High inborn aerobic capacity does not protect the heart following myocardial infarction

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1K.G. Jebsen Center of Exercise in Medicine, Department of Circulation and Medical Imaging, Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway; 2The Norwegian Council on Cardiovascular Disease, Trondheim, Norway; 3Department of Anesthesiology, University of Michigan Medical School, Ann Arbor, Michigan; and 4Institute of Cardiovascular and Medical Sciences, University of Glasgow, Glasgow, United Kingdom

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Hoydal MA, Kaurstad G, Rolim NP, Johnsen AB, Alves M, Koch LG, Britton SL, Stolen TO, Smith GL, Wisløff U. High inborn aerobic capacity does not protect the heart following myocardial infarction. J Appl Physiol 115: 1788–1795, 2013. First published October 31, 2013; doi:10.1152/japplphysiol.00312.2013.—Maximal oxygen uptake (VO2max) is a strong prognostic marker for morbidity and mortality, but the cardio-protective effect of high inborn VO2max remains unresolved. We aimed to investigate whether rats with high inborn VO2max yield cardio-protection after myocardial infarction (MI) compared with rats with low inborn VO2max. Rats breed for high capacity of running (HCR) or low capacity of running (LCR) were randomized into HCR-SH (sham), HCR-MI, LCR-SH, and LCR-MI. VO2max was lower in HCR-MI and LCR-MI compared with respective sham (P < 0.01), supported by a loss in global cardiac function, assessed by echocardiography. Fura 2-AM loaded cardiomyocyte experiments revealed that HCR-MI and LCR-MI decreased cardiomyocyte shortening (39%, and 34% reduction, respectively, both P < 0.01), lowered Ca2+ transient amplitude (37%, P < 0.01, and 20% reduction, respectively), and reduced sarcoplasmic reticulum (SR) Ca2+ content (both; 20%, P < 0.01) compared with respective sham. Diastolic Ca2+ cycling was impaired in HCR-MI and LCR-MI evidenced by prolonged time to 50% Ca2+ decay that was partly explained by the 47% (P < 0.01) and 44% (P < 0.05) decrease in SR Ca2+-ATPase Ca2+ removal, respectively. SR Ca2+ leak increased by 177% in HCR-MI (P < 0.01) and 67% in LCR-MI (P < 0.01), which was abolished by inhibition of Ca2+/calmodulin-dependent protein kinase II. This study demonstrates that the effect of MI in HCR rats was similar or even more pronounced on cardiac- and cardiomyocyte contractile function, as well as on Ca2+ handling properties compared with observations in LCR. Thus our data do not support a cardio-protective effect of higher inborn aerobic capacity.

METHODS

Animals. The rats used in the present study were selected and breed over 22 generations for either HCR or LCR starting from a N:NIH stock obtained from the National Institutes of Health (USA), as previously described (25, 48). In brief, the selection is based on an exercise capacity test at 11 wk of age, where rats with the 20% highest and 20% lowest scores are selected to generate the next generation for each strain. Concurrent breeding of HCR and LCR at every generation allows them to serve as reciprocal controls for unknown environment changes. Female rats were randomized for sham (SH) or MI surgery, giving the following four groups: HCR-SH, HCR-MI, LCR-SH, and LCR-MI. The Norwegian council for Animal Research approved the study, which was in accordance with Use of Laboratory Animals Laboratory Animals by the European Commission Directive 86/609/EEC.
MI surgery. MI was performed by permanent occlusion of LAD during 1.5% isoflurane anesthesia, and the success of inducing an permanent LAD occlusion was evaluated by echocardiography after 4 wk, as earlier described (23). Following MI, the animals remained sedentary in their cages before they were killed after 12 wk. One week before sacrificing the animals, the rats were examined by echocardiography to determine cardiac function. We included rats with MI larger than 40% of the LV to ensure consistency in the study population. There were no differences in the number of excluded animals or infarct size between the HCR and LCR.

V\textsubscript{O2max} All animals remained sedentary for 12 wk following the MI surgical procedure. At the end of the experimental period, we measured V\textsubscript{O2max} during uphill (25°) treadmill running in a metabolic chamber (48) before sacrificing the animals. The V\textsubscript{O2max} test procedure is a previously described and validated method (19, 46).

Echocardiography. Echocardiography was measured by Vevo 770 VisualSonics (Toronto, Canada) during isoflurane (2%) anesthesia. LV end-diastolic diameter (LVEDD) and end-systolic diameter (LVESD) were recorded with parasternal long-axis B-mode echocardiography, which allowed calculation of the fractional shortening (FS) ([% = (LVEDD − LVEDS)/LVEDD] × 100) as an index of systolic LV function.

Cardiomyocyte isolation, Ca\textsuperscript{2+} measurements, and cardiomyocyte dimension measurements. The animals were killed during isoflurane (3%) anesthesia and heparinized (0.2 ml heparin) before removal of the heart. Cardiomyocytes from the LV were isolated by retrograde Langendorff perfusion and enzymatic digestion of collagenase (Worthington Biochemical), as previously described (18). To ensure that we only studied viable cardiomyocytes remote from the ischemic infarct area, we removed both the infarct and the border zone area (~1–2 mm from the border of the infarction). Cell experiments were performed in 1.8 mmol/l Ca\textsuperscript{2+} HEPES-based solution. Cardiomyocytes were field stimulated by bipolar electrical pulses at 1–2 Hz in 1.8 mmol/l Ca\textsuperscript{2+} HEPES-based solution and continuously perfused at 37°C. Cardiomyocyte shortening was measured by video-based sarcomere spacing (SarcomLen Sarcomere Length Acquisition Module, Ionoptix, Milton, MA) using an inverted epifluorescence microscope (Nikon TE-2000E, Tokyo, Japan). Ca\textsuperscript{2+} handling measurements by fura-2 AM (2 μmol/l, Molecular Probes, Eugene, OR) was measured by a photomultiplier tube acquisition add-on module (PMTACQ, IonOptix; Optoscan, Cairn Research, Kent, UK). Rod-shaped cardiomyocytes with no visible damage and response up to 2-Hz stimulation were included.

Cell dimensions were measured with Nikon Eclipse E400 Microscope with a DSFi1 camera (Nikon NIS Elements Basic Research, version 3.00 software, Nikon Instruments).

Quantitative measurements of diastolic Ca\textsuperscript{2+} leak. We determined diastolic Ca\textsuperscript{2+} leak from sarcoplasmic reticulum (SR) by the established Shannon et al. (42) protocol. Briefly, to bring the cellular Ca\textsuperscript{2+} content to a steady state, we stimulated the cardiomyocytes electrically at 1 Hz in normal HEPES-based 1.8 mmol/l Ca\textsuperscript{2+} concentration solution for 60 s. After the last electric stimulus, we rapidly switched the perfusion to a 0 Na+/0 Ca\textsuperscript{2+} containing solution and measured diastolic Ca\textsuperscript{2+} concentration in quiescent nonstimulated cardiomyocytes (1 min) ± tetracaine (1 mmol/l). The 0 Na+/0 Ca\textsuperscript{2+} solution prevents the Na+/Ca\textsuperscript{2+} exchange (NCX), which is the primary Ca\textsuperscript{2+} influx and efflux mechanism at rest, whereas tetracaine blocks the Ca\textsuperscript{2+} leak over the ryanodine receptors (RyR). The quantitative difference between diastolic Ca\textsuperscript{2+} concentration with and without tetracaine determines leak. After the 1-min period in 0 Na+/0 Ca\textsuperscript{2+} ± tetracaine solution, we added caffeine (10 mmol/l) to assess the SR Ca\textsuperscript{2+} content. Diastolic Ca\textsuperscript{2+} leak is presented as diastolic Ca\textsuperscript{2+} concentration in relation to total SR Ca\textsuperscript{2+} content. In a subset of experiments, autacamide-2 related inhibitory peptide (AIP, 1 μM, Sigma Aldrich) was used for specific Ca\textsuperscript{2+}/calmodulin-dependent protein kinase II (CaMKII) inhibition, and H-89 (3 μM, Sigma Aldrich) for protein kinase A (PKA) inhibition. Cardiomyocytes were preincubated with AIP or H-89 1 h before the experiments.

Quantitative measurements of Ca\textsuperscript{2+} removal during diastole. We used the rate constant of Ca\textsuperscript{2+} decline in three different solutions to quantify the contribution from SR Ca\textsuperscript{2+}-ATPase (SERCA-2a), NCX, mitochondrial unipporter, and sarco(remel) Ca\textsuperscript{2+}-ATPase: 1) rate constant of Ca\textsuperscript{2+} decline during electrical stimulation in normal HEPES 1.8 mmol/l Ca\textsuperscript{2+} solution, with all contributors active; 2) addition of 10 mmol/l caffeine in normal HEPES 1.8 mmol/l Ca\textsuperscript{2+} solution that offset the SR and re-uptake by SERCA-2a; and 3) addition of 10 mmol/l caffeine in a 0 Na+/0 Ca\textsuperscript{2+} solution that offset both SERCA-2a and NCX.

Confocal imaging of Ca\textsuperscript{2+} release synchrony. Cardiomyocytes loaded with fluo 3-AM (10 μmol/l, Molecular Probes) were used to determine Ca\textsuperscript{2+} release synchrony. For the Ca\textsuperscript{2+} release synchrony analysis, one to three Ca\textsuperscript{2+} transients/cell were used, and the line scan of each Ca\textsuperscript{2+} transient was divided into ~25 equal strips (~4 μm/strip). Time from stimulation to 50% Ca\textsuperscript{2+} release was measured for each strip, and the standard deviation of the measurement was used as a measure of Ca\textsuperscript{2+} release synchronicity across the cell.

Statistics. Statistical analyses were performed by ANOVA using Bonferroni post hoc test. To determine whether there was a significantly more pronounced effect by MI between the two phenotypes (i.e., HCR and LCR), we performed an independent sample two-tailed t-test on the delta (Δ) change induced by MI between the LCR (LCR-SH – LCR-MI) and HCR (HCR-SH – HCR-MI). The use of “relative effect of MI” in this context is, therefore, used to show the Δ change occurring on each single parameter as a consequence of inducing MI in each line of animals (HCR and LCR). In these analyses, we have used the assumption that the sham animals are the zero point reference within the LCR and HCR groups.

Data are presented as means ± SD. P < 0.05 was considered statistically significant.

RESULTS

Intrinsic aerobic capacity and in vivo cardiac contractility. Aerobic capacity, measured as V\textsubscript{O2max}, was significantly lower in LCR-SH compared with HCR-SH (P < 0.01, Fig. 1). V\textsubscript{O2max} was reduced after MI in both HCR and LCR rats compared with their respective sham-groups (by 20 and 36%, respectively, P < 0.01, Fig. 1). Cardiac function measured by echocardiography demonstrated reduced systolic function after MI in both HCR and LCR rats (Table 1). Compared with respective sham group, LV FS was decreased by 49% in
Table 1. Echocardiography measurements of cardiac function and dimension

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<tr>
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<th>Sham</th>
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<th>Sham</th>
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<td>6</td>
<td>6</td>
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<tr>
<td>HR, beats/min</td>
<td>340 ± 7.1</td>
<td>324 ± 7.1</td>
<td>370 ± 48.5</td>
<td>376 ± 19.6</td>
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<tr>
<td>SV, μl/min</td>
<td>261.3 ± 76.9</td>
<td>162.7 ± 51.2</td>
<td>162.4 ± 74.2</td>
<td>113.6 ± 49.0</td>
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<tr>
<td>CO, ml/min</td>
<td>87.4 ± 22.5</td>
<td>55.2 ± 29.6</td>
<td>59.0 ± 22.7</td>
<td>42.1 ± 16.6</td>
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<td>EF, %</td>
<td>67.1 ± 10.1</td>
<td>36.3 ± 8.0</td>
<td>52.0 ± 8.6</td>
<td>26.4 ± 6.4</td>
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<td>LVEDD, mm</td>
<td>5.3 ± 0.4</td>
<td>7.5 ± 0.7</td>
<td>5.9 ± 0.3</td>
<td>7.8 ± 0.5</td>
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<tr>
<td>LVESD, mm</td>
<td>8.1 ± 0.3</td>
<td>8.9 ± 0.7</td>
<td>7.6 ± 0.5</td>
<td>8.7 ± 0.6</td>
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<td>LVESE, μl</td>
<td>122.0 ± 26.8</td>
<td>280.1 ± 16.3</td>
<td>139.8 ± 14.1</td>
<td>301.5 ± 43.7</td>
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<td>LVEDV, μl</td>
<td>383.4 ± 60.6</td>
<td>442.8 ± 41.1</td>
<td>302.2 ± 80.7</td>
<td>415.1 ± 92.2</td>
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<tr>
<td>Positive dP/dt, mmHg/s</td>
<td>8,222.3 ± 752.6</td>
<td>6,170.3 ± 827.5</td>
<td>7,369.8 ± 701.6</td>
<td>5,363.0 ± 287.5</td>
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<tr>
<td>Negative dP/dt, mmHg/s</td>
<td>-8,481.1 ± 923.1</td>
<td>-6,245.6 ± 772.3</td>
<td>-7,520.7 ± 1,551.6</td>
<td>-5,258.4 ± 347.7</td>
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Values are means ± SD; n, no. of animals. HCR, high-capacity runners; LCR, low-capacity runners; MI, myocardial infarction; HR, heart rate; SV, stroke volume; CO, cardiac output; FS, fractional shortening; EF, ejection fraction; LVESD, left ventricle end-systolic dimension; LVEDD, left ventricle end-diastolic dimension; LVESE, left ventricle end-systolic volume; LVEDV, left ventricle end-diastolic volume; dP/dt, change in pressure over time. Positive dP/dt indicates contractility; negative dP/dt indicates relaxation. *P < 0.05, different from HCR-sham. **P < 0.01, different from HCR-sham. †P < 0.05, different from LCR-sham. ‡P < 0.01, different from MI in the two different phenotypes was statistically different. n = number of animals in each group: HCR-sham, n = 5; HCR-MI, n = 5; LCR-sham, n = 6; and LCR-MI, n = 6; number of cells from each animal: n = 40–50. †P < 0.01, different from HCR-MI and LCR-MI.

HCR-MI (from 33% in HCR-SH to 17% in HCR-MI; P < 0.01, Table 1), and by 62% in LCR-MI (from 26% in LCR-SH to 10% in LCR-MI; P < 0.01, Table 1). MI induced a reduction in ejection fraction (EF) from 67 to 36% in HCR (P < 0.01, Table 1) and from 52 to 26% in LCR rats (P < 0.01, Table 1). LV end-systolic volume (LVESE) was increased after MI in both HCR and LCR by 130 and 116%, respectively (both P < 0.001, Table 1).

Cardiomyocyte size. Cardiomyocyte width and length were not significantly different between sham groups. MI induced a significant increase in cardiomyocyte width and length in both HCR and LCR rats (P < 0.01, Fig. 2, A and B).

Cardiomyocyte contractility and Ca2+ transients. LCR-SH was characterized by depressed cardiomyocyte contractile function and relaxation, as well as impaired Ca2+ handling compared with HCR-SH (Fig. 3). The relative reduction in cardiomyocyte FS was 39% in HCR-MI (P < 0.01, Fig. 3A) and 34% in LCR-MI (P < 0.01, Fig. 3A), compared with respective sham group. The Δ change induced by MI relative to their respective sham groups was significantly larger in HCR than LCR rats (P < 0.01, Fig. 3A). Also, the Δ effect of MI on time to 50% relengthening during diastole was larger in HCR (P < 0.05, Fig. 3B). Despite this larger effect of MI in HCR rats, it is important to note that time to 50% relengthening was still significantly faster than observed in LCR-MI (P < 0.01, Fig. 3B) and not significantly different from LCR-SH. Ca2+ transient amplitude was reduced by 37% in HCR after MI (P < 0.01, Fig. 3C), whereas a 20% reduction was observed in LCR-MI rats (not significant, Fig. 3C).

Δ effect of MI relative to their respective sham was more pronounced in HCR than LCR rats (P < 0.05, Fig. 3C). Similar to time to 50% relengthening, Ca2+ decay during diastole was prolonged after MI in both HCR (P < 0.01, Fig. 3D) and LCR (P < 0.01, Fig. 3D). Ca2+ decay was not different between HCR-MI and LCR-SH. Diastolic Ca2+ levels were not different between any group (Fig. 3E), whereas SR Ca2+ content was higher in HCR-SH compared with all other groups (P < 0.01, Fig. 3F). MI reduced SR Ca2+ content with the same impact in HCR and LCR (both 20%, P < 0.01, Fig. 3F).

To determine Ca2+ release properties, we measured the time to 50% peak Ca2+ amplitude and the Ca2+ release synchrony along the cardiomyocyte length. We did not find any significant difference on time to 50% Ca2+ amplitude between groups (Fig. 4A). The difference for synchrony of Ca2+ release was only different between HCR-MI and LCR-SH (Fig. 4B).

Diastolic Ca2+ handling. Since the transition from normal function to heart failure usually shift the Ca2+ cycling distribution between SR and sarcosomma Ca2+ handling proteins...
(3), we quantified SERCA-2a and NCX rate of Ca\(^{2+}\) removal during diastole. We found that SERCA-2a Ca\(^{2+}\) removal rate was significant higher in HCR-SH compared with all other groups (P < 0.001, Fig. 5A). After MI, SERCA-2a Ca\(^{2+}\) removal decreased by 47% in HCR (P < 0.001, Fig. 5A) and 44% in LCR (P < 0.05, Fig. 5A) compared with respective sham. The ∆ change relative to their respective sham groups on SERCA-2a Ca\(^{2+}\) removal following an MI was largest in HCR (P < 0.05, Fig. 5A). The reduction in SERCA-2a function resulted in a compensatory increase in NCX; the NCX rate constant of Ca\(^{2+}\) removal in HCR was nonsignificantly increased by 27% as a response to MI, whereas it increased by 32% in LCR-MI rats compared with LCR-SH (P < 0.05, Fig. 5B). These observations are consistent with the reduced SR Ca\(^{2+}\) content observed in HCR-MI and LCR-MI cardiomyocytes (Fig. 3F). It is, however, important to note that, despite the alteration in Ca\(^{2+}\) removal rate by SERCA-2a and NCX in HCR-MI rats, these parameters were not significant different from LCR-SH.

**Diastolic SR Ca\(^{2+}\) leak.** SR Ca\(^{2+}\) leak was minor in HCR-SH, but increased by 177% after MI (from 2.6% RyR Ca\(^{2+}\) leak in HCR-SH to 7.2% in HCR-MI, P < 0.01, Fig. 6A). The diastolic SR Ca\(^{2+}\) leak in LCR-MI was significantly larger compared with HCR-SH and was further increased by 67% after MI (from 6.1% in LCR-SH to 10.2% RyR Ca\(^{2+}\) leak in LCR-MI, P < 0.01, Fig. 6A). In heart failure, increased phosphorylation of the RyR by CamKII is shown to be a key mechanism for increased diastolic SR Ca\(^{2+}\) leak [2]; therefore, we incubated the cells with the CaMKII inhibitor, AIP. We found that diastolic SR Ca\(^{2+}\) leak remained unchanged in HCR-SH, whereas it was normalized in HCR-MI, LCR-SH, and LCR-MI (Fig. 6B). In addition, to evaluate the effect of

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**Table 1:** Parameters of LV cardiomyocytes. Fractional shortening (A), time to 50% re-lengthening (B), Ca\(^{2+}\) transient amplitude (C), time to 50% Ca\(^{2+}\) decay (D), diastolic Ca\(^{2+}\) level (E), and sarcoplasmic reticulum (SR) Ca\(^{2+}\) content measured by peak caffeine-induced transient (F) are presented as means ± SD. *P < 0.05 and **P < 0.01, different from all other groups. To determine whether the effect of inducing an MI in the two different phenotypes was statistically different, we compared the delta (∆) difference (HCR-sham − HCR-MI and LCR-sham − LCR-MI) with the respective sham groups as zero point control. Number of animals in each group: HCR-sham, n = 5; HCR-MI, n = 5; LCR-sham, n = 6; and LCR-MI, n = 6; number of cells from each animal: n = 6–8.

**Fig. 3.** Measurements on isolated LV cardiomyocytes. Fractional shortening (A), time to 50% re-lengthening (B), Ca\(^{2+}\) transient amplitude (C), time to 50% Ca\(^{2+}\) decay (D), diastolic Ca\(^{2+}\) level (E), and sarcoplasmic reticulum (SR) Ca\(^{2+}\) content measured by peak caffeine-induced transient (F) are presented as means ± SD. *P < 0.05 and **P < 0.01, different from all other groups.

**Fig. 4.** Ca\(^{2+}\) release properties in LV cardiomyocytes. Time to 50% Ca\(^{2+}\) amplitude (A), and synchronization of twitch Ca\(^{2+}\) release along the cell length assessed by the standard deviation (SD) of Ca\(^{2+}\) release along the line scan over the length of the cardiomyocyte (B) are presented as means ± SD. Number of animals in each group: HCR sham, n = 5; HCR MI, n = 5; LCR sham, n = 6; and LCR MI, n = 6; number of cells from each animal: n = 6–8.
PKA phosphorylation on Ca\(^{2+}\) leak, we also incubated the cells with the PKA inhibitor, H-89, and found no significant effects (Fig. 6C).

**DISCUSSION**

The objective of the present study was to investigate if contrasting for high or low inborn aerobic capacity would yield different remodeling on cardiac contractile function and Ca\(^{2+}\) handling after MI; i.e., potential for cardio-protection in rats with high inborn aerobic capacity and, conversely, more severe deterioration in rats with low inborn aerobic capacity. We found that MI led to a severe reduction in V\(_{O2}\text{max}\) and LV function, as well as cardiomyocyte function and Ca\(^{2+}\) handling in both of the groups. In contrast to our hypothesis, HCR rats with high inborn aerobic capacity suffered the same, or even a greater, loss after an MI than LCR rats on V\(_{O2}\text{max}\), cardiac contractile function, and Ca\(^{2+}\) handling properties when relating to the respective sham groups. However, rats with high inborn aerobic capacity displayed an advantageous end outcome compared with their LCR counterparts as the cardiac function in HCR after MI was comparable to the sham-operated LCR for almost all parameters.

**Aerobic capacity and global cardiac function after MI.** As earlier reported, V\(_{O2}\text{max}\) was lower in LCR-SH compared with HCR-SH rats (48). In the present study, V\(_{O2}\text{max}\) was reduced to the same extent in both animal lines after MI, which was explained by an equal deteriorated global cardiac contraction, assessed by reduced LV FS and EF. However, despite similar reduction on V\(_{O2}\text{max}\) and cardiac contraction when relating to their respective sham after MI in LCR and HCR, the levels from HCR-MI rats were still functionally higher than those of LCR-MI and similar to those of LCR-SH. Low EF is associated with ventricular enlargement after MI, or LV dilatation caused by infarct expansion (21, 35). In addition to EF, LVESV is considered a meaningful prognosticator after MI, as it predicts the extent of LV remodeling (35). We observed an equal increase in LVESV in both HCR and LCR after MI, suggesting similar prognosis. Accordingly, in vivo measurements of cardiac function indicated that high-inborn aerobic capacity was not associated with cardio-protective effects after MI in terms of reduced cardiac function and remodeling of the heart relative to their respective sham.

**Cardiomyocyte contractility, relaxation, and Ca\(^{2+}\) transients.** Impaired global cardiac function is commonly mediated by diminished function of individual cardiomyocytes and dysfunctional Ca\(^{2+}\) handling. Our laboratory has previously reported that cardiomyocytes from LCR rats resemble cardiomyocytes from post-MI failing hearts (19, 48). In line with this, we found that LCR-SH had similar cardiomyocyte contractility and Ca\(^{2+}\) handling as HCR-MI on most parameters. However, in contrast to our hypothesis, we observed that the HCR rats had a larger Δ reduction in cardiomyocyte FS and time to functional relengthening compared with their respective sham groups than the Δ reduction after MI in LCR rats.

The lower FS in HCR-MI, LCR-SH, and LCR-MI compared with HCR-SH was probably caused by the reduced Ca\(^{2+}\) transient amplitude through depletion of the SR Ca\(^{2+}\) content (4). Time to 50% Ca\(^{2+}\) decay during diastole was also prolonged in these groups, leading to impaired cardiomyocyte relengthening.

The reduced time to 50% Ca\(^{2+}\) decay, explained by lower rate constant of SERCA-2a Ca\(^{2+}\) removal, probably partly
caused the observed reduction in SR Ca\(^{2+}\) content, leaving less Ca\(^{2+}\) available for cardiomyocyte contraction (17). Reduced SERCA-2a activity is usually accompanied by increased NCX activity, which is commonly seen in failing hearts (16, 30, 38). Increased NCX activity contributes to Ca\(^{2+}\) removal from cytosol to provide relaxation and to avoid Ca\(^{2+}\) overload in the absence of normal SERCA-2a function. Increased NCX-mediated Ca\(^{2+}\) removal during diastole may have negative inotropic effects, as it facilitates extrusion of Ca\(^{2+}\) across the sarcolemma that, in turn, leads to lower SR Ca\(^{2+}\) content. This may be sufficient to reduce the Ca\(^{2+}\) released from the SR for a given L-type Ca\(^{2+}\) channel current trigger (5, 17, 30, 39). Hence, our data indicate that increased NCX rate constant of Ca\(^{2+}\) removal may contribute to the reduction of SR Ca\(^{2+}\) content displayed in HCR-MI, LCR-SH, and LCR-MI in the present study.

**Diastolic SR Ca\(^{2+}\) leak.** In HCR-SH rats, diastolic SR Ca\(^{2+}\) leak was minor, but increased significantly after MI. Despite this increase, the Ca\(^{2+}\) leak was, however, not different from LCR-SH. In LCR-MI, the SR Ca\(^{2+}\) leak was significantly larger than that in all other groups. The increased Ca\(^{2+}\) leak from SR may, in addition to the shift in Ca\(^{2+}\) across the sarcolemma that, in turn, leads to lower SR Ca\(^{2+}\) content. This may be sufficient to reduce the Ca\(^{2+}\) released from the SR for a given L-type Ca\(^{2+}\) channel current trigger (5, 17, 30, 39). Hence, our data indicate that increased NCX rate constant of Ca\(^{2+}\) removal may contribute to the reduction of SR Ca\(^{2+}\) content displayed in HCR-MI, LCR-SH, and LCR-MI in the present study.

The RyR is directly phosphorylated by the multifunctional CaMKII (31). In heart failure, CaMKII activity is increased, causing phosphorylation of RyR that may lead to spontaneous Ca\(^{2+}\) release and afterdepolarizations of the ventricle (2). By acutely inhibiting CaMKII with AIP in isolated cardiomyocytes, we found that SR Ca\(^{2+}\) leak was abolished. In addition, we inhibited the effect of PKA, previously shown to increase the open probability of the RyR, causing increased Ca\(^{2+}\) leak (32, 41). We did, in contrast to those studies, not find any effect of PKA inhibition on SR Ca\(^{2+}\) leak. The increased SR Ca\(^{2+}\) leak appears, therefore, to be caused by CaMKII in the present study. As emphasized by other studies, CaMKII probably serves as an important target for preventing spontaneous Ca\(^{2+}\) release that may trigger ventricular arrhythmias and additionally for rescuing defective Ca\(^{2+}\) handling and hence cardiac function (43, 44).

**Cardioprotection by aerobic capacity appears to be exercise dependent.** The data from the present study are not consistent with a cardio-protective effect of high inborn aerobic capacity; when comparing the change following MI relative to the respective sham groups the in vivo cardiac function, cardiomyocyte function and Ca\(^{2+}\) handling were similar, or, even more profoundly, affected on several parameters in HCR than in LCR rats.

In 1980, Morris et al. (37) reported greater survival rate in fit compared with sedentary subjects after MI. Later, other experimental animal studies suggested that exercise training preconditioning the heart and protects it against ischemia-reperfusion injury (8, 9, 12, 13, 29, 40, 50), as well as promotes healing of the infarcted area (13, 33). The protective role of a high VO\(_{2}\)max in relation to CVD morbidity and mortality may be such as a result of adaptive responses to exercise training that serve as cardiac preconditioning rather than by VO\(_{2}\)max level alone. Our data indicate, therefore, that high inborn VO\(_{2}\)max without the adaptive response to exercise training do not elicit the expected cardio-protective effect on MI tolerance, even though VO\(_{2}\)max is reported more closely related to mortality risk than physical activity levels per se (28, 34). Thus cardio-protection is likely dependent on acute effects provided by exercise training, as it vanishes at cessation of regular exercise (29).

It is difficult from our data to directly explain why high inborn aerobic capacity does not show the same protective effect as exercise training. A potential explanation for the lack of detectable protection in the present study is that the phenotypic changes developed through generation in rats selected for low aerobic capacity [including characteristics similar to the metabolic syndrome (22)] may have triggered mechanisms that, to some extent, may preclude the heart against ischemic damage. These mechanisms were not explored in the present study, but previous studies with this model have shown that the immune response is exaggerated in LCR (45), and especially TNF-α (14) and interleukin-10 (10), which have been shown to have preconditioning effects on ischemia-reperfusion injury and is significantly upregulated in the LCR rats (26).

**Conclusion.** This study demonstrates that the effect of MI in HCR rats was similar or even more pronounced on cardiac and cardiomyocyte contractile function, as well as on Ca\(^{2+}\) handling properties, compared with observations in LCR. Thus our data do not support a cardio-protective effect of higher inborn aerobic capacity. The cardio-protective role associated with high aerobic capacity seems, therefore, not to depend on inborn characteristics, but rather on acquired aerobic capacity and cardiac preconditioning.

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**DISCLOSURES**

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

**AUTHOR CONTRIBUTIONS**


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