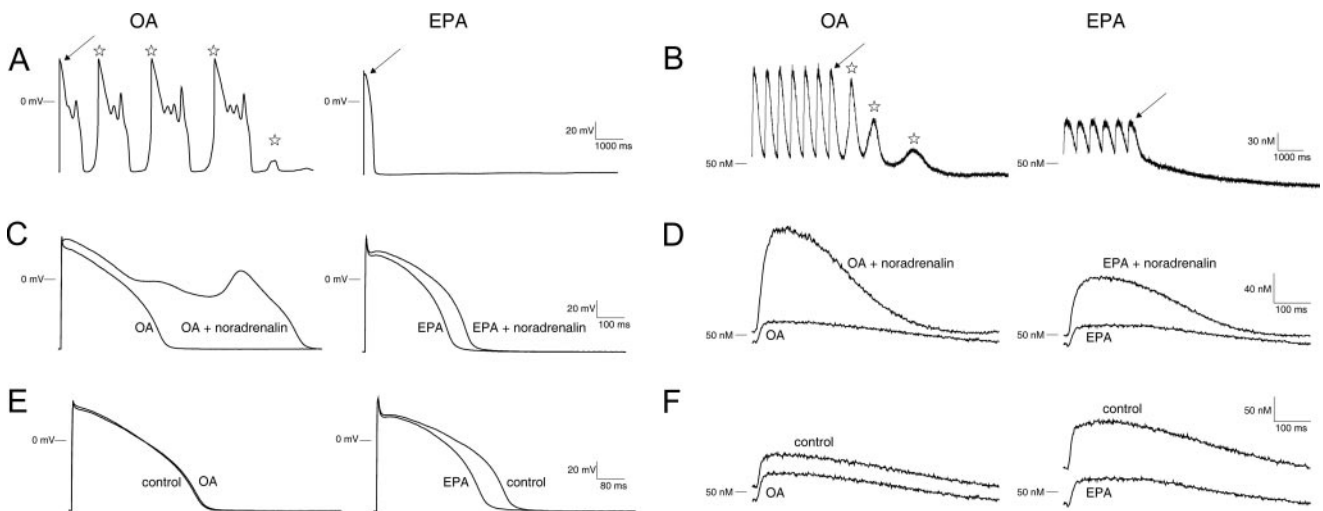


Figure 5. Representative examples in human myocytes. A, Representative example of the last stimulated action potential (arrow) and the tracing period in OA- and EPA-superfused human myocytes. B, Representative examples of the last 7 stimulated calcium transients (arrow) and the tracing period in OA- and EPA-superfused myocytes. C, Action potential recordings at 1 Hz in the absence (control) and presence of OA or EPA. D, Calcium transient recording at 1.5 Hz in the absence (control) and presence of OA or EPA. E, Action potential recordings after 5 minutes of superfusion with OA or EPA in the absence and presence of noradrenalin. F, Calcium transient recordings after 5 minutes of superfusion with OA or EPA in the absence and presence of noradrenalin. The action potential and calcium measurements were performed in different myocytes.

Table 3. Triggered Activity and Calcium Handling in OA- and EPA-Superfused Human Myocytes

Human	OA	EPA
Triggered action potentials, n (n, N)	1.3±0.79 (11, 3)	0.2±0.16 (12, 3)
DADs, n (n, N)	1.0±0.28 (11, 3)	0.3±0.11* (12, 3)
EADs, % (n, N)	73 (11, 3)	17* (12, 3)
Calcium aftertransients, n (n, N)	2.2±1.32 (10, 4)	0.3±0.15 (11, 4)
ΔDiastolic calcium, nmol/L (n, N)	-9±2.7 (10, 4)	-28±6.8* (9, 4)
ΔSystolic calcium, nmol/L (n, N)	-7±8.7 (10, 4)	-46±9.7* (9, 4)

n indicates number of myocytes; N, number of hearts. Values are mean±SEM.

P<0.05, significant differences vs OA (rank-sum test or ANOVA when appropriate).

512±29.9 ms and did not differ from start values (*t*=0 minutes) (*n*=11; *N*=3 *P*=NS; ANOVA). Consistent with previous findings,¹⁹ the human failing ventricular myocytes could not be stimulated at high pacing frequencies (>1 Hz) during action potential recordings. Therefore, APD₉₀ frequency dependency was not obtained in the presence of OA or EPA.

Before the onset of superfusion, diastolic and systolic calcium levels were 83±5.8 and 143±11.7 nmol/L (*n*=19; *N*=4). EPA decreases diastolic and systolic calcium concentrations compared with OA (Table 3; *P*<0.05, ANOVA).

In conclusion, the effects of ω3-PUFAs on human ventricular action potentials and calcium handling correspond closely with those in isolated myocytes from rabbits with HF.

Discussion

Increased intake of fish oil is antiarrhythmic in patients with prior myocardial infarction^{1,2} but not in patients with angina or with sustained ventricular tachycardia.^{21,22} Patients with prior myocardial infarction may suffer from arrhythmias based on DADs resulting from disturbed calcium handling. Therefore, the antiarrhythmic efficacy of fish oil may be specific in HF. To investigate whether ω3-PUFAs reduce arrhythmias in HF, we used isolated myocytes from rabbits with HF and isolated myocytes from patients with end-stage HF. For the rabbit model, we applied combined pressure and volume overload that induced HF over a period of 3 months. In this model, a progressive increase in premature ventricular beats and ventricular tachycardia is observed that appears to have a focal origin.^{6,14,23} Isolated myocytes from these rabbits with HF display high diastolic calcium levels, abnormal SR handling, and action potential prolongation.^{5,24}

Our results show that acute administration of ω3-PUFA DHA and EPA inhibits the formation of noradrenalin-induced triggered activity and reduces the number of DADs and calcium aftertransients compared with control and with monounsaturated ω9 fatty acid OA in isolated myocytes from rabbits with HF. In addition, we confirm these findings in isolated human myocytes from patients with end-stage HF.

The ionic mechanism underlying triggered action potentials and DADs after a spontaneous calcium release is I_{NCX}.²⁵ Inhibition of I_{NCX} by acute administration of fish oil, as described previously,^{26–28} may be an additional mechanism by which acute application of fish oil fatty acids contributed

to the reduction in triggered activity in our study. In addition, a diet rich in fish oil enhances inward rectifier current and shortens the action potential, thereby protecting against DADs in calcium overload conditions.²⁷

Fish Oil Reduces the Sensitivity for Noradrenalin

Our data show that acute administration of ω3-PUFAs reduces the responsiveness to noradrenalin as observed by reduced increases in diastolic and systolic calcium and reduced changes in action potential durations in response to noradrenalin. These reduced responses may be due to altered properties of membrane currents and calcium regulating proteins. However, acute application of ω3-PUFAs also may modulate the β-adrenergic receptors or activation of the G-protein/adenylyl cyclase/cAMP/protein kinase A pathway. Although this may be an interesting additional mechanism by which fish oil reduces sudden death in HF, further experiments are needed to clarify this issue.

Cytoplasmic Calcium

Increased levels of diastolic calcium in HF are directly related to the occurrence of calcium aftertransients.⁵ A decrease in diastolic calcium concentrations by acute administration of ω3-PUFAs may therefore explain the reduced occurrence of calcium aftertransients in both our isolated rabbit and human ventricular myocytes. Systolic calcium concentrations also were markedly reduced by acute application of ω3-PUFAs. The finding that acute application of fish oil leads to decreased levels of intracellular calcium has been described previously in healthy rat myocytes.^{10,13} Reductions in intracellular calcium by ω3-PUFAs may be due to block of I_{Ca,L}¹⁰ and/or inhibition of the calcium release channels of the SR, the ryanodine receptors in the SR membrane.^{12,13,29} Indeed, acute administration of ω3-PUFAs reduced the open probability of the ryanodine receptors in the SR membrane in isolated SR vesicles.^{29,30} In our study, similar mechanisms may account for the reduction in diastolic and systolic calcium levels induced by ω3-PUFAs.

In HF, a decrease in SR calcium content, combined with an increase in diastolic calcium, is related to spontaneous calcium releases from the SR.⁵ The role of the SR calcium content by EPA and DHA in the reduction in triggered activity seen in our study is unknown. However, acute application of ω3-PUFAs increased SR calcium content in healthy rat myocytes.^{12,13} Further experiments are needed to establish the effect of fish oils on SR calcium content in HF.

Fish Oil Shortens the Action Potential Durations

Action potential prolongation is characteristic of HF and contributes to arrhythmogenesis.³ Action potential shortening is an antiarrhythmic intervention for DAD-related arrhythmias.^{4,11} In our study, the action potentials recorded from both rabbit and human myocytes were shortened by acute application of ω3-PUFAs in both rabbit and human myocytes, except at fast (>2 Hz) stimulation rates (Figure 3B). The shortening of the action potential by ω3-PUFAs in human myocytes may have contributed to a reduced number of DADs. In rabbit myocytes, however, susceptibility to DAD

formation was tested with a rapid pacing protocol (3 Hz) in which action potential shortening was absent.

This excludes a role of action potential shortening by ω 3-PUFAs in the reduction of DADs in rabbit myocytes. Our data correspond with previous studies showing that acute application of ω 3-PUFAs shortens the action potential in rat, rabbit, and guinea pig myocytes.^{8,31,32} However, at low concentrations (<10 μ mol/L) of ω 3-PUFAs, prolongation of the rat action potential also has been reported.⁸

Circulating Versus Incorporated ω 3-PUFAs

What are physiological concentrations of free DHA and EPA in humans? Data on free fatty acid concentration in the plasma of patients from the SOFA trial¹⁷ supplemented with 2 g/d fish oil revealed that free EPA and DHA levels range from 5 to \approx 17 μ mol/L. This corresponds to calculations done by Negretti et al,¹³ who estimated that maximum levels of free circulating ω 3-PUFAs are between 8 and 32 μ mol/L. Our study shows that free EPA and DHA inhibit triggered activity in concentrations that are clinically relevant.

A diet rich in fish oil leads not only to circulating free ω 3-PUFAs but also to incorporation of ω 3-PUFAs into membrane phospholipids. In pig and rat myocytes, incorporated ω 3-PUFAs shorten the action potential but do not alter calcium transients and diastolic calcium levels.^{26,27} The relative contribution of circulating versus incorporated ω 3-PUFAs on intracellular calcium handling has yet to be determined.

In this study, oleic acid was used as a control fatty acid. The results indicate that oleic acid reduced diastolic and systolic calcium concentration, although much less than the ω 3-PUFAs. Oleic acid did not inhibit triggered activity. Therefore, monounsaturated fatty acids can be used as a control fatty acid regarding cellular arrhythmogenesis.

Study Limitations and Methodological Considerations

In this study, we used a rabbit model of HF that allows study of the “trigger,” whereas patients with ischemic cardiomyopathy may suffer mainly from arrhythmias that are reentrant. Although maintained by reentry, the arrhythmias in these patients may be initiated by triggered activity.³³ Another difference between the rabbit and human myocytes is that the human myocytes display prolongation of the action potential in response to noradrenalin, whereas the rabbit myocytes display a shortening of the action potential. Because EADs are mainly bradycardia related and depend on a preexisting long action potential,⁴ the noradrenalin-induced prolongation of the action potential may explain the occurrence of EADs in the human myocytes that are not present in the rabbit myocytes. This study described 1 mechanism of arrhythmia; definite answers regarding the efficacy of fish oil in HF in humans who suffer from multiple types of arrhythmias will come from the Gruppo Italiano per lo Studio della Streptococchiosi nell’Infarto Miocardico (GISSI) Heart Failure trial.³⁴

Conclusion

Our data provide evidence for an antiarrhythmic action of free circulating ω 3-PUFAs from fish in HF resulting from a

reduced sensitivity to noradrenalin and a reduction in diastolic calcium.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Fish oil supplementation reduces sudden death in patients with recent myocardial infarction. Arrhythmias in these patients often are due to triggered arrhythmias that arise from early and/or delayed afterdepolarizations resulting from spontaneous intracellular calcium releases. The mechanism by which fish oil reduces sudden death in patients with a preexisting cardiac condition is unknown. In the present study, we tested whether fish oil fatty acids reduce arrhythmias that are evoked by tachycardia and administration of noradrenalin in heart failure. Our study shows that physiological plasma concentrations of fish oil fatty acids inhibit the formation of triggered arrhythmias in isolated myocytes of rabbits and in patients with heart failure. The mechanism underlying the antiarrhythmic action of fish oil fatty acids is a decrease in intracellular calcium and a reduced response to noradrenalin. Therefore, fish oil fatty acids prevent spontaneous intracellular calcium releases and depolarizations. These findings suggest that the mechanism by which circulating fish oil fatty acids prevent sudden death in patients with heart failure is, at least in part, due to inhibition of triggered arrhythmias. Whether fish oil supplements can be safely administered in heart failure to prevent arrhythmias warrants more extensive testing in clinical trials.

Acute Administration of Fish Oil Inhibits Triggered Activity in Isolated Myocytes From Rabbits and Patients With Heart Failure

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