

# Ultrafast sodium channel block by dietary fish oil prevents dofetilide-induced ventricular arrhythmias in rabbit hearts

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**Dujardin KS, Dumotier B, David M, Guizy M, Valenzuela C, Hondeghem LM.** Ultrafast sodium channel block by dietary fish oil prevents dofetilide-induced ventricular arrhythmias in rabbit hearts. *Am J Physiol Heart Circ Physiol* 295: H1414–H1421, 2008. First published August 1, 2008; doi:10.1152/ajpheart.01219.2007.—Several epidemiologic and clinical studies show that following myocardial infarction, dietary supplements of  $\omega$ -3 polyunsaturated fatty acids ( $\omega$ 3FA) reduce sudden death. Animal data show that  $\omega$ 3FA have antiarrhythmic properties, but their mechanisms of action require further elucidation. The effects of  $\omega$ 3FA supplementation were studied in female rabbits to analyze whether their antiarrhythmic effects are due to a reduction of triangulation, reverse use-dependence, instability, and dispersion (TRIA<sub>D</sub>) of the cardiac action potential (TRIA<sub>D</sub> as a measure of proarrhythmic effects). In Langendorff-perfused hearts challenged by a selective rapidly activating delayed rectifier potassium current inhibitor that has been shown to exhibit proarrhythmic effects (dofetilide; 1 to 100 nM),  $\omega$ 3FA pretreatment (30 days;  $n = 6$ ) prolonged the plateau phase of the monophasic action potential; did not slow the terminal fast repolarization; reduced the dofetilide-induced prolongation of the action potential duration; reduced dofetilide-induced triangulation; and reduced dofetilide-induced reverse use-dependence, instability of repolarization, and dispersion. Dofetilide reduced excitability in  $\omega$ 3FA-pretreated hearts but not in control hearts. Whereas torsades de pointes (TdP) were observed in five out of six in control hearts, none were observed in  $\omega$ 3FA-pretreated hearts. Docosahexaenoic acid (DHA) inhibited the sodium current with ultrafast kinetics. Dietary  $\omega$ 3FA supplementation markedly reduced dofetilide-induced TRIA<sub>D</sub> and abolished dofetilide-induced TdP. Ultrafast sodium channel block by DHA may account for the antiarrhythmic protection of the dietary supplements of  $\omega$ 3FA against dofetilide-induced proarrhythmia observed in this animal model.

antiarrhythmia agents; omega-3 fatty acids; ion channels; torsades de pointes

ORAL SUPPLEMENTATION WITH fish oils or ethyl esters of  $\omega$ -3 polyunsaturated fatty acids ( $\omega$ 3FA) are associated in some large clinical studies with significant risk reductions of sudden cardiac death, which, although not directly compared in a head-to-head trial, appear larger than those observed with amiodarone in similar study populations, with less toxicities (1, 2, 9, 11, 14, 27). However, controversy exists about the pro- or antiarrhythmic effects of  $\omega$ 3FA (8, 35). Antiarrhythmic effects of  $\omega$ 3FA have been reported in animal (5, 33) and in cellular models (29). Similar to amiodarone, it has been reported that

$\omega$ 3FA block sodium (42), calcium (41), and potassium channels (17, 24, 26); likewise, they also exhibit antiadrenergic actions (36). In contrast with amiodarone, at therapeutic concentrations,  $\omega$ 3FA do not widen QRS or prolong the QT interval (13). Many class III antiarrhythmic drugs such as dofetilide, as well as noncardiac drugs, can be associated with a prolongation of the QT<sub>c</sub> interval of the electrocardiogram (ECG) and development of polymorphic ventricular arrhythmias torsades de pointes (TdP), an arrhythmia where the ECG exhibits characteristic twisting undulations of the cardiac activations. Therefore, QT prolongation itself is regarded by some as a proarrhythmic liability (37). Changes of the QT interval on the surface ECG generally reflect changes of the plateau and the repolarization phase of the action potential but can also be affected by changes in the conduction and dispersion of the APD. It thus follows that the QT interval can be lengthened by slowed repolarization and sometimes by slowed conduction and dispersion of the action potentials as well. Slowed repolarization gives the action potential a more triangular shape, referred to as triangulation (21). To be effective against tachyarrhythmias, drug-induced prolongation of action potential duration (APD) should be most marked during tachycardia; instead many drugs prolong the APD little during tachycardia, but primarily at slow heart rates. This is termed reverse use-dependence (21), which inherently leads to the instability of APD (38). Finally, all three mechanisms result in spatial and temporal dispersion. Triangulation, reverse use-dependence, instability, and dispersion are referred to as TRIA<sub>D</sub>. APD prolongation without TRIA<sub>D</sub> can be antiarrhythmic, whereas drugs inducing TRIA<sub>D</sub> are proarrhythmic, inducing preferentially TdP when APD is prolonged but ventricular fibrillation when APD is shortened (20, 21, 38).

Therefore, in the present study we investigated the effects of dietary supplements of esterified  $\omega$ 3FA: 1) how do they block sodium channels without widening the QRS, 2) what are their effects upon TRIA<sub>D</sub>, 3) can they antagonize the proarrhythmic effects of drugs that increase TRIA<sub>D</sub> (e.g., dofetilide), 4) by what mechanism(s) might they be considered a powerful antiarrhythmic alternative, and 5) under what conditions might they be less effective.

## MATERIALS AND METHODS

The effects of  $\omega$ 3FA feeding as used in the Gruppo Italiano per lo Studio della Sopravvivenza nell'Infarto miocardico (GISSI Prevenzi-

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one study) (14) were studied in 2.5-kg female rabbits. Rabbits were selected because, as in humans, the main repolarizing current in the ventricles is the rapidly activating delayed rectifier potassium current ( $I_{Kr}$ ). Females were selected because, as in humans, they appear more sensitive to the development of TdP (30). Other species were deemed less appropriate because the rat has the transient outward potassium current ( $I_{To}$ ) current as the primary repolarizing current, whereas dogs and guinea pigs have much slow delayed rectifier potassium current ( $I_{Ks}$ ) in addition to  $I_{Kr}$ , which may obscure any proarrhythmia; dog hearts are also too large for easy Langendorff perfusion. The rabbits were fed with standard Lapina (Quartes, Belgium) at libitum. Six hearts from animals fed with food enriched with 15 mg  $\omega$ 3FA  $\cdot$  kg<sup>-1</sup>  $\cdot$  day<sup>-1</sup> for 30 days [mixing daily 55% eicosapentaenoic acid ester (EPA-EE) and 45% docosahexaenoic acid ethyl ester (DHA-EE) to their food as required; Omacor; Solvay] were compared with six control hearts. The investigation conforms to the *Guide for Care and Use of Laboratory Animals* published by the National Institutes of Health (NIH Publication No. 85-23, Revised 1996) and was approved by the Veterinary Department of the Belgian Government. Following stunning by the captivator bolt, the heart was quickly removed and perfused in the Langendorff mode.

The His-bundle was sectioned, and the distal part was stimulated. Recording electrodes were placed under the left ventricular endocardium and on the epicardium. A grounded potassium-perfused electrode served as the reference. The heart was perfused at a constant pressure of 80 cmH<sub>2</sub>O with a bicarbonate buffer containing (in mM) 118 NaCl, 4 KCl, 22 NaHCO<sub>3</sub>, 1.1 MgCl<sub>2</sub>, 0.4 NaH<sub>2</sub>PO<sub>4</sub>, 1.8 CaCl<sub>2</sub>, 5 dextrose, 2 pyruvate, and 0.038 creatine. The perfusate was equilibrated with 95% O<sub>2</sub>-5% CO<sub>2</sub> adjusted to obtain a pH of 7.35 at 36° to 37°C. Stimulation occurred at 1.5 times the threshold stimulation current. More detailed descriptions of the experimental system were described previously (19).

**Electrophysiological measurements.** The experiment consisted of brief and long protocols. The brief protocols were applied every minute and consisted of a 30-s train at a cycle length of 1,000 ms, followed by 10-s trains at cycle lengths of 750 and 300 ms. The long protocols were executed after 15-min equilibration periods at each concentration studied, 0, 1, 3, 10, 30, and 100 nM dofetilide, and terminated with a 10-min washout period. These concentrations cover the therapeutic range of 1–3 nM as well as proarrhythmic concentrations (10–100 nM).

In the long protocols the following parameters were measured: automaticity (nonstimulated spontaneous heart rate in beats per minute) and escape cycle length (when stopping stimulation, the number of milliseconds passing before a spontaneous beat results), coronary perfusion rate (milliliters per minute), ectopic activity (beats per minute), left ventricular septal and epicardial monophasic action potential (MAP) duration (milliseconds) at 30% (APD<sub>30</sub>), 60% (APD<sub>60</sub>), and 90% (APD<sub>90</sub>) of repolarization (APD<sub>30</sub> and APD<sub>90</sub> are less accurate because repolarization may be very slow, so for quantitative comparisons we used APD<sub>60</sub>), activation time of the ventricles (milliseconds), triangulation (APD<sub>90</sub> to APD<sub>30</sub>), reverse use-dependence, instability (beat-to-beat variability in APD), and dispersion of repolarization (beat-to-beat variability between septal and epicardial APD<sub>60</sub>). Conduction was measured as the duration of significant levels of high-frequency activation following each stimulus (18). Reverse use-dependence is measured as the difference in APD<sub>60</sub> between the first 10 and subsequent 20 action potentials during a train of 30 at 1 Hz. Threshold stimulation current (microamperes) is determined as the minimum 2-ms current pulse that, at a cycle length of 400 ms, results in the activation of the heart. When the maximum available stimulation current of 1,000  $\mu$ A becomes unable to drive the heart, the heart is then declared unexcitable. The effective refractory period was determined by progressively delaying an extra pulse until it is captured at 1 Hz (a walking-out pulse). The development and number of early afterdepolarizations (EADs) and TdPs were detected by an automated computer algorithm. The procedures are described in

more detail elsewhere (21, 22, 38). In addition, the instability of the repolarization time (APD<sub>30</sub> to APD<sub>90</sub>) was measured as a Poincaré plot (PC<sub>30–90</sub>), where the repolarization time is plotted against that of the preceding action potential. If all action potentials have similar repolarization times, then a single point on the diagonal line results (see Fig. 4C, *left inset*); as repolarization varies, deviations from the diagonal increase (Fig. 4C, *right inset*). The sum of the distance to the diagonal was computed at each concentration for 450 action potentials.

A fresh stock solution of 100  $\mu$ M dofetilide in dimethylsulfoxide (DMSO) was made daily and pumped into the bubble trap under computer control at the rate required to achieve the desired dofetilide concentrations. The DMSO concentration remained <0.1% at all times.

**Sodium current measurements.** Human embryonic kidney (HEK)293 cells that do not express endogenously sodium channels or accessory subunits were cultured at 37°C in DMEM supplemented with penicillin-streptomycin (Sigma, London, UK), 10% bovine fetal serum, and 1% nonessential amino acids in a 5% CO<sub>2</sub> atmosphere (15). Transfection of Nav1.5 channels (2  $\mu$ g) and CD8 (1  $\mu$ g) was performed using Lipofectamine2000 (10  $\mu$ l). Before experimental use, the cells were incubated with polystyrene microbeads precoated with anti-CD8 antibody (Dynabeads M450; Dynal Biotech, Oslo, Norway) as described (15).

The intracellular pipette filling solution contained (in mM) 5 NaCl, 5 KCl, 130 CsF, 1 MgCl<sub>2</sub>, 4 Na<sub>2</sub>ATP, 10 HEPES-K, and 5 EGTA and was adjusted to pH 7.2 with CsOH. The bath solution contained (in mM) 140 NaCl, 5 KCl, 1.8 CaCl<sub>2</sub>, 1 MgCl<sub>2</sub>, 10 HEPES-Na, 10 glucose, 10 tetraethylammonium, and 2.8 Na-acetate and was adjusted to pH 7.4 with NaOH. DHA (Sigma, St. Louis, MO) was dissolved as previously described (17). Sodium currents were recorded at room temperature (21–23°C) using the whole cell patch-clamp technique with an Axopatch 1C patch-clamp amplifier (Axon Instruments, Foster City, CA). Micropipettes were pulled from borosilicate glass capillary tubes (GD-1; Narishige, Tokyo, Japan) on a programmable horizontal puller (P-87; Sutter Instrument, San Rafael, CA) and heat polished with a microforge (Narishige). To minimize voltage errors, micropipette resistance was 1 to 2 M $\Omega$ . Data analysis was performed using the CLAMPfit program of pCLAMP 9.0.1 and Origin 7.0.3 (Microcal Software, Northampton, MA). The curve-fitting procedure used a nonlinear least-squares (Gauss-Newton) algorithm; results were displayed in linear and semilogarithmic format, together with the difference plot.

Free  $\omega$ 3FA are generally toxic to cells and are kept at low micromolar concentrations in plasma. However, the plasma-free  $\omega$ 3FA concentration can vary greatly depending on the hormonal, metabolic, and nutritional state of the individual. About 99.9% of free  $\omega$ 3FA are bound to albumin in the plasma (10). The low plasma concentration of free  $\omega$ 3FA is maintained by a competition between binding sites on albumin and cell membrane phospholipids. The range of free DHA human plasma concentration is <2.8  $\mu$ M (10). Since DHA and EPA can compete each other for binding to Nav1.5 channels, we chose to analyze only the effects produced by DHA on the current generated by their activation.

**Statistical analysis.** Data are expressed as means  $\pm$  SE. Comparisons of two means were done using a Student's *t*-test, and *P* < 0.05 was considered significant. Instability and unexcitability were not normally distributed so that nonparametric tests were used instead. Goodness of fit was judged by the  $\chi^2$  criterion (17). Comparison of events was done with a Fisher exact test. Due to the small number of experiments (*n* = 6) and large variation of effects, only major drug effects can become significant.

## RESULTS

**Effects of dietary supplements of  $\omega$ 3FA.**  $\omega$ 3FA significantly prolonged APD<sub>30</sub>, APD<sub>60</sub>, and APD<sub>90</sub> (Table 1). APD<sub>30</sub> was

Table 1. Comparison of electrophysiological characteristics obtained in control and  $\omega$ 3FA-pretreated rabbit hearts

	Group		P Value
	Control	$\omega$ 3FAs	
Action potential duration, ms			
At 30%	165 $\pm$ 3.2	204 $\pm$ 15.0	0.02
At 60%	217 $\pm$ 5.5	248 $\pm$ 10.7	0.02
At 90%	252 $\pm$ 6.2	278 $\pm$ 12.4	0.02
Effective refractory period, ms	216 $\pm$ 22.9	278 $\pm$ 4.9	0.01
Reverse use-dependence, ms	-2 $\pm$ 0.8	-3 $\pm$ 0.9	>0.05
Instability, ms	5 $\pm$ 0.9	4 $\pm$ 0.7	>0.05
Dispersion, ms	19 $\pm$ 5.4	17 $\pm$ 5.0	>0.05
Ectopics per minute	0.5 $\pm$ 0.13	0.4 $\pm$ 0.15	>0.05
Threshold stimulation current, $\mu$ A	146 $\pm$ 41	169 $\pm$ 37	>0.05
Activation time, ms	49 $\pm$ 2.0	51 $\pm$ 3.2	>0.05

Values are means  $\pm$  SE;  $n = 6$ /group.  $P$ , comparison between parameters from  $\omega$ -3 polyunsaturated fatty acids ( $\omega$ 3FA)-pretreated and control rabbits.

prolonged more than APD<sub>90</sub> (Fig. 1), i.e.,  $\omega$ 3FA prolonged the APD without triangulation. As expected, the prolongation of APD resulted in a significant prolongation of the effective refractory period (ERP), but this prolongation was more than twice as long as the prolongation of APD<sub>90</sub> (Table 1). Thus additional electrophysiological change(s) must play an important role (discussed in *Dofetilide leads to unexcitability in  $\omega$ 3FA-pretreated hearts*).

At baseline (i.e., before the administration of dofetilide),  $\omega$ 3FA pretreatment had no significant effects on reverse use-dependence, instability, dispersion, or ectopic activity, and neither group exhibited TdP or ventricular tachycardia (VT). Threshold stimulation current and activation times were also not significantly changed (Table 1).

*Dofetilide leads to unexcitability in  $\omega$ 3FA-pretreated hearts.* All control hearts could fully execute the experiment (including 100 nM dofetilide), whereas none of the  $\omega$ 3FA hearts could; one heart became unexcitable at 10 nM dofetilide, and all others at 30 nM [no data at 100 nM in  $\omega$ 3FA-pretreated hearts (Fig. 2, right)].

Since dofetilide acts by a class III mechanism (40), pretreatment with  $\omega$ 3FA might emphasize the prolongation of the action potential of dofetilide and thereby lead to unexcitability. However, 10 nM dofetilide prolonged APD<sub>90</sub> by 235  $\pm$  75 ms in control but only by 193  $\pm$  47 ms in  $\omega$ 3FA hearts ( $P = 0.64$ ); 30 nM dofetilide prolonged APD<sub>90</sub> by 402  $\pm$  152 ms in control but only by 247  $\pm$  107 ms in  $\omega$ 3FA hearts ( $P = 0.47$ ). Furthermore, APD<sub>60</sub> started to exceed 1,000 ms at 3 nM dofetilide in control hearts (Fig. 2) but only at 30 nM dofetilide in  $\omega$ 3FA-pretreated hearts. In control hearts there were 467 APD<sub>60</sub> measurements exceeding 1,000 ms but only six in  $\omega$ 3FA hearts, whereas 26 APD<sub>60</sub> measurements exceeded 1,500 ms in control hearts, and no such prolongations occurred in  $\omega$ 3FA hearts. Finally, APD<sub>60</sub> >1,000 ms occurred in four control hearts but was seen in only one  $\omega$ 3FA experiment. Thus  $\omega$ 3FA pretreatment clearly does not augment dofetilide-induced APD prolongation and cannot account for the unexcitability in  $\omega$ 3FA-pretreated hearts.

A block of sodium channels can also reduce excitability (34, 43). However, in control hearts dofetilide did not slow conduction, agreeing with reports that dofetilide does not block sodium channels. In control hearts, conduction slowed indi-

rectly when the action potential impinged on the tail of the preceding action potential. However, in all  $\omega$ 3FA experiments, the slowing of conduction was already noted at short diastolic intervals, i.e., before impinging on the tail of the preceding action potential. In fact, no conduction was possible until  $\sim$ 30 ms following the end of the preceding action potential. Interestingly, action potentials triggered about 60 ms after the end of the action potential conducted at virtually normal velocity. Thus if this extension of refractoriness was due to block of sodium channels, then there had to be a marked block at short diastolic intervals but virtually no block at slightly longer intervals. Attempts to characterize such fast recovery appeared impossible with the MAP; the time interval over which the recovery occurred was so brief that only rarely could an intermediate conduction velocity be observed. Furthermore, in the presence of dofetilide, the variability of APD rendered it nearly impossible to hit this brief time period. Therefore, the effects of DHA on sodium current were studied with voltage clamp to be able to analyze this recovery process in a short period of time.

DHA (1  $\mu$ M) reduced the sodium current only slightly when membrane potential was maintained (holding potential) at -120 mV (Fig. 3A). However, at -90 mV, it decreased significantly and at -80 mV the block became 42.0  $\pm$  7.1% ( $n = 6$ ;  $P < 0.05$ ). The block increased sharply with increasing concentrations so that by 10  $\mu$ M, the marked reduction of the current already developed even at -120 mV. Since we were primarily interested in therapeutic concentrations that do not reduce the current at well-polarized potentials ( $\approx$  -120 mV), the kinetic studies were therefore done at 1  $\mu$ M.

Following a 500-ms depolarizing pulse, a 10-ms test pulse was applied after various recovery times and at different holding potentials (Fig. 3B, inset). The Nav1.5 magnitude of the current recorded in the test pulse was plotted versus the time elapsed between the end of the 500-ms prepulse and the beginning of the test pulse. From these data, the recovery time constant ( $\tau_{re}$ ) for the sodium currents was extracted by least-square fitting of the data. At -120 mV,  $\tau_{re}$  was 9.4  $\pm$  2.3 ms and similar to that observed in control cells (9.4  $\pm$  2.0 ms;  $n =$

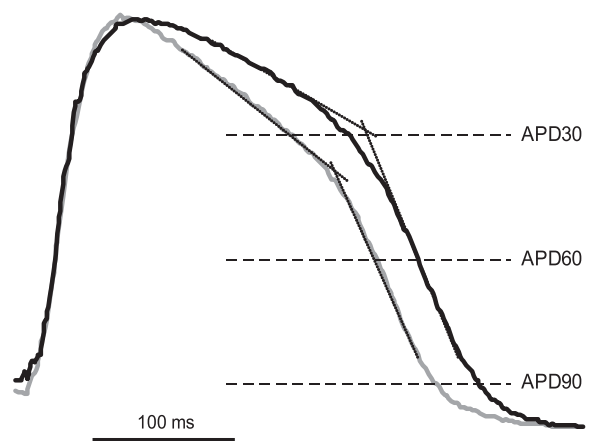


Fig. 1. Comparison of the mean action potential of control (gray) vs.  $\omega$ -3 polyunsaturated fatty acids ( $\omega$ 3FA)-pretreated (black) animals. In the  $\omega$ 3FA-pretreated animals, the plateau phase of the monophasic action potential is flatter and requires more time before the fast, final repolarization begins. APD<sub>30</sub>, APD<sub>60</sub>, and APD<sub>90</sub>, action potential duration at 30%, 60%, and 90%, respectively.

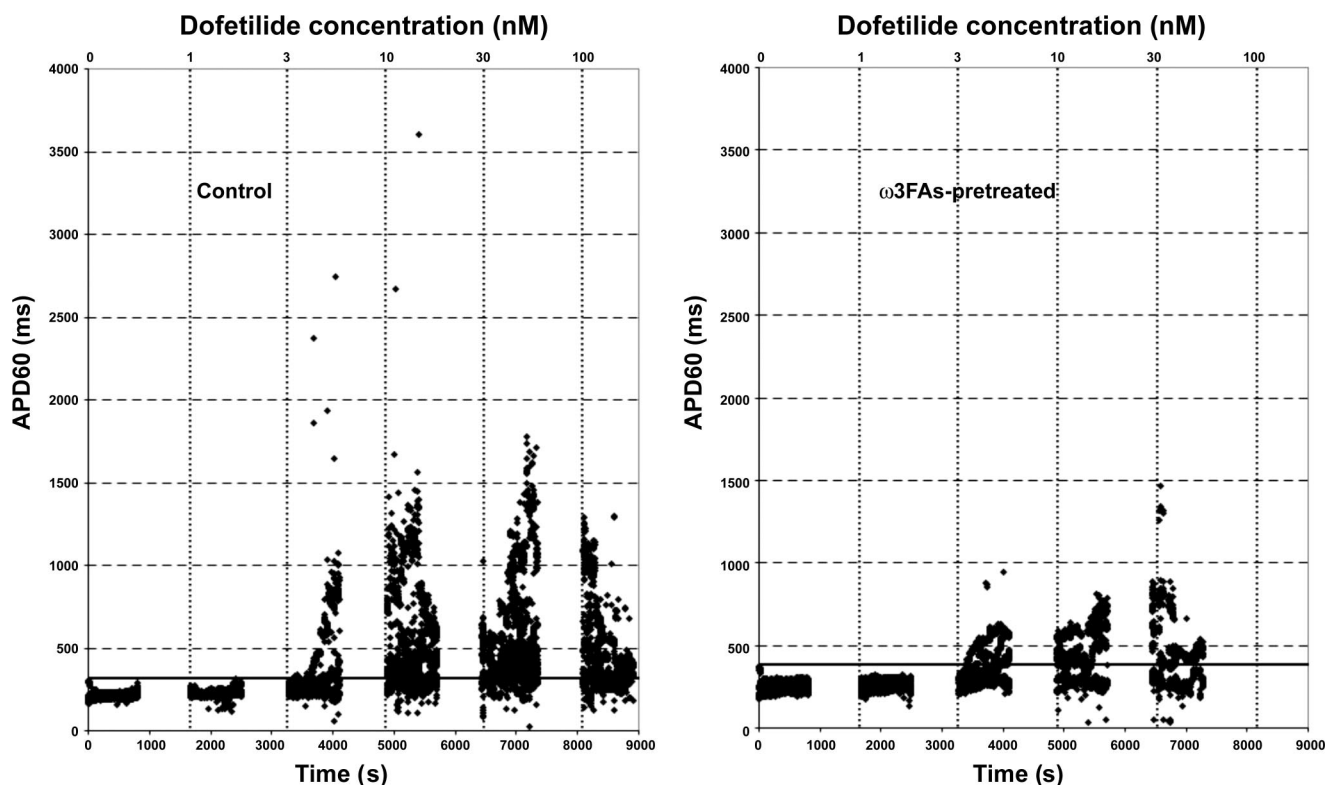


Fig. 2. Comparison of the dofenilide-induced prolongation of APD<sub>60</sub> in control and  $\omega$ 3FA-pretreated hearts. Every minute the hearts were stimulated for 30 s at a cycle length of 1,000 ms, and their APD<sub>60</sub> is plotted as a function of time into the experiment. The first group of data points (0 to 1,700 ms) represents the drug-free period. This is followed by 1 nM (1,700 to 3,300 ms), 3 nM (3,300 to 4,900 ms), 10 nM (4,900 to 6,400 ms), 30 nM (6,400 to 8,100 ms), and finally 100 nM (8,100 to 9,000 ms) dofenilide. In the  $\omega$ 3FA-pretreated group, 1 heart became unexcitable during perfusion with 10 nM dofenilide, and all others during 30 nM, so that, at *right*, no data points could be measured at 100 nM. The 97.5% confidence interval for APD prolongation in control hearts is shown as a solid horizontal line.

5;  $P > 0.05$ ). However, as the holding potential became less negative,  $\tau_{re}$  markedly prolonged; by  $-90$  mV,  $\tau_{re}$  increased from  $25.2 \pm 5.0$  ms in control to  $49.0 \pm 7.3$  ms in  $1 \mu\text{M}$  DHA ( $n = 6$ ;  $P < 0.01$ ). Thus recovery kinetics of Nav1.5 channels in the presence of  $\omega$ 3FA is an ultrafast process, i.e., not much slower than in control.

Thus block induced by  $1 \mu\text{M}$  DHA was measured at the maximum peak of a 10-ms test pulse to  $-10$  mV from different holding potentials applied after a 10- or a 500-ms prepulse. The degree of block measured at the test pulse after the 10- or 500-ms prepulse from the four different holding potentials ( $-120$ ,  $-100$ ,  $-90$ , and  $-80$  mV) was similar:  $12.0 \pm 5.2\%$  vs.  $30.3 \pm 9.7\%$  at  $-120$  mV ( $n = 6$ ;  $P > 0.05$ ),  $16.8 \pm 5.6\%$  vs.  $34.1 \pm 8.9\%$  at  $-100$  mV ( $n = 6$ ;  $P > 0.05$ ),  $18.9 \pm 4.8\%$  vs.  $35.8 \pm 7.2\%$  at  $-90$  mV ( $n = 5$ ;  $P > 0.05$ ), and  $42.0 \pm 7.1\%$  vs.  $35.4 \pm 5.2\%$  at  $-80$  mV ( $n = 4$ ;  $P > 0.05$ ). Thus, upon depolarization, sodium channels quickly become blocked; there is marked block in less than 10 ms, and after only 500 ms most of the channels become blocked. Once the membrane potential becomes sufficiently negative, recovery occurs ultrafast; even at  $25^\circ\text{C}$ ,  $\tau_{re}$  becomes shorter than 50 ms.

*$\omega$ 3FA pretreatment prevents dofenilide-induced TdP.* In control hearts, dofenilide induced TdP in five of the six hearts but in none following  $\omega$ 3FA pretreatment. Since the primary cause for TdP is TRIaD (21, 38), we characterized the effects of  $\omega$ 3FA pretreatment upon these four parameters.

Triangulation increased with the dofenilide concentration (Fig. 4B), but in both groups starting at 10 nM it exceeded the

upper 97.5% confidence limit of 29 ms previously determined in normal rabbit hearts (23). At 30 nM dofenilide, triangulation reached  $222 \pm 109$  ms in control hearts, but it also increased to  $91 \pm 55$  ms ( $P = 0.29$ ; vs. control hearts) in  $\omega$ 3FA-pretreated hearts.

Reverse use-dependence increased significantly starting from 10 nM and exceeded the upper 97.5% confidence limit of 6 ms in both groups (Fig. 4A) (23). However, in control hearts, at 10 nM dofenilide, reverse use-dependence increased to  $32 \pm 11$  ms and only  $8 \pm 6$  ms in  $\omega$ 3FA ( $P = 0.025$ ; vs. control hearts). At 30 nM dofenilide, reverse use-dependence reached  $47 \pm 24$  ms in control hearts but was only  $-1 \pm 25$  ms in  $\omega$ 3FA-pretreated hearts.

Instability increased to  $943 \pm 485$  ms in control hearts and to  $344 \pm 156$  ms in  $\omega$ 3FA-pretreated hearts by 10 nM dofenilide (Fig. 4C). Both values exceeded the proarrhythmic upper 97.5% confidence limit of 136 ms previously determined in normal rabbit hearts (23). At 30 nM, instability further increased to reach  $3,208 \pm 2,013$  ms in control hearts and  $498 \pm 201$  ms in  $\omega$ 3FA-pretreated hearts ( $P = 0.037$ ; vs. control hearts).

Spatial and temporal dispersion of APD<sub>60</sub> increased in control hearts and  $\omega$ 3FA (Fig. 4D). At 10 nM it was  $60 \pm 12$  ms in control hearts and  $41 \pm 18$  ms in  $\omega$ 3FA-pretreated hearts, both values exceeding the proarrhythmic upper 97.5% confidence interval of 23 ms, previously determined in normal rabbit hearts (23). At 30 nM, dispersion further increased to

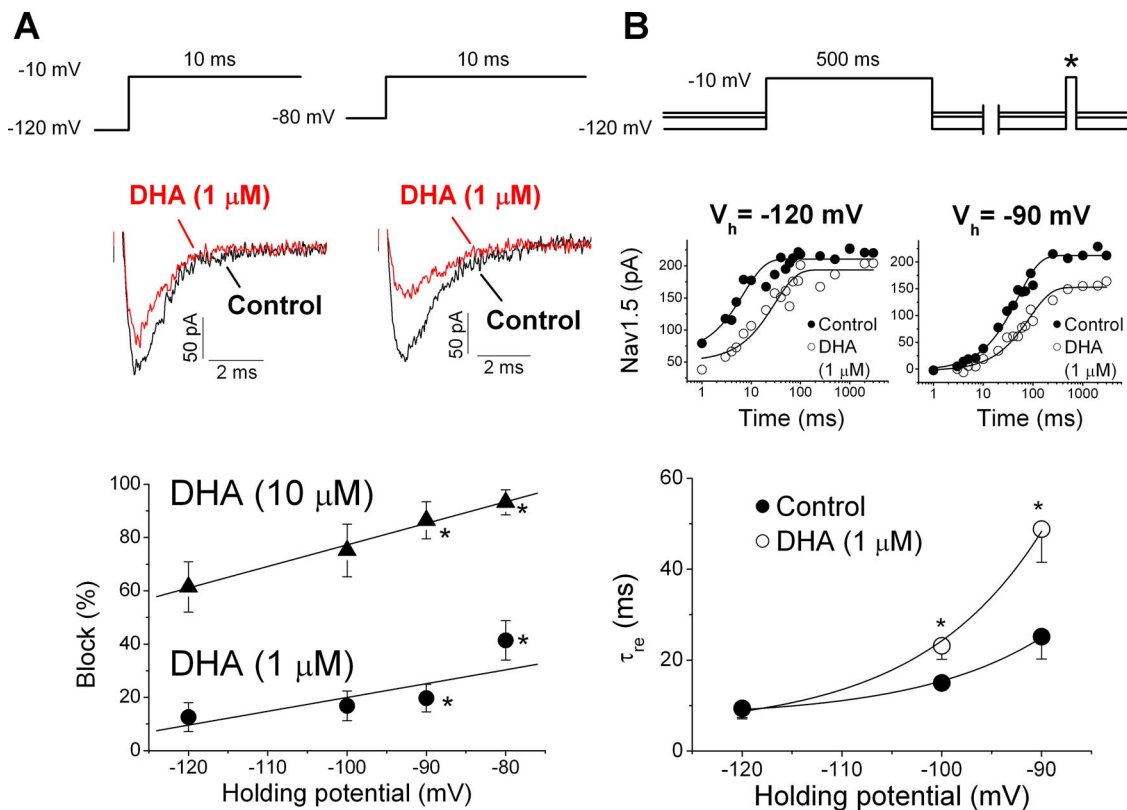


Fig. 3. ω3FA effects on sodium current. *A*: effects of docosahexaenoic acid (DHA; 1 and 10 μM) on Nav1.5 current measured after applying 10-ms depolarizing pulses from different levels of holding potentials (between -120 mV and -80 mV). *B*: a double-pulse protocol consisting in a 500-ms prepulse to -10 mV followed by a 10-ms test pulse to the same level from different holding potentials (-120, -100, and -90 mV) after different recovery time periods. DHA (1 μM) only slowed the recovery process when it was analyzed from a holding potential positive to -100 mV. τ<sub>re</sub>, recovery time constant; V<sub>h</sub>, half-maximal voltage. \**P* < 0.05, changes are significant.

reach 89 ± 24 ms in control hearts and 49 ± 5 ms in ω3FA-pretreated hearts (*P* = 0.13).

**Proarrhythmia.** Stalling of repolarization rate (repolarization rate = 0) (38) started at 1 nM in two of six control hearts, but stalling required 10 nM in ω3FA hearts, where it then

developed in three of six hearts. EADs began at 3 nM in control hearts and at 10 nM in ω3FA. Finally, TdP and VT started to develop at 3 nM dofetilide in control hearts; moreover, dofetilide induced TdP in five out of six control hearts. In contrast with that of the ω3FA-pretreated hearts, there was a

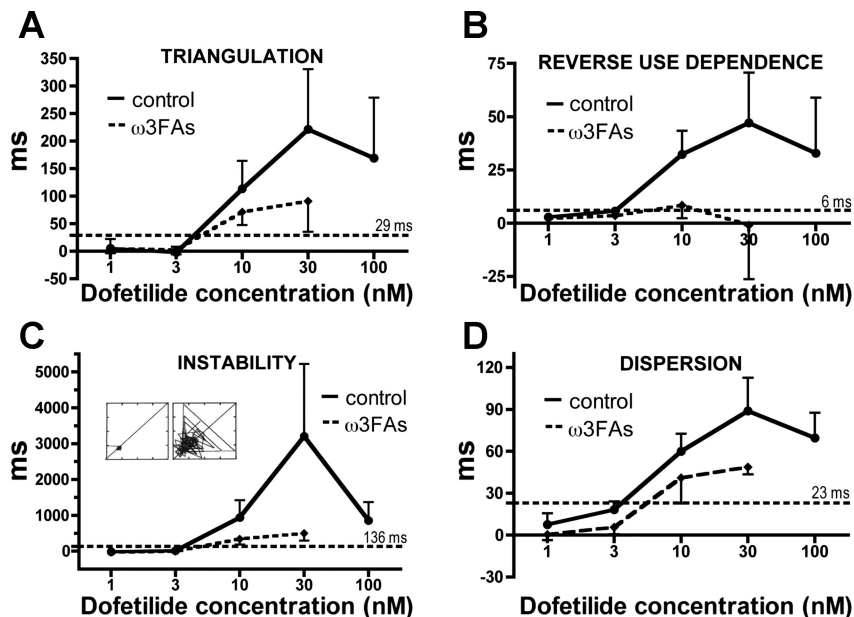


Fig. 4. Comparison of the dofetilide triangulation, reverse use-dependence, instability, and dispersion in control (solid line) and in ω3FA-pretreated animals (dotted line) with upper 97.5% confidence interval limit for normal rabbit hearts (dotted line with tick on y-axis). Different concentrations of dofetilide (x-axis; nM) tested for triangulation (*A*), reverse use-dependence (*B*), instability (*C*), and dispersion (*D*). *C*, inset: a Poincaré plot in drug-free heart (left) with a Poincaré plot in 10 nM dofetilide (right). Axes of the inset panels are 400 ms (experiment, 16,434).

single heart that exhibited VT at 10 and 30 nM dofetilide; most importantly, not a single TdP was observed in the six  $\omega$ 3FA hearts (Fisher exact test;  $P < 0.01$ ).

## DISCUSSION

Our results demonstrate that  $\omega$ 3FA pretreatment completely suppresses dofetilide-induced TdP (which occurred in 5 out of 6 control hearts), does not induce TRIaD, and attenuates dofetilide-induced TRIaD and that DHA (in acute treatments) blocks sodium channels with ultrafast kinetics.

The pharmacological profile of  $\omega$ 3FA strikingly matches that of amiodarone: block of sodium, potassium, and calcium channels as well as  $\alpha$ - and  $\beta$ -adrenoceptors (17, 24, 26, 41, 42). Most importantly, the cardioprotective effects of  $\omega$ 3FA observed in the GISSI Prevenzione study (14) were better than those previously reported with amiodarone (27). Our present observations may account for some of these advantages; whereas amiodarone has been found to induce some triangulation (38),  $\omega$ 3FA do not. Although amiodarone also has fast kinetics of recovery from sodium channel block (32), its onset and offset kinetics are still nearly one order of magnitude slower than those of  $\omega$ 3FA reported in the present study (38). Since clinical use of amiodarone is hampered by its many toxicities (16), the adverse side effects of  $\omega$ 3FA are limited to weak inflammatory and hemostasis disorders (28). Therefore, it could be speculated that  $\omega$ 3FA could be a highly effective and nontoxic adjunct to other strategies for the prevention of myocardial ischemia-related sudden death. However, contrary to reports of the beneficial effects of  $\omega$ 3FA consumption or supplements on cardiac deaths in some populations (14), other trials have suggested a neutral effect in patients with implanted cardioverter defibrillators (7, 35) and even a deleterious effect in men with angina (8). It is unclear whether these contradictory observations on the effects of  $\omega$ 3FA supplementation can be accounted for by different population characteristics (e.g., angina vs. post-myocardial infarction or heart failure), possible toxic effects of mercury exposure associated with fish intake, background fish consumption in the control population, or the type of arrhythmogenic mechanism (e.g., triggered activity vs. reentry arrhythmias) (12).

The ultrafast onset and recovery kinetics of DHA sodium channel block, never observed before with other agents ( $\approx 30$  ms), ensures that conduction is not slowed, except for a brief window early in diastole. Since the recovery of the sodium currents is not much slower than normal, the vulnerable period would not be widened much, as is seen with other sodium channel blockers (34); instead, it would only be delayed. At 25°C and at  $-90$  mV, the  $\tau$  for recovery from sodium channel block is about 50 ms. As the membrane potential is made more negative, recovery becomes increasingly faster, until at  $-120$  mV, recovery closely tracks the reactivation of control sodium channels (Fig. 3B). If this time constant were to exhibit a similar temperature dependence as is the case with other sodium channel blockers (25), then at 37°C recovery of block could easily be three times faster. Furthermore, these recovery characteristics would also be shifted by about 20 mV to more positive potentials (25). Such fast kinetics would well explain our observations that, except for a brief early diastolic window, conduction would not be slowed. However, during and before this early diastolic window, sodium channels would not be

available. Hence throughout the cardiac action potential and specifically during the rapid repolarization period, no inward sodium current activation would be available. Therefore, upon reaching a normal resting potential, recovery would be complete in a time frame similar to that of reactivation in control. Thus the period of slowed conduction (partial availability of sodium channels) would be little or not prolonged, i.e., just delayed.

This profile of sodium channel block has three important clinical consequences. First, whereas in well-polarized tissue sodium channels would behave normally, except for a small delay of their availability, in ischemic tissue recovery would be very slow or may not occur at all. As a result, tissue depolarized by an ischemic event (responsible for proarrhythmia) would remain unavailable for participation in reentry. This mechanism could contribute to the clinical observation that  $\omega$ 3FA reduce sudden death upon reinfarction (14) but might not protect against ventricular arrhythmia in the absence of ischemia (7). Second, much evidence has accumulated that blockers of  $I_{Kr}$  are less torsadogenic provided they also block inward currents (sodium and/or calcium) at lower concentrations than those needed to block  $I_{Kr}$  (6, 23). Our results on the effects of  $\omega$ 3FA on sodium channels, together with those previously reported on L-type  $Ca^{2+}$  channels (41), may account for the complete suppression of dofetilide-induced TdP in  $\omega$ 3FA-pretreated hearts observed in the present study. Finally, it may be of interest to test in a clinical trial whether the danger of sudden death is reduced by  $\omega$ 3FA feeding in specific clinical diseases such as congenital long-QT syndrome type 2, characterized by human *ether-a-go-go*-related gene (HERG) channel dysfunction.

Dofetilide-induced TRIaD was attenuated by pretreatment with dietary supplements of  $\omega$ 3FA. Although these reductions of TRIaD were not large enough to stay within safe limits (21, 23, 38), nevertheless these simultaneous reductions may also have contributed to the observed reduction of dofetilide-induced TdP. However, the fact that TRIaD could not be completely suppressed, whereas TdP was entirely prevented, further supports that the block of sodium channels with ultrafast kinetics potentially plays a key role. If so, then  $\omega$ 3FA might reduce the rare occurrences of TdP and render cardiac as well as noncardiac agents with such liability safer to use.

The effects of  $\omega$ 3FA on the APD and, thus, indirectly on the repolarizing currents observed in this chronic rabbit model are at variance with findings in other animal species (39) in different experimental conditions using isolated cells (39) or  $\alpha$ -linolenic acid (4) and after acute administration at different concentrations of  $\omega$ 3FA (31). The rabbit animal model differs from other species in that the repolarization phase of the APD is exclusively dependent upon  $I_{Kr}$ , and that could explain in part the differences observed between species. In contrast, block of sodium currents by  $\omega$ 3FA is consistently observed in all species.

*Limitations of the present study.* The present paper has several experimental limitations: 1) the effects of  $\omega$ 3FA on sodium channels were analyzed by studying the effects of DHA (not DHA + EPA) on Nav1.5 channels transiently expressed in HEK293 cells; 2) no voltage-clamp studies on myocytes obtained from  $\omega$ 3FA-fed rabbits were done; 3) the beneficial effects of  $\omega$ 3FA were demonstrated after 30 days of pretreatment, so the time course of development of this benefit

is unknown; and 4) specifically, our study cannot rule out that the benefits might not be preceded by untoward effects (which could help account for some of the clinical inconsistencies). The fast sodium channel block with fast kinetics holds for chronic exposure, but preliminary experiments suggest that these effects are more difficult to establish in acute experiments (unpublished observations). Thus additional studies will be needed to address these limitations.

### Conclusions

The cardiac effects of  $\omega$ 3FA resemble those of amiodarone; both block sodium, calcium, and potassium channels, have antiadrenergic properties, and can prolong the APD, reverse TRIaD, and suppress TdP. The main difference is that sodium channel block by  $\omega$ 3FA has a much faster onset and offset kinetics. As a result, the electrophysiological profile of  $\omega$ 3FA appears more desirable; the duration of reduced sodium current (facilitates reentry) is much shorter. These properties, together with the safer profile of  $\omega$ 3FA versus other antiarrhythmic agents, may account for the clinical observation that risk reduction of sudden cardiac death with dietary supplementation of  $\omega$ 3FA, although not directly compared in a head-to-head trial, appears larger than with amiodarone in post-myocardial infarction patients (3, 14, 27). Moreover, if further research confirms that  $\omega$ 3FA or some components of  $\omega$ 3FA may suppress drug-induced TdP, they could be used to salvage certain valuable medications that would otherwise be rejected for medical use. Finally, it would be interesting to investigate the efficacy of the dietary supplementation of  $\omega$ 3FA to reduce the arrhythmic dangers of certain clinical disease states such as the congenital long-QT syndrome type 2, characterized by HERG channel dysfunction.

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