Effects of exercise training on contractile function in myocardial trabeculae after ischemia-reperfusion

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Hwang, Hyosook, Peter J. Reiser, and George E. Billman. Effects of exercise training on contractile function in myocardial trabeculae after ischemia-reperfusion. J Appl Physiol 99: 230–236, 2005. First published March 17, 2005; doi:10.1152/japplphysiol.00850.2004.—Potential protective effects of aerobic exercise training on the myocardium, before an ischemic event, are not completely understood. The purpose of the study was to investigate the effects of exercise training on contractile function after ischemia-reperfusion (Langendorff preparation with 15-min global ischemia/30-min reperfusion). Trabeculae were isolated from the left ventricles of both sedentary control and 10- to 12-wk treadmill exercise-trained rats. The maximal normalized isometric force (force/cross-sectional area; P/VCSA) and shortening velocity (Vo) in isolated, skinned ventricular trabeculae were measured using the slack test. Ischemia-reperfusion induced significant contractile dysfunction in hearts from both sedentary and trained animals; left ventricular developed pressure (LVDP) and maximal rates of pressure development and relaxation (±dP/dt max) decreased, whereas end-diastolic pressure (EDP) increased. However, this dysfunction (as expressed as percent change from the last 5 min before ischemia) was attenuated in trained myocardium (LVDP: sedentary −60.8 ± 6.4% (32.0 ± 5.5 mmHg) vs. trained −15.6 ± 8.6% (64.9 ± 6.6 mmHg); ±dP/dt max: sedentary −54.1 ± 4.7% (1,058.7 ± 124.2 mmHg/s) vs. trained −16.7 ± 8.4% (1,931.9 ± 188.3 mmHg/s); −dP/dt max: sedentary −44.4 ± 2.5% (−829.3 ± 52.0 mmHg/s) vs. trained −17.9 ± 7.2% (−1,341.3 ± 142.8 mmHg/s); EDP: sedentary 539.5 ± 147.6% (41.3 ± 6.0 mmHg) vs. trained 71.6 ± 30.6% (11.4 ± 12.2 mmHg). There was an average 26% increase in P/VCSA in trained trabeculae compared with sedentary controls, and this increase was not affected by ischemia-reperfusion. Ischemia-reperfusion reduced Vo by 39% in both control and trained trabeculae. The relative amount of the β-isofrom of myosin heavy chain (MHC-β) was twofold greater in trained trabeculae as well as in the ventricular free walls. Despite a possible increase in the economy in the trained heart, presumed from a greater amount of MHC-β, ischemia-reperfusion reduced Vo, to a similar extent in both control and trained animals. Nevertheless, the trained myocardium appears to have a greater maximum force-generating ability that may, at least partially, compensate for reduced contractile function induced by a brief period of ischemia.

myosin heavy chain; conditioning; adaptation; protection; contractile proteins

A BRIEF PERIOD OF CORONARY occlusion, followed by flow restoration, can lead to cell damage in the absence of cell death, and this damage can cause a reduction in myocardial contractile function (4). The impaired contractile function is transient, lasting several hours to days or even weeks depending on the severity of the ischemic event. Even though the exact mechanism responsible for the reduction in contractile function is not known with certainty, a depressed myofilament responsiveness to Ca2+ is often associated with the postischemic myocardial dysfunction (9, 16, 20). The depressed Ca2+ responsiveness could result from either alterations in the Ca2+- handling proteins, the myofibrillar proteins, or some combination of both. In fact, changes in both cellular components, Ca2+ handling (15, 33) and myofibrillar proteins (11, 31), have been consistently reported in response to ischemia-reperfusion. Furthermore, alterations in either of these two cellular components can contribute to impaired myocardial pumping activity.

Attenuation of the response to ischemia could reduce ischemia-reperfusion-induced injury. Exercise training has been suggested as a potential approach to modify the severity of ischemia-reperfusion-induced injury, as evidenced by an improvement in functional recovery after ischemia-reperfusion (6–8, 12, 17, 22, 24, 28, 29). Several factors, including an increase in Ca2+ handling (7), endogenous antioxidants (12, 22, 28), and heat shock proteins (17), have been implicated as mechanisms responsible for protective actions of exercise. However, the exact mechanism responsible for this protection remains to be determined.

It is our hypothesis that the beneficial effect of exercise training on ischemia-induced injury depends on the changes in contractile proteins, specifically myosin heavy chain (MHC) composition. Mammalian adult cardiac muscle expresses two isoforms of MHC, MHC-α and -β. A major difference between these two isoforms is the rate of ATPase activity that provides the basis for differential mechanical properties (e.g., shortening velocity and economy) of muscle contraction (5, 27). Specifically, economy of muscle contraction, defined as force produced for a given amount of ATP utilized, is greater in hearts expressing predominantly MHC-β, compared with those expressing predominantly MHC-α (14). In this regard, an intervention that can increase the expression of MHC-β may provide an economical advantage, at the expense of power output, over the myocardium expressing MHC-α and could, thereby, lessen ischemia-reperfusion injury.

The main objective of this study was to investigate potential protective effects of an exercise-training program on the response to myocardial ischemia-reperfusion. Specifically, the following hypotheses were tested: 1) progressively increasing exercise training would increase the expression of MHC-β in the myocardium, and 2) this increase in MHC-β induced by exercise training would attenuate the contractile response to ischemia-reperfusion. In the present study, the protective ef-
fects of exercise training were examined in isolated hearts [left ventricular developed pressure (LVEDP)] and isolated skinned trabeculae. The myofibrillar mechanisms of ischemia-reperfusion-induced alterations in contractile function were investigated in the trabeculae by studying maximal Ca$^{2+}$-activated force generation ($P_0$) and shortening velocity ($V_0$). The results of the present study demonstrate that exercise training can protect against ischemia-reperfusion-induced reductions in contractile function, possibly due to increases in MHC-β expression and the force-generating ability of the muscle. These results are consistent with a very recent report (3) that demonstrated that an increase in MHC-β levels was associated with an improvement in postischemic ventricular function.

**METHODS**

**Animals.** The Ohio State University Institutional Laboratory Animal Care and Use Committee approved the experimental procedures involving animals. Male Sprague-Dawley rats were purchased at the age of 2 mo (body mass 250–280 g). Animals were housed in a temperature- and humidity-controlled room with 12:12-h light-dark cycles and free access to standard rat chow and water.

**Exercise training protocol.** Animals were randomly assigned to one of two groups: an exercise-training group and a sedentary group. Rats in the exercise training group ran on a rodent treadmill for 10–12 wk, 5 days/wk. Exercise intensity and duration were gradually increased from 12 m/min for 15 min to 28 m/min for 60 min. During the first 5 wk, the rats ran at 0% grade. Beginning the sixth week, the animals were trained at 5% grade, and this inclination was maintained for the rest of the training period. Each exercise session included 5-min warm-up and cool-down periods (running at the low intensity and speed). A previous study reported that this training load was at ~74% of maximal O$_2$ consumption (1).

**Ischemia-reperfusion: isolated heart perfusion.** The hearts from all of the trained rats were removed within 48 h after the last training session. Control hearts were obtained from age-matched (within 2 wk) and body mass-matched animals that had not undergone exercise training. Anesthesia was induced by an injection of pentobarbital sodium (60 mg/kg ip), and blood coagulation was prevented by an injection of heparin through a portal vein (0.7 mg). The heart was cannulated through the aorta and connected to the Langendorff perfusion apparatus through which a modified Krebs-Henseleit perfusate, bubbled with 5% CO$_2$-95% O$_2$ gas, was perfused at a constant flow rate (4 ml/min; sedentary) with an improvement in postischemic ventricular function. The pressure signal was digitized at 200 Hz (Origin, Microcal Software, Northampton, MA). The heart was cannulated through the aorta and connected to the Langendorff perfusion apparatus through which a modified Krebs-Henseleit perfusate, bubbled with 5% CO$_2$-95% O$_2$ gas, was perfused in a retrograde manner. The heart was then immersed in a temperature-controlled chamber containing perfusate and stimulated at a frequency of 5 Hz. Cardiac pacing was not maintained during the ischemic period. The temperature and the pH of the perfusate were closely monitored and maintained at 37°C and 7.4, respectively. The composition of the perfusate was the following (in mmol/l): 118 NaCl, 4.63 KCl, 1.2 MgSO$_4$, 1.25 CaCl$_2$, 1.17 KH$_2$PO$_4$, 25 NaHCO$_3$, and 5.5 glucose. After a brief period of washout, a latex balloon filled with water was inserted into the left ventricular cavity through which changes in ventricular developed pressure (peak left ventricular systolic pressure – left ventricular end diastolic pressure) was measured using a pressure transducer (model P23Db, Gould, Cleveland, OH). The pressure signal was digitized at 200 Hz (Origin, Microcal Software, Northampton, MA). The heart was allowed to equilibrate for 15–20 min, during which baseline hemodynamic data were collected. End-diastolic pressure (EDP) was set at 8 mmHg by inflating the latex balloon, and this pressure remained constant throughout the perfusion. Hearts were perfused at a constant flow rate (~15 ml/min; sedentary group 13.4 ± 0.6 vs. exercise-trained group 13.1 ± 0.5 ml/min). This flow rate was maintained throughout the perfusion period, except in the ischemic period, during which switching off the perfusion pump interrupted the myocardial perfusion. One-half of the trained rats and one-half of the sedentary control rats (chosen randomly) served as time perfusion controls (i.e., continuous perfusion for 60 min without flow interruption). The hearts from the other trained and sedentary control rats were subjected to 15 min of perfusion, 15 min of global ischemia (no flow) and 30 min of reperfusion. Baseline hemodynamic data were collected once a stable baseline had been achieved. The baseline hemodynamic data were averaged over the last 5 min before the onset of global ischemia and compared with values obtained during the 30-min reperfusion period.

**MHC analysis.** Transmural samples from the left and right ventricular free walls were obtained by dissection after 60 min of perfusion for the control hearts and after reperfusion in the ischemia-reperfusion hearts and stored at −80°C for later analysis of MHC compositions. The rest of the myocardium was placed in glycinerinating solution (32) for skinning and stored at −22°C until analyzed for measurements of trabecula contractile properties. The frozen transmural samples were thawed and prepared for analysis of MHC isoform composition as described in Blough et al. (2), including the homogenization step. The samples were then stored at −40°C until run on SDS gels. The separating gels (0.75 mm thick), utilized to examine MHC isoform composition, contained 7% (wt/vol) acrylamide-N,N'-methylene-bis-acrylamide (ratio = 50:1), 5% (vol/vol) glycerol, 0.2 M Tris (pH 8.8), 0.1 M glycine, and 0.4% (wt/vol) SDS. The preparation and composition of the stacking gels, as well as the running conditions, electrod buffer, and gel staining and scanning, were as described by Reiser and Kline (25). The amount of MHC-β in each sample was expressed relative to the total amount of MHC. The left, right, and middle portion of each MHC band was scanned, and the results from each sample were obtained by averaging the peak areas from the three scans.

**Contractile properties of trabeculae.** At the end of ischemia-reperfusion, transmural pieces of the left and right ventricular free walls were removed from the heart and stored at −80°C for subsequent biochemical analysis. The rest of the myocardium was placed (and swirled) in a relaxing solution for 15 min (4°C). This tissue was then transferred to a glycinerinating solution to remove membrane compartments. The skinnng process took place overnight at 4°C. After the overnight incubation, the myocardium was stored at −20°C in the same solution until utilized. The myocardium was glycinerinated an average of 22.5 days, which was not significantly different across groups (sedentary control 22 ± 2 vs. exercise trained 23 ± 2 days; $P > 0.05$).

Single trabeculae specimens (~200-μm width and 1-mm length) were dissected from the left ventricular endocardial surface and mounted in the experimental chamber. $P_0$ (normalized with cross-sectional area (CSA)) and $V_0$ were measured, as described in Reiser et al. (26) and Wattanapermpool et al. (32), with the following modifications. The skinned trabeculae were soaked in Triton X-100 [1% vol/
(vol/vol) in relaxing solution] for 5 min before measurement of contractile properties. Images of the trabeculae mounted in the experimental chamber were captured with a video camera (model XC-ST70, Sony). Width, depth, and sarcomere length of the trabeculae were measured using an image-analysis system (Simple PCI, Compix, Cranberry Township, PA). Resting sarcomere length was set to between 1.9 and 2.1 μm. The force transducer output was digitized using a DaqBoard/2000 and Daqview software (Iotech, Cleveland, OH). Force records were viewed and analyzed using DASYLab (version 5.5, DASYTEC, Amherst, NH).

Citrate synthase assay. Tibialis anterior citrate synthase activity was used as an index of the effects of exercise training on oxidative capacity. The enzyme activity was assayed using the modified technique described by Srere (10).

Statistical analysis. Data are expressed as means ± SE. Mechanical properties of isolated hearts were analyzed using two-way analyses of variance with repeated measures on one factor (reperfusion time). Physical and mechanical characteristics of trabeculae were analyzed by using two-way analyses of variance (ischemia-reperfusion × exercise training). The Student’s-t test was applied when morphometric comparisons between groups were made (i.e., exercise trained vs. sedentary control). Within-group comparison of percent MHC-β, i.e., comparison of left and right ventricles and trabeculae, was performed using one-way analysis of variance. If the F-value exceeded a critical value (P < 0.05), Tukey’s post hoc test was used to determine the significance (P < 0.05) of the differences between group means. All of the statistical analyses were performed using SPSS (Windows version 11.5, SPSS, Chicago, IL).

RESULTS

Effects of exercise training on morphometric characteristics. Morphometric characteristics in the sedentary and trained animals are listed in Table 1. There was no significant difference in the body weight between sedentary and trained animals after 10–12 wk of treadmill exercise training. However, exercise training increased (P < 0.05) absolute heart weight by 15% compared with sedentary controls. The heart weight-to-body weight ratio increased by 19% (P < 0.05) in trained rats compared with sedentary rats, indicating that the training protocol in this study induced cardiac hypertrophy. Tibial length was also used to normalize heart weight. The ratio of heart weight to tibial length also increased by 10% (P < 0.05) in the trained compared with sedentary animals, further confirming the effectiveness of the exercise training to induce cardiac hypertrophy. The effectiveness of the exercise training protocol was also shown as an increased citrate synthase activity in the tibialis anterior muscle (10% increase in exer-

Fig. 1. Representative left ventricular pressure tracings obtained from hearts isolated from a sedentary animal (top) and an exercise-trained animal (bottom). After a brief period of washout, a latex balloon filled with water was inserted into the left ventricular cavity through which changes in ventricular developed pressure was measured for 60 min (only last 5 min of baseline are shown; 15-min ischemia; 30-min reperfusion) using a pressure transducer.

Fig. 2. Effects of exercise training and ischemia-reperfusion on cardiac contractile functional recovery. A: left ventricular developed pressure (LVDP). B: left ventricular end-diastolic pressure (EDP). C: maximal rate of pressure development (+dP/dtmax). D: maximal rate of relaxation (−dP/dtmax). Bars represent mean ± SE of percent change from the last 5 min before ischemia. The heart obtained from exercise-trained animals exhibited a better functional recovery compared with sedentary control animals (nonischemia control, n = 5; ischemia control, n = 5; nonischemia trained, n = 5; ischemia trained, n = 5). *Significantly different from control, P < 0.05.
LVDP and maximal rate of pressure development (\( \frac{dP}{dt} \)) among the sedentary control, the trained, and ischemia-reperfusion groups (Fig. 2 and Table 2). As such, the trained hearts would appear to be better able to tolerate an ischemic episode than hearts obtained from sedentary rats. During ischemia, most hearts stopped beating within 3 min (sedentary 2.5 min vs. exercise trained 3 min).

**Contractile and mechanical properties of trabeculae.** Properties of trabeculae that were studied are listed in Table 3. Normalized maximal isometric force, \( \frac{P_o}{CSA} \), was significantly greater (26%; \( P < 0.05 \)) in trabeculae from the hearts of exercise-trained compared with sedentary, animals (Fig. 3). Exercise training had no effect on \( V_o \) (Fig. 4). In contrast, ischemia-reperfusion reduced \( V_o \) by 40 and 37%, respectively (\( P < 0.05 \)), in both sedentary and trained trabeculae. Neither relative resting tension (i.e., relative to active tension) nor normalized resting tension (i.e., normalized with CSA) was different across groups (\( P > 0.05 \)). The peak tension generated during the six activations for the force and velocity measurements did not vary significantly (\( P > 0.05 \)) among the sedentary (with or without ischemia-reperfusion) trabeculae. However, there was a significant reduction (\( P < 0.05 \)) in the peak force generated between the first and last activation in the trained trabeculae (Table 3).

**Expression of MHC-\( \beta \).** The MHC-\( \beta \) composition (expressed as the percentage of the total MHC) in control and trained hearts is presented in Fig. 5. Exercise training increased (\( P < 0.05 \)) the mean MHC-\( \beta \) content in trabeculae (49 ± 5%, trained; 21 ± 2%, control), as well as in the left ventricular free wall (23 ± 3%, trained; 10 ± 1%, control). A training-induced increase (\( P < 0.05 \)) in MHC-\( \beta \) composition also occurred in the right ventricular free wall (15 ± 2%, trained; 5 ± 1%, control). The increase in percent MHC-\( \beta \) was higher (threefold increase) in the right ventricular free wall compared with the other two regions (twofold increase in left ventricular free wall and trabeculae). The trabeculae expressed a significantly higher (one-way ANOVA, \( P < 0.05 \)) percentage of MHC-\( \beta \)-compared with the transmural samples of the left and right ventricular free walls, which was group independent (i.e., the percent change from the average of the last 5 min before ischemia (Fig. 2) or as the actual values (see Table 2) was attenuated (\( P < 0.05 \)) in trained myocardium (LVDP: sedentary −60.8 ± 6.4% vs. trained −15.6 ± 8.6%; \( \frac{dP}{dt}_{max} \): sedentary −54.1 ± 4.7 ± 2.5% vs. trained −16.7 ± 8.4%; \( \frac{dP}{dt}_{max} \): sedentary −44.4 ± 2.5% vs. trained −17.9 ± 7.2%; EDP: sedentary 539.5 ± 147.6% vs. trained 71.6 ± 30.6%). In other words, contractile function recovered to a much greater extent after ischemia-reperfusion in exercise-trained animals (see Fig. 2 and Table 2). As such, the trained hearts would appear to be better able to tolerate an ischemic episode than hearts obtained from sedentary rats. During ischemia, most hearts stopped beating within 3 min (sedentary 2.5 min vs. exercise trained 3 min).

**Table 2. Summary of hemodynamics for rats myocardium from ischemia sedentary and trained animals**

<table>
<thead>
<tr>
<th></th>
<th>Sedentary (( n = 5 ))</th>
<th>Trained (( n = 5 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>LVDP baseline, mmHg</td>
<td>80.2 ± 3.0</td>
<td>77.0 ± 1.7</td>
</tr>
<tr>
<td>5 min</td>
<td>30.7 ± 8.7</td>
<td>40.8 ± 3.5</td>
</tr>
<tr>
<td>10 min</td>
<td>30.4 ± 9.0</td>
<td>52.9 ± 8.5</td>
</tr>
<tr>
<td>15 min</td>
<td>30.3 ± 7.9</td>
<td>56.1 ± 9.8*</td>
</tr>
<tr>
<td>20 min</td>
<td>31.6 ± 8.0</td>
<td>59.9 ± 10.4*</td>
</tr>
<tr>
<td>25 min</td>
<td>31.7 ± 6.2</td>
<td>64.0 ± 6.9*</td>
</tr>
<tr>
<td>30 min</td>
<td>32.0 ± 5.5</td>
<td>64.9 ± 6.6*</td>
</tr>
<tr>
<td>EDP baseline, mmHg</td>
<td>7.2 ± 1.0</td>
<td>7.0 ± 0.8</td>
</tr>
<tr>
<td>5 min</td>
<td>55.0 ± 11.6</td>
<td>13.0 ± 1.1</td>
</tr>
<tr>
<td>10 min</td>
<td>51.7 ± 10.4</td>
<td>12.7 ± 0.7*</td>
</tr>
<tr>
<td>15 min</td>
<td>46.0 ± 7.0</td>
<td>11.3 ± 0.6*</td>
</tr>
<tr>
<td>20 min</td>
<td>42.9 ± 6.0</td>
<td>11.8 ± 1.1*</td>
</tr>
<tr>
<td>25 min</td>
<td>42.3 ± 6.1</td>
<td>11.4 ± 1.2*</td>
</tr>
<tr>
<td>30 min</td>
<td>41.3 ± 6.0</td>
<td>11.4 ± 1.2*</td>
</tr>
</tbody>
</table>

+\( \frac{dP}{dt}_{max} \) baseline, mmHg/s | 2.305 ± 0.94            | 2.325 ± 0.62          |
| 5 min     | 1.003 ± 0.79             | 1.380 ± 0.134         |
| 10 min    | 1.029 ± 0.154            | 1.622 ± 0.194         |
| 15 min    | 1.016 ± 0.130            | 1.698 ± 0.229*        |
| 20 min    | 0.984 ± 0.147            | 1.780 ± 0.262*        |
| 25 min    | 1.023 ± 0.135            | 1.882 ± 1.195*        |
| 30 min    | 1.058 ± 0.124            | 1.931 ± 1.188*        |

−\( \frac{dP}{dt}_{max} \) baseline, mmHg/s | −1.488 ± 0.476           | −1.630 ± 0.769        |
| 5 min     | −0.830 ± 0.458           | −1.143 ± 0.147        |
| 10 min    | −0.834 ± 0.772           | −1.257 ± 0.132        |
| 15 min    | −0.835 ± 0.698           | −1.308 ± 0.160*       |
| 20 min    | −0.802 ± 0.809           | −1.352 ± 0.169*       |
| 25 min    | −0.838 ± 0.689           | −1.378 ± 0.149*       |
| 30 min    | −0.829 ± 0.520           | −1.341 ± 0.142*       |

Values are means ± SE; \( n \), no. of animals. LVDP, left ventricular developed pressure; EDP, left ventricular end-diastolic pressure; +\( \frac{dP}{dt}_{max} \), maximal rate of pressure development; −\( \frac{dP}{dt}_{max} \), maximal rate of pressure relaxation. LVDP was calculated as the difference between left ventricular systolic pressure and EDP. Ischemia/reperfusion induced contractile dysfunction. Exercise training attenuated the depressed contractile function. *Significantly different from sedentary control, \( P < 0.05 \).

**Table 3. Functional characteristics of trabeculae obtained from ischemic and nonischemic myocardium of control and trained animals**

<table>
<thead>
<tr>
<th></th>
<th>Nonischemia Control (( n = 13 ))</th>
<th>Nonischemia Trained (( n = 11 ))</th>
<th>Ischemia Control (( n = 12 ))</th>
<th>Ischemia Trained (( n = 12 ))</th>
</tr>
</thead>
<tbody>
<tr>
<td>( P_o/CSA ), kN/m(^2)</td>
<td>20.6 ± 1.7</td>
<td>26.1 ± 2.9†</td>
<td>20.3 ± 2.3</td>
<td>25.5 ± 1.0†</td>
</tr>
<tr>
<td>Change in ( P_o ), (last activation compared with first activation), %</td>
<td>−6.7 ± 3.7</td>
<td>−14.6 ± 3.4‡</td>
<td>−5.1 ± 1.5</td>
<td>−11.7 ± 1.1‡</td>
</tr>
<tr>
<td>( V_o ), trabecula lengths/s</td>
<td>2.5 ± 0.1</td>
<td>3.0 ± 0.3</td>
<td>1.5 ± 0.3*</td>
<td>1.9 ± 0.2*</td>
</tr>
</tbody>
</table>

Values are means ± SE; \( n \), no. of trabeculae; \( P_o \), active peak tension; \( V_o \), maximal unloaded shortening velocity; CSA, cross-sectional area. Change in \( P_o \) was obtained by comparing the changes that occurred from the first to last tension measurement [(last − first)/(first × 100)]. The \( n \) values for tension and \( V_o \) measurements were 13 nonischemia control, 7 ischemia control; 8, nonischemia trained; and 8 ischemia trained. *Significantly different from nonischemic myocardium; \( P < 0.05 \). †Significantly different from control myocardium, \( P < 0.05 \).
same pattern occurred in both sedentary and trained heart, Fig. 5).

DISCUSSION

The present study investigated the potential protective effect of exercise training on myocardial ischemia and reperfusion-induced contractile dysfunction, as measured by the functional recovery of isolated hearts and mechanical properties of trabeculae. The major findings are the following: 1) 15-min ischemia followed by 30-min reperfusion induced significant contractile dysfunction in hearts from sedentary animals, and this depressed contractile function was attenuated in hearts obtained from exercise-trained animals; 2) there was an average 26% increase in Po in trained trabeculae compared with controls, and this increase was not affected by ischemia-reperfusion; 3) exercise training had no effect on Vo, but, in contrast, ischemia-reperfusion reduced the shortening velocity by ~37% in both control and trained trabeculae; and 4) exercise training increased the expression of MHC-β in trabeculae, as well as in the ventricular free walls.

Training effects on the response to ischemia-reperfusion-isolated hearts. In the present study, hearts obtained from endurance exercise-trained animals exhibited a better recovery of contractile function after a brief period of ischemia and reperfusion than hearts obtained from sedentary rats. In agreement with the present study, some studies have reported that exercise training elicited an improved functional recovery, as measured in whole hearts, in response to ischemia-reperfusion (6, 7, 19, 22, 24), whereas other studies did not (19, 21). These conflicting results may result from diverse experimental protocols (e.g., ischemic duration, global vs. regional ischemia), as well as differences in exercise protocols. For example, Libonati et al. (19) found that low-intensity exercise training (i.e., 20 m/min at 0% grade for 6 wk) had no effect on the functional recovery in whole hearts after 20 min of global ischemia, whereas a significant improvement in myocardial contractile function was observed with high-intensity sprint running. Thus variations in exercise intensity employed might, in part, contribute to the differential findings. Despite these differential findings, a substantial body of evidence shows that exercise training can attenuate the reduction in mechanical function after ischemia-reperfusion (6–8, 12, 17, 22, 24, 28, 29).

Several factors have been proposed as mediators of this protection. There is evidence of an exercise training-induced increase in endogenous antioxidants associated with a protective effect on ischemia-reperfusion injury (22, 24, 28). Specif-
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...ically, Powers et al. (22) reported that 10 wk of treadmill exercise training improved functional recovery of the rat myocardium after a 20-min coronary artery occlusion and this functional recovery was accompanied with enhanced endogenous myocardial antioxidants. This finding suggests that the improvement in functional recovery was due to training-induced enhanced antioxidants and that the scavenging effects on the myocardium subjected to ischemia-reperfusion-induced injury. Several other factors have also been proposed as mediators of this protection, including an increase in heat shock proteins (28), glycolysis (19), high-energy phosphates (6, 7), and/or Ca\(^{2+}\)-handling mechanisms (7). The relative contribution of each of these putative beneficial effects of exercise training remains to be determined.

Several previous studies demonstrated that the attenuated response to ischemia, shown as an improvement in functional recovery, occurs in the absence of an exercise-mediated change in heat shock proteins (12, 29). Similarly, a training-induced improved functional recovery was also observed without an increase in manganese superoxide dismutase activity, a key antioxidant enzyme present in mitochondria (18). Thus the results of these studies suggest that training-induced alterations in either antioxidant or heat shock proteins alone are not sufficient to mediate the protective effect of exercise training on ischemia-reperfusion. These findings further suggest that the putative beneficial effect requires multiple myocardial adaptations (13, 18). In other words, the protection against ischemia-reperfusion results from a combination of multiple training-induced cardioadaptive factors, and it is unlikely that one specific mechanism is responsible for this cardioprotection.

Effects of exercise training on MHC isoform expression and the response to ischemia-reperfusion. In the present study, 10–12 wk of treadmill exercise training increased the expression of myocardial MHC-β in three regions of the heart (i.e., left and right ventricular free walls and trabeculae obtained from the left ventricle). Overall, the increased expression of MHC-β could impart energetic advantages over the myocardium expressing predominantly MHC-α (30). This greater economy may become apparent when O\(_2\) and/or energy supply to the myocardium are not readily available, e.g., during ischemia. In particular, considering the fact that a significant fraction of myocardial ATP is utilized by the Ca\(^{2+}\)-dependent actomyosin Mg\(^{2+}\)-ATPase during muscle activation (23), the presumed economy induced by an increase in MHC-β could possibly leave more ATP available to maintain intracellular Ca\(^{2+}\) homeostasis. The resulting reduction in cellular Ca\(^{2+}\) overload would attenuate the mechanical dysfunction induced by ischemia-reperfusion. In the present study, however, ischemia-reperfusion induced a reduction in V\(_o\), in the presence of enhanced expression of MHC-β. These data suggest that the expected energetic advantage due to the increased MHC-β composition of the trained myocardium was not sufficient to attenuate the ischemia-reperfusion-induced reduction in V\(_o\). These data also suggest that causative mechanisms leading to this ischemia-reperfusion-induced reduction in V\(_o\) are independent of any energetic advantage induced by exercise training, e.g., free radical generation and its damaging effects. However, it is important to note that, despite the absence of a training effect on V\(_o\), the trained myocardium exhibited a better functional recovery. Thus it is possible that the presumed greater economy induced by an increase in MHC-β composition may provide the basis for this improvement in functional recovery. Blunt et al. (3) recently reported that hearts with a greater level of MHC-β, induced by treatment of rats with propylthiouracil for 8 days, exhibited significantly better postischemic function, compared with hearts from nontreated rats, which have relatively higher levels of MHC-α. Additional studies will be required to determine the contribution of this training-induced increase in myocardial contractile economy to the functional recovery after ischemia-reperfusion.

The question remains as to what could account for the differential effects of exercise training on the mechanical response to ischemia-reperfusion in isolated hearts and trabeculae. With regard to V\(_o\), it is important to remember that power output is a function of both shortening velocity and force generation. Thus one might expect that a reduction in V\(_o\) after ischemia-reperfusion would result in a reduced contractile function of the myocardium. However, an increase in P\(_{V}^{o}/CSA\) in the trained myocardium would attenuate the reduced contractile function induced by ischemia-reperfusion. Furthermore, a training-induced increase in the expression of MHC-β may provide an economical advantage for muscle contraction, even in the face of ischemia-reperfusion-induced injury. As noted above, this could compensate for reductions in V\(_o\). The net effect of the cardiac adaptations induced by exercise training is a more complete mechanical recovery (LVDP, +dP/dt\(_{max}\), and −dP/dt\(_{max}\)) after ischemia-reperfusion in the hearts isolated from the exercise-trained animals. Thus exercise training appears to attenuate contractile dysfunction following ischemia-reperfusion.

ACKNOWLEDGMENTS

The research herein described was submitted for the partial fulfillment of the requirements for the Doctor of Philosophy degree (H. Hwang).

GRANTS

The research was supported in part by National Heart, Lung, and Blood Institute Grant HL-68609.

REFERENCES


