Loss of exercise-induced cardioprotection after cessation of exercise

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Lennon, Shannon L., John Quindry, Karyn L. Hamilton, Joel French, Jessica Staib, Jawahar L. Mehta, and Scott K. Powers. Loss of exercise-induced cardioprotection after cessation of exercise. J Appl Physiol 96: 1299–1305, 2004. First published December 12, 2003; 10.1152/japplphysiol.00920.2003.—Endurance exercise provides cardioprotection against ischemia-reperfusion (I/R) injury. Exercise-induced cardioprotection is associated with increases in cytoprotective proteins, including heat shock protein 72 (HSP72) and increases in antioxidant enzyme activity. On the basis of the reported half-life of these putative cardioprotective proteins, we hypothesized that exercise-induced cardioprotection against I/R injury would be lost within days after cessation of exercise. To test this, male rats (4 mo) were randomly assigned to one of five experimental groups: 1) sedentary control, 2) exercise followed by 1 day of rest, 3) exercise followed by 3 days of rest, 4) exercise followed by 9 days of rest, and 5) exercise followed by 18 days of rest. Exercise-induced increases ($P < 0.05$) in left ventricular catalase activity and HSP72 were evident at 1 and 3 days postexercise. However, at 9 days postexercise, myocardial HSP72 and catalase levels declined to sedentary control values. To evaluate cardioprotection during recovery from I/R, hearts were isolated, placed in working heart mode, and subjected to 20.5 min of global ischemia followed by 30 min of reperfusion. Compared with sedentary controls, exercised animals sustained less I/R injury as evidenced by maintenance of a higher ($P < 0.05$) percentage of preischemic cardiac work during reperfusion at 1, 3, and 9 days postexercise. The exercise-induced cardioprotection vanished by 18 days after exercise cessation. On the basis of the time course of the loss of cardioprotection and the return of HSP72 and catalase to preexercise levels, we conclude that HSP72 and catalase are not essential for exercise-induced protection during myocardial stunning. Therefore, other cytoprotective molecules are responsible for providing protection during I/R.

REGULAR PHYSICAL ACTIVITY is associated with multiple cardiovascular benefits, including a decreased incidence of myocardial infarction (MI) and improved survival rate after MI (18). These epidemiological findings have been confirmed by numerous experimental studies indicating that both acute and chronic exercise provides protection against myocardial ischemia-reperfusion (I/R) injury in animals (4, 5, 9, 14–17, 28, 36). Specifically, both short-term (i.e., 1–5 days) and long-term (i.e., weeks to months) exercise decreases I/R-induced cardiac injury resulting from both short-duration (i.e., 5–20 min resulting in myocardial stunning) and long-duration (i.e., >20 min resulting in MI) myocardial ischemia. Although the mechanism(s) responsible for exercise-mediated cardioprotection remain unknown, it has been postulated that exercise-induced increases in myocardial levels of heat shock protein (HSP) 72 and/or antioxidant defenses contribute to this cardioprotection (9, 28, 38, 39, 49). In regard to HSP72 and cardioprotection, transgenic mice that overexpress HSP72 provide convincing evidence that HSP72 imparts cardioprotection. Indeed, compared with wild type, these transgenic animals possess a cardioprotective phenotype as illustrated by improved postischemic contractile function and decreased infarction size (21, 40). Furthermore, it has been shown that enhanced endogenous antioxidants could also be an important component of exercise-induced cardioprotection (49). Therefore, current evidence indicates that, collectively or independently, exercise-induced increases in myocardial levels of HSP72 or antioxidants could lead to improved protection during I/R insults (9, 14, 15, 28, 38, 39).

Although it is clear that exercise provides cardioprotection, it is currently unknown how long the heart retains a cardioprotective phenotype after the cessation of exercise. Therefore, this study investigated the time course of the loss of cardioprotection after cessation of exercise. On the basis of the half-life of putative cardioprotective mediators such as HSP72 (35) that are elevated in response to exercise, we hypothesized that exercise-induced cardioprotection against I/R injury would disappear within 9 days after the cessation of exercise training. To test this postulate, cardioprotection against I/R injury was evaluated at selected time periods after cessation of exercise training by using an isolated working heart model of global ischemia and reperfusion.

METHODS

Animals and Experimental Design

This experimental protocol was approved by the University of Florida Animal Care and Use Committee and followed the guidelines established by the American Physiological Society for the use of animals in research. Male Sprague-Dawley rats (~4 mo old; ~370–400 g) were randomly assigned to one of five experimental groups: 1) sedentary “control” group, 2) exercise followed by 1 day of rest, 3) exercise followed by 3 days of rest, 4) exercise followed by 9 days of rest, and 5) exercise followed by 18 days of rest. These experimental...
groups were then subdivided into sham or in vitro I/R groups. Hearts from the sham groups were used to assess exercise-induced changes in antioxidant enzyme activities. During the study, all experimental groups were maintained on a 12:12-h light-dark photoperiod and provided food and water ad libitum. We selected the rat as an experimental animal because this species is a widely accepted mammalian model for the study of myocardial function and adaptation. Furthermore, choosing male animals avoided variances in blood estrogen levels that occur in female animals. Finally, selection of young adult animals removed confounding experimental variables associated with both development and old age.

Exercise Protocol

Animals assigned to exercise groups performed a total of 8 days of exercise. Treadmill running was selected as our exercise mode to avoid the physiological and psychological issues (e.g., hypoxic exercise and/or fear) associated with swim training in rodents. Exercise training included 5 days of habituation to the treadmill beginning with 10 min of running at 30 m/min, 0% grade, with daily increases of 10 min until 50 min/day were achieved. Animals then performed 3 consecutive days of 60 min running/day (30 m/min, 0% grade) at an estimated work rate of ~70% maximal O2 consumption (26). Note that during the 60 min of daily exercise, the animals were provided three equally spaced 2- to 3-min rest periods. Mild electrical shocks were used sparingly to motivate animals to run. The intensity and duration of the exercise training protocol were selected because this training program has been shown to promote myocardial adaptations and provide cardioprotection against myocardial stunning (9, 14, 15, 37, 38). Exercised animals were killed with a lethal injection of pentobarbital sodium at 1, 3, 9, or 18 days after their last exercise session.

Myocardial Biochemical Measurements

Measurement of myocardial antioxidant capacity. Because exposure of the heart to I/R can decrease antioxidant enzyme activity, left ventricular tissue from sham groups was used to investigate the effects of exercise training on antioxidant capacity. The antioxidants selected were chosen because each plays an important role in regulation of myocardial redox balance and has a key responsibility in providing myocardial protection against oxidative injury. The hearts from sham animals were rapidly removed, rinsed in antioxidant buffer (50 mM NaHPO4, 0.1 mM butylated hydroxytoluene, 0.1 mM EDTA; pH 7.4), and quickly frozen in liquid nitrogen. Sections of the left ventricular and quickly frozen in liquid nitrogen. Sections of the left ventricular NaHPO4, 0.1 mM butylated hydroxytoluene, 0.1 mM EDTA; pH 7.4), animals were rapidly removed, rinsed in antioxidant buffer (50 mM were chosen because each plays an important role in regulation of sure of the heart to I/R can decrease antioxidant enzyme activity, left

Measurement of myocardial HSPs. To determine the effects of exercise on induction of myocardial HSPs, we performed polyacryl-

amide gel electrophoresis and immunoblotting by using the tech-
niques as previously described (14, 28, 38). Briefly, left ventricular
samples from I/R animals were homogenized, and one-dimensional sodium dodecyl sulfate-polyacrylamide (12%) gel electrophoresis was performed to separate proteins by molecular weight. After separation, proteins were transferred to nitrocellulose membranes (Bio-Rad, Hercules, CA). Nitrocellulose membranes were blocked for 2 h with (0.5%) bovine serum albumin. Blots were incubated for 2 h with monoclonal antibodies for HSP72 (SPA-810, StressGen, Victoria, Canada). After chemiluminescence detection (Amersham Biosciences, Piscataway, NJ) and computerized densitometry, results were expressed as a percentage of sedentary controls.

In Vitro I/R Experiments

To evaluate the time course of exercise-associated protection against I/R contractile dysfunction, we used an isolated working heart preparation. At the appropriate time span after cessation of exercise training, animals were anesthetized with 100 mg/kg pentobarbital sodium (i.p.). To facilitate removal of blood from the heart and to prevent clot formation, 100 IU of heparin were injected directly into the hepatic vein. Hearts were then rapidly excised, placed in ice-cold saline, and weighed on an electronic scale (gross wet weight). The aorta was quickly secured on a stainless steel catheter, and the heart was retrograde perfused by using a modified Krebs Henseleit buffer (1.5 mM CaCl2, 130 mM NaCl, 5.4 mM KCl, 11 mM glucose, 2 mM pyruvate, 0.5 mM MgCl2, 0.5 mM NaH2PO4, and 25 mM NaHCO3, pH 7.4) aerated with 95% O2-5% CO2 at a constant perfusion pressure of 80 cmH2O and a temperature of 37°C. Excess tissue was trimmed, weighed, and subtracted from the gross weight to get a final wet weight of the heart. All subsequent values were normalized to this final heart weight.

After 15 min of retrograde perfusion, the hearts were switched to the working heart mode and preischemic heart function was evaluated at 13 ± 0.5 cmH2O (atrial filling pressure) with an 80-cm-high aortic column. Coronary and aortic flows were determined by timed collections of the coronary effluent and aortic column overflow, respectively. Cardiac output was determined as the sum of coronary and aortic flow.

Global, normothermic ischemia was then induced by simultaneously cross-clamping the atrial inflow and the aortic outflow for 20.5 min. During ischemia, hearts remained in a water-jacketed chamber maintained at 37°C. After ischemia, hearts were initially perfused in the retrograde mode for 15 min at a perfusion pressure of 80 cmH2O and then switched to the working heart mode for 15 min for a total of 30 min of reperfusion. The initial reperfusion period in the retrograde mode is necessary to stabilize the hearts before working heart reperfusion. Coronary effluent was collected between minutes 5 and 7 of reperfusion, and lactate dehydrogenase (LDH; EC 1.1.1.27) activity in the effluent was assayed as a marker of myocardial injury (2). Furthermore, cardiac output was monitored at selected time periods during this final 15 min of postischemia recovery in the working heart mode.

We exposed these hearts to 20.5 min of global ischemia because this level of myocardial injury results in a “stunned myocardium” with a ~40% decrease in cardiac working capacity. Myocardial stunning is defined as a nonlethal form of I/R injury that results in impaired cardiac function for 24–48 h after I/R without death of cardiac myocytes. We selected the isolated working heart model in these experiments for several reasons. First, this preparation closely resem-bles the in vivo working heart, and isolated working heart experiments are highly reproducible (44). Second, when contrasted to in vivo models, an advantage of the isolated heart preparation is the ability to...
control afterload and preload of the heart. Third, this model is capable of detecting small changes in cardiac function (50). Finally, because of the collective aforementioned advantages, it has been argued that the working heart is the “model of choice” to study myocardial stunning (50).

**Statistical Analyses**

Myocardial contractility and biochemical measurements were analyzed by using a one-way analysis of variance. When appropriate, a Tukey’s post hoc test was applied. Significance was established at \( P < 0.05 \).

**RESULTS**

**Animal Characteristics**

Animal characteristics are presented in Table 1. No differences existed in animal body weights between the experimental groups. Furthermore, no differences existed in heart weights or the ratio of body weight to heart weight between the five experimental groups.

**Exercise-Induced Changes in Myocardial Antioxidants and HSP72**

Exercise did not significantly alter myocardial GPX or SOD activities or cardiac levels of glutathione. The observation that exercise-related increases in MnSOD activity did not reach statistical significance is not consistent with our previous work and appears to be due to large interanimal variability. In contrast, exercise significantly elevated myocardial Cat activity at both 1 and 3 days postexercise. By day 9, myocardial Cat activity returned to a level not different from sedentary control animals (Table 2).

Compared with the sedentary control animals, exercise resulted in significant increases in myocardial HSP72 levels at 1 and 3 days postexercise (Fig. 1). Similar to exercise-induced changes in Cat activity, however, these training-induced changes were not evident at 9 days after cessation of exercise. Finally, because myocardial levels of HSP72 and antioxidant activity returned to untrained levels by 9 days after cessation of exercise, biochemical measurements were not performed on hearts obtained from animals subjected to 18 days of detraining.

**Exercise Training and Cardiac Function During Recovery From Ischemia**

Successful I/R protocols were completed on 11 animals from the control group along with 10, 9, and 9 animals from the 1, 3, and 9 days postexercise groups, respectively. Animals were excluded from data analysis if they did not complete the full exercise protocol or if their hearts did not demonstrate stable baseline performance during preischemia data collection. The effects of exercise training on pre- and postschemic cardiac function at each time point postexercise are presented in Table 3 and Fig. 2. Note that, before ischemia, no differences existed between experimental groups in heart rate, coronary flow, cardiac output, or cardiac work. Furthermore, no differences existed between any of the experimental groups in heart rate or coronary flow at 30 min of reperfusion. Also, no group differences existed in aortic diastolic pressure at 30 min of reperfusion (data not shown). Reperfusion after 20.5 min of global ischemia resulted in a significant contractile dysfunction in sedentary control animals. For example, compared with preischemic values, percent recovery of cardiac work was depressed by \( >30\% \) in sedentary control animals at 30 min of reperfusion. In contrast to control, exercise-trained animals studied at 1, 3, and 9 days postexercise maintained significantly higher relative cardiac outputs and cardiac work at 30 min of reperfusion (Table 3, Fig. 2). Note that cardiac work increased rapidly during reperfusion and that a steady state was achieved within 5 min after reintroduction of the working heart mode. Therefore, only values from minute 30 of reperfusion are reported.

Absolute LDH activity in the coronary effluent (preischemia) ranged from 12 to 41 mU·min \(^{-1} \)·g wet weight \(^{-1} \) across the five experimental groups. Postischemic LDH activity in the

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control (n = 11)</th>
<th>1 Day (n = 10)</th>
<th>3 Days (n = 9)</th>
<th>9 Days (n = 9)</th>
<th>18 Days (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Body weight, g</td>
<td>382.5 ± 2.44</td>
<td>382.5 ± 2.94</td>
<td>381 ± 2.74</td>
<td>392.8 ± 3.74</td>
<td>372.6 ± 2.6</td>
</tr>
<tr>
<td>Heart weight, g</td>
<td>1.13 ± 0.01</td>
<td>1.12 ± 0.02</td>
<td>1.1 ± 0.02</td>
<td>1.15 ± 0.02</td>
<td>1.11 ± 0.02</td>
</tr>
<tr>
<td>Heart-to-body weight ratio, mg/g</td>
<td>2.96 ± 0.03</td>
<td>2.92 ± 0.06</td>
<td>2.89 ± 0.07</td>
<td>2.93 ± 0.05</td>
<td>2.98 ± 0.03</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. No differences existed in any measurement between experimental groups.

**Table 1. Postexercise training animal body and heart weights for animals used in working heart experiments**

<table>
<thead>
<tr>
<th>Enzyme Activity (per mg protein)</th>
<th>Control (n = 10)</th>
<th>1 Day (n = 10)</th>
<th>3 Days (n = 10)</th>
<th>9 Days (n = 10)</th>
</tr>
</thead>
<tbody>
<tr>
<td>GPX, μmol/min</td>
<td>0.21 ± 0.01</td>
<td>0.244 ± 0.009</td>
<td>0.235 ± 0.006</td>
<td>0.221 ± 0.007</td>
</tr>
<tr>
<td>Cat, units</td>
<td>1.04 ± 0.03</td>
<td>1.21 ± 0.03†</td>
<td>1.17 ± 0.02†</td>
<td>1.06 ± 0.03</td>
</tr>
<tr>
<td>Total SOD, units</td>
<td>42.77 ± 1.79</td>
<td>44.84 ± 2.58</td>
<td>48.03 ± 2.52</td>
<td>46.19 ± 3.17</td>
</tr>
<tr>
<td>MnSOD, units</td>
<td>18.17 ± 1.63</td>
<td>20.82 ± 1.26</td>
<td>22.12 ± 1.73</td>
<td>19.64 ± 1.99</td>
</tr>
<tr>
<td>Cu/ZnSOD, units</td>
<td>24.60 ± 1.79</td>
<td>23.72 ± 1.82</td>
<td>25.91 ± 2.05</td>
<td>26.54 ± 2.65</td>
</tr>
<tr>
<td>Total glutathione, mmol</td>
<td>1.36 ± 0.08</td>
<td>1.38 ± 0.10</td>
<td>1.44 ± 0.12</td>
<td>1.47 ± 0.11</td>
</tr>
</tbody>
</table>

Values are means ± SE; n, no. of animals. GPX, glutathione peroxidase; Cat, catalase; SOD, superoxide dismutase; MnSOD, manganese SOD; Cu/ZnSOD, copper-zinc SOD. Cat units were calculated by using the rate constant of a first-order reaction (k), K = (2.303)/log(10 A1/A2), where t is time in min, A1 is initial absorbance reading − blank absorbance, and A2 is absorbance reading at 1 min − blank absorbance. One unit of SOD activity is defined as the amount of SOD required for a 50% decrease in cytochrome c reduction rate. *Different from control, P < 0.05. †Different from 9 days, P < 0.05.
coronary effluent ranged from 20 to 264 mU·min⁻¹·g wet weight⁻¹ across the experimental groups; these values are consistent with I/R insults that result in myocardial stunning. For clarity, LDH activities were normalized (percent) to preischemic values. Consistent with the cardiac performance measures, analyses revealed LDH activity in the coronary effluent collected during reperfusion was significantly higher from control hearts compared with exercise trained hearts at 1, 3, and 9 days postexercise (Fig. 3).

Because we observed protection against I/R-induced cardiac dysfunction and LDH release that persisted through 9 days postexercise, we further assessed the time course of exercise-induced cardioprotection by adding another exercise-trained group that was evaluated at 18 days postexercise. As illustrated in Table 3 and Fig. 2, exercise-induced preservation of posts ischemic cardiac output and cardiac work was lost at 18 days postexercise. Similarly, I/R-induced LDH release from the heart at 18 days postexercise was not different from controls (Fig. 3). Collectively, these findings indicate that exercise-induced cardioprotection against myocardial stunning remains for at least 9 days after cessation of exercise training.

**DISCUSSION**

**Overview of Principal Findings**

To our knowledge, this is the first investigation of the relationship between the loss of protection against myocardial stunning after cessation of exercise and the reduction in putative cardioprotective proteins in the heart. Our data indicate that exercise-induced cardioprotection against myocardial stunning remains for at least 9 days after cessation of exercise training. Importantly, we observed that cardioprotection persists in the absence of elevated myocardial levels of HSP72 and antioxidants (e.g., Cat) at 9 days postexercise. These findings indicate that exercise-associated cardioprotection is not critically dependent on increases in cardiac levels of these cytoprotective proteins.

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**Table 3. Effects of exercise training intensity on pre- and posts ischemic cardiac function**

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 11)</th>
<th>1 Day (n = 10)</th>
<th>3 Days (n = 9)</th>
<th>9 Days (n = 9)</th>
<th>18 Days (n = 8)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Preischemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>342.6 ± 7.9</td>
<td>347.2 ± 7.9</td>
<td>336.5 ± 10.4</td>
<td>337.6 ± 5.7</td>
<td>311.2 ± 16.3</td>
</tr>
<tr>
<td>CF, ml·min⁻¹·g</td>
<td>14.9 ± 0.47</td>
<td>16.5 ± 0.55</td>
<td>17.9 ± 0.92†</td>
<td>15.5 ± 0.44</td>
<td>14.3 ± 0.5</td>
</tr>
<tr>
<td>CO, ml·min⁻¹·g</td>
<td>43 ± 0.45</td>
<td>43.6 ± 1.07</td>
<td>42.7 ± 1.1</td>
<td>43 ± 0.9</td>
<td>41.5 ± 1.6</td>
</tr>
<tr>
<td>SP, mmHg</td>
<td>90.7 ± 1.53†</td>
<td>89.4 ± 1.4†</td>
<td>91.3 ± 1.9†</td>
<td>91.9 ± 1.6†</td>
<td>100.4 ± 3.1†</td>
</tr>
<tr>
<td>Cardiac work, SP × CO</td>
<td>3,900 ± 75</td>
<td>3,900 ± 124</td>
<td>3,889 ± 81</td>
<td>3,948 ± 87</td>
<td>4,158 ± 178</td>
</tr>
<tr>
<td>RPP, HR × SP</td>
<td>30,953 ± 256</td>
<td>30,946 ± 314</td>
<td>30,585 ± 563</td>
<td>30,974 ± 338</td>
<td>30,976 ± 1,228</td>
</tr>
</tbody>
</table>

| **Postischemia**     |                 |              |               |               |                |
| HR, beats/min        | 328.7 ± 10.5    | 328 ± 8.9    | 341 ± 13.8    | 336 ± 5.8     | 310 ± 16       |
| CF, ml·min⁻¹·g       | 14.5 ± 0.99     | 16.5 ± 0.97  | 16.1 ± 1.1    | 15.2 ± 0.78   | 12.2 ± 0.75    |
| CO, ml·min⁻¹·g       | 33.6 ± 1.6      | 39.3 ± 1.5*  | 41 ± 1.2*     | 38.5 ± 1.4*   | 33.2 ± 2.9     |
| SP, mmHg             | 82.9 ± 2.7      | 85.3 ± 1.4   | 85.2 ± 2.4    | 84.7 ± 2.3    | 87.3 ± 3.4     |
| Cardiac work, SP × CO| 2,818 ± 212     | 3,354 ± 146  | 3,462 ± 71    | 3,255 ± 134   | 2,916 ± 278    |
| Cardiac work, %preischemia | 68 ± 4      | 86 ± 3*      | 89 ± 2*       | 83 ± 4*       | 74 ± 3         |
| RPP, HR × SP         | 27,006 ± 477    | 27,881 ± 395 | 28,699 ± 551  | 28,394 ± 510  | 26,806 ± 112   |

Values are means ± SE; n, no. of animals. Postischemia values represent 30 min of reperfusion measured in the working heart mode. HR, heart rate; CF, coronary flow; CO, cardiac output; SP, systolic pressure; RPP, rate-pressure product. *Significantly different from control, P < 0.05. †Significantly different from 18 days, P < 0.05.
associated with small (e.g., 10 g) amounts of work in our laboratory, have reported that exercise is protective against I/R injury (9, 14, 15, 37, 38). In contrast, several studies, including reports (3, 14, 17, 38, 46), have suggested that exercise does not elevate myocardial levels of glutathione or the activities of GPX or two intracellular forms of SOD. These findings agree with previous data that short-term exercise training does not elevate myocardial glutathione or the activities of GPX or two intracellular forms of SOD. Furthermore, our data show that acute exercise training is associated with threefold increases in myocardial levels of HSP72. Exercise-induced increases in myocardial levels of HSP72 are widely reported in the literature, and an increase in this stress protein has been postulated to play a role in exercise-induced cardioprotection (14–16, 28, 46).

It is well established that both ischemia and reperfusion promote the formation of radicals/oxidants and that oxidative injury contributes to myocardial stunning (3). It follows that exercise-induced increases in myocardial antioxidants could provide cardioprotection against this type of I/R injury. Therefore, to determine the effects of exercise on myocardial antioxidant capacity, we measured glutathione levels along with the activities of key antioxidant enzymes (Table 2). Our results indicate that short-term exercise training does not elevate myocardial glutathione or the activities of GPX or two intracellular forms of SOD (i.e., CuZnSOD or MnSOD). The finding that acute exercise does not elevate myocardial levels of glutathione, GPX, and CuZnSOD agrees with previous reports (9, 14, 15, 37, 38). In contrast, several studies, including work in our laboratory, have reported that exercise is associated with small (e.g., 10–20%) but significant increases in myocardial MnSOD activity (9, 14, 15, 17, 38). Among this work are studies indicating that exercise-induced increases in MnSOD may be critical for protection against MI (17, 49). Nonetheless, in the present study, although the mean MnSOD activity tended to be higher in exercise-trained hearts compared with control, these levels did not reach significance.

In contrast to other antioxidant enzymes, exercise training elevated myocardial Cat activity. Although some controversy exists, others have reported exercise-induced increases in cardiac Cat activity in both short- and long-duration exercise training (16, 19, 23, 25, 41, 42, 47). In contrast, myocardial glutathione levels are typically not elevated after short-term exercise, but most long-term training studies report that several weeks of training elevates cardiac glutathione levels (9, 19, 20, 27, 34). On the basis of these observations, it is tempting to speculate that the myocardial strategy to eliminate hydrogen peroxide changes over time in response to continuous exercise training. That is, the increased ability to remove hydrogen peroxide early during training adaptation is achieved by elevating Cat activity alone, whereas as exercise training continues, there is a gradual increase in glutathione levels. Increasing cardiac levels of glutathione with chronic exercise training would improve the ability of the heart to eliminate hydrogen peroxide because glutathione works in tandem with GPX to remove hydrogen peroxide and other organic hydroperoxides. The biological rationale for this changing adaptive strategy is unclear but could be linked to the fact that exercise-induced increases in Cat activity can be achieved rapidly via gene expression, whereas myocardial glutathione synthesis is a relatively slow process limited by the availability of cellular levels of cysteine (13).

**Exercise-Induced Myocardial Adaptation**

Our results confirm previous data that short-term endurance exercise training results in cardioprotection against an in vitro I/R insult that produces severe myocardial stunning. This agrees with previous reports in which both in vivo and in vitro I/R models were used to study exercise-induced cardioprotection (14–17, 28, 38, 46). Furthermore, our data show that acute exercise training is associated with threefold increases in myocardial levels of HSP72. Exercise-induced increases in myocardial levels of HSP72 are widely reported in the literature, and an increase in this stress protein has been postulated to play a role in exercise-induced cardioprotection (14–16, 28, 46).

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**Postexercise Loss of Cardioprotection**

The mechanism(s) responsible for exercise-induced cardioprotection remains controversial. It has been suggested that exercise-induced increases in cardiac levels of HSP72 and/or key antioxidants are potential mechanisms to explain the cardioprotection associated with physical activity (8, 17, 22, 28–30, 32, 49). Our hypothesis that exercise-induced cardioprotection against myocardial stunning would disappear within 9 days after the cessation of exercise training was formulated by reports indicating that the half-life of putative cardioprotective proteins (e.g., HSP72) is approximately 2–3 days (35). The half-life of circulating levels of some of the isoforms of antioxidant enzymes, however, can be as short as a few hours (12). Therefore, after cessation of exercise training, the postexercise decline in HSP72 would result in an approximately 88% reduction in myocardial levels within 9 days. It follows that, if HSP72 and/or Cat is essential for exercise-induced cardioprotection, the protection would disappear after myocardial levels of these protective proteins decline to sedentary control levels. Our results reveal that cardiac levels of both HSP72 and Cat decline over time and reach sedentary control values at day 9 postexercise. Nonetheless, the finding that cardioprotection remains at 9 days postexercise indicates that mechanisms other than HSP72 and Cat are involved in exercise-related protection against I/R-induced myocardial stunning.

**Potential Mechanisms of Exercise-Induced Protection Against Myocardial Stunning**

If exercise-induced increases in myocardial Cat and HSP72 are not essential to achieve exercise-mediated protection against myocardial stunning, other mechanism(s) must be responsible for this form of cardioprotection. The two primary theories to explain the pathogenesis of myocardial stunning are the radical hypothesis and the calcium hypothesis (3). The radical hypothesis postulates that myocardial stunning results from oxidative injury by radicals and other reactive species produced during ischemia and reperfusion. This oxidative...
injury could impair myocardial contractility due to a reduced responsiveness of contractile filaments to calcium (3). The calcium hypothesis is based on evidence that I/R results in a transient calcium overload in the myocardium that triggers calcium-activated proteases (i.e., calpains) to degrade key cytoskeletal proteins. The radical and calcium hypotheses are not mutually exclusive because calcium overload in cells can contribute to radical production and oxidation of calcium transport proteins can promote disturbances in calcium homeostasis (3). Therefore, it is feasible that the mechanism responsible for exercise-induced protection against myocardial stunning is linked to exercise provoked expression of antioxidants and/or calcium handling proteins.

As mentioned previously, others have concluded that exercise-induced increases in MnSOD may be critical for protection against myocardial infarction (17, 49). Nonetheless, in the present study, although the mean MnSOD activity tended to be higher in exercise-trained hearts compared with control, these levels did not reach significance. Furthermore, work in our laboratory using antisense oligonucleotides indicates that exercise-induced increases in MnSOD are not essential for protection against myocardial stunning (16, 24). However, it seems likely that other antioxidants may be involved in cardioprotection. In this regard, preliminary experiments in our laboratory suggest that exercise elevates cardiac mRNA levels of several other antioxidant proteins (e.g., thioredoxin reductase, metallothionen) in the heart (unpublished observations). Future experiments are required to determine whether one or more of these antioxidants are essential to achieve exercise-induced protection against myocardial stunning.

Related to the calcium hypothesis of myocardial stunning, several laboratories have investigated exercise-induced changes in myocardial calcium handling. For example, although exercise does not alter the number of L-type calcium channels in the heart (33), exercise training does increase the rate of calcium transport by calcium ATPase in the sarcoplasmic reticulum of rat hearts (45). At present, other than sarcoplasmic reticulum calcium ATPases, limited information exists regarding exercise-mediated changes in other calcium handling proteins in the heart. It seems possible that exercise training may alter calcium-handling proteins resulting in protection against I/R-induced calcium overload. This could provide an exciting area for future research.

Summary and Conclusions

These experiments investigated the rate of decline of exercise-induced cardioprotection after the cessation of exercise training. Our data indicate that exercise-induced cardioprotection remains for 9 days after cessation of exercise training but that this protection is absent at 18 days postexercise. The fact that cardioprotection persists in the absence of elevated myocardial levels of HSP72 and Cat at day 9 postexercise implies that cytoprotective molecules other than HSP72 and Cat provide cardioprotection against myocardial stunning. Although the mechanism(s) responsible for exercise-induced cardioprotection remains elusive, continuing advances in biotechnology (i.e., microarray analysis) will permit the measurement of transcription in a large number of genes and will greatly accelerate research progress in our laboratory and others (7, 10).

REFERENCES

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