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Effects of dietary n-3 fatty acids on contractility, Na^+ and K^+ currents in a rat cardiomyocyte model of arrhythmia

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The n-3 polyunsaturated fatty acids (PUFAs) have been reported to prevent ventricular fibrillation in human clinical studies and in studies involving experimental animals and isolated cardiomyocytes. This study aimed to determine whether dietary n-3 PUFAs could prevent isoproterenol and free radical-induced arrhythmic (asynchronous) contractile activity in adult rat cardiomyocytes and whether whole-cell Na^+ and K^+ currents measured by patch-clamp techniques were affected. Dietary supplementation with fish oil for 3 weeks significantly increased the proportion of total n-3 PUFAs in ventricular membrane phospholipids compared with saturated fat supplementation (18.8 ± 0.6% vs. 8.1 ± 1.0%, respectively). Cardiomyocytes from the fish oil group were less susceptible to isoproterenol-induced asynchronous contractile activity than were those from the saturated fat group [EC_{50} values: 892 ± 130 nM, n = 6 and 347 ± 91 nM, n = 6 ($P < 0.05$), respectively]. Fish oil supplementation also prolonged the time taken to develop asynchronous contractile activity induced by superoxide and hydrogen peroxide. The voltage dependence of inactivation of Na^+ currents were significantly altered (−73.5 ± 1.2 mV, n = 5 vs. −76.7 ± 0.7 mV, n = 5, $P < 0.05$, for saturated fat and fish oil treated groups, respectively). The voltage dependence of activation of Na^+ and K^+ currents was not significantly affected by the dietary fish oil treatment. These results demonstrate the antiarrhythmic effects of dietary fish oil in a cardiomyocyte model of arrhythmia. (J. Nutr. Biochem. 11:382–392, 2000)

Keywords: ion currents; polyunsaturated fatty acids; reperfusion; docosahexaenoic acid; free radicals

Introduction

Recent evidence suggests that the consumption of polyunsaturated fatty acids (PUFAs) of the omega 3 or n-3 class reduce both the incidence of and mortality from coronary heart disease.[1–3] Experimental animal studies have also shown that dietary n-3 PUFAs lead to changes in myocardial phospholipid fatty acid composition and to protection from the development of ischemic and reperfusion-induced arrhythmias in both rats and marmosets.[4,5] The results of these epidemiologic and experimental animal studies strongly suggest that the beneficial effect of the dietary n-3 PUFAs is mediated in part by their potent, intrinsic, antiarrhythmic activity.

At the cellular level, evidence of the antiarrhythmic action of acutely applied n-3 PUFAs has been derived from studies using heart cells (cardiomyocytes) isolated from neonatal[6] and from adult rats.[7–9] In the above studies n-3 PUFAs were only antiarrhythmic if added in the free fatty acid form to facilitate infusion into the site of action in the sarcolemmal membrane. Other lipid forms of acute administration of n-3 PUFAs were ineffective in terms of antiarrhythmic activity.[10] In recent studies we have reported that n-3 PUFAs acutely added to cardiomyocytes isolated from the hearts of young adult rats also displayed antiarrhythmic properties, with antiarrhythmic activity being associated

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with an increase in cardiomyocyte sarcolemmal lipid fluidity.[7,8,11] In a manner similar to that reported by Xiao et al.,[12] we also reported that whole-cell sodium currents in adult rat cardiomyocytes could be inhibited by the acute addition of n-3 PUFAs.[13] Acute addition of n-3 PUFAs to adult rat cardiomyocytes have also been reported to inhibit potassium currents,[14] and collectively, these results support the notion that the n-3 PUFAs exert potent antiarrhythmic effects.

This study therefore aimed to establish whether an antiarrhythmic and a proarrhythmic effect of dietary fish oil and saturated fat, respectively, could be observed in isolated rat cardiomyocytes by studying the effects of dietary lipid supplementation on various cardiomyocyte contractility parameters associated with the maintenance of synchronous contractile activity in a cellular model of arrhythmogenesis. Catecholamine and reactive oxygen species (ROS) were used as arrhythmogenic stressors to induce arrhythmic (asynchronous) contractile activity in isolated adult rat cardiomyocytes. Importantly, because ion channel function plays a central role in arrhythmogenesis, this study was also designed to establish whether dietary lipid supplementation influenced cellular Na^+ and K^+ ion current activity, as has been shown to occur with respect to the acute addition of n-3 PUFAs to both adult[13,14] and neonatal cardiomyocytes.[12] This could provide evidence that a dietary lipid induced change in ion channel activity was central to the changes observed in cell contractility in a myocyte model that mimics, in part, certain cardiac arrhythmias.

Materials and methods

Animals and dietary oils

Ethics approval for this study was obtained from the CSIRO Health Sciences and Nutrition Animal Ethics Committee. Male Sprague-Dawley rats were obtained from the University of Adelaide, Central Animal House (Adelaide, Australia). All animals were housed in the CSIRO small animal colony in groups of up to five per cage with food [modified laboratory rat chow containing 0.7% α-linolenic acid, 7% fat total (equivalent to 65 kJ energy/day, supplied by the addition of Sunola Oil, Meadow Lea Foods, Ryde, NSW, Australia)] and water provided ad libitum. Room temperature was maintained at 23°C with constant (55%) humidity, and lights were maintained on a 12-hour light (8:00 AM to 8:00 PM)/dark cycle. Animals were maintained on the modified diet from 4 weeks of age and for the duration of the experiment. At 9 weeks of age the rats were gavaged with either a sham gavage using glycerol (REF group) or 2 mL of lard fat containing a high proportion of saturated fat (SF group; source of beef and mutton fat, Metro Quality Foods, Greenacres, NSW, Australia) or fish oil (FO group; RoPUFA, Hoffmann La Roche, Basel, Switzerland, source of n-3 PUFA) once daily for 3 weeks (an additional 74 kJ fat/day, therefore a total 29% energy obtained from fat). The fatty acid composition of the colony rat diet and the dietary oils administered by gavage is given in *Table 1*.

Table 1 Fatty acid composition (wt%) of the reference diet and dietary lipid supplements

Major FAME	Reference (REF)	Saturated fat (SF)	High n-3 PUFA (FO)
14:0	0.29	6.67	8.07
16:0	4.79	30.61	15.50
16:1	0.11	1.89	10.13
18:0	4.07	18.53	2.64
18:1[1]	75.25	35.97	15.58
18:2(n-6)	13.27	1.78	2.89
18:3(n-3)	0.68	0.65	1.13
20:4(n-6)	n/d	0.06	1.26
20:5(n-3)	n/d	0.07	24.33
22:5(n-3)	n/d	n/d	2.28
22:6 (n-3)	n/d	n/d	12.14
Σ Saturated	10.43	58.04	27.31
Σ Monounsaturated	75.50	39.39	28.16
Σ Polyunsaturated	13.96	2.56	44.57
Σ n-6	13.27	1.84	4.45
Σ n-3	0.68	0.72	40.00
n-6/n-3	19.38	2.55	0.11

Data shown are the means from diet/lipid supplement samples. Computational parameters (Σ and n-6/n-3) are derived from the full fatty acid set.
[1] 18:1 contains n-9 and n-7 isomers.
FAME—fatty acid methyl esters.
PUFA—polyunsaturated fatty acids.
n/d—not detected.

Perfusion and culture media

Calcium-free Tyrode perfusion media contained (in mM): 137.7 NaCl, 4.8 KCl, 1.2 KH_2PO_4, 1.2 $MgSO_4$, 11 glucose, and 10.0 N-[2-hydroxyethyl]piperazine-N′-[2-ethanesulfonic acid] (HEPES), pH 7.40. $CaCl_2$ was added prior to use to give the appropriate concentrations indicated. Tyrode solution was prepared using ultra-pure (Milli-Q) water and was filtered through a 0.22 μm Millipore filter (Bedford, MA USA) prior to use and gassed with 100% oxygen (O_2). Dulbecco's Modified Eagle Medium (DMEM) culture medium was supplemented with 1 mM Ca^{2+}, 10 mM HEPES, 25 mM $NaHCO_3$, 100 U/mL Penicillin G, 100 μg/mL Streptomycin, 2 mM carnitine, 5 mM creatine, 5 mM taurine, and 1 mg/mL delipidated bovine serum albumin (BSA).

Preparation of ventricular myocytes

Hearts were excised and perfused retrogradely on a Langendorff apparatus (nonrecirculating) with Tyrode solution containing 1.5 mM Ca^{2+} for 4 min as described previously.[8] The heart was then perfused with nominally Ca^{2+}-free Tyrode solution (nonrecirculating) for 2 min. Buffers were maintained at 37°C and gassed with 100% O_2 during the Langendorff perfusion. The heart was further perfused in a recirculating manner for 20 min with Tyrode solution supplemented with 20 μM Ca^{2+}, 45 U/mL collagenase, 0.2 U/mL protease, and 0.1% (w/v) delipidated BSA at 37°C. After perfusion with collagenase, the ventricles were removed, minced with scissors, and agitated in Tyrode solution containing 40 μM Ca^{2+}, 1.5% (w/v) BSA, and 30 mM 2,3-butane-dione monoxime at 25°C. The suspension was filtered through 250 μm nylon-mesh gauze to a final volume of 50 mL. The concentration of Ca^{2+} was increased stepwise to 1 mM over 45 min. For Na^+ current determination, aliquots of the cell suspension were added to petri dishes containing 6 × 17 mm glass coverslips. For contractility measurements, aliquots of the cardiomyocyte suspension (approximately 2 mL) were added to petri dishes containing 12 mm (diameter) round glass coverslips coated with laminin (50 μg/mL). Rod-shaped cardiomyocytes adhered to coverslips within 60 min at room temperature. Nonadhering cardiomyocytes were removed by gentle suction with a disposable plastic Pasteur pipette. Coverslips were washed twice with DMEM culture medium (1 mM Ca^{2+}) pre-equilibrated with 5% carbon dioxide (CO_2):95% O_2. Cardiomyocytes were maintained in DMEM in a humidified incubator at 37°C and gassed with 5% CO_2 in air until use.

Fatty acid analysis

Total lipids were extracted from the ventricular tissue, the standard colony diet, and dietary oils using a slight modification of the method of Bligh and Dyer.[15] Ventricular tissue was homogenized in 1 mL water using a Tenbroeck hand-held homogenizer (10 passes). Four milliliters of 2-propanol was added and the mixture boiled for 30 sec. After cooling, 8 mL of chloroform was added, the mixture shaken, and the organic phase collected. After re-extracting the aqueous phase with an additional 4 mL of chloroform, the organic phases were combined and evaporated to dryness under nitrogen and then further dried using anhydrous sodium sulphate. The phospholipids were separated from the other lipid classes by thin layer chromatography (TLC) on silica gel 150A-LK5D plates (Whatman, Clifton, NJ USA) and developed in a solvent system of petroleum ether:acetone (3:1 [v/v]). The phospholipids remaining at the origin were scraped from the plate. Fatty acid methyl esters (FAMEs) were prepared by heating the samples at 50°C overnight in 1% (v/v) H_2SO_4 in methanol. FAMEs were extracted using hexane and contaminants removed using a Biosil (silicic acid) column. All solvents used for lipid extraction, TLC, and preparation of FAMEs contained the antioxidant butylated hydroxytoluene (0.05% w/v). FAMEs from lipid extracts of the experimental oils, standard diet, and the myocardial phospholipid extracts were analyzed by gas-liquid chromatography (GLC). GLC was performed using a Hewlett Packard HP 5710 gas chromatograph (Hewlett Packard, Palo Alto, CA USA) fitted with a 50 meter BPX70 capillary column (Scientific Glass Engineering, Melbourne, Victoria, Australia). The FAMEs were separated using a carrier gas (hydrogen) flow of 35 cm/sec with a temperature gradient of 130°C to 230°C at 4°C per min. A cold on-column injector was used with the flame ionization detector temperature set at 250°C. FAMEs were identified using authentic lipid standards (Nu-Chek-Prep Inc., Elysian, MN USA) by GLC. The proportions of the total fatty acids were normalized to a value of 100%.

Measurement of cardiomyocyte contraction and response to isoproterenol

Cardiomyocytes on coverslips were placed in a custom designed superfusion chamber and superfused with Tyrode buffer containing 1 mM Ca^{2+} at 37°C and allowed to equilibrate for 2 min. Cell length was determined as described previously.[8] Briefly, a video camera mounted on an inverted Olympus microscope housed in a perspex chamber maintained at 37°C transferred images to a computer connected between the camera and the monitor. An on-line, real-time computer program was used (LabVIEW, National Instruments, Victoria, Australia), which allowed for continuous observation of changes in cell length during contractile activity. Contractility was induced in the presence of electrical-field stimulation using a Grass S4 stimulator. The cells were routinely stimulated with a pulse duration of 5 ms at a frequency of 1 Hz using two platinum wire electrodes located at either end of the superfusion chamber. Cellular arrhythmia, measured as asynchronous contractile activity, was induced by superfusion with progressively increasing isoproterenol concentrations (0.01 μM to 3 μM) in the presence of electrical-field stimulation (1 Hz). Asynchronously contracting cells (in the presence of electrical-field stimulation) were defined as those cells that exhibited a contraction rate exceeding the rate of applied electrical stimulation (i.e., cells not contracting in synchrony with the applied electrical stimulus). Asynchronous contracting cells usually contracted at 2 to 4 contractions/sec.

Free radicals and cardiomyocyte contractility

To investigate the effect of free radicals on the development of asynchronous contractile activity in adult rat cardiomyocytes, cells were exposed to a free radical generating system (FRGS) of 2.3 mM purine and 7 mU/mL xanthine oxidase[16] generating both superoxide and hydrogen peroxide. Cardiomyocytes adhering to coverslips were initially superfused at 37°C with Tyrode buffer containing 2 mM Ca^{2+} at a flow rate of 2 mL/min and gassed with 100% O_2. Following a 3-min equilibration period, cells were stimulated at a frequency of 1 Hz. After an additional 2 min, cells were superfused with the FRGS (preincubated together for 3 min prior), pH 7.3, and gassed with 100% O_2. The contractile activity of the cells was monitored every 2 min for 20 min, recording the number of cells displaying asynchronous contractile activity.

Electrophysiologic recording of cardiac Na^+ current

Electrodes were prepared from borosilicate glass using a two-stage puller (Narishige Scientific Instruments, Tokyo, Japan) and resistances were typically between 1 and 3 MΩ when containing the pipette solution. Whole-cell currents were recorded 5 min after achievement of a whole-cell patch clamp configuration. Current recording was performed using an EPC9 HEKA amplifier (HEKA Electronik, Lambrecht/Pfalz, Germany). Whole cell capacitance and series resistance compensation was achieved using the controls within the on-line computer program (EPC9 HEKA patch clamp); recording was only performed if a series resistance compensation of at least 90% could be achieved. Satisfactory voltage control was indicated by the following criteria: (1) The negative limb of the current-voltage curve spanned at least 25 mV; (2) there were no abnormal notches in the current-voltage curve; and (3) there was no crossover between recordings at different voltages of the inactivation curve. Whole-cell sodium currents were evoked by voltage steps generated by the computer program, which outputs the waveform via a digital to analogue converter connected to the command input of the amplifier. The resulting currents were filtered at 5 kHz and recorded through an analogue to digital converter operating at 20 kHz. Up to six cell recordings per rat were carried out. All values obtained were then averaged for each rat. To determine the voltage dependence of Na^+ current activation, data points were fitted by the equation:

$$I = [G_{max} \cdot (V - E_{rev})] \cdot [1/1 + e^{(V-V')/k}] \quad (1)$$

using a least squares fitting algorithm, where G_{max} is the maximum conductance, V' (or V_{50}) is the membrane potential for half activation of the channels, V is the test membrane potential, E_{rev} is the reversal potential for the current, and k is a slope factor. For voltage dependence of inactivation, the data points were fitted using the Boltzmann equation:

$$I = I_{max} \cdot [1/1 + e^{(V-V')/k}] \quad (2)$$

where I_{max} is the maximum current, V is the test membrane potential, V' (or V_{50}) is the membrane potential at which half of the channels are inactivated, and k is a slope factor.

Solutions used for Na^+ current measurements

The standard external (bath) solution used for sodium current measurements contained (in mM): NaCl, 20; N-tris-(hydroxymethyl)-methyl-2-aminoethanesulphonic acid (TES), 10; KCl, 5; $MgCl_2$, 1; $CaCl_2$, 2; $CoCl_2$, 5; CsCl, 5; glucose, 10; cholineCl, 110, pH adjusted to 7.4 with 5.0 M NaOH. The pipette solution for all experiments contained (in mM): CsF, 120; TES, 10; $MgCl_2$, 2; Na_2-EGTA, 20; $CaCl_2$, 2; pH adjusted to 7.4 with 5.0 M KOH. These solutions are designed to block all ionic currents other than sodium currents. In addition, the low extracellular sodium concentration (20 mM) is designed to reduce the peak sodium current and hence minimize series resistance errors in the clamp potential.

Electrophysiologic recording of cardiac K^+ current

The peak transient outward current (I_{to}) amplitude was measured as the difference between peak current and the steady-state current at the end of a 300 ms voltage step. For the steady-state activation protocol, the cells were prepulsed at −90 mV for 50 ms then held at −120 mV for 300 ms and stepped to various potentials between −90 and +50 mV for 300 ms. For the inactivation protocol, the cells were prepulsed at −90 mV for 50 ms and stepped from various potentials between −120 mV and +20 mV (held for 300 ms) to a holding potential of +30 mV for 300 ms. Up to six cell recordings per rat were carried out. For both activation and inactivation data, the data points were fitted using the Boltzmann equation (equation 2).

Solutions used for K^+ current measurements

All experiments were conducted at room temperature (21–23°C). The bath solution contained (in mM): KCl, 5; $MgCl_2$, 1; $CaCl_2$, 1; $CoCl_2$, 5; TES, 10; choline chloride, 130; with pH adjusted to 7.35 using KOH. The solution in the recording pipette contained (in mM): KCl, 115; KF, 10; $MgCl_2$, 5; EGTA, 20; HEPES, 10; with pH adjusted to 7.35 with KOH. These solutions are designed to block all ionic currents other than potassium currents. Under these experimental conditions, only the Ca^{2+}-independent component of I_{to} was studied because EGTA was included in the pipette solution and $I_{Ca,L}$ was blocked.

Chemicals

DMEM culture medium, BSA (fraction V), carnitine, creatine, taurine, 2,3-butane-dione monoxime, protease (type XIV), laminin, purine, xanthine oxidase, and isoproterenol were from Sigma Chemical Co. (Castle Hill, NSW, Australia). Collagenase was from Yakult Honsha Co., Ltd. (Tokyo, Japan). Penicillin/Streptomycin was from GIBCO-BRL (Melbourne, Victoria, Australia). All other chemicals were of the highest grade available. BSA was delipidated by washing in acetone, petroleum spirit, and diethyl ether (50 g BSA was washed in 200 mL of each solvent three times, filtered, and vacuum dried).

Statistics

Where necessary, statistical analyses were performed using the computer software programs Instat version 3.0 (GraphPad Software, San Diego, CA USA) or SPSS v9.0 (Chicago, IL USA). One-way or two-way analysis of variance (ANOVA) with Bonferroni multiple comparison test was used to compare differences between effects of the dietary treatments. Results are expressed as the mean ± SEM. For each comparison, the level of significance was set at $P < 0.05$ for the indicated number of animals per dietary group. Where data appear as (n = x, y), x is the number of rat hearts and y is the number of cells studied.

Results

Phospholipid fatty acid analysis

Ventricular phospholipid fatty acid composition for rats supplemented by gavage with either saturated fat (SF) or fish oil (FO) or from the REF (sham gavage) group are shown in *Table 2*. Ventricular phospholipid fatty acids of animals maintained on the REF diet alone exhibited a relatively low level of 22:6 n-3, 22:5 n-3, and 20:5 n-3 compared with the other two dietary groups (*Table 2*). Three weeks of daily gavage with the FO supplement significantly increased the proportion of total n-3 PUFAs due to increases in the proportions of 20:5 n-3, 22:5 n-3, and 22:6 n-3. Concomitant with n-3 PUFA supplementation was a reduction in the proportions of 18:1, 18:2 n-6, and 20:4 n-6, and hence a significant reduction in the ratio of n-6:n-3 PUFAs in this group. There was also an increase in the proportion of the total n-3 PUFAs in the SF group compared with the REF group, but not to the extent evident for the FO group. For the SF group, only the proportions of 22:5 n-3 and 22:6 n-3 were increased, with little change in the proportion of 20:5 n-3 in comparison with the REF group. For the proportions of all the other major fatty acids, the SF group was similar to the REF group and significantly different from the FO group. The proportion of total saturated fatty acids in the ventricular phospholipids was not significantly different across all dietary groups even following SF supplementation.

Table 2 Fatty acid comparison (wt%) of the ventricular phospholipids after dietary lipid supplementation

Major FAME	REF (n = 8)	SF (n = 6)	FO (n = 6)
14:0	0.13 ± 0.02	0.27 ± 0.09	0.21 ± 0.02
16:0	16.48 ± 0.34	16.06 ± 0.51	15.89 ± 0.39
16:1	0.32 ± 0.04	0.76 ± 0.47	0.25 ± 0.07
17:0	0.23 ± 0.01[b,d]	0.36 ± 0.04	0.41 ± 0.04
18:0	24.75 ± 0.49	24.45 ± 0.71	25.28 ± 0.63
18:1[1]	15.38 ± 0.92[b]	14.36 ± 0.56[a]	10.13 ± 1.11
18:2(n-6)	6.29 ± 0.19[c,d]	5.37 ± 0.16[c]	3.72 ± 0.25
18:3(n-3)	0.34 ± 0.27	0.11 ± 0.05	n/d
20:2	0.63 ± 0.05	0.51 ± 0.04	tr.
20:3(n-6)	tr.	0.21 ± 0.03	0.21 ± 0.02
20:4(n-6)	29.6 ± 0.36[b]	28.66 ± 1.17[d]	24.47 ± 1.34
20:3(n-3)	0.21 ± 0.00	tr.	0.20 ± 0.00
22:0	0.30 ± 0.01	tr.	0.25 ± 0.06
20:5(n-3)	0.18 ± 0.07[c]	0.32 ± 0.17[c]	3.25 ± 0.79
24:0	0.76 ± 0.16	0.54 ± 0.08	tr.
22:5(n-3)	0.41 ± 0.14[c,e]	1.32 ± 0.22[c]	2.45 ± 0.07
22:6 (n-3)	3.67 ± 0.65[c,d]	6.70 ± 0.87[c]	13.11 ± 0.53
Σ Saturated	43.12 ± 0.53	41.76 ± 0.85	42.29 ± 0.73
Σ Monounsaturated	15.87 ± 0.93[b]	15.38 ± 0.98[a]	10.50 ± 1.13
Σ Polyunsaturated	41.01 ± 0.48[b]	42.85 ± 0.99[a]	47.21 ± 1.53
Σ n-6	36.08 ± 0.45[c]	34.24 ± 1.17[b]	28.36 ± 1.14
Σ n-3	4.30 ± 0.82[c,d]	8.10 ± 1.030[c]	18.84 ± 0.64
n-6/n-3	8.39 ± 1.43[b]	4.23 ± 1.05	1.50 ± 0.06

Data shown are mean ± SEM for six to eight animals per dietary group (indicated in brackets). Computational parameters (Σ and n-6/n-3) are derived from the full fatty acid set.
[1]18:1 contains n-9 and n-7 isomers. Superscripts indicate significant differences at [a]$P < 0.05$, [b]$P < 0.01$, [c]$P < 0.001$ vs. FO and [d]$P < 0.05$, [e]$P < 0.01$ vs. SF (by ANOVA with Bonferonni multiple comparison test).
FAME—fatty acid methyl esters. REF—reference diet. SF—saturated fat diet. FO—fish oil diet. n/d—not detected.
tr.—less than 0.2%.

General features of dietary supplemented rats and cardiomyocyte contractile parameters

Body weights were not significantly different in rats supplemented with SF or FO or in the REF group [425 ± 12 g (n = 7), 436 ± 11 g (n = 6), and 429 ± 14 g (n = 7), respectively]. Cell viability was determined by counting the number of rod-shaped cells from each preparation. The viability of cardiomyocytes immediately after isolation was

Table 3 Summary of cardiomyocyte contractile parameters

	Reference	Saturated fat	Fish oil
V[1]	21.3 ± 1.7 (6, 31)	20.0 ± 0.5 (7, 37)	19.2 ± 0.5 (6, 34)
Diastolic cell length (μm)[2]	140.3 ± 3.3 (7, 35)	139.3 ± 3.9 (7, 36)	148.7 ± 3.8 (6, 29)
Systolic cell length (μm)[3]	135.3 ± 3.1 (7, 35)	130.3 ± 5.9 (7, 36)	143.9 ± 4.0 (6, 29)
ΔCell length (%)[4]	3.9 ± 1.0 (7, 35)	3.7 ± 0.5 (7, 36)	3.3 ± 0.7 (6, 29)
PRP (%)[5]	12.4 ± 0.7 (7, 35)	12.6 ± 0.5 (7, 36)	12.7 ± 0.7 (6, 29)

Data represent mean ± SEM (n = x, y: x = number of rat hearts and y = number of cells studied).
[1]The voltage required to stimulate 90% of cells in a field to contract.
[2]Resting (diastolic) cell length.
[3]Cell length at peak (systolic) cell shortening during steady-state.
[4]Percent change in cell length [(sytolic-diastolic)/diastolic]*100 during steady-state.
[5]Post-rest potentiation (PRP): (post-rest contraction length/steady-state contraction length)x100.

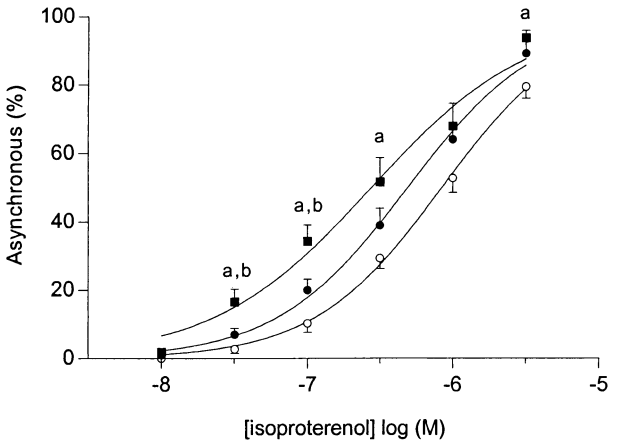

Figure 1 Percentage of asynchronously contracting cardiomyocytes in response to increasing concentrations (0.01–3 μM) of isoproterenol. Diets were reference (●), saturated fat (■), or fish oil (○). Data are means ± SEM for cardiomyocytes isolated from n = 6 animals per dietary group. Significant differences at $P < 0.05$ are designated as a, FO versus SF and b, REF versus SF by two-way analysis of variance using Bonferonni multiple comparison test. EC_{50} values for REF, SF, and FO groups were 531 ± 91 nM, 347 ± 91 nM and 892 ± 130 nM, respectively ($P < 0.05$).

70.2 ± 2.0%, 63.1 ± 2.9%, and 63.0 ± 5.1% for the REF, SF, and FO groups, respectively (not significant). However, after plating, greater than 90% of adhered cells were found to be viable by the same method. Our previous studies involving acute application of n-3 PUFAs (eicosapentaenoic acid and docosahexaenoic acid) to electrical-field stimulated cardiomyocytes demonstrated that an increase in voltage was required to maintain cellular contractions[8,9,17] and that this may be due to the inhibition of Na^+ currents.[13] Therefore, we determined the voltage required to stimulate contractions in each cardiomyocyte studied. There was no significant difference between the groups. In response to electrical-field stimulation cardiomyocytes contracted in synchrony with the applied pulse at 1 Hz. The contractile data are shown in *Table 3*. The resting cell length (diastolic) was similar in all three groups of cardiomyocytes studied. In response to steady-state electrical-field stimulation cardiomyocytes shortened by approximately 3.5% and was similar for all groups. Because studies of post-rest contractile behavior help the overall understanding of intracellular Ca^{2+} handling,[18] we measured the change in contraction amplitude following a 2-min rest (no stimulation) before reapplying electrical stimulation to initiate the post-rest potentiation (PRP) contraction. As shown in *Table 3*, the PRP contraction was always greater than the amplitude of contractions at steady-state (approximately 12.5% in all groups), because the rest period allows the sarcoplasmic reticulum to accumulate Ca^{2+}.

Effect of dietary lipid supplementation on isoproterenol-induced asynchronous contractile activity

The protocol developed to determine the effect of dietary lipids on cardiomyocyte contractility and the development of asynchronous contractile activity utilized quiescent, calcium-tolerant cells, in which contractile activity was synchronous with the applied electrical-field stimulation over a period of many hours. Asynchronous contractile activity could be elicited by the addition of increasing concentrations of the β-adrenergic receptor agonist isoproterenol (3 min for each concentration). Asynchronous contractile activity first became evident within approximately 5 min of isoproterenol addition, and then became the predominant form of contractile activity after approximately 15 min (>3 μM isoproterenol). Using the above protocol, *Figure 1* shows that asynchronous contractile activity was evident at significantly lower isoproterenol concentrations in cardiomyocytes isolated from the SF group in comparison with the REF group, with the EC_{50} values being 347 ± 91 nM (n = 6) and 531 ± 91 nM (n = 6; $P < 0.05$, by two-way ANOVA), respectively. As shown in *Figure 1*, dietary n-3 PUFA supplementation (FO) significantly reduced and delayed the development of isoproterenol-induced asynchronous contractile activity compared with the REF and SF groups (EC_{50} = 892 ± 130 nM, n = 6). The rank order potency for the effect of these dietary supplements on the development of asynchronous cardiomyocyte contractility was SF, REF, FO.

Effect of dietary lipid supplementation on free radical-induced asynchronous contractile activity

Cardiomyocytes from rats fed the REF, SF, and FO diets were all contracting synchronously for the first 4 min of superfusion with the FRGS. However, following prolonged exposure to the FRGS, more cardiomyocytes hypercontracted or developed asynchronous contractile activity. As evident from *Figure 2*, cardiomyocytes from the FO group remained synchronous for a longer time course, although the rate (slope of the sigmoid curve at its midpoint) at which the cardiomyocytes became asynchronous was the same as

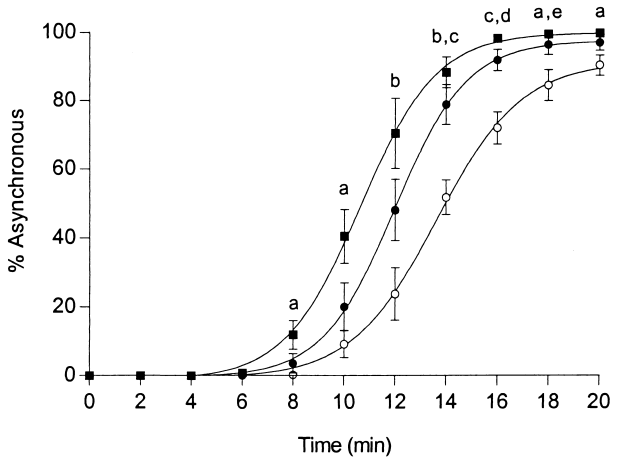

Figure 2 The effect of superoxide free radicals and hydrogen peroxide on contractility of cardiomyocytes isolated from rats fed a reference (●), saturated fat (■), or fish oil (○) diet. Data are means ± SEM of experiments using cardiomyocytes isolated from $n = 6$ to 9 animals per group. Significant differences are designated as a, FO versus SF ($P < 0.05$); b, FO versus SF ($P < 0.01$); c, FO versus REF ($P < 0.01$); d, FO versus SF ($P < 0.001$); and e, FO versus REF ($P < 0.05$) by two-way analysis of variance using Bonferonni multiple comparison test.

those of the REF and SF groups. The time taken until 50% of cardiomyocytes displayed asynchronous contractile activity was 11.8 ± 0.5 min for the REF group compared with 10.7 ± 0.6 min for the SF group (not significantly different). However, cardiomyocytes from FO-treated rats showed a significant increase in the time taken until 50% of cardiomyocytes contracted in an asynchronous manner (t = 13.6 ± 0.5 min, $P < 0.01$ vs. SF). Furthermore, cardiomyocytes from the SF group showed a significant left shift of the curve (i.e., a more sensitive response) to the FRGS compared with the REF and FO groups (*Figure 2*). Indeed, the FO group was more resistant to the external FRGS-induced asynchronous contractions over the entire time course compared with either the REF or SF groups.

Cardiomyocyte Na^+ currents

Voltage-dependence of activation. The average whole-cell capacitance of all cardiomyocytes from dietary lipid supplemented rats was 130.8 ± 4.7 pF ($n = 28$), 120.6 ± 6.1 pF ($n = 28$), and 134.3 ± 6.3 pF ($n = 28$) for REF, SF- and FO-supplemented rats, respectively. The effect of the diets on the voltage-dependence of activation was determined by evoking currents by voltage steps to various potentials between −90 mV and +30 mV from a holding potential of −140 mV (as depicted in upper left panel of *Figure 3A*). *Figure 3A* (bottom left panel) shows an example of typical sodium currents evoked in a single cardiomyocyte by a step in membrane potential from a holding potential of −140 mV to potentials of 0, −10, −20, −30, −40, and −50 mV. The current density (maximum amplitude of the Na^+ currents/whole-cell capacitance) was plotted against the holding potential, as shown in *Figure 3A* (right) for cardiomyocytes isolated from rats supplemented for 3 weeks with either REF, SF, or FO. The means of all the above parameters for the least-squares fit of equation 1 are given in *Table 4*. There was no significant difference between any of the dietary lipid supplemented groups for any of the parameters examined. Sodium currents in all cells were activated at approximately −60 mV and reached a maximum current at approximately −30 mV (*Figure 3A*). The maximum sodium current densities elicited by a voltage step from −140 mV to −30 mV for REF, SF- and FO-supplemented rats were −14.8 ± 1.6 pA · pF^{-1} ($n = 5$), −15.5 ± 0.8 pA · pF^{-1} ($n = 5$), and −15.4 ± 0.9 pA · pF^{-1} ($n = 5$), respectively (*Figure 3A, right*).

Voltage-dependence of inactivation. The effect of the dietary lipid supplementation on the voltage-dependence of inactivation of the sodium current was investigated by stepping the membrane potential to a test potential of −30 mV from holding potentials that varied between −140 and −35 mV (upper left panel of *Figure 3B*). *Figure 3B* (bottom left panel) shows an example of typical sodium currents superimposed from a single cardiomyocyte by a step in membrane potential from holding potentials of −130, −110, −90, −70, and −50 mV to a test potential of −30 mV. The current density (maximum amplitude of the Na^+ currents/whole-cell capacitance) was plotted against the holding potential, as shown in *Figure 3B* (right), for cardiomyocytes isolated from rats supplemented for 3 weeks with either REF, SF, or FO. The mean values for the parameters I_{max}, V_{50}, and k, for the least-squares fit of equation 2 are given in *Table 4*. Maximum sodium current densities (I_{max} at −140 mV) in all cells from REF and SF rats were −13.4 ± 1.3 pA · pF^{-1} and −13.5 ± 0.9 pA · pF^{-1}, respectively. For FO supplemented rats the maximum current density of −12.7 ± 0.4 pA · pF^{-1} was lower compared with both the REF and SF groups; however, this was not statistically significant. *Figure 3B* (right) shows that the voltage-dependence of inactivation was left shifted in the FO group compared with either the REF or SF groups. The values for the membrane potential at which half of the Na^+ channels were inactivated (V_{50}) was −74.0 ± 0.6 mV and −73.5 ± 1.2 mV for the REF and SF groups, respectively (not significantly different). The V_{50} for the voltage-dependence of inactivation for the FO group was significantly more negative compared with the SF group (−76.7 ± 0.7 mV, $P < 0.05$). Kinetic analysis of the rates of current activation for REF, SF, and FO groups were 0.330 ± 0.030 ms ($n = 5, 28$), 0.290 ± 0.010 ms ($n = 5, 28$), and 0.350 ± 0.020 ms ($n = 5, 27$), respectively (not significantly different). Inactivation of whole-cell Na^+ currents (current decay) for the REF, SF, and FO groups were 1.51 ± 0.11 ms, 1.21 ± 0.09 ms, and 1.28 ± 0.10 ms, respectively (not significantly different). Using a two-pulse protocol as described previously[19] (with holding potential −90 mV and the test potential −20 mV), we determined the time-dependent reactivation (recovery) rates of the Na^+ inward current, which was well fitted by a single exponential function: $I_2/I_1 = 1 - e^{(-t/\tau)}$. No significant difference was observed when comparing the time constants (τ) between the REF, SF, and FO groups (20.50 ± 1.74 ms, 17.01 ± 2.88 ms, and 21.92 ± 2.88 ms, respectively).

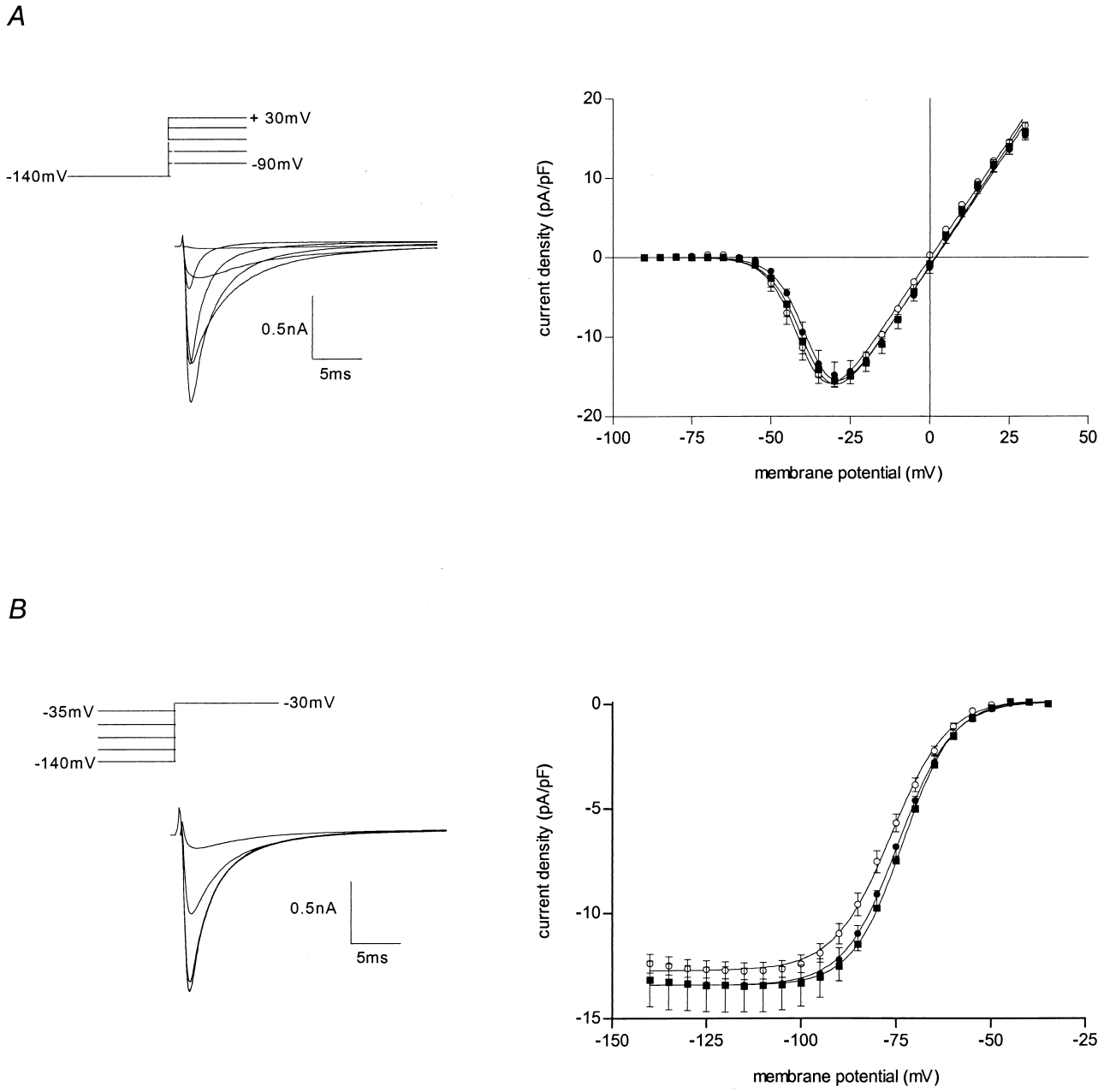

Figure 3 The voltage-dependence of (A) activation or (B) inactivation of Na^+ currents in adult rat ventricular myocytes following dietary lipid supplementation. (A) Sodium currents were evoked by voltage steps from a holding potential of −140 mV to various voltages between −90 mV and +30 mV as depicted (upper left). Plotted below are superimposed currents evoked at 0, −10, −20, −30, −40, and −50 mV. The peak current density [peak current amplitude/cell capacitance (pA · pF^{-1})] was plotted against the test potential and is shown on the right. The solid lines show the least-squares best fit of the equation: $I = [G_{max} \cdot (V - E_{rev})] \cdot [1/1 + e^{(V-V')/k}]$. The parameters for the best fit in each case are shown in *Table 4*. (B) Sodium currents were evoked by voltage steps to −30 mV from various holding potentials between −140 and −35 mV, as depicted (upper left). Plotted below are currents evoked at −130, −110, −90, −70, and −50 mV, shown superimposed. The peak current density (pA · pF^{-1}) was plotted against the pulse potential. The solid line shows the least-squares best fit of the equation: $I = I_{max} \cdot [1/1 + e^{(V-V')/k}]$. The parameters for the best fit in each case are shown in *Table 4*. The V_{50} for the fish oil (FO) group was significantly more negative compared with the saturated fat (SF) group ($P < 0.05$, respectively). Data represent mean ± SEM from animals fed a reference (●; n = 5, 28), SF (■; n = 5, 28), or FO (○; n = 5, 28) diet.

Cardiomyocyte transient outward (I_{to}) K^+ currents

Voltage-dependence of activation. The average whole-cell capacitance of all cardiomyocytes from dietary lipid supplemented rats was 119.3 ± 4.8 pF (n = 29), 118.1 ± 5.0 pF (n = 29), and 132.3 ± 4.3 pF (n = 28) for REF, SF- and FO-supplemented rats, respectively. I_{to} in all cells were activated at approximately −20 mV and reached a maximum current at approximately +50 mV (*Figure 4A*). The effect of the dietary treatments on the voltage-dependence of activation was determined by evoking currents by voltage steps to various potentials between −90 mV and +50 mV from a holding potential of −120 mV (as depicted in upper left panel of *Figure 4A*). *Figure 4A* (lower left panel) shows

Table 4 Na^+ current activation and inactivation parameters from equations 1 and 2, respectively

Activation parameters	G_{max} (pS/pF)	V_{50} (mV)	E_{rev} (mV)	k (mV^{-1})
REF ($n = 5$)	0.62 ± 0.05	−36.5 ± 0.9	2.2 ± 0.7	4.8 ± 0.3
SF ($n = 5$)	0.61 ± 0.03	−38.6 ± 1.6	1.7 ± 1.6	4.1 ± 0.2
FO ($n = 5$)	0.60 ± 0.04	−40.0 ± 1.0	0.5 ± 1.2	4.2 ± 0.2
Inactivation parameters	I_{max} (pA/pF)	V_{50} (mV)	k (mV^{-1})	
REF ($n = 5$)	−13.4 ± 1.3	−74.0 ± 0.6	6.4 ± 0.2	
SF ($n = 5$)	−13.5 ± 0.9	−73.5 ± 1.2	6.2 ± 0.2	
FO ($n = 5$)	−12.7 ± 0.4	−76.7 ± 0.7[a]	6.6 ± 0.3	

Data represent mean ± SEM (numbers in parentheses represent the number of hearts, $n = 28$ cells examined for each dietary group).
[a]$P < 0.05$ vs. SF.
REF—reference diet. SF—saturated fat diet. FO—fish oil diet.

an example of typical I_{to} currents evoked in a single cardiomyocyte by a step in membrane potential from a holding potential of −140 mV to potentials of −70, −10, 0, +10, +20, +30, and +40 mV. The current density (maximum amplitude of the I_{to} currents/whole-cell capacitance) was plotted against the test potential, as shown in *Figure 4A* (right), for cardiomyocytes isolated from rats from the REF, SF, or FO groups. The maximum I_{to} current densities elicited by a voltage step from −120 mV to +50 mV for cardiomyocytes from the REF, SF, and FO groups were 2.07 ± 0.17 ($n = 5$), 2.34 ± 0.16 ($n = 5$), and 1.92 ± 0.19 ($n = 5$) $pA \cdot pF^{-1}$, respectively (*Figure 4A*). Although the FO group had a marginally lower maximum current density (at +50 mV) compared with either either REF or SF, the difference was not significant. The means of all the above parameters for the least squares fit of equation 2 are summarized in *Table 5*.

Voltage-dependence of inactivation. The effect of the dietary lipid supplementation on the voltage dependence of inactivation of the I_{to} currents was investigated by stepping the membrane potential to a test potential of +30 mV from holding potentials that varied between −120 and +20 mV (upper left panel of *Figure 4B*). *Figure 4B* (lower left panel) shows an example of typical I_{to} currents evoked in a single cardiomyocyte by a step to +30 mV from various holding potentials of −120, −80, −50, −40, −30, −20, and −10 mV. The current density (maximum amplitude of the I_{to} currents/whole-cell capacitance) was plotted against the holding potential, as shown in *Figure 4B* (right), for cardiomyocytes isolated from rats supplemented with either SF or FO or from the REF group. The mean values for the parameters I_{max}, V_{50}, and k, for the least-squares fit of equation 2 are given in *Table 5*. There was no significant difference between any of the groups for any of the parameters examined, however, there was a slightly lower current density in the FO group than in the RF or SF groups. Kinetic analysis of the rates of I_{to} inactivation for the REF, SF, and FO groups were 49.1 ± 2.6 ms ($n = 5, 29$), 41.3 ± 3.2 ms ($n = 5, 27$), and 42.9 ± 4.4 ms ($n = 5, 27$), respectively (not significantly different). Using a two-pulse protocol (with holding potential −90 mV and the test potential +30 mV) we determined the time-dependent reactivation (recovery) rates of the I_{to} for the SF and FO groups. The data were well fitted by the single exponential function: $I_2/I_1 = 1 - e^{(-t/\tau)}$. The time constants ($\tau$) were not significantly different between the SF and FO groups [16.8 ± 3.2 ms ($n = 3, 15$) and 16.9 ± 5.5 ms ($n = 3, 15$), respectively].

Discussion

In the present study we show that cardiomyocytes isolated from the hearts of rats supplemented with either n-3 PUFAs or a predominantly saturated fatty acid mix exhibited distinct changes in phospholipid fatty acid composition with respect to the proportion of n-3 and n-6 PUFAs. Cardiomyocyte contractile properties were also modulated in a manner consistent with the effects reported for these different dietary lipids at the experimental animal level.[4,5] Although effects were observed on both the incidence of isoproterenol-induced and free radical-induced asynchronous contractile activity, which at the cellular level partly resemble ischemic and reperfusion arrhythmias in the myocardium, respectively, little effect was seen on cardiomyocyte ion current activity.

The reference diet provided a relatively low, but not deficient, level of 18:3 n-3 in the diet to minimize the extent of conversion of 18:3 n-3 to the longer chain n-3 PUFAs, particularly 22:6 n-3. As a result the proportion of 22:6 n-3 in the ventricular phospholipid fatty acids of the REF dietary animals was approximately 3.7% of the total fatty acids, with the total proportion of n-3 PUFAs being 4.3%. These proportions are significantly lower than those reported in previous publications,[4,20] and indicate that the level of dietary 18:3 n-3 dramatically influences the proportions of both n-3 and n-6 PUFAs in ventricular phospholipids, presumably due to the competition between 18:3 n-3 and 18:2 n-6 for further metabolism to the longer chain PUFAs by the Δ6 desaturase pathway.[21] Supplementation of the REF diet by gavage with an n-3 PUFA enriched fish oil diet, mainly enriched with 20:5 n-3, resulted in a substantial increase in the proportion of total n-3 PUFAs (including 22:6 n-3). These changes in membrane lipid composition may influence cardiomyocyte contractile activity in terms of susceptibility to the development of asynchronous contractions, with higher proportions of 22:6 n-3 underlying the antiarrhythmic effects, and the higher proportion of 20:4 n-6 underlying the proarrhythmic effects via the production of pro- and antiarrhythmic prostanoids and thromboxanes.[22,23] Dietary induced differences in cardiomyocyte arrhythmia susceptibility were not attributable to differences in the proportion of the total ventricular phospholipid fatty acid saturated fatty acids, which remained similar in all three groups.

In this study we employed two intervention strategies to elicit asynchronous contractile activity. Increasing concentrations of the β-adrenergic receptor agonist isoproterenol induced a steady increase in the percentage of cells undergoing asynchronous contractile activity. We have previously reported that isoproterenol-induced asynchronous

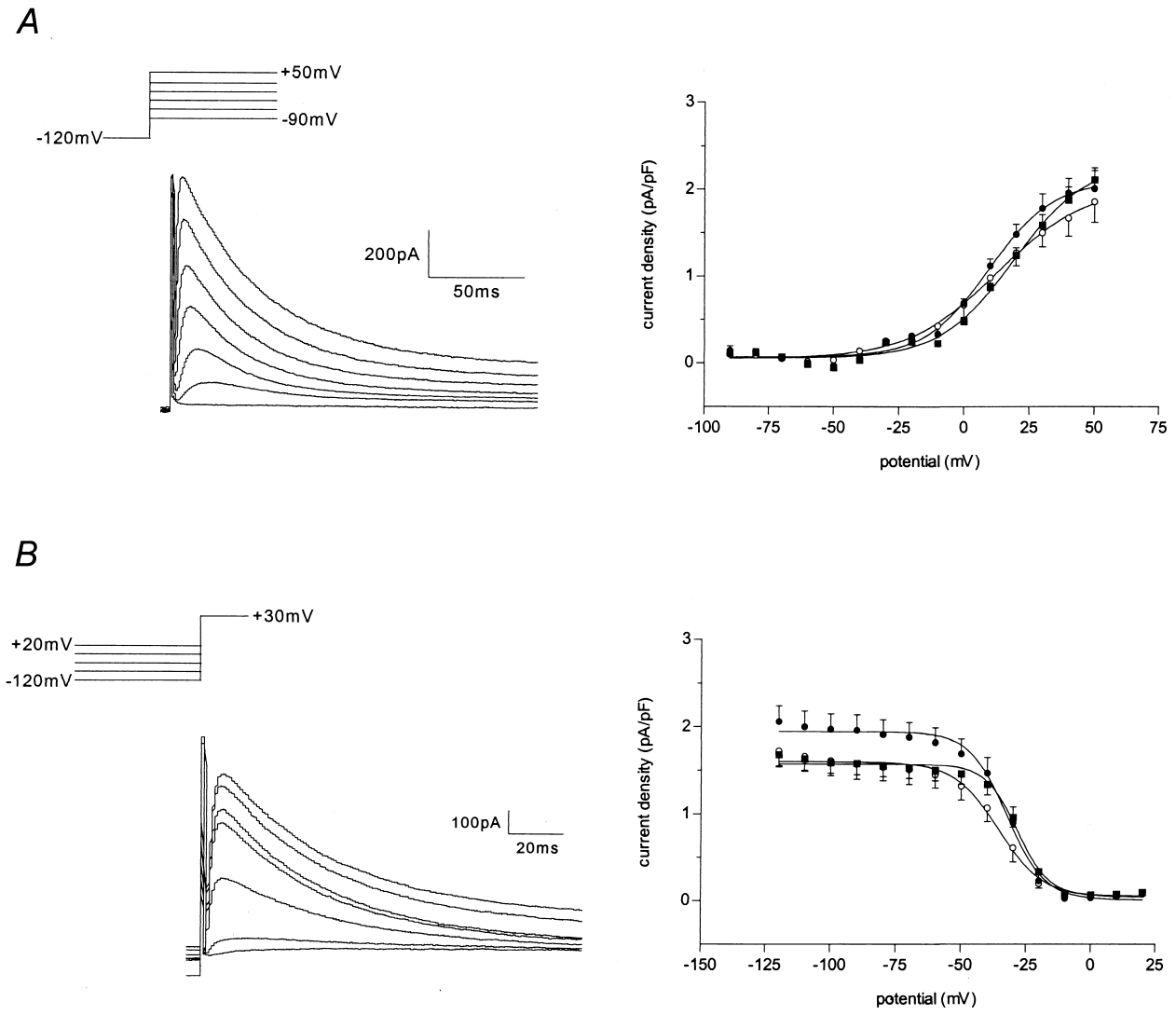

Figure 4 The voltage-dependence of (A) activation or (B) inactivation of I_{to} in adult rat ventricular myocytes following dietary lipid supplementation. (A) Transient outward currents (I_{to}) were evoked by voltage steps from a holding potential of −120 mV to various voltages between −90 mV and +50 mV as depicted (upper left). Plotted below are superimposed currents evoked at −70, −10, 0, 10, 20, 30, and 40 mV. The peak current density [peak current amplitude/cell capacitance (pA · pF^{-1})] was plotted against the test potential and is shown on the right. The solid lines show the least-squares best fit of the Boltzmann equation. The parameters for the best fit in each case are shown in *Table 5*. (B) I_{to} was evoked by voltage steps to +30 mV from various holding potentials between −120 and +20 mV, as depicted (upper left). Plotted below are currents evoked at −120, −80, −50, −40, −30, −20, and −10 mV, shown superimposed. The peak current density (pA · pF^{-1}) was plotted against the pulse potential. The solid line shows the least-squares best fit of the Boltzmann equation. The parameters for the best fit in each case are shown in *Table 5*. Data represent mean ± SEM from animals supplemented with reference (●; n = 4, 17), saturated fat (■; n = 5, 24), or fish oil (○; n = 5, 28).

contractile activity could be rapidly prevented or terminated by the acute addition of low micromolar concentrations of n-3 PUFAs, but not n-6 PUFAs or saturated fatty acids.[8,9] At the cellular level this form of contractile behavior in part reflects the behavior of the whole myocardium to arrhythmogenic stressors associated with ischemic arrhythmias, excessive β-adrenergic receptor activation, and calcium overload. Given the reported involvement of ROS in reperfusion arrhythmias, our second intervention involved subjecting cardiomyocytes to the effects of a FRGS involving exposure of cells to superoxide radicals and hydrogen peroxide.[24,25]

This study has demonstrated that dietary n-3 PUFA supplementation delayed the onset of free radical-induced damage and also reduced the number of cells developing asynchronous contractile activity after a 20-min superfusion with a FRGS. Dietary n-3 PUFAs have been reported to not only prevent ischemic arrhythmias in the coronary artery ligated rat model, but also to have potent antiarrhythmic effects on reperfusion arrhythmias in such a model.[20]

At the cellular level, ROS stimulate lipid peroxidation chain reactions in which the fatty acid side chains of the membrane phospholipids, preferentially those with several double bonds, are attacked.[26] It would appear that membranes enriched with unsaturated fatty acids (namely from rats supplemented with FO) would be more prone to lipid peroxidation, a fact reported in numerous studies.[27–30] One explanation of this apparent paradox is the reported increase

Table 5 Transient outward (I_{to}) current activation and inactivation parameters from the Boltzmann equation

Activation parameters	I_{max} (pA/pF)	V_{50} (mV)	k (mV^{-1})
REF (n = 4)	2.11 ± 0.21	8.80 ± 1.70	12.23 ± 0.72
SF (n = 5)	2.34 ± 0.16	17.67 ± 1.85	13.55 ± 0.31
FO (n = 5)	1.92 ± 0.19	7.39 ± 4.52	13.50 ± 0.80
Inactivation parameters	**I_{max} (pA/pF)**	**V_{50} (mV)**	**k (mV^{-1})**
REF (n = 4)	1.94 ± 0.17	−32.72 ± 1.29	−5.25 ± 0.22
SF (n = 5)	1.57 ± 0.12	−29.03 ± 0.41	−5.70 ± 0.50
FO (n = 5)	1.56 ± 0.16	−33.98 ± 3.40	−6.30 ± 0.44

Data represent mean ± SEM (numbers in parentheses represent the number of hearts, n = 17–28 cells examined for each dietary group). REF—reference diet. SF—saturated fat diet. FO—fish oil diet.

in levels of the endogenous myocardial antioxidant enzymes, namely superoxide dismutase,[31] catalase, and glutathione peroxidase, induced by n-3 PUFA supplementation.[32,33] This increase would be expected to help protect against the free radical attack and delay the onset of asynchronous contractile activity.

Using the above cellular model we were able to demonstrate that dietary lipid supplementation with n-3 PUFA enriched FO for a period of 3 weeks resulted in significant protection of asynchronous contractile activity induced by either isoproterenol superfusion or by the extrinsic generation of ROS, particularly superoxide and hydrogen peroxide. In this regard the effect of dietary n-3 PUFAs at the cellular level are similar to those reported when using a whole animal model of ischemic and reperfusion-induced cardiac arrhythmias,[20] indicating the relevance of this cellular model to whole animal models in terms of dietary lipid studies.

Because Na^+ and K^+ currents are important for the initiation and progression of the excitation-contraction coupling process in cardiomyocytes, it was plausible that dietary supplementation with FO as a means of increasing the incorporated n-3 PUFAs in cardiac membrane phospholipids may also alter these major ion currents, as has been shown in other cells.[34,35] Indeed, our previous study demonstrated potent effects of acute application of the n-3 PUFAs on cardiac Na^+ currents, which included inhibition of whole-cell Na^+ currents and a shift in the voltage-dependence of inactivation to more negative potentials.[13] Similarly, in the present study, FO supplementation resulted in a significant shift of the voltage-dependence of inactivation of Na^+ currents to more negative potentials. These data indicate that more Na^+ channels may be residing in an inactivated state in cardiomyocytes from FO-treated rats at membrane potentials close to the physiological resting membrane potential (−80 mV). However, significant decreases in the peak Na^+ currents in the FO group were not observed. Therefore, it is likely that at resting membrane potential, a lower fraction of Na^+ channels are available for activation following incorporation of n-3 PUFAs into membrane phospholipids. This voltage-dependent effect is consistent with the mechanism of action of certain antiarrhythmic agents such as lidocaine. Although previous investigators have shown that acute addition of n-3 PUFAs can inhibit cardiac K^+ currents (I_{to}),[14] this study demonstrates that, under the conditions used, dietary supplementation of n-3 PUFAs (and subsequent incorporation into membrane phospholipids) did not significantly effect whole-cell outward K^+ currents. It is likely that I_{to} is not directly involved in the antiarrhythmic action of the n-3 PUFAs when they are incorporated into membrane phospholipids.

The evidence so far suggests that the sensitivity of cardiomyocytes to β-adrenergic receptor stimulation, and therefore the extent of arrhythmogenesis (as measured by asynchronous contractile activity), is modulated in part by a dietary lipid effect on cell membrane n-3 PUFA levels that influence β-adrenergic receptor sensitivity. Furthermore, it would also appear that arrhythmias, which may arise due to the involvement of free radicals, are also modulated by the nature of the dietary lipid supplement. For both types of asynchronies, the effects of dietary lipid supplementation on cell contractile activity appear mainly independent of their effects on Na^+ and K^+ current activity. Our cell model offers a unique opportunity to further explore the relationship between nutritional factors, particularly the omega-3 fatty acids and heart health.

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