Exercise attenuates inflammation and limits scar thinning after myocardial infarction in mice

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Exercise attenuates inflammation and limits scar thinning after myocardial infarction in mice. Am J Physiol Heart Circ Physiol 309: H345–H359, 2015. First published May 22, 2015; doi:10.1152/ajpheart.00683.2014.—Although exercise mediates beneficial effects in patients after myocardial infarction (MI), the underlying mechanisms as well as the question of whether an early start of exercise after MI is safe or even beneficial are incompletely resolved. The present study analyzed the effects of exercise before and reinitiated early after MI on cardiac remodeling and function. Male C57BL/6N mice were housed sedentary or with the opportunity to voluntarily exercise for 6 wk before MI induction (ligation of the left anterior descending coronary artery) or sham operation. After a 5-d exercise-free phase after MI, mice were allowed to reexercise for another 4 wk. Exercise before MI induced adaptive hypertrophy with moderate increases in heart weight, cardiomyocyte diameter, and left ventricular (LV) end-diastolic volume, but without fibrosis. In sedentary mice, MI induced eccentric LV hypertrophy with massive fibrosis but maintained systolic LV function. While in exercised mice gross LV end-diastolic volumes and systolic function did not differ from sedentary mice after MI, LV collagen content and thinning of the infarcted area were reduced. This was associated with ameliorated activation of inflammation, mediated by TNF-α, IL-1β, and IL-6, as well as reduced activation of matrix metalloproteinase 9. In contrast, no differences in the activation patterns of various MAPKs or adenosine receptor expressions were observed 5 wk after MI in sedentary or exercised mice. In conclusion, continuous exercise training before and with an early reonset after MI ameliorates adverse LV remodeling by attenuating inflammation, fibrosis, and scar thinning. Therefore, an early reonset of exercise after MI can be encouraged.

exercise; remodeling; scar thinning; magnetic resonance imaging; fibrosis; myocardial infarction

NEW & NOTEWORTHY

Our data provide new insights into the exercise-mediated cardioprotection by implying that an early reinitiation of exercise after myocardial infarction attenuates left ventricular fibrosis and scar thinning, presumably by attenuating the coordinated proinflammatory response involving TNF-α, IL-6, and IL-1β. These findings could translate into clinical benefits in patients.

CORONARY ARTERY DISEASE is the single most frequent cause of death worldwide. In Europe, every sixth man and every seventh woman will die of myocardial infarction (MI) (71). Those patients who survive MI frequently develop systolic heart failure due to the infarct-induced loss of functional myocardium per se and remodeling of the remainder of the left ventricular (LV) myocardium, which is frequently associated with thinning of the LV wall in the area of MI and its border zone, LV dilation, and systolic dysfunction. These parameters of myocardial remodeling are important prognostic markers for the long-term outcome of these patients. For instance, regional wall thinning is associated with postinfarction ventricular arrhythmia (36) and adverse outcome in patients undergoing coronary artery bypass surgery (87).

Scar formation occurs secondary to ischemia-induced cell death. After an acute MI, cardiomyocytes can undergo necrosis and/or apoptosis. Enhanced activity of matrix metalloproteinases (MMPs) and, in particular MMP-9, breaks down collagen, contributing to myocyte slippage and infiltration of inflammatory cells (50, 67, 68). This leads to phagocytosis of the necrotic myocardium and to the release of profibrotic factors such as TNF-α (74). The resulting fibroblast infiltration and proliferation induce the formation of scar tissue. At the same time, cardiomyocytes can undergo either apoptosis or hypertrophy in the ischemic border zones. Whereas the latter can be viewed as an attempt to compensate for the loss of viable myocardium by maintaining LV contractility, the former leads to infarct expansion and further aggravation of LV wall thinning (11, 74). The final remodeling phase is characterized by fibrosis and LV dilatation (29, 51, 67). Recent clinical studies involving cardiac MRI have indicated that the amount of scar tissue in the thinned region of the heart inversely correlates with the improvement of regional and global systolic function after revascularization of such chronic ischemic areas (65), indicating that, in particular, the nonfibrotic tissue may consist of hibernating myocytes that become revitalized and functional upon the restoration of blood flow (64). In fact, regional wall thinning can be reversible upon revascularization, presumably by the reversal of hibernation, and this reverse remodeling is associated with a favorable prognosis in patients after MI (9, 65).

Based on these clinical observations, basic and clinical research have been directed at preventing scar formation, wall thinning, and adverse cardiac remodeling after MI. The mechanisms of cardiac hypertrophy are complex and controlled by a myriad of partly redundant signaling pathways (27). Furthermore, a distinction can be made between physiological and pathological cardiac hypertrophy. In contrast to pathological hypertrophy, physiological hypertrophy, induced by regular and moderate endurance training, is neither associated with systolic or diastolic dysfunction nor with fibrosis (46, 49). Indeed, exercise improves cardiac performance and myocardial

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perfusion (48). Among others, the INTERHEART study (24) demonstrated that physical activity lowers the risk of MI. Therefore, it may become therapeutically important to reproduce physiological hypertrophy to mitigate maladaptive remodeling of the left ventricle (LV) after cardiac injury. However, various studies in animals and humans have shown conflicting results concerning the effect of endurance training after MI on LV remodeling, including beneficial (35, 55, 66, 83, 88), neutral (2, 21, 41, 56), and adverse (19, 34, 37) effects. European Society of Cardiology guidelines recommend exercise-based rehabilitation after an acute MI (class Ib). Indeed, exercise has been shown to reduce all-cause mortality and reinfarction and to improve quality of life after infarction (25, 39). In contrast, a recent study (72) failed to confirm the benefits of exercise-based rehabilitation. Besides the investigation of therapeutic effects of exercise on the remodeling process after MI, more recently, researchers have become interested in the effect of endurance training before MI and reported protective but also neutral effects (5, 7, 12, 70, 75).

To gain further insight into the mechanisms of how exercise can impact on scar formation and LV remodeling after MI in physically active individuals, we conducted a study in mice that underwent endurance training for 6 wk before and 4 wk after anterior MI. Using MRI analyses, we performed an indepth analysis of myocardial structure and function, with a special emphasis on wall thinning and remodeling of the anterior wall of the LV, and observed an exercise-mediated protection against infarct-induced thinning of the LV anterior wall. This was associated with downregulation of the proinflammatory cytokines TNF-α, IL-6, and IL-1β as well as a lack of MMP-9 activation, reduced collagen content in the LV, and slightly ameliorated reactivation of the fetal gene program. These data indicate that endurance training in patients before and after MI may have a beneficial effect on scar formation processes, which may contribute to the benefits provided by exercise in these patients.

### MATERIALS AND METHODS

**Model of MI.** Sedentary and runner groups were subjected to permanent ligation of the left anterior descending coronary artery (LAD) using a 6-0 thread to induce MI or sham operation, respectively. Under deep anesthesia [ketamine (10 mg/kg body wt) and xylazine (1 mg/kg body wt)], the thorax was opened in the third intercostal room, and the LAD was ligated ~2 mm below the left atrium. Occlusion of the LAD was confirmed microscopically by discoloration of the ischemic area below the ligation knot. The intercostal space, muscle layers, and skin were closed with continuous sutures with 6-0 silk. Sham animals underwent the same procedure without occlusion of the LAD. After surgery, sedentary groups were again kept under the usual care, whereas runner groups were housed after a 5-day exercise-free phase again with the opportunity of voluntary exercise for 4 wk before being characterized.

**MRI.** MRI examinations were performed with a horizontal-bore 9.4-T MRI animal scanner (94/20, BrukerBioSpin, Ettlingen, DE) using a high-performance water-cooled gradient system. Animals were subjected to general anesthesia initiated for 1 min in an induction chamber using a mixture of 3% isoflurane and 97% O₂ at a flow rate of 1.5 l/min. During the MRI procedure, anesthesia was applied with a reduced concentration of 1.0–1.5% isoflurane and 98.5–99% O₂ at a flow rate of 1.5 l/min using an animal nose mask. Core temperature, respiration rate, and ECG were monitored throughout the procedure. All animals were placed in a prone position in a dedicated animal cradle containing a four-element (2 × 2) phased-array surface coil (BrukerBioSpin). Physiological data were processed and monitored using an external computer with dedicated software (PC-SAM32, Sa Instruments, Stony Brook, NY). For cine imaging of the heart, prospectively triggered FLASH (fast low angle shot, repetition time: 5.9 ms, echo time: 2.0 ms, matrix: 192 × 192 pixels, field of view: 2 × 2 cm, section thickness: 1 mm, pixel size: 102 × 102 × 1,000 μm) sequences in two-chamber and four-chamber views were obtained. The maximum number of acquired movie frames was manually adjusted to the corresponding length of the RR interval obtained from the ECG. For the assessment of cardiac function, six to eight consecutive sequences/sections were recorded in the short-axis orientation covering the ventricles from base to apex. The acquired image data were transferred to an external workstation and analyzed using segment software (Segment Medviso AB, S). For the assessment of cardiac function, LV end-systolic volume (LVESV), LV end-diastolic volume (LVEDV) were measured by manual contouring the subendocardial interface to the intracavitary blood. Cardiac parameters were calculated as follows: stroke volume (SV) = LVEDV − LVESV, cardiac output = SV × heart rate, and ejection fraction = SV/LVEDV.

To investigate the impact of exercise before and after MI on cardiac architecture, two-dimensional MRI analyses of ventricular wall thickness in the whole LV were performed at the study baseline and endpoint. Depending on the heart size, due to the particular cardiac phenotype, six to eight consecutive sequences in short-axis orientation with a section thickness of 1 mm/sequence were required to cover the LV from the base (equal to sequence 1) to the apex (equal to sequences 6, 7, or 8). After the definition of end diastole and end systole in LV short-axis projections, the epicardium and endocardium were manually framed in each sequence, and the transverse projections were divided into wall regions. The particular wall thickness was measured during a whole cardiac cycle and averaged per sequence.

**Echocardiography.** Echocardiography was performed using the VisualSonicsVevo 770 imaging system (scanhead: RMV701B, 15–45 MHz, cardiac mouse). Anesthesia was induced for 1 min (3% isoflurane mixed with 97% O₂ at a flow rate of 1 l/min) in an induction chamber and maintained via inhalation of 1.0–1.5% isoflurane and 98.5–99% O₂ at a flow rate of 1 l/min using a nose mask. Core temperature and ECG signals were monitored throughout the procedure. Two-dimensional (B-mode) images were recorded in parasternal long- and short-axis projections with guided one-dimensional M-mode recordings at the midventricular level, apical of the papillary...
A: Study design, survival, and verification of the prohypertrophic animal model. Male C57Bl/6 mice (6–8 wk), housed sedentary or with an opportunity of voluntary exercise (runner group) for 6 wk and characterized via MRI and echocardiographic baseline measurements, were subjected to myocardial infarction (MI) induction (MI-sedentary or MI-runner groups) or sham operation (sham-sedentary or sham-runner groups). Sedentary mice were again kept under the usual care, and exercising animals were allowed to resume voluntary exercise after a 5-day exercise-free phase after surgery. After the 4-wk end point, MRI and echocardiography were performed before mice were euthanized and characterized ex vivo.

B: Effect of start point of exercise reonset after MI induction on the percent survival rate in MI-runner and MI-sedentary groups.

C: Effect of permanent MI on cardiac phenotype in wild-type runner and sedentary mice as assessed morphologically by heart weight (i), heart weight-to-tibia length ratio (HW/TL; ii), and left ventricular (LV) end-diastolic internal diameter (LVEDD; iii) and histologically by cardiomyocyte (CMY) diameter (iv).

D: Extent of functional and structural post-MI remodeling in runner and sedentary mice with small, medium, and large MIs as assessed by fractional shortening (FS) and LVEDD. Sham mice served as controls. Values are shown as means ± SE; experiment numbers are indicated in bars. *P < 0.05; **P < 0.01.
Excercise limits post-MI scar thinning

Muscle in both views. Standard measurements of LV end-systolic diameter (LVESV) and LV end-diastolic diameter (LVEDD) were performed in parasternal long-axis projections during at least three beats from this projection and averaged. ECG and respiration gating were used to suppress movement artifacts.

**Tissue sampling and conservation.** Mice were euthanized by an intraperitoneal injection of a mixture of ketamine hydrochloride (100 mg/kg) and xylazine hydrochloride (10 mg/kg). After a left thoracotomy, hearts were explanted, the atria were removed, and the myocardium was washed, weighed, and split for biochemical and histological analyses (midventricular slice). LV tissue was frozen in liquid N₂ immediately after explantation for biochemical analyses and then stored at −80°C before RNA and protein isolation. Midventricular slices for histological analyses were fixed in 4% formaldehyde within 1 min after explantation of the heart and stored at 4°C.

**Quantitative PCR analysis.** For PCR analysis of whole cell lysates, LV tissue was pelleted, lysed, and prepared as previously described (22). cDNA gained from 1 µg mRNA was diluted 10-fold for quantitative PCR analysis and 5-fold for TaqMan PCR. PCRs were run in triplicates. mRNA expression was normalized per sample against Gapdh, and relative quantitation of gene expression was calculated using the comparative ΔΔCₜ method (where C₀ is threshold). All data based on expression analyses are expressed as fold changes over the sham-sedentary group.

Primers for mouse cDNA, purchased from TIB Molbiol (Berlin, DE), were as follows: α-myosin heavy chain (MHC), forward 5′-AATCTACCACTTCCTAC-3′ and reverse 5′-TAGTGTAGTGTTGTC-3′; β-MHC, forward 5′-GCTTTAGGGCCGACATTTG-3′ and reverse 5′-GTTGCTATTCCGAACCTGTTTTG-3′; atrial natriuretic factor (ANF), forward 5′-GGTCTGCTTCTCATCACC-3′ and reverse 5′-CGGCTCTTCCTCCATCAG-3′; adenosine A₁ receptor (A₁R), forward 5′-ATCCCCTCTCGGATACAGCTG-3′ and reverse 5′-ACTAGGGTTCTTCACGAAAAC-3′; adenosine A₂a receptor (A₂aR), forward 5′-CCTATCCCTGTTACTGAGATC-3′ and reverse 5′-CAGTTGTTCCAGCAGAACAT-3′, adenosine A₂b receptor (A₂bR), forward 5′-TCTTCTCTCCGCTGTTG-3′ and reverse 5′-CCAGTGGACAAACCTTTATACCTGGA-3′; adenosine A₃ receptor (A₃R), forward 5′-ACTCTGATGGTTCTTCACGAAAAC-3′, and reverse 5′-CAACCACTCCTCCACCTTGGT-3′; and Gapdh, forward 5′-CAACCACTCCTCCACCTTGGT-3′ and reverse 5′-CAACCACTCCTCCACCTTGGT-3′.

**Western blot analysis.** For Western blot analysis of whole cell lysates, LV tissue was pelleted, lysed, and prepared as previously described (43). Primary antibodies were purchased from Santa Cruz Biotechnology (Santa Cruz, CA; A₂aR, A₂bR, A₃R, and p70S6K, 1:1,000), Alpha Diagnostics (Brussels, BE; α-R, 1:500), Dionova [Hamburg, DE; calsequestrin (CASQ2), 1:2,500], R&D (Wiesbaden, DE; GSK3β, MMP-2, and MMP-9, 1:500). Secondary antibodies were purchased from Sigma-Aldrich. Protein expression was quantified densitometrically using Labworks 4.5 software and normalized against CASQ2. All data based on expression analyses are expressed as fold changes over the sham-sedentary group.

**Histological analysis.** For morphometric analyses, transverse midventricular tissue slices (5 µm) of formaldehyde-fixed and paraffin-embedded heart tissue were stained with hematoxylin and eosin or 0.1% sirius red as previously described (52). LUCIA software (Nikon) was used for the quantification of collagen in the infarction area (as a measure for scar size) and of interstitial fibrosis in the remote LV in at least 15 sections/midventricular tissue slice. Mice were categorized into groups with collagen content of 15%–40% (small infarction), 41%–55% (medium infarction), and 56%–85% (large infarction) in the LV anterolateral wall. For measurements of cardiomyocyte diameter and cross-sectional area in vertically oriented hematoxylin and eosin-stained cardiomyocytes, at least 50 cardiomyocytes were measured per heart using LUCIA software (Nikon).

**Statistics.** Continuous data are presented as means ± SE. To determine differences between two groups, an unpaired or paired Student’s t-test was used, as appropriate. Differences between four groups were determined using one-way ANOVA (group comparison involving one independent variable) or two-way ANOVA (group comparison involving two independent variables, each with two conditions), as appropriate, followed by Tukey’s posttest. All calculated P values are two-sided. Differences with P values of <0.05 were considered to be statistically significant. All analyses were performed with Microsoft Excel 2007 and GraphPad Prism 6.0 software.

**RESULTS**

Exercise does not affect the MI-induced hypertrophic response. Mice were exposed to voluntary exercise on a treadmill for 6 wk before MI or sham operation and for 4 wk after the intervention (Fig. 1A). In preliminary experiments, we observed excess mortality when mice returned to voluntary exercise only 1 day after MI and, therefore, a 5-day exercise-free interval was included before reexposure to voluntary exercise (Fig. 1, A and B). Exercise per se induced physiological LV hypertrophy in the sham-runner group with an increase in heart weight, heart weight-to-tibia length ratio, and LVEDD compared with sedentary mice (P < 0.05; Fig. 1, C,i–iii, and Table 1). This physiological hypertrophy occurred in the absence of any fibrosis. MI also led to a hypertrophic response (organ weight and cardiomyocyte diameter in histological analyses) in both running and sedentary mice, whereas only sedentary mice showed a significant MI-induced increase in fractional shortening and heart rate.

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sedentary Group</th>
<th>Runner Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Number of animals/group</td>
<td>26</td>
<td>22</td>
</tr>
<tr>
<td>LV end-diastolic diameter, µm</td>
<td>4.34 ± 0.55</td>
<td>4.05 ± 0.10†</td>
</tr>
<tr>
<td>LV end-systolic diameter, µm</td>
<td>3.42 ± 0.06</td>
<td>3.84 ± 0.06‡</td>
</tr>
<tr>
<td>Fractional shortening, %</td>
<td>21.9 ± 0.74</td>
<td>17.75 ± 0.75</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>467 ± 8</td>
<td>468 ± 10</td>
</tr>
</tbody>
</table>

Values are means ± SE. MI, myocardial infarction; LV, left ventricular. †P < 0.01 vs. baseline; ‡P < 0.05 and §P < 0.01 vs. the sedentary group.
In contrast, ANF expression did not increase in running of the adult mice with large MIs but was unaffected in exercised mice (Fig. 2). In the MI-sedentary group, mRNA expression of ANF increased infarct size dependently (Fig. 2A). In light of these rather small effects of exercise on hemodynamic parameters, we performed a more detailed analysis of the morphological remodeling of the LV. Exercise led to a robust reduction in the collagen content of the whole LV and in the scar region after MI, with unchanged collagen content in the remote areas (Fig. 3A). MI led to a significant reduction of the anterior wall thickness in the infarct area as a sign of scar formation (Fig. 3B). This wall thinning through scar formation was more pronounced in mice with large MIs (Fig. 2). Furthermore, LVESD, which integrates LV dilation and systolic dysfunction, increased infarct size dependently, independent of the size of the MI (Fig. 2).

**Moderate attenuation of MI-induced fetal gene reexpression through exercise.** In the MI-sedentary group, mRNA expression of ANF increased infarct size dependently (Fig. 2A). Although no differences in the expression of the adult α-MHC isoform were observed (Fig. 2C), there was an infarct size-dependent shift of the β-MHC-to-α-MHC ratio toward the fetal isoform in sedentary mice and was most pronounced in mice with large MIs (Fig. 2A). In agreement with echocardiographic and morphometric data (Table 1 and Fig. 1), LV mass, LVEDV, and LVESV increased after MI in sedentary mice, whereas in exercised mice, no increases were observed, although this lack of increase was rather related to an exercise-induced increase in these parameters after 6 wk of exercise before MI, with no differences in dimensions compared with sedentary mice at the end of the study (Table 2). Again, no significant differences in LV ejection fraction or cardiac output were observed across all groups and time points.

**Exercise reduces LV fibrosis after MI.** In light of these rather small effects of exercise on hemodynamic parameters, we performed a more detailed analysis of the morphological remodeling of the LV. Exercise led to a robust reduction in the collagen content of the whole LV and in the scar region after MI, with unchanged collagen content in the remote areas (Fig. 3A). MI led to a significant reduction of the anterior wall thickness in the infarct area as a sign of scar formation (Fig. 3B). This wall thinning through scar formation was more pronounced in mice with large MIs (Fig. 2). Furthermore, LVESD, which integrates LV dilation and systolic dysfunction, increased infarct size dependently, independent of the size of the MI (Fig. 2).

**Exercise does not influence LV dilatation or cardiac function after MI.** To assess the effect of exercise on cardiac morphology and function more thoroughly, we performed cardiac MRI shortly before MI or sham operation (referred to as baseline, which was already 6 wk after the initiation of exercise before MI in the runner group; Fig. 1A). In agreement with echocardiographic and morphometric data (Table 1 and Fig. 1), LV mass, LVEDV, and LVESV increased after MI in sedentary mice, whereas in exercised mice, no increases were observed, although this lack of increase was rather related to an exercise-induced increase in these parameters after 6 wk of exercise before MI, with no differences in dimensions compared with sedentary mice at the end of the study (Table 2). Again, no significant differences in LV ejection fraction or cardiac output were observed across all groups and time points.

**Exercise limits post-MI scar thinning**

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**Fig. 2.** Exercise attenuates reexpression of fetal genes after small and medium MI. A–D: effect of different infarction sizes on mRNA expression of atrial natriuretic factor (ANF; A), β-myosin heavy chain (MHC; B), α-MHC (C), and the β-MHC-to-α-MHC ratio (D) in sedentary (i) and runner (ii); 6 wk before and 4 wk after MI induction) mice. Values are normalized over sham-sedentary mice and shown as means ± SE; experiment numbers are indicated in bars. *P < 0.05; **P < 0.01; ***P < 0.005.
EXERCISE LIMITS POST-MI SCAR THINNING

Table 2. Exercise before and after MI does not influence LV dilation or cardiac function as assessed by MRI

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Sedentary Group</th>
<th>Runner Group</th>
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</thead>
<tbody>
<tr>
<td></td>
<td>Baseline</td>
<td>5 wk after MI</td>
</tr>
<tr>
<td>Number of animals/group</td>
<td>6</td>
<td>7</td>
</tr>
<tr>
<td>LV mass, mg</td>
<td>61.62 ± 2.91</td>
<td>89.03 ± 7.99*</td>
</tr>
<tr>
<td>LV mass, µl</td>
<td>58.69 ± 2.77</td>
<td>84.79 ± 7.61*</td>
</tr>
<tr>
<td>LV end-diastolic volume, µl</td>
<td>53.97 ± 1.34</td>
<td>103.53 ± 17.29*</td>
</tr>
<tr>
<td>LV end-systolic volume, µl</td>
<td>29.15 ± 2.63</td>
<td>74.55 ± 18.70*</td>
</tr>
<tr>
<td>Stroke volume, µl</td>
<td>24.82 ± 1.79</td>
<td>36.44 ± 1.68*</td>
</tr>
<tr>
<td>Cardiac output, ml/min</td>
<td>13.45 ± 0.94</td>
<td>16.38 ± 1.02</td>
</tr>
<tr>
<td>Ejection fraction, %</td>
<td>46.40 ± 4.14</td>
<td>41.35 ± 5.82</td>
</tr>
<tr>
<td>Heart rate, beats/min</td>
<td>548 ± 9</td>
<td>449 ± 16‡</td>
</tr>
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</table>

Values are means ± SE. *P < 0.05, †P < 0.01, and ‡P < 0.005 vs. baseline; §P < 0.05 vs. the sedentary group.

The main findings of the present study are that endurance training before and after MI reduced the inflammatory response mediated by TNF-α, IL-6, and IL-1β, which was associated
with reduced activation of MMP-9, ameliorated LV fibrosis, and the prevention of scar thinning in the infarct area. Furthermore, the reactivation of the fetal gene program was moderately reduced. In contrast, MAPK activation patterns were not differentially regulated after MI in sedentary and exercised mice, whereas upregulation of adenosine receptors after exercise and before MI may have contributed to the initiation of the anti-inflammatory effects of exercise. Our results suggest that the reinitiation of exercise can be recommended to patients relatively early after MI.

A

**A** i

LV collagen content [%]

Sham-Sedentary  Mi-Sedentary  Mi-Runner

Sham-Sedentary  Mi-Sedentary  Mi-Runner

ii

Collagen content in the infarct area [%]

Mi-Sedentary  Mi-Runner

Mi-Sedentary  Mi-Runner

iii

Collagen content in the remote area [%]

Mi-Sedentary  Mi-Runner

Mi-Sedentary  Mi-Runner

iv

B

**B** i

LVAW; Sedentary

Baseline (n=6)  5 weeks post MI (n=7)

LVAW; Runner

Baseline (n=6)  5 weeks post MI (n=8)

***  *  *  *

ii

Average wall thickness in the infarct area [mm]

LVAW

Sedentary

Runner

Baseline  5 weeks post MI

iii

C

**C**

End-diastole

Sedentary

Runnner

End-systole

Baseline  5 weeks post MI

Baseline  5 weeks post MI

Baseline  5 weeks post MI
Effects of exercise on LV hypertrophy and structural remodeling. MI-induced cardiomyocyte hypertrophy and dilatation of the LV remained largely unaffected by physical activity. However, it is unclear to what extent the hypertrophic response in the MI-runner group represented an adaptive or maladaptive response, since LV systolic function was not altered by MI, which limits the conclusions about adaptive and maladaptive hypertrophy. These observations concur with previous studies investigating the effect of exercise after MI on the hypertrophic response in rodents (13, 14, 33) and humans (21, 32, 56). In contrast, Rengo et al. (62) documented increased LV hypertrophy after 10-wk endurance training after MI in mice. These diverging observations may be attributed to different training protocols. In studies that have documented no aggravation of hypertrophic features as a result of exercise, the voluntary and therefore moderate training period was initiated immediately after MI or no later than 7 days after surgical intervention. In contrast, Rengo et al. (62) made use of a daily treadmill training of defined duration and O2 consumption, starting much later (4 wk) after MI induction, when the maladaptive remodeling process may already have been established. Some studies in animal models and humans have reported exercise-mediated adverse effects on LV dilatation when started early after a large MI (19, 37) and neutral or beneficial effects when initiated late after large MI (41, 55, 62). However, it has to be taken into consideration that adverse effects of endurance training on LV dimensions were only documented in mice subjected to swimming, which may be a more intense (and potentially harmful) stress compared with voluntary treadmill running (19). In our study, 6 wk of exercise before and 4 wk after MI did not affect systolic function after MI. Other experimental studies have reported an improvement of LV ejection fraction after 8–10 wk of exercise (13, 33) when initiated early after MI and no effect when initiated late after MI (62). Taken together, conflicting results, including beneficial, neutral, and adverse effects, of exercise on after MI

Fig. 4. Exercise attenuates matrix metalloproteinase (MMP)-9 activation after MI. A: effect of permanent MI in wild-type sedentary and runner mice on mRNA expression of the fibrosis markers transforming growth factor (TGF)-β (i), connective tissue growth factor (CTGF; ii), and collagen type Iα2 (iii). B and C: effect of permanent MI in wild-type sedentary and runner mice on protein expression of latent (i) and active (ii) isoforms of MMP-2 (B) and MMP-9 (C). Representative Western blots and the corresponding housekeeping protein calsequestrin (CASQ2) expression are shown below. Values are normalized over sham-sedentary mice and are shown as means ± SE; experiment numbers are indicated in bars. *P < 0.05.
Fig. 5. Exercise reduces the inflammatory response after MI. A–C: effect of permanent MI on wild-type sedentary and runner mice on mRNA expression of the proinflammatory factors TNF-α (A), IL-6 (B), and IL-1β (C) independent of infarct size (i), dependent on infarction size (ii and iii), and in subgroups with large infarctions (iv). Values are normalized over sham-sedentary mice, expressed as log_{10} values, and shown as means ± SE; experiment numbers are indicated in bars. *P < 0.05; **P < 0.01. D: correlation between TNF-α mRNA expression and IL-1β (i), IL-6 (ii), and collagen type 1α2 (iii) mRNA expression and echocardiographically determined LV end-systolic volume (LVESV; iv).
Fig. 6. No obvious role for phosphatidylinositol 3-kinase (PI3K) or MAPK activation in exercise-mediated protection. A–E: effect of permanent MI in wild-type sedentary and runner mice on total protein expressions (i), phosphorylation profiles (ii), and phosphorylation profile-to-total expression ratios (iii) of Akt (A), p70S6K (B), glycogen synthase kinase (GSK3β) (C), and ERK44 (D)/ERK42 (E). p, phosphorylated. Representative Western blots and corresponding housekeeping protein CASQ2 expression are shown (iv). Sham mice served as controls. Values are normalized over sham-sedentary mice and shown as means ± SE; experiment numbers are indicated in bars. *P < 0.05.
remodeling appear to be related to 1) variations in the training methods, 2) the exercise-free interval immediately after MI, and 3) the training durations before and after MI.

Effects of exercise on fibrosis and scar thinning. Despite only minor effects of exercise on LV hemodynamics and the reactivation of fetal genes, a key morphological observation was that scar thinning, detected by advanced MRI techniques, was reduced in exercised compared with sedentary mice. Whereas a similar observation was made previously by histological analyses (12), imaging studies using one-dimensional echocardiographic projections have failed to detect this subtle yet clinically potentially important information (2, 19, 34, 85). In fact, regional wall thinning predicts ventricular arrhythmias and adverse outcomes in patients with coronary artery disease (36, 87). Furthermore, while scar thinning may potentially induce lethal ventricular ruptures after MI, we do not have evidence that this occurred in our mice after MI. The fact that LV systolic function was largely preserved despite LV dilatation indicates that, overall, infarct sizes may have been moderate and, therefore, rupture did not occur.

Exercise reduced the inflammatory response after MI. As a potential underlying mechanism of decreased scar thinning, LV collagen synthesis in the infarct area was significantly attenuated in exercised mice. This is in accordance with other reports (70, 86). Matrix turnover is regulated by several factors, among which MMPs play a major role (51, 61, 67, 78). In fact, we observed that exercise prevented the activation of MMP-9, whereas the activation of MMP-2 as well as the expression of both MMPs were not affected by exercise. As an upstream event to induce fibrosis, inflammation is well characterized, and, in fact, we observed strong activation of inflammatory cytokines such as TNF-α, a potent paracrine activator.

Fig. 7. Exercise upregulates adenosine receptors. A and B: effect of permanent MI in wild-type sedentary and runner mice on mRNA (A) and protein (B) expression of the following adenosine receptor subtypes: A<sub>1</sub> (A<i>1</i>R; i), A<sub>3</sub> (A<i>3</i>R; ii), A<i>2a</i> (A<i>2a</i>R; iii) and A<i>2b</i> (A<i>2b</i>R; iv). Representative Western blots of receptors and corresponding housekeeping protein CASQ2 expression are shown below. Sham mice served as controls. Values are normalized over sham-sedentary mice and shown as means ± SE; experiment numbers are indicated in bars. *P < 0.05; **P < 0.01.
of MMP-9, and also IL-6 and IL-1β in sedentary mice after MI, whereas this coordinated proinflammatory response was substantially reduced in exercised mice, particularly in those with large MIs. The fact that TNF-α upregulation correlated with the upregulation of ANF, collagen type 1α2, and the increase in LVESV suggests that inflammation is an important signaling mechanism that contributes to LV remodeling processes.

Anti-inflammatory effects of exercise per se have been already widely discussed (28, 60). In the context of myocardial injury, it has been previously reported that endurance training reduces TNF-α levels in plasma of patients with heart failure (1) and in the soleus muscle of infarcted rats (3). It has been suggested that IL-6 produced in skeletal muscle during exercise triggers the production/release of systemic anti-inflammatory cytokines, such as IL-10 and IL-1 receptor antagonists that prevent TNF-α secretion (6, 54). Reactivation of fetal genes, cardiomyocyte hypertrophy, apoptosis, and the subsequent LV dilatation and dysfunction, all contributing to the transition from compensated hypertrophy into decompensated heart failure, are associated with an initial increase in proinflammatory factors (44). Based on our observations, we suggest that attenuated fibrosis formation and scar thinning in the infarcted LV anterior wall could be explained by the exercise-mediated prevention of MMP-9 activation, which, in turn, could be due to the inhibition of its potent activators TNF-α, IL-6, and IL-1β, and vice versa, the MMP-9-mediated feedback mechanism facilitating further release of active TNF-α would be impaired (20). This hypothesis is supported by the fact that proinflammatory and profibrotic signaling cascades share several components, such as NF-κB activation in cells infiltrating the infarct area (neutrophils during the first 3 days after MI followed by macrophages on days 3–7), leading to increased expression and the release of proinflammatory and growth-promoting factors, such as TNF-α, ILs, and TGF-β. Subsequently, elevated MMP activity, in particular MMP-9, scar formation, prominent fibrosis, and ventricular dilatation characterize the transition from proinflammatory events into reparative responses (15). One possible mediator of the beneficial effects on LV remodeling induced by exercise could be adenosine. In the rat heart, it has been shown that endurance training leads to increased activity of 5′-ectonucleotidase, a key regulator of adenosine metabolism (38). Adenosine is known to reduce infarct size, increase coronary flow, and attenuate myocardial fibrosis (23, 42, 45) in the context of ischemic preconditioning and cardiac remodeling and to prevent TNF-α-induced cardiomyocyte hypertrophy (40). Furthermore, we have previously identified adenosine as an important endogenous regulator of TNF-α expression and MMP-9 secretion, probably by activation of its A2aR subtype, involving the cAMP/PKA pathway (18, 77, 79). Therefore, it may be speculated that the exercise-mediated increase in the expression of A2aR (observed in the sham-runner group) may have provided a "preconditioning"-like effect in mice before MI, preventing TNF-α upregulation and the subsequent activation of maladaptive signaling pathways, such as NF-κB-mediated MMP-9 transcription and proinflammatory cytokine production.

No discernible role of MAPK activation in exercise-mediated protection. Expression analyses of well-known mediators of prohypertrophic responses, such as ERK (47) and downstream targets of the phosphatidylinositol 3-kinase (PI3K) pathway, did not show any differences between the groups. We observed that Akt/GSK3β/p70S6K signaling contributes to the development of the physiologically and pathologically hypertrophied phenotype, whereas ERK seems to be mainly activated by maladaptive hypertrophy. Kemi and colleagues (31) have suggested that Akt activation diverges physiological hypertrophy induced by treadmill running from pathological hypertrophy, induced by transverse aortic constriction. In our hands, pAkt/Akt was upregulated in pathologically hypertrophied hearts of MI-sedentary mice as well as in physiologically hypertrophied hearts in sham-runner mice compared with sham-sedentary mice. The lack of an increased activation in the combined MI/exercise group might be explained by the different pathways induced by the different hypertrophy triggers. In fact, physiological stimuli like exercise act via IGF-I/PI3K and Akt isoforms and their roles in mediating physiological versus pathological remodeling (69).

Adenosine receptors are upregulated by exercise: a potential link to anti-inflammation? The expression of all four adenosine receptors was enhanced after exercise. However, at the protein level, only the A2aR subtype was upregulated. Surprisingly, the expression levels of all adenosine receptors, and in particular A2aR, were reduced back to baseline after MI. Under physiological conditions, A2aR is involved in regulating energy demand and supply by mediating vasodilatation, angiogenesis, vasculogenesis, inhibition of inflammation (e.g., by repressing TNF-α), and modification of adrenergic signal transduction (23). Since these are all typical features of exercise-induced adaptive remodeling, it is plausible that A2aR stimulation may have contributed to these effects. Increased activity of CD73 (5′-ectonucleotidase), the main regulator of adenosine metabolism, has been documented in exercising rats (38). We and others have shown that adenosine receptor expression can rapidly be increased in the presence of adenosine itself or stress induced by the addition of lipopolysaccharide (16, 17, 53, 76, 80).

This is the first report showing that adenosine receptors are upregulated by continuous exercise. However, the stimulus may not be strong enough to persist after a major stress, such as MI. The exact role of adenosine and its receptors in the setting of exercise-mediated cardioprotection needs to be further evaluated. It has been previously reported that stimulation of A2aR early after MI attenuates inflammation (73) and preserves cardiac function and that prereperfusion stimulation reduces infarct size (58) and attenuates contractile dysfunction in the remote LV. Subsequently, increased A2aR signal due to pre-MI exercise could be associated with cardioprotective effects in the early remodeling stages and may have paved the way for further antifibrotic mechanisms in the progressive remodeling progress. Even though at 5 wk
after MI, adenosine receptor expression was not different between exercised and sedentary mice with MI, we do not know the exact time course of downregulation and, therefore, we cannot rule out that adenosine receptor signaling may have persisted at least for some time in the early phase after MI and, therefore, may have contributed to the beneficial effects.

Another potential link between exercise and protection from maladaptive remodeling may be β3-adrenergic receptors (β3ARs) and endothelial nitric oxide synthase (eNOS) signaling. The endogenous activation of β3ARs was associated with reduced myocardial injury during MI; however, no analysis of fibrosis was provided in this study (8). Moreover, coupling of β3ARs to eNOS provided protection from isoproterenol-induced hypertrophy and fibrosis (4). Finally, studies from our own laboratory have revealed that exercise-mediated protective effects on telomere regulation and protection from doxorubicin-induced cardiac damage were mediated by eNOS (82), and eNOS deletion aggravated fibrosis and maladaptive remodeling after pressure overload (30). Since adenosine receptor activation mediates cardiac protection also via eNOS activation in the context of ischemic preconditioning (26), it may be speculated that either β3AR and/or adenosine receptor activation before and during MI may have contributed to the reduction in inflammation, fibrosis, and scar thinning. These interactions should be addressed by future studies.

Limitations of the study. All results presented in this study were obtained from analyzing male mice and can therefore not directly be translated to female mice. To our knowledge, there is currently no information available regarding potential sex-specific exercise-related differences in post-MI remodeling.

Conclusions. Continued exercise after MI reduces LV fibrosis and scar thinning, presumably by attenuating the coordinated proinflammatory response involving TNF-α, IL-6, and IL-1β. Based on the clinical observations that cardiac fibrosis (84) and wall thinning are associated with adverse outcomes (36, 87), our data imply that an early reinitiation of exercise after MI may presumably translate into clinical benefits in patients as well.

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DISCLOSURES

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

AUTHOR CONTRIBUTIONS

Author contributions: S.-L.P., Y.D., M.B., D.R.W., and C.M. conception and design of research; S.-L.P., A.M., and M.W. performed experiments; S.-L.P. and A.M. analyzed data; S.-L.P. and C.M. interpreted results of experiments; S.-L.P. prepared figures; S.-L.P. drafted manuscript; S.-L.P. and C.M. edited and revised manuscript; C.M. approved final version of manuscript.

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