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Early exercise training after myocardial infarction prevents contractile but not electrical remodelling or hypertrophy

Virginie Bito[1], Monique C. de Waard[2], Liesbeth Biesmans[1], Ilse Lenaerts[1], Semir Ozdemir[1,3], Elza van Deel[2], Yousra Abdel-Mottaleb[1], Ronald Driesen[1], Patricia Holemans[1], Dirk J. Duncker[2], and Karin R. Sipido[1]*

[1]Laboratory of Experimental Cardiology, University of Leuven, KUL, Campus Gasthuisberg O/N 7th floor, Herestraat 49, B-3000 Leuven, Belgium; [2]Experimental Cardiology, Department of Cardiology, University Medical Center Rotterdam, Thoraxcenter, Erasmus MC, Rotterdam, The Netherlands; and [3]Department of Biophysics, Akdeniz University, Antalya, Turkey

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Aims Exercise started early after myocardial infarction (MI) improves *in vivo* cardiac function and myofilament responsiveness to Ca^{2+}. We investigated whether this represents partial or complete reversal of cellular remodelling.

Methods and results Mice with MI following left coronary ligation were given free access to a running wheel (MI_{EXE}, $N = 22$) or housed sedentary (MI_{SED}, $N = 18$) for 8 weeks and compared with sedentary sham-operated animals (SHAM, $N = 11$). Myocytes were enzymatically isolated from the non-infarcted left ventricle. Myocytes in MI were significantly longer and even more so with exercise (165 $\pm$ 3 μm in MI_{EXE} vs. 148 $\pm$ 3 μm in MI_{SED} and 136 $\pm$ 2 μm in SHAM; $P < 0.05$, mean $\pm$ SEM); cell width was not different. Contraction was measured during electrical field stimulation at 1, 2, and 4 Hz. Unloaded cell shortening was significantly reduced in MI_{SED} (at 1 Hz, L/L_0=4.4 $\pm$ 0.3% vs. 6.7 $\pm$ 0.4% in SHAM; $P < 0.05$, also at 2 and 4 Hz). Exercise restored cell shortening to SHAM values (MI_{EXE}, L/L_0=6.4 $\pm$ 0.5%). Membrane currents and $[Ca^{2+}]_i$ were measured via whole-cell patch clamping, with Fluo-3 as Ca^{2+} indicator, all at 30°C. Ca^{2+} transient amplitude, I_{CaL} and sarcoplasmic reticulum Ca^{2+} content were not different between the three groups. Diastolic Ca^{2+} levels at 4 Hz were significantly elevated in MI_{SED} only, with a trend to increased spontaneous Ca^{2+} release events (sparks). Action potential duration was increased and transient outward K^+ currents significantly reduced after MI; this was unaffected by exercise.

Conclusions Early voluntary exercise training after MI restores cell contraction to normal values predominantly because of changes in the myofilament Ca^{2+} response and has a beneficial effect on diastolic Ca^{2+} handling. However, the beneficial effect is not a complete reversal of remodelling as hypertrophy and loss of repolarizing K^+ currents are not affected.

Keywords Calcium • Exercise • Arrhythmias • Myocardial infarction • Potassium currents

1. Introduction

Ventricular remodelling after a large myocardial infarction (MI) is one of the most common causes of heart failure. Loss of myocardial tissue and consequently increased haemodynamic load on the remaining myocardium induces hypertrophy of the myocytes. Matrix remodelling, apoptosis, and re-arrangement of the myocytes all contribute to cavity remodelling with dilatation, eventually evolving to heart failure, a process that involves multiple signalling pathways.[1] This remodelling process has been termed maladaptive because of the eventual loss of function and the cellular correlate has been characterized in animal models. In an early stage, 1 week after MI, the hypertrophic response can be accompanied by an improved function of the isolated myocytes.[2] However, in a later stage, very often reduced contraction and altered Ca^{2+} handling contribute to the deteriorating cardiac function *in vivo*. In contrast to this maladaptive remodelling, exercise has been associated with cardiac hypertrophy that is mostly devoid of contractile dysfunction, but rather associated with improved contractile function.[3,4] Different underlying signalling cascades can account for the different types of remodelling and

* Corresponding author. Tel: +32 16 347 153; fax: +32 16 345 844, Email: karin.sipido@med.kuleuven.be

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raise considerable interest as targets to reverse maladaptive remodelling, or slow progression to heart failure.[1,5,6]

In the clinical setting, a careful regime of exercise training can improve signs and symptoms of heart failure due to combined effects on skeletal muscle, oxygen uptake and transport as well as cardiac function. Animal experiments indicate that differences in training intensity and modality lead to different effects at the level of the cardiac myocytes.[7]

Recently, we showed in mice that voluntary treadmill running started early after a large MI resulted in a small but significant improvement of cardiac function assessed *in vivo*.[8] Myofilament response to Ca^{2+} in isolated skinned myocytes returned to normal and myocytes of exercised mice had a better contraction than those of sedentary mice with MI though global Ca^{2+} transients remained largely unchanged. However, several questions remained such as (i) the extent of myocyte functional improvement compared with normal hearts, and (ii) whether the beneficial effects of exercise extended also to other remodelling processes such as myocyte hypertrophy and electrical remodelling. To answer the latter question we characterized in detail the properties of intact single myocytes isolated from sedentary mice with MI and mice with MI exercising voluntarily. To answer the first question and establish whether there was a full reversal or prevention of remodelling, data were compared with those of sham sedentary mice. Lastly, we included a comparison between sham sedentary and sham exercising mice to identify whether exercise remodelling was additive or modulatory in the setting of post-MI remodelling.

2. Methods

Experiments complied with the 'Guide-for-Care-and-Use-of-Laboratory-Animals' of the National Institutes of Health (NIH Publication No. 86-23, Revised 1996), and were approved by the Erasmus MC Animal Care Committee and the Ethical Committee of the University of Leuven.

2.1 Animals

MI was produced by permanent ligation of the left-anterior-descending-coronary-artery (LAD) with a 7-0 silk suture (Braun, Aesculap AG&CO. KG, Germany) as described before.[8] Mice were weighed, sedated with 4% isoflurane, intubated, and ventilated under pressure control with O_2-N_2 (1:2, vol/vol) containing ~2.5% isoflurane for anaesthesia. SHAM animals underwent the operation without infarct induction. A total of 51 C57Bl/6J mice of either sex (10–12 weeks old at the time of entry) were studied for cellular remodelling, 8 weeks after LAD ligation or sham intervention.

Sham-operated mice (SHAM, $N = 11$) and a group of mice with MI had a sedentary lifestyle (MI_{SED}, $N = 18$) while another group of mice with MI had free access to a treadmill with voluntary exercise training (MI_{EXE}, $N = 22$). The ratio M:F was between 1:2 and 1:3, and was not different between the three experimental groups.

In a separate series of experiments, we compared two additional groups of mice, SHAM undergoing EXE for 8 weeks ($SHAM_{EXE}$, $N = 8$) and matched $SHAM_{SED}$ mice ($N = 8$). In the present study the mice that were given free access to the running wheel, MI_{EXE} and $SHAM_{EXE}$, ran on average 11.4 ± 1.6 and 9.7 ± 0.6 km/day, respectively (P = NS).

2.2 Cell isolation

Eight weeks after entering the study, mice were injected with heparin (1000 U/kg i.p.) and killed by injection of a lethal dose of pentobarbital (0.1 mg/g i.p.). The heart was excised and weighed. Single LV cardiomyocytes were obtained by enzymatic dissociation through retrograde perfusion of the aorta.[9] The RV and the atria were discarded; for the mice with MI, the scar area and adjacent zone was also removed to obtain cells from the non-infarcted area only.

2.3 Solutions

During field stimulation and patch-clamp experiments cells were superfused with normal Tyrode solution (in mmol/L: 137 NaCl, 5.4 KCl, 0.5 $MgCl_2$, 1 $CaCl_2$, 11.8 HEPES, and 10 glucose, pH adjusted to 7.40 with NaOH). The pipette solution for whole-cell patch clamp contained (in mmol/L): 120 K-aspartate, 20 KCl, 10 NaCl, 10 K-HEPES, 5 MgATP, and 0.05 K_5-fluo-3, pH 7.2. For measurements of K^+ currents, K_5-fluo-3 was omitted and 10 mM EGTA was added to the pipette solution; the [$CaCl_2$] in the external solution was lowered to 0.18 mmol/L. For measurement of L-type Ca^{2+} current, K^+ was replaced with Cs^+ in the external Tyrode and in the pipette solution.

2.4 Experimental protocols

Cells were placed in a perfusion chamber on the stage of an inverted microscope (Nikon Diaphot). Cardiomyocyte length and width were measured in 40 cells per animal. T-tubule density was determined in myocytes stained with di-8-ANNEPS, as previously described.[10] From each animal, at least six cells were studied. From a full Z-stack of images of each cell, the 3–5 central slices were analysed and the signal density of di-8-ANNEPS is expressed as fraction of the total image surface within the external sarcolemmal boundaries.

Unloaded cell shortening of intact cardiomyocytes was measured with a video-edge detector (Crescent Electronics, USA) at different frequencies of stimulation (field-stimulation) and data are presented as fractional shortening (% diastolic length, L/L_0).

Ca^{2+} transients and currents were recorded using the whole-cell ruptured patch clamp technique. Calibration of Ca^{2+} was performed as before[9] using maximal fluorescence values, F_{max}, measured at the end of the experiment; with this method, resting $[Ca^{2+}]_i$ can be measured. Global Ca^{2+} was measured in current-clamp mode, during action potential (AP) recordings at a range of frequencies. Additional data on specific Ca^{2+} transport were obtained in voltage clamp mode.

For the measurement of L-type Ca^{2+} current, I_{CaL}, the voltage-clamp protocol consisted of a 600 ms pre-pulse from −70 to −50 mV, followed by 250 ms depolarizing voltage steps ranging between −40 and +60 mV. I_{CaL} was measured as peak current minus the current obtained at the end of the pulse.

SR Ca^{2+} content was estimated by integrating the Na/Ca current during release of available Ca^{2+} by a 10 s application of 10 mM caffeine.

The total of repolarizing K^+ currents was measured during depolarizing pulses from −70 to +60 mV for 5 s. The decay phases of the K^+ currents could be fitted by the sum of two exponentials and a steady-state component, reflecting the inactivating currents $I_{to,f}$ and $I_{K,slow}$ and the non-inactivating current I_{SS}.[11] Voltage dependence of current activation and inactivation were obtained during a range of depolarizing potentials preceded by a step to −50 mV, inactivating the Na^+ current and most of $I_{to,f}$.

2.5 Statistics

Data were analysed using one- or two-way ANOVA, followed by *post hoc* testing, as appropriate. Data were considered significant for $P < 0.05$. Data are shown as mean ± SEM; '*N*' refers to the number of animals, '*n*' to the number of cells. For cellular data, each group contains cells from at least five animals.

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3. Results

3.1 Myocyte hypertrophy and T-tubule density

Total HW: BW was significantly larger after MI compared with SHAM and was not different between MI_{SED} and MI_{EXE} (*Figure 1A*). Isolated cells were significantly longer after MI, and even slightly longer in MI_{EXE} than in MI_{SED}; cell width was not different between the groups (*Figure 1B* and *C*). Cellular hypertrophy after MI was also evident from the increase in cell capacitance, reflecting the increase in membrane surface area though there was no difference between MI_{SED} and MI_{EXE} (SHAM 146 $\pm$ 4 pF, $N = 11$, $n = 144$; MI_{SED} 205 $\pm$ 6 pF, $N = 18$, $n = 148$; MI_{EXE} 195 $\pm$ 6 pF, $N = 21$, $n = 169$). T-tubule signal density was unchanged (SHAM 28 $\pm$ 1%, $N = 5$, $n = 31$; MI_{SED} 28 $\pm$ 2%, $N = 14$, $n = 84$; MI_{EXE} 30 $\pm$ 5%, $N = 8$, $n = 48$).

3.2 Cell contraction and systolic Ca^{2+} handling

MI in sedentary animals significantly reduced the amplitude of cell shortening at all frequencies of stimulation, without affecting the time to peak contraction; exercise fully reversed the reduction in cell shortening (*Figure 2A*). Ca^{2+} transient amplitude was unaffected, but there was a trend to lengthening of the time to peak in the MI_{SED}, with intermediate values in MI_{EXE} (*Figure 2B*). In all groups, increasing the frequency had but a small effect on the amplitude of shortening and of Ca^{2+} transients, but the rate of contraction increased at the higher frequency. This behavior was similar for the three groups.

A further analysis of the Ca^{2+} fluxes showed no change in L-type Ca^{2+} current density (*Figure 3A*). In a subgroup of cells, Ca^{2+} transients were measured simultaneously (*Figure 3B*). A trend to larger Ca^{2+} transients in MI_{EXE} correlated with a trend to larger I_{CaL} but this did not reach significance and may be related to the lower cell number. SR Ca^{2+} content was not significantly different, though there was a trend towards reduction after MI (*Figure 3C*).

3.3 Relaxation and diastolic Ca^{2+} handling

There was no difference in the rate of cell re-lengthening (data not shown). The time to 50% decline of the Ca^{2+} transient was not different either between SHAM and MI, sedentary or exercised (*Figure 4A*). The half-time of decline decreased with increasing frequency of stimulation, and this behaviour was not different between the groups. However, the level of Ca^{2+} between beats at higher frequencies of stimulation was higher in MI_{SED} than in SHAM, which was not observed in MI_{EXE} (*Figure 4B*). The capacity of NCX for Ca^{2+} removal was assessed by the rate of relaxation of Ca^{2+} transient during caffeine application; it was significantly higher in myocytes from MI_{EXE} (*Figure 4C*).

The propensity for diastolic Ca^{2+} loss from the SR was assessed from the properties of spontaneous release events or sparks during 10 s resting periods following stimulation at different frequencies (*Figure 5A*). The frequency of sparks increased with increasing rate of stimulation (*Figure 5B*). At the highest frequency it tended to be higher in MI_{SED} than in SHAM, but not in MI_{EXE} as suggested by the data shown in *Figure 5C*, though this failed to reach statistical significance. Spark amplitude, time course or spatial width was not different between the groups (data not shown).

3.4 Electrical remodelling

Myocyte remodelling in hypertrophy includes ion channel changes that often lead to AP prolongation. Channels most commonly affected are the family of K^+ channels underlying the transient outward K^+ current. The maximal peak transient outward K^+ current was significantly reduced after MI, independent of whether the mice exercised or not (*Figure 6A*). We did a further analysis of underlying current components by fitting of the decay of the current.[11] The mean values for the time constants, both slow and fast were not different between the groups (in ms, Tau slow 769 $\pm$ 61, 816 $\pm$ 59, 734 $\pm$ 54 and Tau fast 104 $\pm$ 13, 80 $\pm$ 8, 100 $\pm$ 7, for SHAM, MI_{SED} and MI_{EXE}, respectively). The most significant downregulation in MI_{SED}, and in relative terms the largest effect, was seen for the $I_{K,slow}$ component. In addition there was a small but significant reduction of the steady-state component. With exercise there was no recovery of the loss of the $I_{K,slow}$ component, but the reduction of the steady-state component was not present any more. There was after exercise a trend to reduction of $I_{to,f}$, but this was not significant.

We further examined voltage dependence in a protocol including a prepulse to -50 mV, inactivating most of the $I_{to,f}$ component (*Figure 6B* and *C*). The I–V curves confirm the significant reduction of $I_{K,slow}$. The voltage dependence of inactivation was similar in all groups (data not shown).

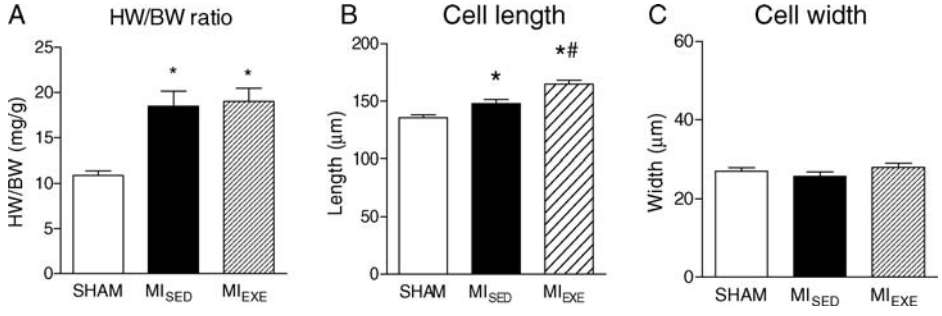


Figure 1 Hypertrophy after MI and exercise. (*A*) Heart weight-body weight ratio in SHAM ($N = 11$), MI_{SED} ($N = 18$), and MI_{EXE} ($N = 22$). (*B* and *C*) Cell length and cell width measured from >40 cells per animal in SHAM ($N = 7$), MI_{SED} ($N = 15$) and MI_{EXE} ($N = 14$). $^*P < 0.05$ for MI_{EXE} and MI_{SED} vs. SHAM, $^{\#}P < 0.05$ for MI_{SED} vs. MI_{EXE}.

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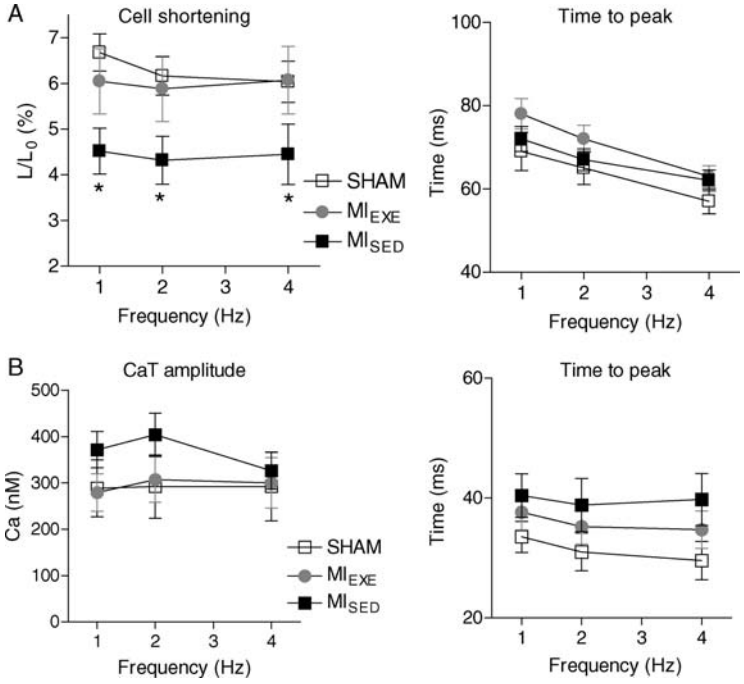


Figure 2 Exercise restores cell contraction without affecting systolic Ca^{2+}. (*A*) Average data of fractional shortening and time to peak shortening at 1, 2, and 4 Hz, in SHAM ($n = 52$), MI_{SED} ($n = 50$), and MI_{EXE} ($n = 39$). Cell shortening (ΔL) is normalized to cell length (L_0) and was measured during steady state. $^*P < 0.05$ SHAM vs. MI_{SED}. (*B*) Average data of Ca^{2+} transient amplitude and time to peak obtained in current-clamp in SHAM ($n = 12$), MI_{SED} ($n = 13$), and MI_{EXE} ($n = 17$). F/F_0 in a large and independent sample of field-stimulated myocytes was also not different between groups: at, respectively, 1, 2, and 4 Hz 1.91 $\pm$ 0.07, 1.71 $\pm$ 0.04, and 1.40 $\pm$ 0.03 in SHAM ($n = 38$); 2.28 $\pm$ 0.11, 1.92 $\pm$ 0.09, and 1.47 $\pm$ 0.03 in MI_{SED} ($n = 24$); 1.90 $\pm$ 0.11, 1.66 $\pm$ 0.10, and 1.34 $\pm$ 0.06 in MI_{EXE} ($n = 42$).

APs in myocytes from hearts with MI also had a significant prolongation, independent of whether the mice exercised or not (*Figure 6D*). This was prominent on the early and mid-repolarization, as illustrated in the figure by the APD_{50}, but the full duration of the AP was not different (no significant difference in APD_{90}).

3.5 Effects of exercise in sedentary mice without MI

The results above suggest a modulatory effect of exercise on the remodelling processes following MI. Alternatively, they are the result of added effects with independent remodelling processes due to exercise. We therefore performed an independent study comparing $SHAM_{SED}$ mice with $SHAM_{EXE}$.

The results of *Figure 7* indicate that this level of exercise had only minor effects in itself. There was a trend to increase in HW:BW, despite BW being comparable. Myocytes were only mildly hypertrophied, in width, but not length. Contractile parameters and Ca^{2+} handling were not different. There was no effect on AP duration or amplitude of K^+ currents.

4. Discussion

4.1 Contraction and myofilament response normalize with exercise

We have previously reported that the severely compromised cardiac function after MI can be improved with exercise.[8] This beneficial effect observed *in vivo* was associated with reduced fibrosis and improved shortening of myocytes from MI mice after exercise. The latter was ascribed to normalization of the Ca^{2+} response of the myofilaments, in particular the restoration of the maximal force development capacity; the underlying molecular mechanism remains currently unexplained. The present study extends these previous findings and shows that the unloaded shortening of intact isolated myocytes of mice that exercised voluntarily after MI, is not different from that of sham-operated mice. This preserved function is remarkable and occurs in the absence of significant changes in the amplitude of the Ca^{2+} transient in field stimulation. If anything, the Ca^{2+} transient amplitude and I_{CaL} tend to be larger in MI_{EXE} than in either SHAM or MI_{SED}. However, data did not reach significance because of a large variability within the MI_{EXE} group. This observation may be due to a restricted number of recordings but shows that if anything, exercise improves Ca^{2+} influx and Ca^{2+} release, but that this mechanism is not the main effect of exercise to restore cell function. These data confirm that a major change underlying the normalized contraction is in the myofilament response, consistent with studies in the rat.[12,13] The observed changes during unloaded shortening correspond with the restitution towards normal values in MI_{EXE} of the downward shift of the pCa-F curve of skinned cardiac myocytes from MI_{SED}.[8]

The normal amplitude of the Ca^{2+} transient is not immediately reconciled with earlier data of a loss of SERCA in MI_{SED} and also reported in other models of post-MI remodelling.[14] It is possible that a reduction in Ca^{2+} binding in the cytosol leads to an

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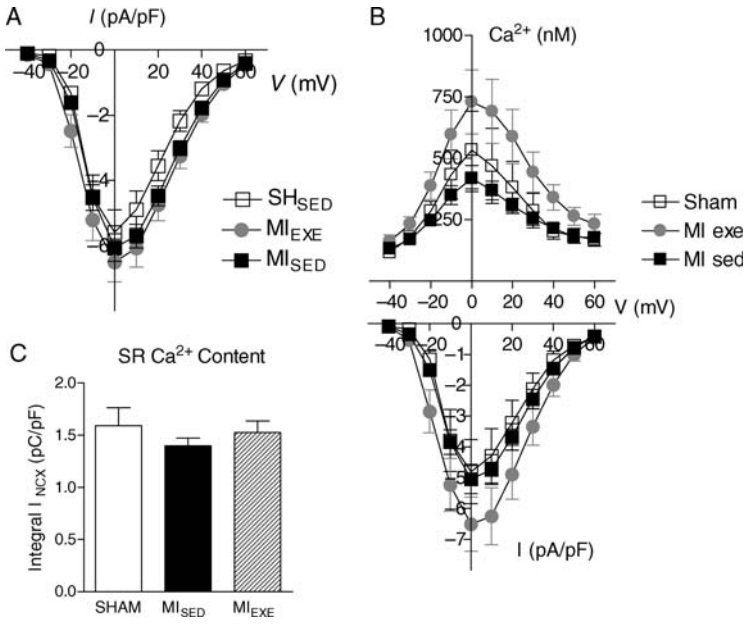


Figure 3 L-type Ca^{2+} current, I_{CaL}, Ca^{2+} transients and SR Ca^{2+} content are unchanged. (*A*) Current–voltage relation I_{CaL} L-type Ca^{2+} current density, I_{CaL}, measured as peak-end of pulse current normalized to cell capacitance, in SHAM ($n = 15$), MI_{SED} ($n = 30$), and MI_{EXE} ($n = 21$). (*B*) Current–voltage relation in a subset of cells with matching Ca^{2+} transient amplitude data in the right panel, SHAM ($n = 9$), MI_{SED} ($n = 12$), and MI_{EXE} ($n = 9$). (*C*) SR Ca^{2+} content measured as the integral of the NCX current obtained during a 10 mM caffeine application in SHAM ($n = 15$), MI_{SED} ($n = 17$), and MI_{EXE} ($n = 20$).

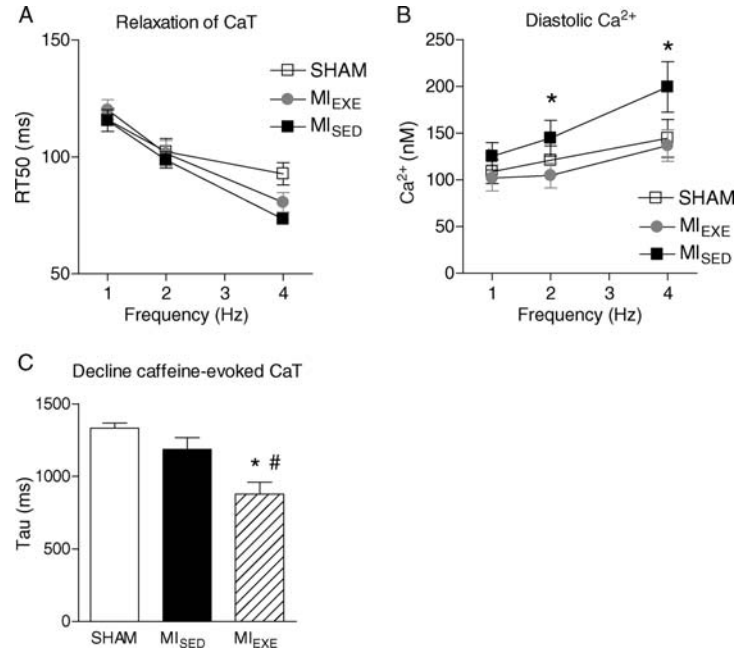


Figure 4 Higher diastolic Ca^{2+} in MI is reduced with exercise. (*A*) Half-time of decline of Ca^{2+} transient during current-clamp experiments at 1, 2, and 4 Hz in SHAM ($n = 12$), MI_{SED} ($n = 13$), and MI_{EXE} ($n = 17$). The absence of differences was also confirmed in an independent large group of field-stimulated myocytes [data not shown, SHAM ($n = 50$), MI_{SED} ($n = 38$), and MI_{EXE} ($n = 52$)]. (*B*) Diastolic Ca^{2+} measured in current-clamp at 1, 2, and 4 Hz in SH_{SED} ($n = 12$), MI_{SED} ($n = 13$), and MI_{EXE} ($n = 17$). (*C*) Na/Ca exchange function assessed as the time constant, tau, of the decline of the caffeine-evoked Ca^{2+} transient in SHAM ($n = 14$), MI_{SED} ($n = 14$), and MI_{EXE} ($n = 14$).

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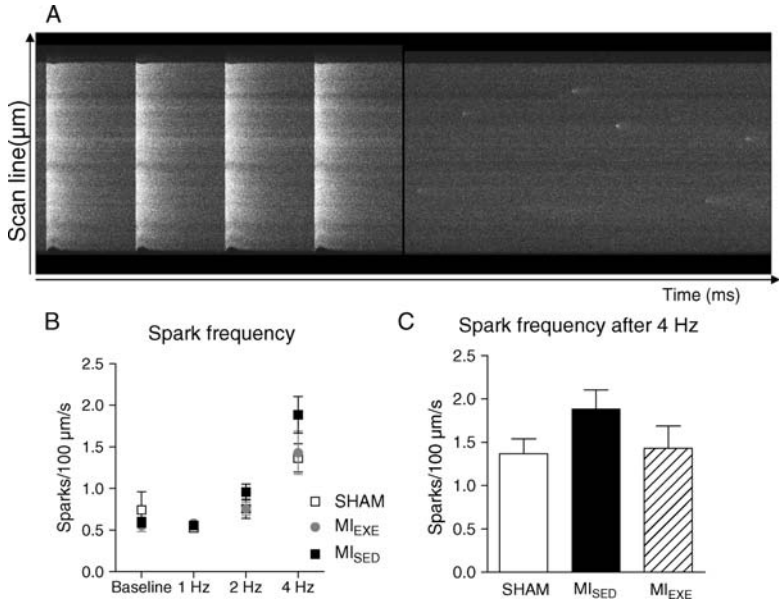


Figure 5 Sparks occurrence and properties are unchanged. (*A*) Example of a line scan image during pacing at 1 Hz followed by a rest period. (*B*) Sparks frequency occurring at rest or after 1, 2, or 4 Hz stimulation in SHAM ($n = 50$), MI_{SED} ($n = 38$), and MI_{EXE} ($n_{cells} = 52$). (*C*) Frequency of sparks observed after 4 Hz stimulation.

overestimation of the available Ca^{2+} when we look at the free cytosolic values. Preliminary data on Ca^{2+} buffering indeed suggest that the maximal Ca^{2+} binding capacity of the myocyte is reduced in MI_{SED} and recovers with exercise.

A reduction in SERCA pumps and/or a reduction in myofilament Ca^{2+} binding in MI_{SED} could both contribute to a reduced cytosolic Ca^{2+} buffering. Exercise can restore reduced SERCA expression,[12,13] though in a rat model with sprint training after MI, SERCA was not increased but further reduced, and SR uptake improved because of PLB reduction.[15]

Reduction in myofilament density can be a cause of reduced cellular Ca^{2+} binding capacity. Reduced density can occur as a consequence of glycogen accumulation as reported in the pig after MI and also in chronic ischaemia in the pig and humans.[16,17] We have no data to support that this is important in the mouse model of post-MI remodelling. Histological analysis of remote myocardium using PAS staining in an additional four hearts of each group showed very low levels of glycogen, present only in the subendocardium of 3 out of 12 hearts, without relation to MI_{SED} or MI_{EXE} (data not shown). Whether this reflects a species-dependence is not clear at the moment.

4.2 Diastolic Ca^{2+} handling after MI and effect of exercise

A decrease in SERCA is usually associated with a slower decline of the depolarization-induced Ca^{2+} transient. We could not document such a slower relaxation after MI when we analysed the tau of decline during stimulation at different frequencies. However, at the higher frequencies, relaxation was incomplete, since the inter-beat diastolic Ca^{2+} levels were higher in the MI_{SED} myocytes.

This is likely the manifestation of reduced SR Ca^{2+} removal. In addition, another element that could contribute to elevated diastolic Ca^{2+} levels is enhanced loss of Ca^{2+} from the SR through RyR. This cannot be measured during the short interbeat interval, but can be inferred from measurements during a long diastolic interval, either as the tetracaine-sensitive change in diastolic Ca^{2+}[18], or as the frequency of spontaneous RyR release events.[19] In the present study, there was a trend to higher release events in MI_{SED}, normalized in MI_{EXE}. Increased leak of Ca^{2+} from the SR was reported before in rat after MI[19] and in the rabbit with non-ischaemic heart failure,[20] but not MI.[21] So far, to our knowledge, there are no data on exercise published. However, in the last series of experiments we performed, we found no difference in Ca^{2+} spark frequency or morphology after exercise in SHAM animals (data not shown).

Our data indicate that exercise can reduce the diastolic Ca^{2+} levels, presumably through a beneficial effect on leak and Ca^{2+} removal by NCX. The evidence for an enhanced capacity of NCX is clear from the faster decline of the caffeine-induced Ca^{2+} transients and consistent with a higher expression level.[8] Though higher expression of NCX could 'drain' Ca^{2+} from the cell as postulated in heart failure, if SERCA function is preserved this may not occur.[22] Indeed, the actual Ca^{2+} removal from the cytosol will be a balance between RyR leak, Ca^{2+} reuptake by SERCA, and NCX. In MI_{SED}, with a higher RyR leak and less SERCA uptake, this balance may shift towards a larger fraction being removed during the decline of the Ca^{2+} transient. Measurements of the NCX current shortly after the peak of the Ca^{2+} transient show the highest values of current density in MI_{SED} (data not shown), probably reflecting this less favourable balance. To explain that the further upregulation of NCX with exercise does not lead to depletion of the cell of Ca^{2+}, we postulate that SERCA function is actually better after exercise[23] and/or that the reduced Ca^{2+} leak from the SR helps to retain SR Ca^{2+}.

4.3 Electrical remodelling after MI and exercise

The loss of the transient outward K^+ current is considered a hallmark of electrical remodelling in heart failure (reviewed in[24,25]). In post-MI

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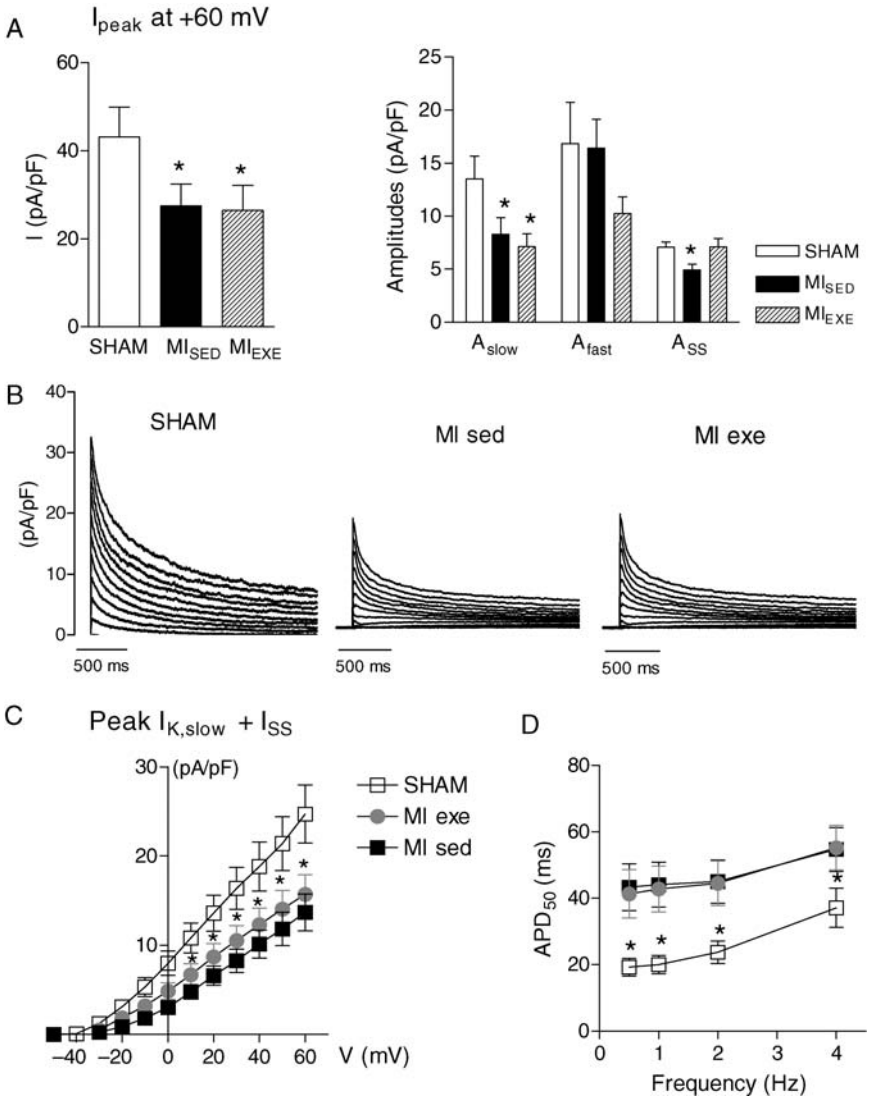


Figure 6 Electrical remodelling after MI is not reversed with exercise. (*A*) Total peak repolarizing K^+ currents (I_{peak}) measured at +60 mV from −70 mV in SHAM ($n = 15$), MI_{SED} ($n = 17$) and MI_{EXE} ($n = 10$). The decay phase was fitted with two exponentials and a non-inactivating component. Average data of amplitudes of the fast, the slow, and the steady-state current A_{fast}, A_{slow}, A_{SS}. (*B*) Representative K^+ current family evoked during 3 s voltage steps ranging from −40 to +60 mV, preceded by a prepulse to −50 mV. (*C*) Current–voltage relation of peak outward K^+ current ($I_{K,\ slow}$ + I_{ss}) in SHAM ($n = 17$), MI_{SED} ($n = 16$), and MI_{EXE} ($n = 21$). (*D*) AP duration at 50% repolarization (APD_{50}) at 1, 2, and 4 Hz in SHAM ($n = 20$), MI_{SED} ($n = 25$), and MI_{EXE} ($n = 25$). $^*P < 0.05$ MI_{SED} and MI_{EXE} vs. SHAM.

remodelling, most data have been gathered in the rat model,[26,27] but also in the dog[28] and in the mouse.[29] In the rat this loss of K^+ current is linked to prolongation of the AP,[26] while in larger mammals with a pronounced AP plateau, the role of K^+ currents in AP prolongation is more complex.[30] Our data are consistent with a downregulation of the I_{Kslow} component after MI and the AP was also significantly prolonged. A downregulation of (components of) transient outward K^+ currents has been reported in a number of other mouse models of cardiac hypertrophy and heart failure, e.g. in a calcium-dependent genetic model[31] and after pressure overload.[32] In the case of angiotensin-induced hypertrophy, opposite changes in current components may result in an apparently unchanged total K^+ current.[33] These reports illustrate the variation depending on the stimulus for hypertrophy and also the regional variation. This latter aspect was not addressed in the current study.

Exercise did not reverse the loss of K^+ currents, but neither did it further reduce them. In contrast to the rescue of changes in myofilament and Ca^{2+} handling after MI by exercise, there thus seems to be no additive or rescue effect of exercise on the electrical remodelling post-MI in the present study. This suggests that different mechanisms control the Ca^{2+} handling and the K^+ channel expression. In contrast to our data, in rats with an intense exercise (sprint training) program after MI, prolongation of the AP and K^+ current downregulation with MI were rescued by the training.[34] To our knowledge this is the only other study examining the effects of exercise on electrical remodelling after MI. The differences with our data could be species-dependent, but may also relate to different exercise regimes, as suggested for differences in exercise-induced changes in excitation–contraction coupling.[3] Only few studies examined AP and K^+ current remodelling in chronic exercise training in the absence of MI, but these report a

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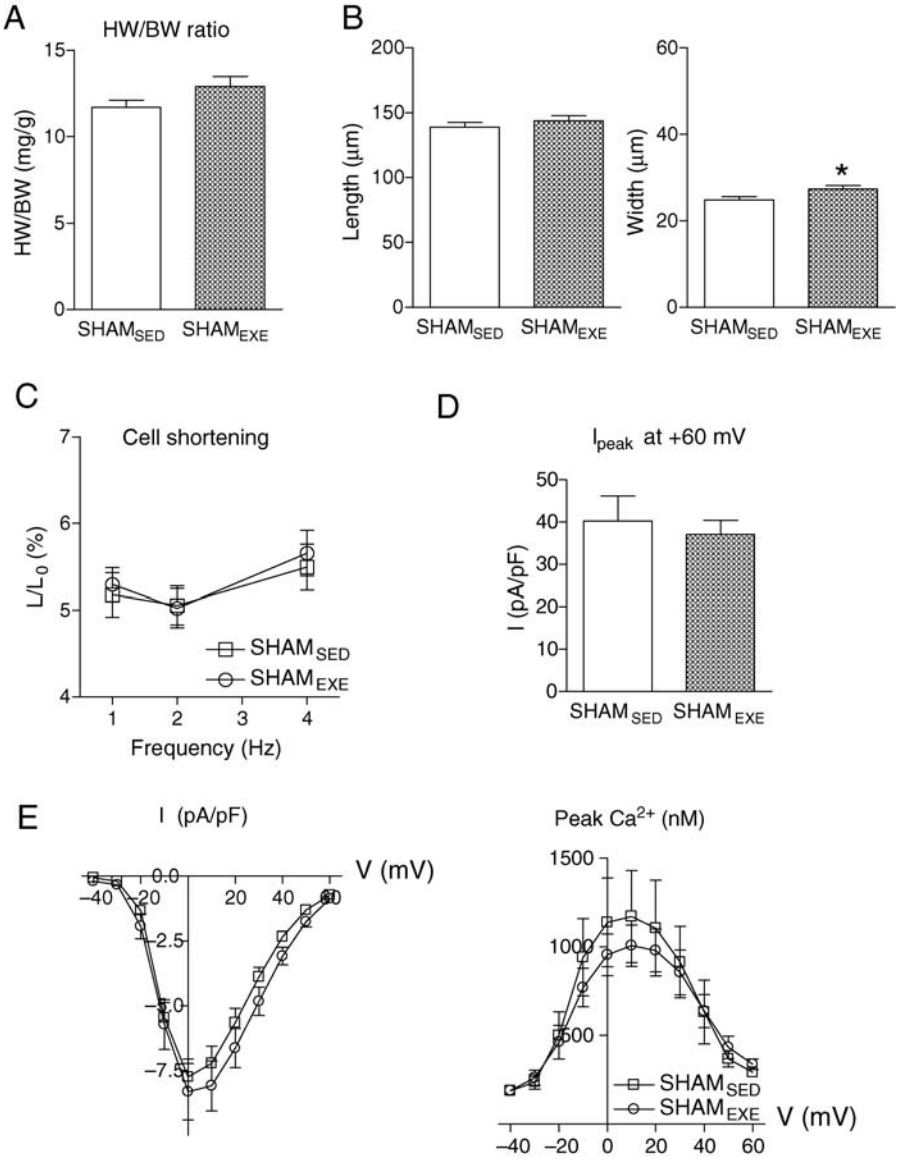


Figure 7 Exercise does not have a major effect *per se*. (*A*) Heart weight–body weight ratio in $SHAM_{SED}$ ($N = 6$) and in $SHAM_{EXE}$ ($N = 8$). (*B*) Cell length (left panel) and cell width (right panel) measured in >40 cells per animal in $SHAM_{SED}$ ($N = 6$) and $SHAM_{EXE}$ ($N = 8$). (*C*) Unloaded cell shortening measured at 1, 2, and 4 Hz in $SHAM_{SED}$ ($n = 41$) and in $SHAM_{EXE}$ ($n = 56$). (*D*) Total peak repolarizing K^+ currents measured during a single pulse to +60 mV in $SHAM_{SED}$ ($n = 17$) and $SHAM_{EXE}$ ($n = 32$). (*E*) L-type Ca^{2+} current density and the associated Ca^{2+} transient measured in $SHAM_{SED}$ ($n = 13$) and $SHAM_{EXE}$ ($n = 20$).

small reduction in I_{sus} and prolongation of the AP[35] or regional AP prolongation,[36] suggesting that exercise *per se* is not always beneficial.[37] The level of exercise used in the present study had no effect on K^+ currents in itself.

Studies in rats undergoing moderate or high-intensity exercise have shown that vigorous exercise leads to more pronounced hypertrophy, contractility, and remodelling compared with moderate exercise.[7] In the same line, randomized studies in heart failure patients have shown that moderate exercise improves LV remodelling but is less efficient than high-intensity exercise, especially for increased aerobic capacity.[38] The same was shown in patients with type 2 diabetes.[39] Whether this might be more advantageous to electrical remodelling remains to be studied.

4.4 Cell hypertrophy and relation to different exercise regimes

Although it is thought that exercise, as a physiological stimulus, leads to a proportional increase in cell length and width, this is not consistently reported in the literature. In female rats trained on a treadmill,[7] moderate exercise increased myocyte length but not myocyte width, while high-intensity exercise increased both. The hypertrophy observed during voluntary exercise might be quite specific and region-dependent.[36,40,41] In female rats, moderate voluntary exercise induced an increase in HW:BW ratio, due to an increase in cell width but not cell length, though this increase was observed only in cells from the sub-endocardial layer and not from the sub-epicardial layer. In the

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present study, likewise, voluntary exercise *per se* induced a small increase in cell width, that however did not have significant impact on HW:BW. In the setting of MI, this was translated in a small increment of the cell lenghtening induced by MI, indicating the interaction between the two hypertrophic stimuli.

4.5 Conclusions

Moderate, voluntary exercise after MI in the mouse can fully rescue the contractile dysfunction of myocytes and restore diastolic Ca^{2+} handling, but not electrical remodelling or hypertrophy. The beneficial effect on function may therefore be mitigated by the persistence of a potentially arrhythmogenic substrate. Diverging signalling pathways are likely to underlie this difference. Post-MI remodelling has been linked to calcineurin,[42–44] and exercise rather to Akt-dependent pathways,[45] but the interaction between them remains to be studied. The specific exercise regime may also be an important factor determining the (reverse) remodelling. The current study may closely resemble a change in lifestyle of patients after MI from sedentary to moderate exercise, and complements earlier studies that mostly relied on intense and forced exercise regimes.[3] However, a recent small clinical study suggests the superior benefit of high-intensity interval training.[38] The type of exercise thus clearly needs further investigation. In addition, the lack of benefit on electrical remodelling, suggested in the present study, or even potential harm, suggested by some literature data, should be evaluated in the clinical setting.

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