Endurance exercise training normalizes repolarization and calcium-handling abnormalities, preventing ventricular fibrillation in a model of sudden cardiac death

Ingrid M. Bonilla,1 Andriy E. Belevych,2 Arun Sridhar,1 Yoshinori Nishijima,1 Hsiang-Ting Ho,2 Quanhua He,4 Monica Kukielka,2 Dmitry Terentyev,2 Radmila Terentyeva,2 Bin Liu,5 Victor P. Long,1 Sandor Györke,2 Cynthia A. Carnes,2,1 and George E. Billman2

1College of Pharmacy, The Ohio State University, Columbus, Ohio; and 2Department of Physiology and Cell Biology and the Dorothy M. Davis Heart and Lung Research Institute, The Ohio State University, Columbus, Ohio

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Address for reprint requests and other correspondence: C. A. Carnes, College of Pharmacy, Ohio State Univ., 500 W. 12th Ave., Columbus, OH43210 (e-mail: carnes.4@osu.edu).

SUDDEN CARDIAC DEATH (SCD) remains the leading cause of death in industrialized countries. The risk of SCD is increased in patients who have survived a myocardial infarction (MI) (25, 29). Multiple lines of evidence suggest that abnormalities in autonomic nervous system balance are associated with an increased risk of sudden death (40, 57). Specifically, reduced parasympathetic or increased sympathetic activity increases the risk of ventricular tachyarrhythmias (55, 56).

Endurance exercise training is well known to alter autonomic nervous system activity favorably (8, 38), by increasing parasympathetic regulation and by reducing sympathetic nervous system activity, actions that could be antiarrhythmic (8). Endurance exercise training has previously been reported to improve cardiac autonomic balance and to prevent malignant arrhythmias in a canine model of post-MI sudden death (8, 9). Similar results have been reported in other animal models (22, 34). Importantly, exercise training has also been shown to improve autonomic balance and reduce the incidence of sudden death in patients with MI or coronary artery disease (28). The mechanisms that mediate the possible antiarrhythmic effects of endurance exercise training remain to be elucidated fully.

Abnormal repolarization (due to altered potassium currents) or intracellular calcium (Ca2+) dysregulation may occur individually or in concert to decrease cardiac electrical stability and increase the propensity for SCD. It is well established that MI induces repolarization abnormalities that can provide an electrophysiological substrate for ventricular arrhythmias (43, 57). In a similar manner, abnormal Ca2+ regulation during acute myocardial ischemia or following MI elicits elevations in cytosolic Ca2+ (Ca2+ overload) that can provoke oscillations in membrane potential (delayed afterdepolarizations) that, if of sufficient magnitude, can trigger extrasystoles (21).

The effects of endurance exercise training on repolarization abnormalities or ventricular myocyte Ca2+ regulation have not been extensively studied. There are only a few studies providing indirect evidence that exercise training can alter repolarization. Aerobic conditioning has been shown to reduce QT interval in young patients with long QT syndrome (36). Exercise training has also been shown to reduce regional differences in repolarization in heart failure patients (1) and prolong the effective refractory period in rabbits (47). There is a similar paucity of information concerning the effects of exercise training on cardiac Ca2+ regulation. Furthermore, the few published studies have often yielded conflicting results. For example, similar exercise training protocols have both increased and decreased sodium/calcium exchanger (NCX) activity (20). Thus the anti-arrhythmic effects of endurance exercise training on repolarization abnormalities or ventricular myocyte Ca2+ regulation remain to be elucidated.

Our laboratory previously reported that ventricular myocytes obtained from post-MI animals susceptible to ventricular fibrillation (VF+) exhibited both repolarization abnormalities...
(43) and intracellular Ca$^{2+}$ alternans (4). It is possible that exercise training could reverse these abnormalities and, as a consequence, protect against VF. Therefore, it was the purpose of the present study to test the hypothesis that exercise training would improve cardiac electrophysiological properties and Ca$^{2+}$ handling and thereby protect against VF in a well-characterized canine model of sudden death.

**MATERIALS AND METHODS**

**In Vivo Studies**

All animal procedures were approved by The Ohio State University Institutional Animal Care and Use Committee and conformed with the Guide for the Care and Use of Laboratory Animals published by the US National Institute of Health (National Institutes of Health publication no. 85-23, revised 1996).

**Surgical preparation.** Mixed breed dogs were anesthetized and instrumented, as has been previously described (7, 14, 15). Briefly, a 20-MHz pulsed Doppler flow transducer and a hydraulic occluder were placed around the left circumflex coronary artery. Two bipolar insulated stainless steel pacing electrodes were also sutured on the epicardial surface of the heart and used to obtain a ventricular electrogram. One bipolar electrode was placed in the potentially ischemic area (posterolateral surface of the left ventricle, an area supplied by the left circumflex artery) and a nonischemic region (anterior left ventricle proximal to the occluder). A two-stage occlusion of the left anterior descending artery was then performed distal to the first large diagonal branch to produce an anterior wall MI. This vessel was partially occluded for 20 min and then tied off.

**Exercise plus ischemia test: selection for susceptibility to malignant arrhythmias.** The studies began 3–4 wk after the production of the MI. The susceptibility to VF was tested as previously described (7, 14, 15). Briefly, the animals ran on a motor-driven treadmill while workload progressively increased, until a heart rate (HR) of 70% of maximum (~210 beats/min) had been achieved. During the last minute (on average during the 18th min) of exercise, the left circumflex coronary artery was occluded, the treadmill stopped, and the occlusion maintained for an additional minute (total occlusion time = 2 min). The exercise plus ischemia test reliably induced ventricular flutter that rapidly deteriorated into VF in susceptible animals. Therefore, large defibrillation electrodes (Stat-padz, Zoll Medical, Burlington, MA) were placed across the animal’s chest so that electrical defibrillation (Zoll M series defibrillator, Zoll Medical) could be achieved with a minimal delay, but only after the animal was unconscious (~10–20 s after the onset of VF). The occlusion was immediately released if VF occurred. This exercise plus ischemia test was repeated, using the same exercise intensity, after the completion of the 10-wk exercise training or 10-wk sedentary time period (see below).

**Exercise training protocol.** Forty-one mixed-breed dogs (male/female 2–3 yr old), weighing 19.8 ± 0.5 kg, developed VF during the exercise plus ischemia test. These susceptible (VF+) dogs were then randomly assigned to either a 10-wk exercise training period (VF+ exercise, n = 21) or an equivalent sedentary period (VF+ sedentary, n = 20). The dogs in the VF+ exercise group ran on a motor-driven treadmill for 10 wk, 5 days/wk, at ~70–80% of maximum HR (13). The exercise intensity and duration progressively increased as follows: 1st wk, 20 min at 4.8 kph/0% grade; 2nd wk, 40 min at 5.6 kph/10% grade; 3rd wk, 40 min at 6.4 kph/10% grade; 4th wk, 60 min at 6.4 kph/10% grade; 5th wk, 60 min at 6.4 kph/12% grade; 6th wk, 75 min at 6.4 kph/12% grade, 7th wk, 90 min at 6.4 kph/12% grade; 8th–10th wk, 90 min at 6.4 kph/14% grade. Each exercise session included 5-min warm-up and 5-min cool-down periods (running at a low intensity, 0% grade and speed, 4.8 kph). The dogs in the VF+ sedentary group were placed in a transport cage for equivalent time periods but without exercise.

The ECG data were digitized (1 kHz) and recorded using a Biopac MP-100 data acquisition system (Biopac Systems, Goleta, CA). The exercise data were averaged over the last 30 s of each exercise level. The coronary occlusion data were averaged over the last 5 s before, and at the 60-s time point (or VF onset) after, occlusion onset. The ECG variables assessed were HR, HR variability, PR interval, QRS duration, QTc (Bazett’s correction factor), and descending portions of the T wave (Tpeak-Tend), an index of the dispersion of repolarization (35, 53). HR variability (0.24- to 1.04-Hz component of R-R interval variability; an index of cardiac vagal tone index) was obtained using a Delta-Biometrics vagal tone monitor triggering off the electrocardiogram R-R interval (Urbana-Champaign, IL). This device employs the time-series signal processing techniques, as developed by Porges, to estimate the amplitude of respiratory sinus arrhythmia (37). Details of this analysis have been described previously (10).

**In Vitro Studies**

**Myocyte isolation.** At the end of the 10-wk study period, the VF+ sedentary (n = 14) and VF+ exercise (n = 19) dogs were anesthetized with pentobarbital sodium (50 mg/kg iv; Nembutal, Lanabeck, Deerfield, IL). The heart was rapidly removed and perfused with cold cardioplegia [containing the following (in mM) 110 NaCl, 1.2 CaCl$_2$, 10 mM NaHCO$_3$, 16 KCl, and 16 of MgCl$_2$] injected into the coronary ostia. The left circumflex coronary artery was cannulated for myocyte isolation, as previously described (42, 43). After washout of blood from the heart, collagenase (Worthington type 2, 1.15 mg/ml) was added to the perfusate. After 30–45 min of enzyme perfusion, the digested midmyocardial section of the left ventricle was separated from the epicardial and endocardial sections, avoiding the infarcted area and the “border zone”. The typical yield of this procedure is 30–70% rod-shaped myocytes with sharp margins and staircase ends. The myocytes were suspended and stored at room temperature in standard incubation buffer containing the following (in mM): 118 NaCl, 4.8 KCl, 1.2 KH$_2$PO$_4$, 0.68 glutamine, 10 glucose, 5 pyruvate, 1 CaCl$_2$, 1 μM insulin, and 1% BSA, pH adjusted to pH 7.35. A group of five mixed-breed dogs (male/female, 2–3 yr old) were used as a source of control myocytes. All myocyte experiments were conducted within 10 h of isolation.

**Protein expression.** Potassium channel subunits and calcium-handling proteins were assessed by immunoblot analysis. Protein (20 mg) from tissue homogenates was subjected to 4–15% SDS-PAGE (Bio-Rad Laboratories), blotted onto nitrocellulose membranes (Bio-Rad Laboratories). Voltage-gated K$^+$ (Kv) channel interacting protein 2 (KChip2), anti-DPP6, and Kv4.3 antibodies were from Santa Cruz Biotechnology (Santa Cruz, CA). Anti-inward rectifier K$^+$ (Kir) channel 2.1, Kir2.2, and Kir2.3 antibodies were from Alomone Labs (Jerusalem, Israel). Anti-ryanodine receptor 2 (RyR2) antibody was from ThermoFisher Scientific; anti-NCX1 antibody from Milipore; anti-sarc(endo)plasmic reticulum Ca$^{2+}$-ATPase (SERCA) and anti-phospholamban (PLN) were a gift from Dr. Mark Ziolo (The Ohio State University, Columbus, OH), anti-GAPDH antibody was used to measure GAPDH as a loading control (Abcam, Cambridge, MA). Phosphorylation status of RyR2 was examined using phosphor-specific antibodies to S2808 and S2814 (Badrilla) and normalized to total RyR2. Blots were developed with super Signal West Pico (Pierce) and quantified using ImageJ (National Institutes of Health) and Origin 8 (OriginLab, Northampton, MA) software.

Electrophysiological studies. Amphotericin-B perforated patch-clamp technique was used with a bath temperature of 36 ± 0.5°C, as previously described (42, 43). Myocytes were placed in a laminin-coated cell chamber (Cell Microcontrols, Norfolk, VA) and superfused with bath solution containing the following (in mM): 135 NaCl, 5 MgCl$_2$, 5 KCl, 10 glucose, 1.8 CaCl$_2$, and 5 HEPES, with pH adjusted to 7.40 with NaOH at temperature of 36 ± 0.5°C. Borosilicate glass micropipettes with tip resistance of 1.5–3 MΩ were filled with pipette solution containing the following (in mM): 100 potassium
um-aspartate, 40 KCl, 5 MgCl₂, 5 EGTA, 5 HEPES, pH adjusted to 7.2 with KOH. Action potential duration (APD) data were obtained as the average of the last 10 traces (steady state) from 25 elicited at each stimulation rate. The standard deviation of the APD at 90% repolarization (APD₉₀) for the last 10 traces (i.e., from traces 15–25) was used to evaluate repolarization variability (43).

For potassium current measurements, nifedipine (2 μM) was added to the superfusate to block the L-type calcium current. For voltage clamp experiments, only cells with access resistance <20 MΩ were included in the analysis. Inward rectifier K⁺ current (Iₖᵣₑₜ) was elicited with a holding potential of −40 mV and 100-ms voltage steps from −140 mV to +40 mV and measured as the steady-state current. Inward slope conductance was calculated from the inward current–voltage relationship between −140 and −100 mV (19, 44). Transient outward K⁺ current (Iₒ) was elicited from a holding potential of −60 mV by a series of 100-ms test potentials from −20 to +50 mV, as previously described (42). Rapidly activating delayed rectifier K⁺ current (Iₖₑ₅) and slowly activating delayed rectifier K⁺ current (Iₖₑ₇₋₅) were elicited by voltage steps from −20 to +50 mV from a holding potential of −60 mV; Iₖₑ₇₋₅ was separated pharmacologically by superfusion with 0.5-sotalol (100 μM) (42, 43).

Ca²⁺ handling measurements. Calcium currents were recorded using conventional whole cell configuration of voltage clamp technique. For the calcium current recordings, the external solution contained the following (mmol/l): 140 NaCl, 5.4 CsCl, 2.0 CaCl₂, 0.5 MgCl₂, 10 HEPES, and 5.6 glucose (pH 7.4). Patch pipettes were filled with a solution that contained the following (in mmol/l): 123 CsCl, 20 TEA Cl, 5 MgATP, 5 NaCl, 1 MgCl₂, 0.1 Tris GTP, 10 HEPES, and 0.1 Fluo-3 K-salt (pH 7.2). Ca²⁺ currents were evoked by 300-ms depolarizing steps from a holding potential of −50 mV to 0 mV at 1 Hz to measure the time-dependent profile of Ca²⁺-activated cytosolic Ca²⁺ transients. Intracellular Ca²⁺ imaging was performed using an Olympus Fluoview1000 confocal microscope in line-scan mode. Ca²⁺ alternans amplitude was defined as 100 – (A2/A1) × 100 (%), where A1 and A2 are the amplitudes of two consecutive cytosolic Ca²⁺ transients, evoked as described above, with the addition of 100 nM isoproterenol.

Calcium sparks were studied in myocytes permeabilized with saponin using an intracellular solution of the following (mmol/l): 120 potassium creatine phosphokinase, 0.5 EGTA (pCa 7), and 20 HEPES (pH 7.2). Statistical analysis. All data are presented as means ± SE. The ECG data were analyzed using a three-factor [group (2 levels: sedentary vs. exercise trained); prepost (2 levels: i.e., before and after either exercise or sedentary time period), and time (3 levels: preexercise onset, exercise, and occlusion)] mixed-designed ANOVA with repeated measures on two factors (prepost and time). In a similar fashion, the HR and HR variability responses to submaximal exercise were analyzed using a three-factor ANOVA [prepost (2 levels) × group (sedentary vs. exercise trained) × exercise (7 levels) with repeated measures on two factors (prepost and time)]. Since repeated-measures ANOVA depends on homogeneity of covariance, this sphericity assumption (i.e., the assumption that the variance of the difference scores in a within-subject design is equal across the groups) was tested using Mauchley’s test. If the sphericity assumption was violated, then the F-ratio was corrected using Huynh-Feldt correction (NCSS, Kaysville, UT). If the F-ratio was found to exceed the critical value (P < 0.05), then the difference between the means was determined using Scheffé’s test (NCSS). The effect of exercise training on susceptibility to VF was evaluated using Fisher’s exact test.

Electrophysiological data were analyzed using Clampfit 10.2 (Axon Instruments) and Origin 8.0 (OriginLab). Currents were normalized to the cell capacitance and expressed as (pA/pF). APD and current densities and Ca²⁺ spark frequency were analyzed by ANOVA with post hoc least significant difference testing as appropriate (SAS for Windows version 9.1, Cary, NC). Based on normality of Iₖᵣₑₜ and calcium transient alternans amplitude data distribution, the Kruskall-Wallis test was used to test for differences between the groups.

RESULTS

Confirmation of Exercise Training

The effects of the 10-wk exercise training on the HR and HR variability responses to submaximal exercise training are displayed in Fig. 1. In agreement with previous studies (8), the exercise training program elicited significant (P < 0.01) decreases in HR in response to submaximal exercise after the 10-wk treatment period that were accompanied by significant increases in HR variability, an indicator of vagal tone index (6, 8). In contrast, these variables did not change in the sedentary group (data not shown). These data are consistent with an exercise training-induced bradycardia, a well-established hallmark of an effective training program (8, 24).

Effect of Exercise Training on Susceptibility to VF

Of the 41 dogs randomized to either exercise training or a matched duration sedentary period, two dogs in the VF+ exercise group died suddenly (2–3 wk after training began), while six dogs in the VF+ sedentary group died suddenly during the 10-wk sedentary period (P = 0.130). Six dogs (2 in the exercise group; 4 in the sedentary group) did not complete the 10-wk treatment period that were accompanied by significant increases in HR variability, an indicator of vagal tone index (6, 8). In contrast, these variables did not change in the sedentary group (data not shown). These data are consistent with an exercise training-induced bradycardia, a well-established hallmark of an effective training program (8, 24).

Fig. 1. The effect of the 10-wk exercise training (n = 19) on the heart rate and the heart rate variability responses to submaximal exercise in animals susceptible to ventricular fibrillation (VF). Exercise elicited significantly smaller increases in heart rate (A), and smaller reductions in the heart rate variability (cardiac vagal tone index 0.24- to 1.04-Hz frequency component of R-R interval variability; (B) after exercise training. The variables were not altered in the sedentary group (data not shown). Values are means ± SE. Pre, before beginning the 10-wk exercise training or sedentary period; Post, after the completion of the 10-wk study period. *P < 0.01, Pre vs. Post. Exercise levels: 1 = 0 kph/0% grade; 2 = 4.8 kph/0% grade; 3 = 6.4 kph/0% grade; 4 = 6.4 kph/4% grade; 5 = 6.4 kph/8% grade; 6 = 6.4 kph/12% grade; 7 = 6.4 kph/16% grade.
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Table 1. Effect of exercise training on ECG parameters at baseline, during exercise, and during coronary artery occlusion

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Pre</th>
<th>Exercise</th>
<th>Occlusion</th>
<th>Post</th>
<th>Exercise</th>
<th>Occlusion</th>
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<tr>
<td>Ex Train</td>
<td>123.5 ± 6.1</td>
<td>207.9 ± 5.6</td>
<td>229.8 ± 9.8*</td>
<td>119.4 ± 6.9</td>
<td>187.9 ± 5.6#</td>
<td>211.4 ± 6.6#</td>
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<td>Sed</td>
<td>128.8 ± 6.2</td>
<td>198.1 ± 7.4</td>
<td>218.5 ± 9.9*</td>
<td>120.9 ± 5.6</td>
<td>202.0 ± 4.3</td>
<td>227.9 ± 6.5*</td>
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<td>PR interval, ms</td>
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<tr>
<td>Ex Train</td>
<td>104.3 ± 3.1</td>
<td>84.7 ± 2.6</td>
<td>82.6 ± 3.4</td>
<td>102.6 ± 2.9</td>
<td>82.9 ± 3.0</td>
<td>84.0 ± 4.3</td>
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<tr>
<td>Sed</td>
<td>99.5 ± 3.5</td>
<td>82.1 ± 2.8</td>
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<td>92.8 ± 3.5</td>
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<td>80.6 ± 2.1</td>
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<td>82.3 ± 2.8</td>
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<td>82.0 ± 2.4</td>
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<td>Ex Train</td>
<td>299.3 ± 4.9</td>
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<td>328.2 ± 5.8*</td>
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<td>316.3 ± 8.4#</td>
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<td>Tpeak−Tend (corrected), ms</td>
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<td></td>
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<tr>
<td>Ex Train</td>
<td>63.2 ± 5.4</td>
<td>78.2 ± 5.6</td>
<td>114.8 ± 8.9*</td>
<td>63.0 ± 4.6</td>
<td>67.4 ± 5.4</td>
<td>72.5 ± 5.2#</td>
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<tr>
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<td>107.6 ± 8.3*</td>
<td>72.2 ± 6.1</td>
<td>84.2 ± 8.6</td>
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</table>

Values are means ± SE. Pre, before exercise training (Ex Train) or sedentary (Sed) period; Post, after 10 wk of Ex Train or equivalent Sed period. Tpeak−Tend, a measure of the ventricular repolarization dispersion (35, 53), was also increased during ischemia before the onset of the 10-wk study period (Fig. 2A). In a similar manner, Tpeak−Tend, a measure of the ventricular repolarization dispersion (35, 53), was also increased during ischemia before the onset of the study period in both the sedentary and the exercise training groups, but only in the sedentary group at the end of the 10-wk training period (P < 0.0002) (Fig. 2C).

Fig. 2. Exercise training normalized repolarization abnormalities in dogs susceptible to VF. Note that ischemia provoked increases in QTc in both the exercise-trained (A) and sedentary (B) group at Pre. These increases were abolished by exercise training (A), but unchanged in the sedentary group (B). C: exercise training had a significant effect on the ischemia-induced increase of the descending portion of the T wave (Tpeak−Tend), an index of ventricular repolarization dispersion (35, 53) evaluated in response to the exercise plus ischemia test in susceptible animals Pre and Post (n = 17). Conversely, in the sedentary group, there was no difference in the ischemia-induced increase of the descending portion of the T wave (Tpeak−Tend) after the 10-wk sedentary period (n = 10). Values are means ± SE. *P < 0.01, occlusion vs. exercise. #P < 0.01, Pre vs. Post treatment period.

the protocol due to occluder failure. Exercise training prevented exercise plus ischemia-induced tachyarrhythmias in 16 of 17 dogs tested, while, in the sedentary group, 10 of the 10 dogs tested continued to have VF after the observation period (P < 0.00001).

**ECG Analysis**

The effects of exercise training or the 10-wk sedentary period on ECG variables in response to the exercise plus ischemia test are displayed in Table 1. The coronary artery occlusion provoked similar changes in HR in the sedentary and exercise-trained groups before and at the end of the 10-wk study period. The baseline QTc interval significantly (P < 0.02) increased during ischemia in both the VF+ sedentary and VF+ exercise-trained groups before the onset of the 10-wk study period. However, in marked contrast, QTc still increased during ischemia in the VF+ sedentary group, but not in the VF+ exercise dogs at the end of the 10-wk training period (Fig. 2A). In a similar manner, Tpeak−Tend, a measure of the ventricular repolarization dispersion (35, 53), was also increased during ischemia before the onset of the study period in both the sedentary and the exercise training groups, but only in the sedentary group at the end of the 10-wk training period (P < 0.0002) (Fig. 2C).
Neither QRS duration nor PR interval were altered by the coronary occlusion in either the sedentary or the exercise-trained groups (Table 1).

**Electrophysiological Recordings**

In agreement with our laboratory’s previous study (43), ventricular myocytes from VF+/ sedentary animals had significant prolongations of APD50 and APD90 at both 0.5 and 1 Hz and increased beat-to-beat variability in APD compared with control myocytes ($P < 0.05$). Exercise training reduced APD50 and APD90 ($P < 0.05$, Fig. 3) compared with the sedentary VF+ group, to values no different from control myocytes. In a similar manner, exercise training normalized the beat-to-beat variability (measured by standard deviation) in APD90 ($P < 0.05$, Fig. 3 B).

The resting membrane potential in the myocytes isolated from the VF+/ exercise group had a more negative resting potential compared with VF+/ sedentary group ($-82.3 \pm 0.8$ vs. $-79.6 \pm 0.8$ mV, $P < 0.05$), returning to values no different from those seen in control (i.e., no MI, $-81.7 \pm 0.3$ mV) dogs. Inward $I_{K1}$ conductance in both VF+ groups was reduced to a similar extent relative to controls ($P < 0.05$), while peak outward $I_{K1}$ did not differ between the three groups (Fig. 4). $I_{K1}$ was significantly reduced in myocytes from VF+/ sedentary dogs compared with the control group and was further reduced in the VF+/ exercise group ($P < 0.05$, Fig. 4). Finally, $I_{Kr}$ was significantly reduced in myocytes from the VF+/ sedentary group compared with controls. Exercise training significantly increased $I_{Kr}$ ($P < 0.05$ vs. VF+/ sedentary group) to levels no different from control, although it is notable that this increase was highly heterogeneous (Fig. 4). No change in $I_{Ks}$ was found between the three groups (Fig. 4).

Ventricular cell capacitance was significantly increased in myocytes from VF+/ sedentary group compared with the myocytes from the control group ($204.7 \pm 9.7$ vs. $151.7 \pm 11.4$ pF, $P < 0.05$), while myocytes from the VF+/ exercise group did not differ from either controls or the VF+/ sedentary group ($183.5 \pm 13.1$ pF, $P =$ nonsignificant).

**Protein Expression**

Potassium channel subunit expression was measured to assess possible contributions to current alterations. $Kv4.3$, which contributes to $I_{to}$, and the $Kv4.3$-associated auxiliary proteins DPP6 and KCHIP2 were measured (39); KChIP2 was significantly reduced ($P < 0.05$) in VF+/ sedentary samples compared with either control or VF+/ exercise groups, while $Kv4.3$ and DPP6 expression did not differ between groups. $K_{ir}2.1$, $K_{ir}2.2$, and $K_{ir}2.3$ analyses showed that $K_{ir}2.1$ was significantly ($P < 0.05$) reduced in both sedentary and exercised-trained VF+ groups, while the expression of $K_{ir}2.2$ and $K_{ir}2.3$ proteins did not differ among the three groups ($P =$ nonsignificant) (Fig. 5).

**Calcium Handling**

Our laboratory previously reported that ventricular myocytes isolated from VF+/ dogs displayed increased susceptibility to Ca2+ transient alternans (4). This effect was attributed, at least partially, to increased activity of the RyRs (4). In the present study, the effect of exercise on RyR activity was evaluated by measuring Ca2+ alternans and spark frequency in permeabilized myocytes. As shown in Fig. 6, myocytes from the VF+/ sedentary group exhibited a significant ($P < 0.05$) increase in Ca2+ spark frequency compared with controls, while exercise...
treatment almost completely restored Ca\(^{2+}\) spark frequency to control levels. Furthermore, endurance exercise training significantly reduced the amplitude of Ca\(^{2+}\) alternans (\(P < 0.05\)), while the incidence of Ca\(^{2+}\) alternans was returned toward control values (Fig. 7). Steady-state peak Ca\(^{2+}\) current density (1 Hz) was not different between the groups (control: 6.2 ± 0.7 pA/pF, \(n = 11\); VF+ sedentary: -5.0 ± 0.3 pA/pF, \(n = 11\); VF+ exercise: -5.1 ± 0.6 pA/pF, \(n = 14\)).

Western blot analyses showed that, in the sedentary VF+ group, protein levels of RyR, PLN, and SERCA2a were significantly (\(P < 0.05\)) reduced compared with control (Fig. 8). Exercise training increased PLN and SERCA2a to values no different than control, while RyR2 density remained reduced in the VF+ exercise group relative to control (\(P < 0.05\)). As shown in Fig. 8, the PLN-to-SERCA2a ratio was not altered in the exercise VF+ group relative to control (\(P < 0.05\)). Exercise training results in a further decrement in \(I_{K1}\) (\(P < 0.05\)). Values are means ± SE; \(N = 7–28\) cells/group.

**DISCUSSION**

The present study investigated the effects of endurance exercise training on myocyte electrophysiology and Ca\(^{2+}\) handling in a post-MI, ischemia-induced model of VF. The primary findings of our study are as follows: 1) in agreement with previous studies (13, 15, 20, 27), endurance exercise training prevented malignant arrhythmias in a canine post-MI model of SCD; 2) in post-MI VF+ animals, there were significant increases in QTc interval and the descending portion of the T wave (\(T_{\text{peak}}-T_{\text{end}}\)), which were significantly attenuated by a 10-wk endurance exercise training program; 3) in agreement with previous studies (43), ventricular myocytes from dogs was unchanged among the three groups. There was hyperphosphorylation of S2814, the CAMKII-dependent site in the VF+ sedentary group; exercise training reduced this to levels no different from control (Fig. 9). Our laboratory has previously reported (3) that this modification contributes to cellular arrhythmias and suggest that much of the improved arrhythmia phenotype after exercise training is attributable to reduced CAMKII-dependent phosphorylation.
susceptible to VF exhibited prolonged APD and increased beat-to-beat variability in APD, changes that were attenuated by a 10-wk endurance exercise program; and 4) myocytes from the animals susceptible to VF also exhibited abnormalities in RyR activity [increased Ca$^{2+}$/H$^{+}$ leak from the sarcoplasmic reticulum (SR), and Ca$^{2+}$ alternans, attributable to CAMKII-dependent phosphorylation of RyR2] (4), changes that were abrogated by exercise training. Notably, these exercise-induced alterations were not evident in the sedentary group, suggesting that exercise training rather than time-dependent healing was responsible for the observed effects. Thus endurance exercise normalized both in vivo and in vitro repolarization abnormal-

Fig. 5. Western blot analyses of potassium channel subunit expression. A: representative Western blots of left ventricular potassium channel expression. B: the I$_{K_{A}}$ auxiliary protein voltage-gated K$^{+}$ (Kv) channel interacting protein 2 (KChip2) was reduced in sedentary VF+ group and normalized after exercise training. C: no change in the I$_{K_{S}}$ pore-forming subunit Kv4.3 was observed between groups. D: protein subunits inward rectifier K$^{+}$ (K$_{ir}$) channel 2.1 (K$_{ir}$2.1) were similarly reduced in the sedentary and exercise-trained VF+ groups, relative to control. K$_{ir}$2.2 and K$_{ir}$2.3 did not differ among groups. Values are means ± SE; n = 4 dogs per group. *P < 0.05 vs. control. #P < 0.05 vs. VF+ sedentary.

Fig. 6. Effect of exercise training on calcium spark frequency. A: representative examples of calcium sparks from the three groups. B: calcium spark frequency was increased in the sedentary VF+ group and restored toward control by exercise training. F/F$_{0}$, fluorescence ratio. Values are means ± SE. *P < 0.05 vs. *control and #sedentary VF+.
ities, improved myocyte Ca\(^{2+}\) handling, and prevented malignant arrhythmias in post-MI animals previously shown to be susceptible to VF induced by ischemia.

**Effect of Exercise Training on Cellular Electrophysiology: Normalization of Repolarization Abnormalities**

In agreement with the present study, most animal studies report that exercise training can prevent arrhythmias particularly those following ischemia or MI (8, 23, 31). Epidemiological studies indicate that high levels of physical activity may protect against coronary artery disease and reduce cardiac mortality (48). Endurance exercise training favorably alters autonomic balance, enhancing cardiac parasympathetic regulation (12, 17), and decreasing sympathetic activity (41, 52). Exercise training has been shown to improve both cardiac parasympathetic function and to reduce cardiac mortality in patients recovering from MI (32). Furthermore, our laboratory recently demonstrated that exercise training normalized abnormal β-adrenergic receptor activity (13) and enhanced cardiac parasympathetic regulation in dogs that were susceptible to VF before exercise training (11). However, this protection did not appear to result solely from improved cardiac vagal regulation, as treatment with the cholinergic antagonist atropine did not reintroduce ventricular arrhythmias (11). Thus we postulated that additional factors were likely to contribute to the protection observed following exercise training. Consistent with this conclusion, exercise training normalized both cellular electrophysiology and calcium handling in isolated cardiac myocytes that would lack vagal modulation.

Our laboratory previously reported that myocytes from dogs susceptible to VF exhibited abnormal repolarization (43). Specifically, APD\(_{90}\), APD alternans, and APD variability (beat-to-beat variability) were increased in VF+ myocytes compared with VF− or control myocytes (4, 43). Similarly, in the present study, exercise training reversed these cellular abnormalities in repolarization. In the present study, exercise training abolished ischemically induced changes in QTc and T\(_{\text{peak}}\)-T\(_{\text{end}}\) data, consistent with improved ventricular repolarization. Furthermore, as QRS duration was not altered by either ischemia or exercise training, the improvement in QTc interval did not result as a consequence of changes in ventricular electrical conduction. One potential mechanistic explanation for this finding is increased expression and/or activity of sarcolemmal K\(_{\text{ATP}}\) channels, which could modulate the ischemia-induced changes observed in vivo; previous studies have demonstrated a role for these channels in exercise-induced cardioprotection (18, 54). Similarly, exercise training decreased the repolarization abnormalities observed in long QT syndrome patients and heart failure patients and prolonged the refractory period in rabbit hearts (1, 36, 47).

In contrast to our findings, Bito et al. (16) found that APD was similar in myocytes from either exercise-trained or sedentary post-MI mice. Furthermore, Such and colleagues (47) reported that physical training in normal (noninfarcted) rabbits...
prolonged ex vivo ventricular refractory period accompanied by a decrease in VF susceptibility. Species differences (rabbit or mouse vs. dog), absence of MI (rabbit study), or differences in the intensity of the exercise training (uncontrolled voluntary wheel running vs. supervised progressively increasing exercise) most probably accounts for differences noted between these studies and the present investigation.

In contrast to our results of normalization of the APD_{90} by endurance exercise training, it has been reported that exercise training causes a prolongation in the APD_{90} in epicardial monophasic action potential in female rats (46). Reductions in I_{Kr} in response to exercise have also been reported in rats (33, 46) and may explain exercise-induced prolongation of APD_{90} in this species, given the strong modulatory effect of I_{Kr} on overall APD in rat ventricle. These findings in rats are congruent with the further reduction in I_{Kr} that we observed following endurance exercise training, relative to the sedentary group. Although K_{V4.3} was unchanged by exercise, the K_{V4.3} modulatory protein KChIP2 was normalized, which is unexpected considering the further reduction in I_{Kr}, we observed. Thus we suggest that posttranslational modifications or altered channel trafficking, or alterations of other channel subunits (e.g., K_{V1.4}) contributing to I_{Kr} expression may explain the observed exercise training-induced decrement in I_{Kr}, possibilities that will require further investigation. Additionally, this result demonstrates that I_{Kr} reduction in isolation is not a critical determinant of APD_{90} in canine ventricular myocytes, as our laboratory has previously observed in VF+ post-MI canine ventricular myocytes (44). This is congruent with studies examining the role of the I_{Kr}-dependent notch on calcium current using an action potential clamp, where shallower APD notches resulted in less reactivation of I_{Ca,L} (an effect that reduces height of the plateau of the action potential and the duration of the action potential) (2). Thus the modulatory effect of I_{Kr} in canine ventricular myocytes is highly complex due to the interplay with other ion currents.

Our laboratory previously reported that I_{Kr} is decreased in the VF+ canine model (43). In the present study, this was...
confirmed in the VF+ sedentary group, and we found that endurance exercise training increased $I_{Kr}$, although there was significant variability in response to the exercise training. Notably, exercise training did not increase outward $I_{K1}$, $I_{Kr}$, or $I_{wo}$; thus the shortening of the action potential we observed is at least partially attributable to alterations in $I_{Ks}$ and possibly to other currents (late Na+ current) not measured in the present study. It is notable that we observed a small shift to a more negative resting potential after exercise training, a change that would increase bathmotropy and improve diastolic electrical stability. The basis of this observation cannot be attributed to $I_{K1}$ (49) (as this current was unaltered by exercise training); it is possible that some other change (e.g., Na-K-ATPase) may have resulted in the exercise-induced improvement in resting potential.

**Effect of Exercise Training on Intracellular Calcium Handling**

Abnormalities in intracellular Ca$^{2+}$ handling in disease states may lead to contractile dysfunction and/or triggered tachyarrhythmias (40, 57). Several disease states, such as heart failure and MI, are well known to result in dysregulated SR Ca$^{2+}$ release, which can trigger malignant arrhythmias, especially when accompanied by prolongation of the action potential, effects that act in concert to increase the formation of after-depolarizations (40, 57).

Our laboratory previously reported in this canine model that susceptibility to VF is associated with abnormal Ca$^{2+}$ handling, manifested as increased frequency and amplitude of Ca$^{2+}$ alternans compared with controls (4). This is notable, as Ca$^{2+}$ alternans is associated with an increased vulnerability to cardiac arrhythmias (50). In the present study, exercise training reduced the amplitude and incidence of Ca$^{2+}$ alternans, which may contribute to the attenuation of VF observed after exercise training. Several factors are known to contribute to Ca$^{2+}$ alternans, including slowed SERCA-mediated SR Ca$^{2+}$ uptake (51); however, in the VF model used in the present study, myocyte Ca$^{2+}$ alternans are associated with dysregulated RyR function manifested as increased frequency of Ca$^{2+}$ sparks in the absence of notable changes in SR Ca$^{2+}$ uptake (5). In the present study, a normalization of spark frequency was observed, suggesting normalization of RyR function (Fig. 6). Altered RyR function in various disease states, including this model of VF, has been attributed to posttranslational modification of the channel protein by CAMKII phosphorylation (26). Notably, we found that exercise training reduced CAMKII-dependent hyperphosphorylation of RyR at S2814 (but not PKA-dependent phosphorylation of S2808, Fig. 9). Normalization of CAMKII-dependent phosphorylation of RyRs could contribute to normalization of calcium-dependent arrhythmogenic alterations in VF myocytes and to the beneficial effects of exercise in VF animals. This finding is consistent with work by Stolen et al. (45), who demonstrated that normalization of Ca$^{2+}$ handling by exercise training in a mouse model of diabetic cardiomyopathy involves a reduction of CaMKII-dependent phosphorylation of RyR.

**Limitations**

The QRS duration was used to assess ventricular conduction and lacks the sensitivity to assess potential regional variations in conduction velocity. In the present study, we studied only midmyocardial myocytes isolated remote from the infarct. Thus any regional differences in APD or ion currents (33) were not examined. Isolated myocytes are, by definition, uncoupled and may not fully reflect the electrophysiology of in vivo, coupled, myocytes. We cannot exclude the contributions of other ion currents (e.g., KATP current) or channel subunits (e.g., Kv1.4) to our findings; future studies will be required to explore these possibilities more fully.

**Conclusion**

In summary, endurance exercise training in a post-MI SCD canine model decreases APD, beat-to-beat variability, and the incidence and amplitude of Ca$^{2+}$ alternans. In addition, it normalizes the Ca$^{2+}$ spark frequency, $I_{Ks}$, QTc interval, and the descending portion of the T wave ($T_{peak}-T_{end}$). At the molecular level, normalization of calcium handling can be attributed to modulation of CAMKII-dependent phosphorylation of the RyR. Collectively, our data suggest that the arrhythmogenic effects of endurance exercise training in the post-MI setting can be attributed to normalization of in vivo and in vitro ventricular repolarization, as well as improved Ca$^{2+}$ handling.
In addition to the improved autonomic balance observed after exercise training, alterations in repolarization and calcium regulation may be antiarrhythmic following MI.

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DISCLOSURES
No conflicts of interest, financial or otherwise, are declared by the author(s).

AUTHOR CONTRIBUTIONS

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