Exercise training preserves coronary flow and reduces infarct size after ischemia-reperfusion in rat heart

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Among diseases in North America, cardiovascular disease exacts economic and humanitarian costs that are unparalleled by any other single disease. Among Americans who suffer from cardiovascular disease, >1.1 million will experience a myocardial infarction each year (24). Chronic exercise training has been shown to reduce many risk factors relating to cardiovascular disease, including high blood pressure, high cholesterol, obesity, and insulin resistance. Although chronic exercise training can increase the chance of survival after a myocardial infarction (23), it is not clear whether this is due to a training-induced reduction in the amount of tissue injury after nonlethal ischemia and reperfusion or some other mechanism.

Only two studies have directly examined the infarct-sparing influence of endurance exercise training. McElroy et al. (21) examined the effects of swimming on myocardial infarct size after irreversible coronary artery ligation in the rat. They found a decrease in infarct size after training and hypothesized that the cardioprotective effect of exercise was mediated by increased myocardial vascularity that would, in theory, act to minimize the area of myocardium at risk during coronary artery occlusion. However, this concept was not experimentally verified. Hamilton et al. (9) recently reported that a 5-day program of exercise training was sufficient to confer infarct sparing in hearts subjected to a transient regional ischemia and subsequent reperfusion. They postulated that the improvement in tolerance to ischemia-reperfusion insult was due, at least in part, to exercise-induced increases in myocardial antioxidant defenses. Because coronary flow was not measured in these studies, it is not known whether exercise-induced adaptations in coronary vascular function were associated with the infarct sparing.

In this study, we sought to determine whether a prolonged program of exercise training protects the heart against myocardial infarction. In addition, because coronary flow was not assessed in the earlier studies reporting exercise training-induced infarct sparing (9, 21), we designed studies to determine whether alterations in myocardial perfusion, during and after transient regional ischemia-reperfusion, were associated with training-induced cardioprotection against mechanical dysfunction and infarction.

METHODS

Animal model. Female Sprague-Dawley rats (age 2–3 mo) were randomly assigned to a chronic exercise training (Tr) group (n = 9) or a sedentary (Sed) group (n = 10). All animals were housed in the same facility with a 12:12-h light-dark cycle, with food and water provided ad libitum. Rats in the Tr group were run on a treadmill at a 10% grade for >20 wk.
The first 2 wk involved a familiarization period where animals ran 20 m/min, with running duration gradually increased from 5 to 30 min over this time. Treadmill speed and running duration were increased over the next 6 wk to 20 m/min (10 min), 28 m/min (30 min), and 35 m/min (20 min), and this protocol was maintained until death. At the time of death, tibia length was measured, and the plantaris muscle was frozen for subsequent citrate synthase assay (29). The study was conducted under the guidelines accepted by the American Physiological Society and received prior approval from the Institutional Animal Care and Use Committee at the University of Colorado at Boulder.

Whole heart preparation. At the time of death, all animals were 6–7 mo of age. Before death, rats were deeply anesthetized with pentobarbital sodium (35 mg/kg ip injection). After diminution of animal reflexes, hearts were excised, placed in ice-cold saline, and rapidly hung by the aorta on the cannula of a modified Langendorff apparatus. Hearts were perfused (76.5 mmHg perfusion pressure) with heated (37.5°C) Krebs buffer containing 117.4 mM NaCl, 4.7 mM KCl, 1.9 mM CaCl₂, 1.2 mM MgSO₄, 1.2 mM KH₂PO₄, 5 mM pyruvate, 11 mM glucose, 25 mM NaHCO₃, and 1,200 U/ml heparin and equilibrated with 95% O₂-5% CO₂. Similar to the methods of Skeeihan et al. (28), a 3-F pressure-transducing catheter (Millar Instruments, Houston, TX) was placed through the cannula and aortic valve into the chamber of the left ventricle (LV), and developed pressure waveforms were acquired with a personal computer connected to the transducer (SONOlab, Sonometrics, London, ON, Canada). After a 5-min stabilization period, baseline pressure was measured, and coronary flow rate was obtained by collection of the coronary effluent for 1 min.

Ischemia-reperfusion protocol. After baseline measurements, a suture was threaded through the tissue surrounding the left anterior descending coronary artery 3–5 mm distal to the aorta. Both ends of the suture were inserted into a small polyethylene tube that was used as a snare, and ischemia was induced by tightening and clamping the snare so that the artery was fully compressed. Pressure and coronary flow measurements were recorded every 15 min for 1 h during ischemia. Hearts that did not exhibit a decrease of coronary flow after the tightening of the coronary artery snare were not included in the study (1Tr and 2 Sed hearts). After 1 h, the snare was loosened, and reperfusion ensued for 2 h. Coronary flow and pressure data were recorded at 1, 3, 5, and 10 min after the onset of reperfusion and then every 15 min until the end of the 2-h reperfusion period.

Nonischemic time controls. A separate group of hearts from Tr (n = 5) and Sed (n = 7) animals were excised, cannulated, and perfused as described above, without the ischemic bout, to observe how the mechanical and flow measurements change as a function of time. Pressure and flow were recorded in these hearts at the same time points as in the hearts that experienced ischemia-reperfusion. At the end of the 3-h protocol, hearts were cut down, sliced, and weighed in a manner consistent with the groups that underwent ischemia-reperfusion (see below).

Measurement of infarct size. Infarct size was measured using methods similar to those previously described (25, 34). After the reperfusion period, the snare was retightened around the coronary artery, and 100 µl of 0.05% Evans blue solution was injected into the aortic cannula and perfused through the heart. Once the dye passed through the heart (after 3 min), the heart was cut down, the right ventricle was trimmed off, and the LV was sliced transversely from base to apex into four slices of equal width. Each of the slices was then immersed in phosphate-buffered saline containing 10 mM butanedione monoxime and photographed with a digital camera connected to a simple microscope. After both sides of each slice were photographed, each slice was placed in 100 mM phosphate buffer with 0.1% triphenyltetrazolium chloride and incubated for 10 min at 37°C in a covered water bath. After incubation, each side of every slice was again photographed, and the slices were weighed. LV weight was obtained by summation of the slice weights for each heart. The microscope lighting and magnification were kept constant throughout the procedure for experimental consistency.

Images of the slices (Fig. 1) were transferred from the digital camera to a personal computer equipped with ImageJ imaging software (23a) for morphometric analysis, and analyses were performed in a single-blind manner to avoid experimenter bias. Total slice area (TA), zone at risk (ZAR), and infarct area (IA) were measured. ZAR was identified as the area of each slice that did not turn blue (i.e., not perfused with dye) after perfusion with the solution containing Evans blue dye. The portion of the ZAR that did not turn red in response to triphenyltetrazolium chloride incubation and remained white was classified as IA. ZAR and IA were obtained by summation of all slices from each side of the heart. A section of the myocardium that stained red (after perfusion with the solution containing Evans blue dye) after perfusion with the solution containing Evans blue dye was used as the representative ZAR and IA for that slice. ZAR weight was quantified by dividing the mean ZAR by the TA of each slice and multiplying by the slice weight (slice wt × ZAR/TA). The fraction of each LV that was ZAR was calculated by dividing the sum of all ZAR weights by the sum of all slice weights for that LV. Infarct weight was calculated by dividing the IA by the TA and multiplying by the weight of each slice; total LV infarct weight was determined by summation. IA was expressed as a fraction of ZAR by taking the sum of all infarct weights and dividing them by the sum of all ZAR weights. All fractional IA and ZAR data are reported as percentages.

Western blots for CuZn- and Mn-SOD. In a separate group of experiments, hearts from the same animal population were excised, and the LV free wall was dissected for homogenization. The heart samples were washed with buffer containing (in mM) 100 KCl, 50 MOPS, 5 MgCl₂, 1 ATP, and 1 EGTA (pH 7.4) and minced with scissors. Samples were washed further and then homogenized using a motorized homogenizer (Polytron, Brinkmann Instruments, Westbury, NY) for 10 s at medium speed in 16 volumes (i.e., 1 g of tissue in 16 ml of buffer) of the aforementioned buffer containing 2.5 mg/ml BSA and protease cocktail (Complete, Roche Diagnostics, Mannheim, Germany). The homogenate was immediately frozen at −80°C and used for subsequent Western blot assay. Protein concentrations of homogenate were determined using the bicinchoninic acid assay (Pierce Biotechnology, Rockford, IL). Western blots were performed using SDS-PAGE in a 15% polyacrylamide gel with 30 µg of homogenate protein per lane. Blots were first probed using a polyclonal antibody against CuZn-SOD (Stressgen Biotechnologies, Victoria, BC, Canada) at a dilution of 1:45,000. After a secondary horseradish peroxidase-conjugated goat anti-rabbit antibody (Santa Cruz Biotechnology, Santa Cruz, CA) and then a chemiluminescent substrate (Western Lighting, Perkin-Elmer Life Sciences, Boston, MA) were applied, blots were developed on film. Blots were then stripped using standard methods and reprobed for Mn-SOD (Stressgen, 1:100,000) with the same secondary antibody and chemiluminescent substrate. All blots were scanned into a computer, and band density was analyzed using ImageJ software (23a).

Data analysis. Pressure waveforms were analyzed using a custom-made computer program (Matlab, version 6.1.0.450, Release 12.1, Mathworks, Natick, MA). All statistical comparisons were made using SPSS software (version 11.0,
Pressure and flow data were statistically analyzed using repeated-measures analysis of variance. Between-group comparisons of infarct size data were made using a one-tailed Student’s t-test to test the a priori hypothesis that training would result in a decreased myocardial infarct size. A two-tailed Student’s t-test was used to examine between-group contrasts in SOD protein expression and absolute changes in LV developed pressure and coronary flow at the onsets of ischemia and reperfusion, respectively. Values are means ± SE, and significance is reported at \( P < 0.05 \).

**RESULTS**

**Animal model.** Morphological data from Tr and Sed animals are presented in Table 1. There were no differences in age; tibia length; baseline heart rate; baseline peak systolic, minimum diastolic, and developed LV pressure; or baseline coronary flow between groups. Body weight \( (P < 0.05) \) and citrate synthase activity \( (P < 0.01) \) were significantly greater in Tr than in Sed animals.

**Infarct size.** There were no differences in ZAR between groups when expressed as area of the whole slice at risk \((36 \pm 3 \text{ and } 40 \pm 4\% \text{ for Tr and Sed, respectively})\) or as absolute mass of tissue at risk \((0.26 \pm 0.02\text{ g for Tr and Sed, respectively})\). IA, normalized for ZAR, was significantly smaller in hearts from Tr than in hearts from Sed animals \((24 \pm 3\% \text{ vs. } 32 \pm 2\%, P < 0.05; \text{Fig. 2})\).

**Nonischemic time controls.** No between-group differences in developed or diastolic pressure were observed

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**Table 1. Effects of run training on animal morphology and muscle biochemical characteristics**

<table>
<thead>
<tr>
<th></th>
<th>Sed</th>
<th>Tr</th>
<th>( P ) Value</th>
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<tr>
<td>Age, mo</td>
<td>7.0 ± 0.1</td>
<td>6.9 ± 0.1</td>
<td>0.59</td>
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<tr>
<td>Final body wt, g</td>
<td>266 ± 6</td>
<td>285 ± 6</td>
<td>(&lt; 0.05)</td>
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<tr>
<td>Tibia length, mm</td>
<td>36.5 ± 0.6</td>
<td>37.2 ± 0.4</td>
<td>0.35</td>
</tr>
<tr>
<td>LV wt/tibia length, mg/mm</td>
<td>18 ± 1</td>
<td>19 ± 1</td>
<td>0.28</td>
</tr>
<tr>
<td>Citrate synthase activity, ( \mu \text{mol}\cdot g \text{ wet wt}\cdot \text{min}^{-1} )</td>
<td>16.6 ± 0.8</td>
<td>22.0 ± 1.2</td>
<td>(&lt; 0.01)</td>
</tr>
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Values are means ± SE. LV, left ventricle; Sed, sedentary; Tr, endurance trained.
at any time point in the nonischemic time control measurements (Fig. 3, A and B). Coronary flow was significantly greater in the Tr animals only 65 and 75 min after baseline recordings were obtained (0 min; Fig. 3C).

**Pressure development.** There were no differences in baseline LV developed pressure (Fig. 4). After 30 min of ischemia, LV developed pressure was significantly higher in hearts from the Tr than in hearts from the Sed animals. This difference persisted until 45 min into the reperfusion period ($P < 0.05$). The mean decline in LV developed pressure was significantly greater after ischemia in the Sed than in the Tr animals ($P < 0.05$, Student’s t-test). The time- and group-dependent changes in LV developed pressure could be accounted for by elevations in diastolic pressure (Fig. 5). Animals from the Tr group showed significantly lower diastolic pressure from 45 min of ischemia through 30 min of reperfusion ($P < 0.05$).

![Fig. 3](image-url)  
Fig. 3. Nonischemic time control data for Tr (○, $n = 5$) and Sed (●, $n = 7$) animals. There were no significant between-group differences in developed (A) or diastolic (B) pressure at any time point. Coronary flow (C) was significantly higher in the Tr group at 65 and 75 min after baseline recording. *$P < 0.05$.

![Fig. 4](image-url)  
Fig. 4. LV developed pressure (LVDP) during regional ischemia (I) and subsequent reperfusion (R). Data point to the left of 0 min on abscissa represents a preischemic baseline reading; coronary artery occlusion commenced at 0 min. LVDP was significantly greater in Tr (○, $n = 8$) than in Sed (●, $n = 8$) hearts from 45 min of ischemia through 30 min of reperfusion. *$P < 0.05$. Dotted and dashed lines represent nonischemic time control measurements (from Fig. 3A) for Tr and Sed, respectively. Inset: LVDP data during transition from ischemia to reperfusion on an expanded time scale. Heart rates in Tr and Sed hearts were 255 ± 3.8 and 260 ± 2.3 beats/min, respectively ($P = 0.28$).

![Fig. 5](image-url)  
Fig. 5. Minimum diastolic pressure during regional ischemia (I) and subsequent reperfusion (R). Data point to the left of 0 min on the abscissa indicates a preischemic baseline reading; coronary artery occlusion commenced at 0 min. Significant difference in LVDP (Fig. 3) was due primarily to significantly greater diastolic pressure in Sed (●) than in Tr (○) hearts. *$P < 0.05$. Dotted and dashed lines represent nonischemic time control measurements (from Fig. 3B) for Tr and Sed, respectively. Inset: minimum LV diastolic pressure data during transition from ischemia to reperfusion on an expanded time scale.
Coronary flow data. Coronary effluent was used as an index of coronary flow and is reported per gram of heart tissue (Fig. 6). There were no differences in baseline coronary flow between groups. After the onset of ischemia, coronary flow in Tr and Sed hearts decreased similarly. However, after 45 min of ischemia, coronary flow was significantly higher in Tr than in Sed animals, and this difference persisted for the duration of the protocol ($P < 0.05$). In addition, Tr hearts experienced a significantly greater mean increase in flow on reperfusion than did Sed hearts ($P < 0.05$, Student’s $t$-test), indicating the presence of a training-induced hyperemic response (see DISCUSSION).

SOD protein expression. Western blot analysis demonstrated that antioxidant enzyme protein levels were elevated after exercise training. CuZn-SOD (19-kDa band) and Mn-SOD (25-kDa band) protein levels increased significantly in Tr animals compared with Sed animals (Fig. 7; $P < 0.05$ for both isoforms).

**DISCUSSION**

Infarct sparing. In 1978, McElroy et al. (21) reported that exercise training reduced the size of myocardial infarction that occurred in response to irreversible coronary artery occlusion in the rat. They found that chronic swimming increased the ventricular myocardial capillary-to-fiber ratio, and they concluded that increased myocardial vascularization was the most likely mechanism underlying the exercise-induced reduction in infarct size after coronary artery occlusion. If this was the case, then it is likely that the reduced infarct size observed in hearts from Tr animals was due to a training-induced reduction in the amount of myocardial tissue in the ischemic zone distal to the coronary artery occlusion. However, without a direct assessment of the size of the ischemic zone at risk, this conclusion is purely speculative. Furthermore, the issue of whether training increases the resistance of myocardium to tissue injury within an ischemic region was not addressed.

Since the study of McElroy et al. (21), there have been relatively few studies (9, 33, 34) directly examining the effect of exercise, acute and repetitive training, on the resistance of the heart to infarction in the face of ischemia and/or reperfusion challenge. Although the exercise paradigms and the experimental models used in these studies have been quite varied (Table 2), it seems clear that, within a well-defined ischemic ZAR, “late-phase” infarct sparing (24–36 h after a bout of...
exercise (33, 34), short-term (5 days) exercise training (9), and 20 wk of exercise training (present study). From a clinical perspective, these data are significant, in that they indicate that the exercise-induced increase in the resistance of the heart to myocardial infarction is persistent and sustainable over very long periods of time.

Our observation regarding the sustainability of training-induced infarct sparing is relevant for another reason. In a wide variety of late-phase cardioprotection models (pharmacological and nonpharmacological), a tight correlative link appears to exist between Mn-SOD induction and infarct sparing (see Ref. 3 for review). Causality has been inferred in experiments where suppression of Mn-SOD protein expression (with the use of antisense oligonucleotides) has been shown to abolish the infarct-sparing late-phase preconditioning effects of adenosine A1 receptor agonist, heat stress, and acute exercise (6, 34, 35). A review of the literature suggests that late-phase infarct sparing in response to exercise (acute and prolonged) or other preconditioning stimuli has not been clearly dissociable from elevations in Mn-SOD activity and/or protein expression. Although endurance training has been previously shown to elicit increases in myocardial Mn- and CuZn-SOD activities (9, 11, 26), a number of studies have shown that the activities of these SOD isoforms are not increased in young animals by training (15, 19, 30) or that the alteration in SOD activity (Mn-SOD) observed in response to the endurance exercise stimulus is only transient in nature and is not observable after ~9 wk of training (10). Our findings indicate that infarct sparing and elevated Mn-SOD expression are persistent over 20 wk of training and add to the circumstantial case for a functional link between exercise-induced infarct sparing and Mn-SOD expression (Table 2). It may be relevant to note that a putative explanation for the observed differences in the effect of training on SOD activity or protein expression may be differences in duration and intensity of exercise protocols. Specifically, the training protocol in this study is very similar to that used by Hamilton et al. (9) and Powers et al. (26), where animals ran at ≥30 m/min on a ≥10% incline. In studies where training elicits transient changes or no changes in SOD activity (10, 19), training intensity was significantly lower (≥25 m/min and ≥10% grade).

**Coronary flow.** Another mechanism that may have contributed to training-induced infarct sparing in our studies is an improved hyperemic response to severe ischemia-reperfusion. On review of the existing work on exercise-induced infarct sparing, we found only two studies (33; present study) that examined coronary flow in experimental settings in which exercise-induced infarct sparing has been demonstrated (Table 2). In the first study, infarct sparing after an acute bout of exercise occurred concomitantly with a small but significant elevation in coronary flow at one time point at the end of a 2.5-h ischemia-reperfusion protocol (33). In the present study, training-induced changes in coronary flow were quite pronounced throughout our ischemia-reperfusion protocol. Key features of our coronary flow data are as follows. 1) Before ischemia-reperfusion intervention, no between-group difference in baseline coronary flow was apparent (Fig. 5). This finding is consistent with earlier work using this model of exercise training (13, 18). 2) The magnitude of the drop in coronary flow after coronary artery occlusion was identical in Sed and Tr hearts. This observation indicates that our coronary artery occlusion protocol was reproducible across both groups of hearts and is internally consistent with our observation that the absolute size of the ZAR, as determined by Evans blue dye exclusion, was the same in Tr and Sed hearts. 3) During the 1 h of ischemia, coronary flow outside the ZAR was preserved in Tr hearts, whereas it fell in Sed hearts. To our knowledge, this finding is unique insofar as it demonstrates that, during zonal ischemia, coronary flow in tissue outside the ZAR is better preserved in Tr hearts. Although this phenomenon could not have con-
ported to infarct sparing in our study, it could well have been causally related to the preservation in LV mechanical function that we observed in Tr hearts. 4) Immediately on reversal of coronary artery occlusion, coronary flow in Tr hearts rebounded to preischemia levels, whereas in Sed hearts, it did not. Furthermore, the absolute increase in coronary flow when flow was reestablished to the ZAR was significantly greater in Tr than in Sed hearts. The more robust postischemic hyperemic response in the ZAR of Tr hearts may well have contributed to infarct sparing in our study.

Overall, the training-induced augmentation in the hyperemic reactivity of the coronary vasculature to ischemia-reperfusion stress in our study is consistent with previous findings that postischememia hyperreperfusion is observed after global ischemia in hearts from treadmill-trained male rats (4, 5). In addition, it has been suggested that training may lead to a decrease in responsiveness to vasoconstrictor stimuli (31), which may explain the sustenance of coronary flow during ischemia in the Tr animals. The extent to which these types of adaptations, as opposed to adaptations at the level of the ventricular myocardium, contribute to training-induced infarct sparing remains to be determined.

Preserved LV pressure development. Our results showed a significant training-induced preservation in LV developed pressure and diastolic function during and after regional ischemia-reperfusion. Specifically, our ischemia-reperfusion protocol resulted in significant elevations in minimum diastolic pressure in Sed hearts that were markedly attenuated by chronic exercise training. Improved mechanical performance in response to ischemia-reperfusion challenge after chronic exercise training has been a common finding over the last ~30 years and has been observed by numerous investigators (4, 9, 22, 26, 27). However, the physiological basis for training-induced cardioprotection against LV mechanical dysfunction after ischemia-reperfusion is not known.

Although our observation that chronic training elicited improved LV mechanical function in the face of ischemia-reperfusion challenge is not new, several elements of our study are worthy of comment. As is the case with regard to infarct sparing, the training-induced preservation of mechanical function observed in this study may have been due to an intrinsic resistance of the myocardium to the transient ischemia and/or improved coronary flow to regions of the heart during and after the ischemic episode. Given the temporal relation between training-induced preservation in LV mechanical function and coronary flow outside the ZAR during ischemia and to the ZAR during reperfusion (Figs. 4–6), it would appear that the case in support of a causal link between the two phenomena is strong.

In addition to the training-induced sustenance of flow during ischemia and the augmented hyperemic response to reperfusion, intrinsic myocardial mechanisms may have contributed to the preservation of LV mechanical function throughout ischemia and reperfusion. In our female rat model, we have demonstrated that endurance training elicits significant increases in the protein expression of Mn- and CuZn-SOD (present study) and heat shock protein 70 (13) and a marked diminution in the activation of a repolarizing ATP-sensitive K+ current in response to severe hypoxic challenge (14). Although these intrinsic myocardial adaptations to training are often observed, the role they play in the cardioprotection against mechanical stunning is not clear, because previous work has shown that elevations in myocardial heat shock protein 70 and Mn- and CuZn-SOD activities are not required for a training-induced preservation in mechanical function in the face of ischemia-reperfusion stress (10, 32).

Whether an exercise training-induced preservation in LV mechanical function after a severe bout of ischemia can occur in the absence of an improved myocardial hyperemic response has yet to be determined.

Experimental model. As shown in Table 2, the experimental models used to address the issue of exercise-induced cardioprotection against infarction have been quite varied with respect to the exercise paradigms and experimental heart preparations that have been used. The exercise paradigm issue has been addressed in this study. With regard to heart preparations, several issues are worthy of comment. Two general approaches have typically been used to study ischemia-reperfusion injury in the heart. In this study, we used an in vitro Langendorff-perfused heart preparation to study the mechanical and tissue injury aspects of ischemia-reperfusion. Among the advantages of this commonly employed model (1, 2, 7, 8, 16, 17, 20, 33) is that key elements of cardioprotection (i.e., infarct sparing and preserved mechanical function) can be studied in the absence of cardiac innervation, blood-borne inflammatory cell types, or other factors extrinsic to the myocardium that may influence the response of the heart to ischemia. These advantages are gained at the expense of the physiological context that is eliminated. Exercise-induced infarct sparing has also been studied using in situ preparations (9, 34), and these possess the integrated extrinsic factors identified above. The reports of infarct sparing produced by acute exercise (34) and prolonged training (present study) in vitro preparations clearly indicate that factors intrinsic to the heart are involved in the cardioprotection.

Finally, a key difference between the in situ and in vitro approaches is worth considering. In the in vitro Langendorff-perfused model, coronary artery perfusion pressure is constant, and alterations in coronary flow are reflective of changes in coronary vascular resistance. In in situ preparations (9, 34), coronary flow is primarily dependent on the mechanical function of the heart. Because training has been shown to preserve LV mechanical function in the face of ischemia-reperfusion stress, it would be expected that preserved mechanical function in an in situ setting would also preserve coronary perfusion and improve tissue survivability. From an integrated physiological and/or clinical perspective, this is certainly quite relevant. From a mechanistic standpoint, the in situ approach has not been amendable to determining how training actually af-
fects postischemic coronary flow or how exercise-induced changes in coronary flow might be due to intrinsic coronary vascular vs. LV functional adaptations during ischemia-reperfusion stress. Clearly, each approach has its strengths and weaknesses.

Summary and conclusion. In this study, we have demonstrated that prolonged endurance training confers a cardioprotective effect against infarction in a defined region of myocardium subjected to severe ischemia and subsequent reperfusion. In addition, a novel observation in our study was that, during severe ischemia, coronary flow to regions of the myocardium outside the ischemic zone was better maintained in hearts isolated from endurance-trained rats. This may have contributed to improved LV mechanical function during ischemia. On reperfusion of the ZAR, the increase in flow to the previously ischemic region of the heart was markedly higher in hearts isolated from trained rats. In addition, endurance training elicited a significant increase in the protein expression of myocardial Mn- and CuZn-SOD. It seems reasonable to speculate that these latter adaptations, along with the improved ZAR reperfusion, contributed to the training-induced infarct sparing that we observed. More work is required to determine the relative contributions of these myocardial and coronary vascular adaptations to infarct sparing in hearts previously conditioned by endurance training.

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DISCLOSURES

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REFERENCES