Early Exercise Training Normalizes Myofilament Function and Attenuates Left Ventricular Pump Dysfunction in Mice With a Large Myocardial Infarction

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***Abstract*—**The extent and mechanism of the cardiac benefit of early exercise training following myocardial infarction (MI) is incompletely understood, but may involve blunting of abnormalities in Ca^{2+}-handling and myofilament function. Consequently, we investigated the effects of 8-weeks of voluntary exercise, started early after a large MI, on left ventricular (LV) remodeling and dysfunction in the mouse. Exercise had no effect on survival, MI size or LV dimensions, but improved LV fractional shortening from 8±1 to 12±1%, and $LVdP/dt_{P30}$ from 5295±207 to 5794±207 mm Hg/s (both $P<0.05$), and reduced pulmonary congestion. These global effects of exercise were associated with normalization of the MI-induced increase in myofilament Ca^{2+}-sensitivity ($\Delta pCa_{50}=0.037$). This effect of exercise was PKA-mediated and likely because of improved β_1-adrenergic signaling, as suggested by the increased β_1-adrenoceptor protein (48%) and cAMP levels (36%; all $P<0.05$). Exercise prevented the MI-induced decreased maximum force generating capacity of skinned cardiomyocytes (F_{max} increased from 14.3±0.7 to 18.3±0.8 kN/m^2 $P<0.05$), which was associated with enhanced shortening of unloaded intact cardiomyocytes (from 4.1±0.3 to 7.0±0.6%; $P<0.05$). Furthermore, exercise reduced diastolic Ca^{2+}-concentrations (by ~30%, $P<0.05$) despite the unchanged SERCA2a and PLB expression and PLB phosphorylation status. Importantly, exercise had no effect on Ca^{2+}-transient amplitude, indicating that the improved LV and cardiomyocyte shortening were principally because of improved myofilament function. In conclusion, early exercise in mice after a large MI has no effect on LV remodeling, but attenuates global LV dysfunction. The latter can be explained by the exercise-induced improvement of myofilament function. **(*Circ Res*. 2007;100:1079-1088.)**

Key Words: cardiac function ■ cardiomyocytes ■ exercise training ■ heart failure

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Left ventricular (LV) remodeling after myocardial infarction (MI) is a compensatory mechanism that serves to restore LV pump function. Despite the apparent appropriateness of LV remodeling to maintain cardiac pump function early after MI, remodeling is an independent risk factor for the development of congestive heart failure.[1] The mechanism underlying the progression from LV remodeling to overt heart failure remains incompletely understood, but recent evidence indicates that abnormalities in myofilament function and Ca^{2+}-handling contribute to the LV dysfunction in the porcine heart, early after MI.[2]

In contrast to pathological LV remodeling after MI, LV remodeling produced by regular dynamic exercise is associated with a decreased risk for coronary artery disease and heart failure.[3] Exercise training is associated with an increased myocardial perfusion capacity and with normal or even increased contractile function in the normal heart.[4,5] There is also clinical evidence that exercise after MI has a beneficial effect on disease progression and survival.[6,7] For example, physical conditioning in patients with LV dysfunction results in an increased exercise capacity which has been ascribed, at least in part, to skeletal muscle adaptations.[8]

The effects of exercise on LV remodeling and function are still incompletely understood, as several studies in humans reported contradictory effects of training on LV remodeling after a MI.[9–18] Careful inspection of these studies suggests that after a *small* MI, exercise has no detrimental effect[11,13] or even improves[15,17,18] LV geometry and function, independent of whether exercise was started late, ie, ≈1 year,[17,18] or early, ie, <2 months,[11,13,15] after MI. In contrast, in patients with a

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large MI, exercise had either no,[14] or a beneficial[18] effect on ejection fraction (EF) and LV volumes but only when started late after MI. However, when exercise after a large MI is started at a time when LV remodeling is still ongoing (<3 to 4 months after MI), the majority of studies reported that exercise has either no,[11–13] or even a detrimental[9,10] effect on LV volume and EF.

Similar to these clinical studies, studies in rats indicate that exercise started late (>3 weeks) after a moderate to large MI, encompassing 35% to 50% of LV mass, at a time when infarct healing is complete, does not aggravate,[19,20] or even blunts[21–23] LV dilation and hypertrophy. In contrast, when started <1 week after a moderate to large MI,[24–27] exercise resulted in variable outcomes with beneficial,[28] no,[24,27] or detrimental[25,26] effects on LV remodeling. These rodent studies lend further support to the concern that early exercise may have detrimental effects on LV remodeling after a large MI, although interpretation is hampered by the fact that late exercise studies in rats principally used treadmill running,[18,19,21,22] whereas early exercise studies predominantly used swimming.[23–25,27,28] This is important because the exercise responses to swimming are markedly different from those to treadmill running.[29,30]

In light of these observations, the first aim of the present study was to assess the effects of exercise by voluntary treadmill running, started within 24 hour after a large MI, on LV remodeling and dysfunction in the mouse. The results indicated that exercise attenuated the MI-induced LV dysfunction, without a detrimental effect on LV remodeling. Consequently, we tested the hypothesis that exercise early after a large MI is able to reverse the MI-induced abnormalities in β_1-adrenergic receptor and Ca^{2+}-handling protein expression, phosphorylation status of contractile proteins, Ca^{2+}-handling and myofilament function, within the noninfarcted remodeled myocardium.

Materials and Methods

For detailed description see the expanded Materials and Methods in the online data supplement available at http://circres.ahajournals.org. 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.

Experimental Groups

147 C57Bl/6J mice of either sex (≈12-weeks old) entered the study and were randomly assigned to one of four experimental groups. Sham-operated mice (SH) and mice with MI were housed sedentary (SH_{SED}, MI_{SED}) or subjected to voluntary exercise training (SH_{EX}, MI_{EX}) for 8-weeks.

Experimental Procedures

MI was produced by permanent ligaton of the left-anterior-descending-coronary-artery (LAD).[31,32] Eight weeks after entering the study, mice were anesthetized and instrumented for hemodynamic measurements.[32] M-mode LV echocardiography was performed and LV diameters at end-diastole (LV_{EDD}) and end-systole (LV_{ESD}) were measured, and fractional-shortening (FS) calculated. Pressure-diameter relations were obtained from M-mode images synchronized with LV-pressure by simultaneous ECG recording.[32]

Tissue Analysis

Right (RVW) and left (LVW) ventricular weight, tibial-length (TL) and lung-fluid weight were determined in each animal. Masson's trichrome-staining was used for analysis of LV collagen volume fraction and cardiomyocyte cross-sectional area (CSA) (n=8/group). Endocardial and epicardial infarct-circumference, infarct-thickness, and infarct-surface-area were determined.[32] In 8 mice, infarct-size was determined 24 hour after LAD ligation. Skeletal muscle samples were obtained to determine maximal citrate-synthase (CS) activity in 12 SH_{SED} and 12 SH_{EX}.[33]

Force Measurements in Single Permeabilized Cardiomyocytes

Isometric force was measured in single permeabilized cardiomyocytes of 5 mice per group at different $[Ca^{2+}]$ and a sarcomere length of 2.2 μm.[2,34] Rate-of-force redevelopment (Ktr) was determined at pCa values ranging from 4.5 to 5.8 using the release-restretch method.[2,34] After obtaining a complete force–pCa series, myocytes were incubated in relaxing solution containing the exogenous catalytic subunit of protein-kinase A (PKA) and a second force–pCa series was obtained.[2,34] Force-pCa relations were fit to the Hill equation.[2,34]

Myosin-Heavy-Chain Composition

Myosin-heavy-chain (MHC) isoform composition was analyzed by 1-dimensional SDS-PAGE.[35]

Myofilament Proteins Phosphorylation Status

LV samples were separated on gradient gels and stained with Pro-Q Diamond phosphoprotein gel stain in conjunction with SYPRO Ruby staining. The phosphorylation signals for myofilament proteins were normalized to the intensities of the SYPRO Ruby stained myosin binding protein-C (MyBP-C) bands and analyzed.

β_1-Adrenergic Signaling

cAMP levels were measured in LV samples, homogenized in 100 μL frozen 0.1 mol/L HCl, using an enzyme immunoassay kit.[2] PKA levels were measured in LV samples, homogenized in 100 μL frozen PKA extraction buffer, using PepTag Assay kit.[2]

Western Immunoblotting

LV samples were homogenized and protein concentrations were determined. Proteins were separated by SDS-PAGE and blots were stained reversibly with Ponceau Red, and incubated overnight at 4°C with diluted primary antibodies. Signals were visualized using Supersignal West Femto Maximum Sensitivity Substrate and Hyperfilm ECL and quantified.

Contractile Properties of Intact Cardiomyocytes

Single LV cardiomyocytes were obtained from the noninfarcted part of the LV in 11 additional mice (6 MI_{SED} and 5 MI_{EX}) by enzymatic dissociation. Unloaded cell shortening and intracellular Ca^{2+}-concentrations $[Ca^{2+}]_i$ were studied using field stimulation and ruptured patch clamp recording techniques.[36]

Statistics

Data were analyzed using two-way ANOVA, followed by post hoc testing with Student-Newman-Keuls, or using unpaired t-testing, as appropriate. Survival was analyzed by Kaplan-Meier method and log-rank (Mantel-Cox) test. Significance was accepted when $P<0.05$. Data are means±SEM.

Results

Exercise and Survival

MI_{EX} initially ran shorter distances per day compared with SH_{EX} (Figure 1), but total distance over the 8-week period and hence daily distance was similar in SH_{EX} (5.9±0.2 km/d) and

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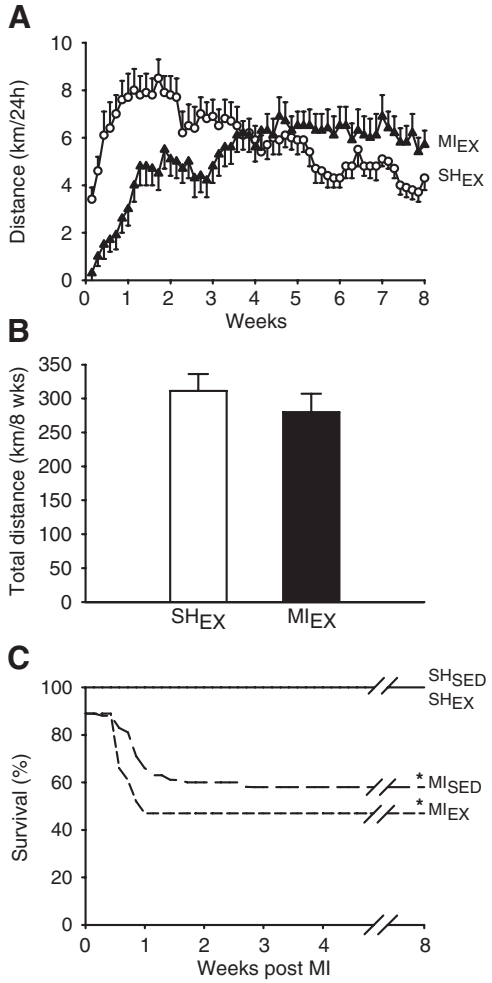


Figure 1. A, Daily running distance in MI (n=21) and SH (n=18) mice that survived the entire 8-week follow-up period. B, Total distance run over 8 weeks. No significant differences were observed. C, Kaplan-Meier survival curves for all four groups. Time point zero represents the immediate postoperative survival of 89% after MI and 100% in SH mice. Numbers of mice entering the study: SH_{SED} (n=34), SH_{EX} (n=18), MI_{SED} (n=61), MI_{EX} (n=34). *$P<0.05$ vs corresponding SH.

MI_{EX} mice (5.2±0.2 km/d). Exercise increased skeletal muscle CS-activity in SH_{EX} (371±28 μmol-CS/g-protein) compared with SH_{SED} (290±21 μmol-CS/g-protein; $P<0.05$). MI was associated with 40% mortality; exercise had no significant effect on survival in MI mice.

LV Remodeling

Twenty-four hours after LAD ligation, 88±2% of the area-at-risk had become infarcted, corresponding to 43±3% of the LV. This resulted in marked LV dilation and hypertrophy, reflected in the increased LV diameter, relative LVW (LVW/TL), and increased cardiomyocyte-CSA and LV collagen volume fraction within the remote myocardium 8 weeks later (Figure 2 and Figure 3A through 3C).

Exercise had no significant effect on cardiomyocyte-CSA and LVW/TL in either Sham or MI mice. However, exercise tended to decrease LV end-diastolic diameter and significantly reduced LV collagen content in both Sham and MI mice (Figure 3).

Global LV Function

MI resulted in lower LV systolic pressure, $LVdP/dt_{P30}$, fractional shortening, $LVdP/dt_{min}$ and increased τ, but had no apparent effect on LV end-diastolic pressure (Table). Exercise had minimal effects on LV systolic and diastolic function in Sham mice, but increased both $LVdP/dt_{P30}$ and FS after MI.

MI resulted in a marked rightward shift as well as a narrowing of the LV pressure-diameter relation, indicating LV dilation and depressed FS (Figure 3D). Exercise caused a small leftward shift in Sham and MI animals (both $P<0.02$). Lung-fluid weight and RVW/TL were increased after MI, indicative of pulmonary congestion and RV hypertrophy (Figure 3E and 3F), which were abolished by exercise.

Force Development in Single Permeabilized Cardiomyocytes

Passive force was similar in SH_{SED} (3.0±0.4 kN/m^2) and MI_{SED} (2.9±0.3 kN/m^2), but maximal isometric force (F_{max}) was significantly lower in MI_{SED} than in SH_{SED} (Figure 4A). The normalized force-pCa curves also showed a leftward shift in MI animals, indicating greater Ca^{2+}-sensitivity in MI_{SED} than in SH_{SED}, which was accompanied by an increased steepness (nHill) of the force-pCa curves in MI compared with Sham (2.4±0.1 versus 2.6±0.1 respectively, $P=0.05$). Treatment with the catalytic subunit of PKA decreased pCa_{50} in both MI_{SED} and SH_{SED}, reflecting a PKA-induced decrease in myofilament Ca^{2+}-sensitivity. Importantly, after PKA the Ca^{2+}-sensitivity was no longer different between MI_{SED} and SH_{SED} (Figure 4), suggesting that loss of PKA-mediated myofilament protein phosphorylation contributed to the increased myofilament Ca^{2+}-sensitivity after MI. PKA had no effect on F_{max} in MI_{SED} and SH_{SED} (not shown).

Exercise had no effect on passive force of cardiomyocytes from either Sham or MI mice (not shown). Exercise had also no effect on contractile properties of cardiomyocytes from Sham mice (Figure 4). However, exercise restored F_{max}, Ca^{2+}-sensitivity and nHill (2.4±0.1 versus 2.7±0.1 in MI_{SED} and MI_{EX} respectively, $P=0.02$) in MI mice. The effects of PKA on Ca^{2+}-sensitivity were now similar in Sham and MI animals.

MHC composition (% α-MHC) was not altered following MI, consistent with the maintained Ktr (Figure 5). Exercise had no effect on either MHC composition or Ktr.

Myofilament Proteins Phosphorylation Status

There were no significant differences in phosphorylation status of myofilament proteins MyBP-C, troponin I (TnI), myosin light chain (MLC-2) (Figure 4), troponin T (TnT) and desmin (not shown), between SH_{SED} and MI_{SED}. Exercise had no significant effect on the phosphorylation status of MyBP-C, TnI, TnT, and desmin, but increased MLC-2 phosphorylation in MI mice.

β_1-Adrenergic Signaling

Total PKA levels were not affected by MI and exercise training (Figure 4). Surprisingly, cAMP was also not different in MI_{SED} compared with SH_{SED}. However, exercise after MI significantly increased cAMP levels from 4.5±0.3 pmol/mg

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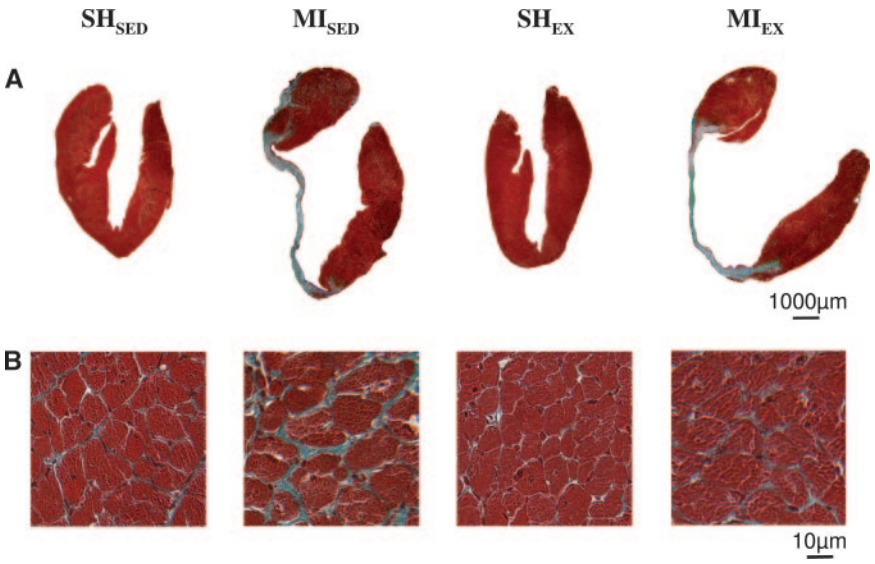


Figure 2. A, Masson's trichrome staining showing the green/blue infarct area in longitudinal cross-section of the LV, 8 weeks after permanent LAD ligation. MI includes the anterior wall and apical part of the LV. B, Magnifications of the Masson's trichrome image showing collagen and myocyte CSA in viable myocardium (remote zone in MI mice).

protein in MI_{SED} to 6.1±0.8 pmol/mg protein in MI_{EX} ($P<0.05$).

Western Immunoblotting

Protein level of the β_1-adrenergic receptor decreased after MI, but did not change in SH_{EX} mice. The decrease was not accompanied by significant changes in GRK2 and $G\alpha_{i\text{-}3}$ expressions (Figure 6). Protein levels of SERCA2a decreased in the remodeled myocardium after MI, but were not altered in SH_{EX} mice. PLB levels were maintained in both MI_{SED} and SH_{EX}. PLB phosphorylation at the Ser16 site did not change in both MI and exercise mice, whereas phosphorylation at the Thr17 site was decreased in both groups. Na^+/Ca^{2+}-exchanger levels did not change in MI_{SED} and SH_{EX} mice.

Exercise after MI increased β_1-adrenergic receptor levels and Na^+/Ca^{2+}-exchanger levels, but had minimal effects on the expression of GRK2, $G\alpha_{i\text{-}3}$, SERCA2a, and PLB and PLB phosphorylation at both Ser16 and Thr17 sites (Figure 5).

Contractile Properties of Isolated Intact Cardiomyocytes

To further investigate the mechanism by which exercise improved LV function in MI mice, we performed additional experiments in enzymatically isolated intact cardiomyocytes obtained from MI_{SED} and MI_{EX}. Unloaded cell shortening in MI_{EX} was significantly higher compared with MI_{SED} mice (Figure 7). Preliminary cell shortening data of 3 SH_{SED} mice showed values comparable to MI_{EX} (not shown). Basal $[Ca^{2+}]_i$

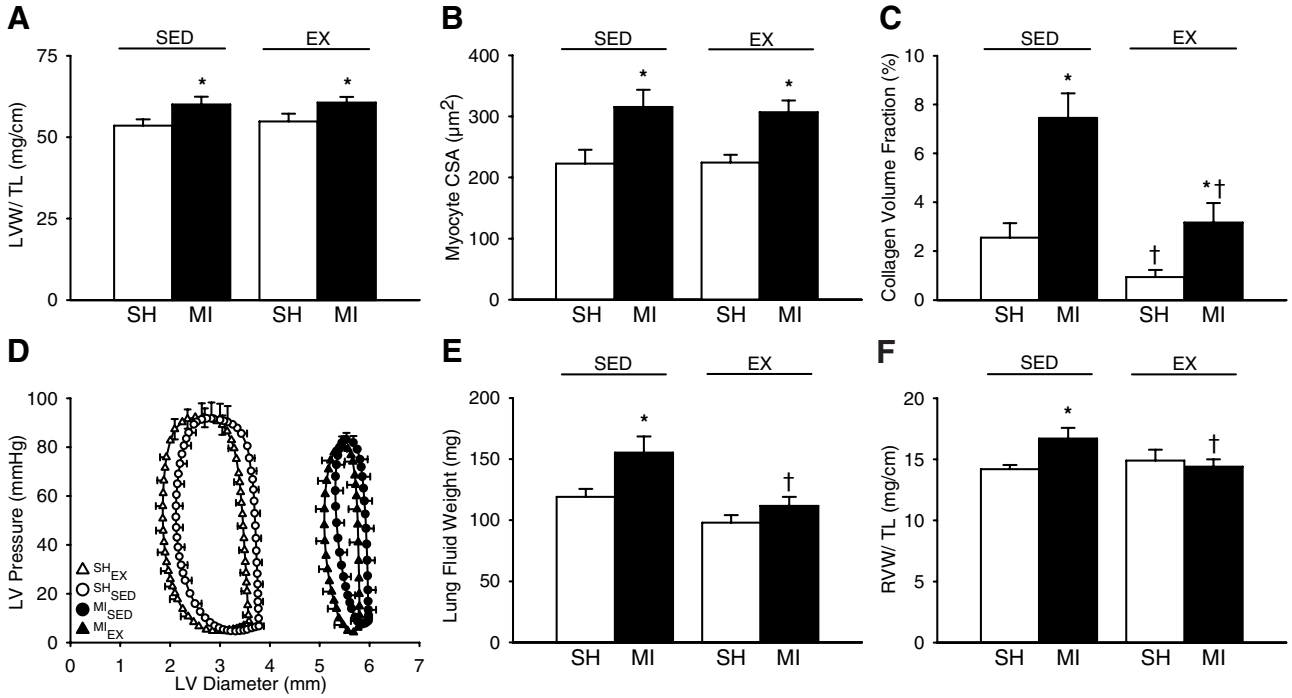


Figure 3. Effects of MI and exercise on relative LVmass (A), myocyte CSA (B), collagen volume fraction (C), LV pressure-diameter relation (D), lung fluid weight (E), and relative RVmass (F). *$P<0.05$ vs corresponding SH; †$P<0.05$ vs corresponding SED.

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LV Anatomical and Functional Data

	Sedentary	Exercise
Anatomical data		
Body weight, g		
Sham	26±1	25±1
MI	26±1	24±1
LV weight, mg		
Sham	96±4	95±4
MI	109±4*	110±4*
Endocardial infarct length, mm		
MI	3.8±0.3	3.5±0.4
Epicardial infarct length, mm		
MI	4.6±0.3	4.2±0.4
Infarct thickness, mm		
MI	0.11±0.01	0.13±0.02
Infarct area, mm^2		
MI	1.54±0.14	1.59±0.08
Hemodynamic data		
Heart rate, bpm		
Sham	537±8	555±12
MI	520±7	528±9
MAP, mm Hg		
Sham	70±2	79±5†
MI	69±2	71±1*
LVSP, mm Hg		
Sham	93±2	100±4
MI	83±2*	82±2*
LV dP/dt_{P30}, mm Hg/s		
Sham	7189±239	7669±267
MI	5295±207*	5891±204*†
Fractional shortening, %		
Sham	39±2	47±3†
MI	8±1*	12±1*†
LV dP/dt_{min}, mm Hg/s		
Sham	−7879±462	−9329±568
MI	−5184±285*	−5203±410*
Tau, ms		
Sham	7.9±0.5	8.1±1.0
MI	10.6±0.8*	10.5±1.1*
LVEDP, mm Hg		
Sham	6.4±0.6	4.3±0.9
MI	7.9±1.0	5.7±0.8

MAP, mean arterial pressure; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end diastolic pressure. SH_{SED} (n=27), SH_{EX} (n=11), MI_{SED} (n=27), and MI_{EX} (n=14). *$P<0.05$ vs corresponding Sham; †$P<0.05$ vs corresponding Sedentary.

was lower in MI_{EX} than MI_{SED}, but the $[Ca^{2+}]_i$ transient amplitudes were similar in the 2 groups.

Discussion

The present study investigated the impact of 8 weeks of voluntary exercise training, started early after a large MI, on LV remodeling and dysfunction in mice at the in vivo, cellular and molecular level. The main findings were that: (1) exercise had no adverse effect on LV dimensions and hypertrophy, while ameliorating LV dysfunction and backward failure; (2) exercise normalized MI-induced myofilament dysfunction, which likely contributed to the exercise-induced improvement in unloaded shortening of isolated intact cardiomyocytes, as the $[Ca^{2+}]_i$ transient amplitude was not altered by exercise. In addition, basal $[Ca^{2+}]_i$ was reduced by exercise; and (3) exercise likely mediated these effects via increased β_1-adrenoceptor protein and cAMP levels, and Na^+/Ca^{2+}-exchanger protein levels.

Pathophysiology of MI-Induced LV Dysfunction in Mice

In agreement with previous reports,[31,32,37] permanent LAD ligation in mice resulted in LV remodeling, characterized by LV dilation, hypertrophy, and increased collagen deposition in remote noninfarcted myocardium, and resulted in marked LV dysfunction, characterized by decrements in LV pump function (fractional shortening) and decrements in indices of global LV contractility (dP/dt_{P30}) and relaxation (dP/dt_{min} and τ), which was associated with LV backward failure reflected in pulmonary edema and RV hypertrophy. The mechanism for LV dysfunction after MI remains incompletely understood, but has been proposed to be the consequence of alterations in LV geometry with no effect on cardiomyocyte function or β_1-adrenergic responsiveness.[38] Conversely, other investigators reported that alterations in β_1-adrenergic signaling[21] and Ca^{2+}-handling[22,23] of the remote myocardium also contribute to global LV dysfunction. In agreement with the latter notion, we observed downregulation of β_1-adrenergic receptor levels and reductions in SERCA2a after MI, whereas no clear changes were found in protein levels of GRK2, $G\alpha_{i\text{-}3}$, PLB and Na^+/Ca^{2+}-exchanger. Unexpectedly, cAMP levels were not depressed in MI mice, which is consistent with the observation that cAMP-dependent PKA mediated phosphorylation of PLB-Ser16 was also not decreased after MI, but suggests that the β_1-adrenergic signaling in MI mice is not a simple function of the β_1-receptor density. PLB-Thr17 phosphorylation, which is mediated by CaM-kinase II, was attenuated following MI. Reduced phosphorylation of PLB-Thr17 will inhibit SERCA2a function in the mouse heart particularly at higher heart rates.[39] The latter could, in conjunction with decreased β1-adrenoceptor and SERCA2a expression, contribute to perturbations in cardiomyocyte Ca^{2+}-handling in MI mice, particularly during increased activity.

In agreement with observations in swine,[2] remodeling of noninfarct myocardium in mice was associated with altered myofilament function, characterized by decreased F_{max} and increased Ca^{2+}-sensitivity of tension development in single permeabilized cardiomyocytes. The small increase in Ca^{2+}-sensitivity after MI was likely the result of the minor reduction of PKA-mediated phosphorylation of TnI and MyBP-C,[2,40] as treatment with exogenous PKA abolished the difference in Ca^{2+}-sensitivity between Sham and MI animals. However, Pro-Q Diamond analysis did not reveal significant decreases in phosphorylation status of myofilament proteins in MI_{SED} compared with SH_{SED}. It is possible that a small

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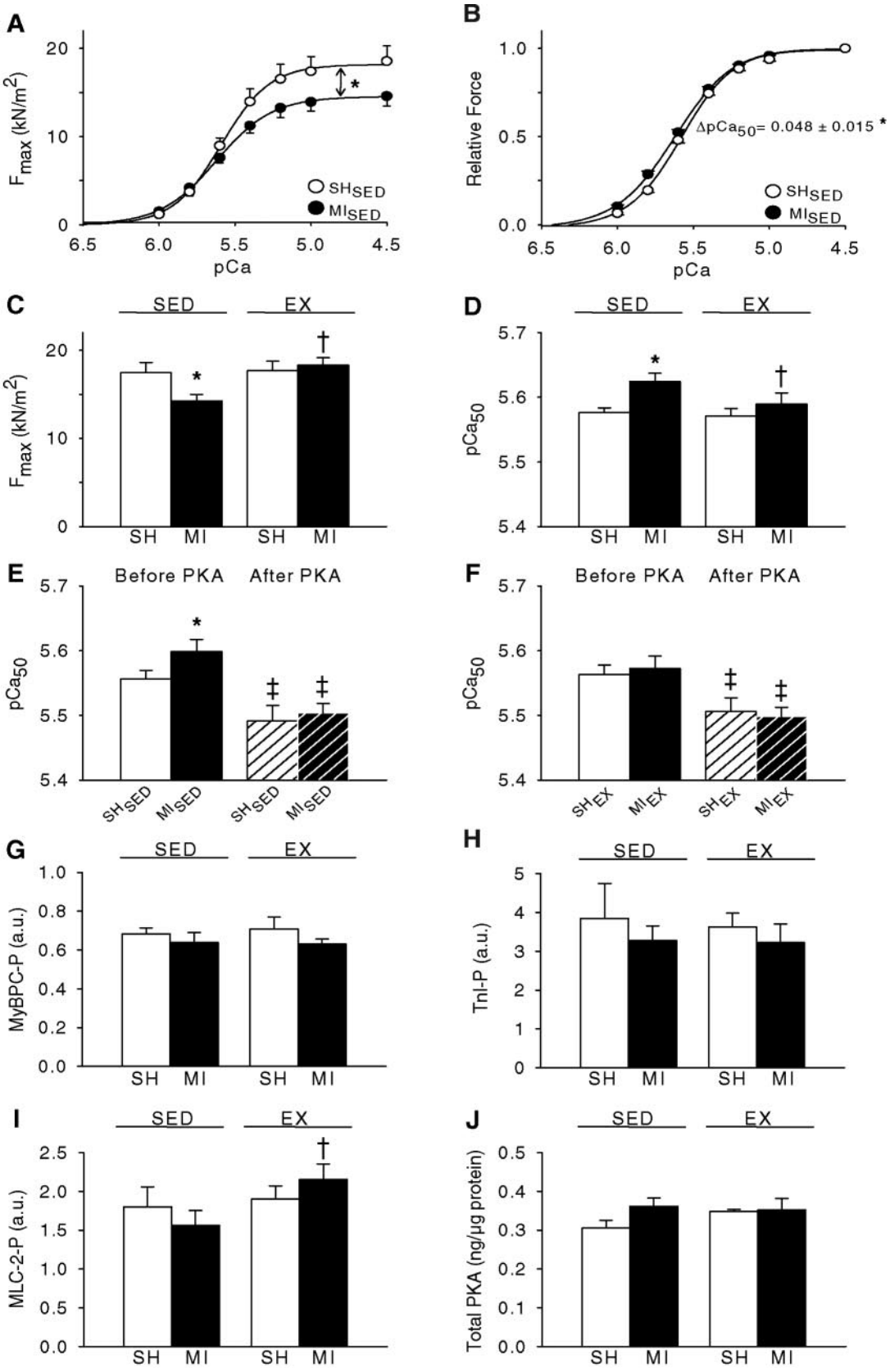


Figure 4. Absolute (A and C) and normalized (B and D) force-pCa curves and bar charts. The pCa_{50} before and after treatment with exogenous PKA (E and F). The panels (A, B, and E) show the effects of MI in SED mice. The panels (C, D, and F) show the effects of EX in SH and MI. Myofilament protein phosphorylation of MyBP-C (G), TnI (H), MLC-2 (I), and total PKA (J) in LV remote myocardium. *$P<0.05$ vs corresponding SH; †$P<0.05$ vs corresponding SED; ‡$P<0.05$ after PKA vs before PKA.

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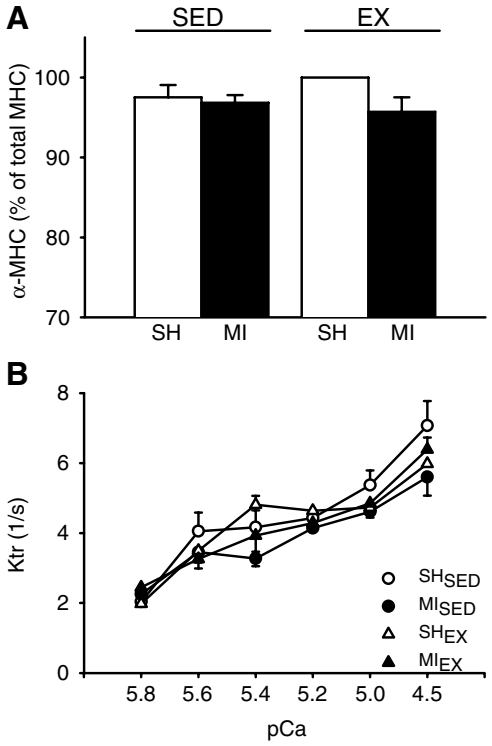


Figure 5. Effects of MI and exercise on relative α-myosin heavy chain (MHC) levels (A) and rate of force redevelopment (Ktr) at different Ca^{2+}-levels (B). No significant differences were observed.

decrease in PKA-mediated phosphorylation of TnI and MyBP-C was obscured by increased PKC-mediated phosphorylation of these myofilament proteins as increased PKC activity was observed in rat hearts within 1 to 8 weeks after MI.[41]

The mechanism underlying the MI-induced reduction in F_{max} is less clear. A role for degradation of TnI in reducing F_{max}, as suggested in pigs,[2] is unlikely in the post-MI remodeled mouse heart. First, no degradation products were observed in remodeled myocardium. Second, Narolska et al recently demonstrated that exchange of truncated TnI in human cardiomyocytes had no effect on F_{max}.[42] A likely alternative candidate responsible for the reduction in F_{max} is increased PKC-mediated phosphorylation, because the absence of a change in TnI phosphorylation in MI animals is consistent with increased PKC activity. Studies in rodent myocardium indicated a central role for PKC-mediated phosphorylation of TnI[43] and TnT[44] in decreasing F_{max}. Based on our Pro-Q analysis PKC-mediated TnT phosphorylation can be excluded as possible cause for the reduction in F_{max}, because there were no differences in TnT phosphorylation among the groups. Although speculative, our myofilament force measurements and phosphoprotein data could be interpreted to suggest that the increase in Ca^{2+}-sensitivity and the decrease in F_{max} are because of reduced PKA-mediated and increased PKC-mediated phosphorylation of TnI, respectively.

Surprisingly, we did not observe a shift from α-MHC to β-MHC protein expression, which is a post-MI hypertrophy marker in rats[45] and mice.[29] In contrast, protein levels of another hypertrophy marker ANP were elevated in remote LV of MI_{SED} (5.41±2.39 a.u.) compared with SH_{SED} (0.10±0.09 a.u.; $P=0.016$), correlating well with lung-fluid weight ($R^2=0.55$; $P=0.014$). An explanation for this unexpected lack of MHC-isoform shift is not readily found, but it should be noted that our observations are consistent with the unchanged Ktr after MI.

In conclusion, the present study supports the concept that alterations at the cellular level in remote noninfarcted myocardium contribute to decreased global LV function after MI. Future studies, using catecholamine challenges, are required to determine in greater detail the importance of perturbations in kinase-phosphatase signaling cascades in post-MI remodeled mouse heart.

Mechanism of Beneficial Effects of Exercise Training After MI

Rat studies on exercise after MI have reported no changes in parameters of LV function such as LV dP/dt_{max},[23,25] fractional shortening,[23] PV-relation[24] or cardiac output,[46] irrespective of whether exercise was started early[24,25] or late,[25,46] irrespective of a small[24,25] or large[25,46] MI, and irrespective of treadmill[23,46] or swim[24,25] training. Nevertheless, exercise was reported to improve cardiomyocyte function.[21–23,47] Thus, exercise after healed MI (>3 weeks after MI) attenuates β-MHC expression,[21] and restores cardiomyocyte Ca^{2+}-handling and Ca^{2+}-responsiveness, and SERCA2a and Na^+/Ca^{2+}-exchanger levels.[23] Furthermore, exercise was reported to blunt cardiomyocyte hypertrophy, and to restore Ca^{2+}-transients, and SERCA2a and Na^+/Ca^{2+}-exchanger expression.[22,47] Thus, beneficial effects of exercise on LV remote myocardium in rats are clearly observed when exercise is initiated late after MI. To date no study has investigated the effects of exercise started early after MI on β_1-adrenergic signaling, Ca^{2+}-handling and myofilament function.

In view of the concern that early exercise may aggravate LV remodeling after a large MI, we investigated the effects of exercise started immediately after MI on LV remodeling and dysfunction in the mouse. The results indicate that in mice, even after a large MI (comprising ≈43% of LV mass), 8 weeks of voluntary exercise does not aggravate LV remodeling, as relative LV mass and cardiomyocyte size as well as infarct geometry were unchanged, whereas exercise decreased collagen content and actually tended to decrease LV end-diastolic diameter. These observations are in agreement with a recent study by Konhilas et al[48] who reported that 8 weeks of moderate exercise in mice with hypertrophic cardiomyopathy reversed collagen deposition with little effect on cardiac hypertrophy. Interestingly, exercise also reversed expression of hypertrophy markers and components of apoptosis pathways. In view of the minimal effects of exercise on LV remodeling and the pronounced effects on LV dysfunction, we elected to focus on the effects of exercise training on myofilament function and Ca^{2+}-handling. However, the study by Konhilas et al warrants future studies that include the analysis of hypertrophy and survival signaling pathways in the model of post-MI remodeling.

Exercise attenuated LV dysfunction and ameliorated LV backward failure, which were likely because of improved cardiomyocyte function, as shortening of isolated cardiomyo-

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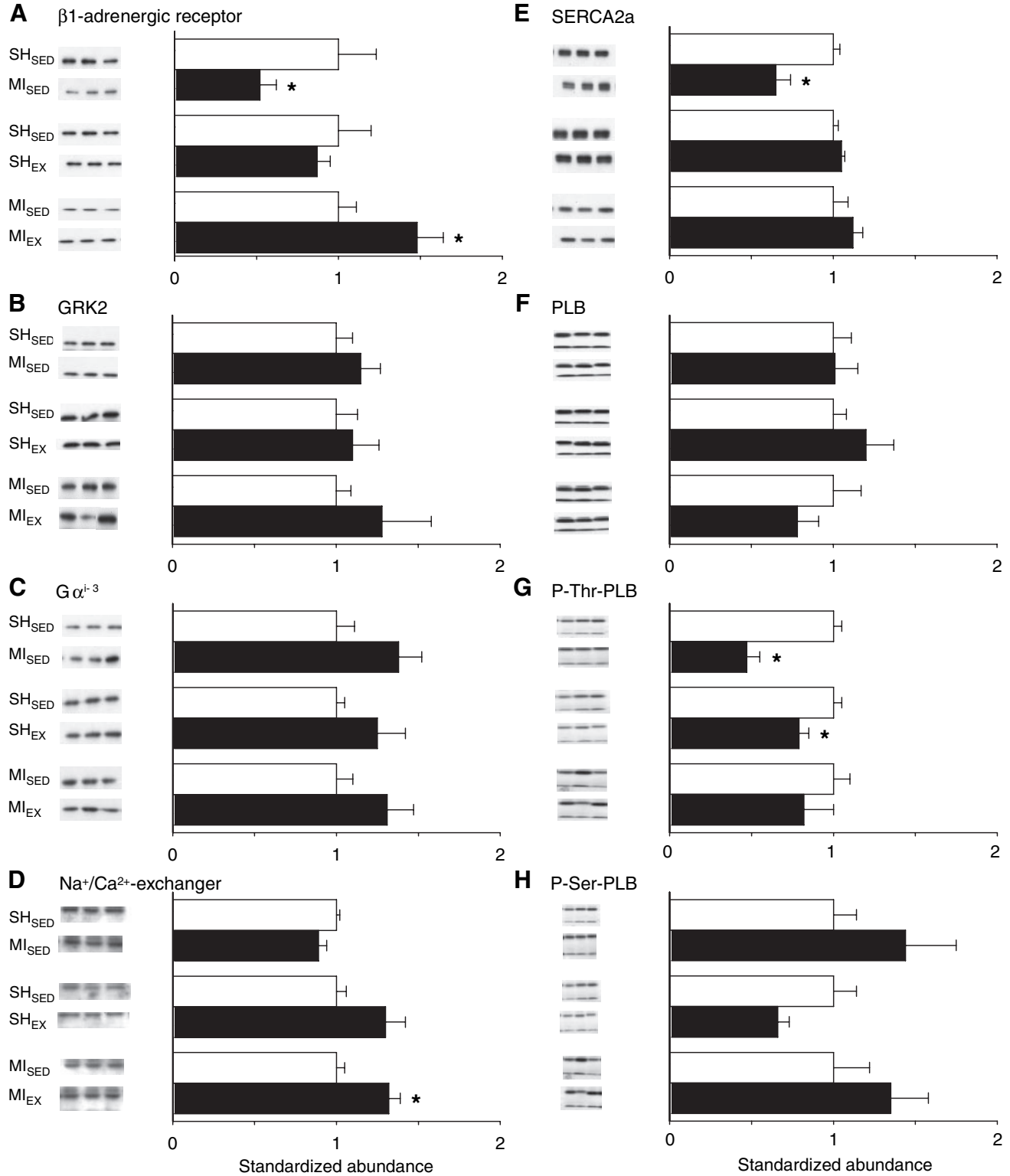


Figure 6. Immunoblot analyses of β_1-adrenergic signaling and Ca^{2+}-handling proteins of the four groups. Representative Western blots are shown, with summary quantification in bar graphs (n=5 for each bar). Comparisons were made in a pair-wise fashion (normalized within each pair). *$P<0.05$ vs corresponding white bar.

cytes was increased by exercise. Ca^{2+}-transient amplitude remained unaltered, consistent with the lack of effect of exercise on SERCA2a and PLB expression. However, basal (diastolic) calcium concentrations were reduced by exercise after MI. In the absence of changes in SERCA2a and PLB protein levels or phosphorylation, a potential explanation for the reduction in basal $[Ca^{2+}]_i$ is the increased expression of Na^+/Ca^{2+}-exchange. However, future studies are needed to determine whether this small increase in Na^+/Ca^{2+}-exchanger protein levels is indeed responsible for the exercise-induced

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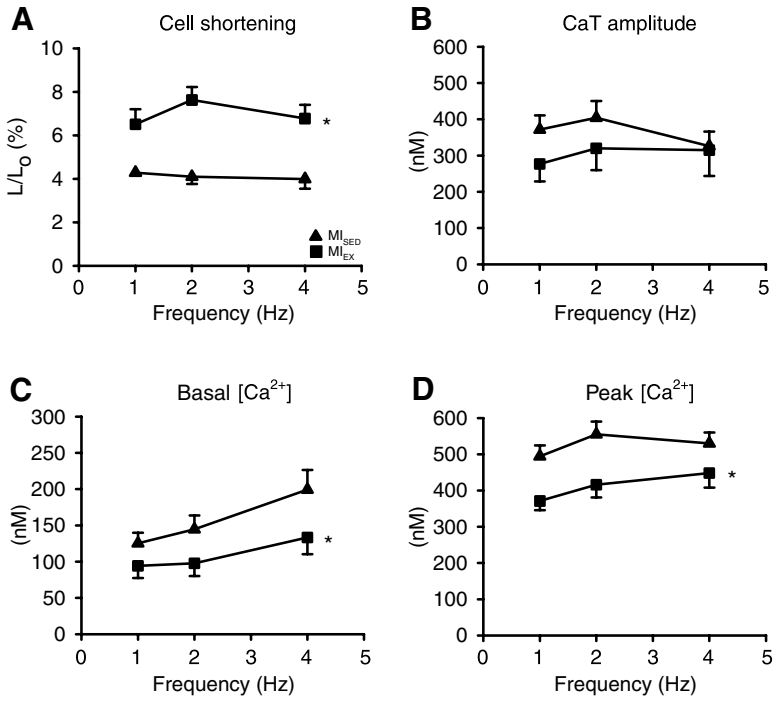


Figure 7. Single cell shortening during field stimulation at the indicated frequencies (A), $[Ca^{2+}]_i$ transient amplitude (B), basal (C), and peak (D) $[Ca^{2+}]_i$ values during whole cell current clamp recording at the same frequencies. *$P<0.05$ MI_{EX} vs MI_{SED}.

reduction in diastolic Ca^{2+} levels, or whether other mechanisms, including increased sarcolemmal Ca^{2+}-ATPase activity, also contribute.

Importantly, our findings indicate that the improved cell shortening was not because of increased Ca^{2+}-transient amplitude, suggesting that the exercise-induced normalization of myofilament function was principally responsible for the improved isolated myocyte shortening in vitro and global LV fractional shortening in vivo. Exercise restored myofilament Ca^{2+}-sensitivity after MI, which was likely mediated via increased β_1-adrenergic signaling as suggested by the increased β_1-adrenoceptor and cAMP levels and by the normalization of the Ca^{2+}-sensitivity response to PKA. Restoration of β_1-adrenergic signaling also acts to increase MLC-2 phosphorylation via PKA-mediated inhibition of protein phosphatase 1.[49] Indeed, MLC-2 phosphorylation was increased by exercise. It is however unlikely that the exercise-induced increase in MLC-2 phosphorylation is involved in either the increased F_{max} or reduced Ca^{2+}-sensitivity in MI_{EX}, because phosphorylation of MLC-2 increases myofilament Ca^{2+}-sensitivity without an effect on F_{max}.[50,51] Future studies are required to further delineate the mechanism underlying the exercise-induced normalization of F_{max}.

Clinical Implications

The present study indicates that exercise training started early after a large MI is beneficial, resulting in improved LV function and molecular phenotype, without adverse effects on LV remodeling. The beneficial effects appear to be the result of improved β_1-adrenergic signaling and myofilament function. Because some of these cellular adaptations to exercise are also observed following chronic β_1-adrenoceptor blockade,[52,53] future studies should be aimed at investigating whether combined β_1-adrenoceptor blockade and exercise yield added benefit.

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Disclosures

None.

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