Exercise training improves myocardial tolerance to in vivo ischemia-reperfusion in the rat

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Powers, Scott K., Haydar A. Demirel, Heather K. Vincent, Jeff S. Coombes, Hisashi Naito, Karyn L. Hamilton, R. Andrew Shanely, and James J. Essup. Exercise training improves myocardial tolerance to in vivo ischemia-reperfusion in the rat. Am. J. Physiol. 275 (Regulatory Integrative Comp. Physiol. 44): R1468–R1477, 1998.—Experimental studies examining the effects of regular exercise on cardiac responses to ischemia and reperfusion (I/R) are limited. Therefore, these experiments examined the effects of endurance exercise training on myocardial biochemical and physiological responses during in vivo I/R. Female Sprague-Dawley rats (4 mo old) were randomly assigned to either an exercise training group or to an exercise training group. After a 10-wk endurance exercise training program, animals were anesthetized and mechanically ventilated, and the chest was opened by thoracotomy. Coronary occlusion was achieved by a ligature around the left coronary artery; occlusion was maintained for 20 min, followed by a 10-min period of reperfusion. Compared with untrained, exercise-trained animals maintained higher (P < 0.05) peak systolic blood pressure throughout I/R. Training resulted in a significant (P < 0.05) increase in ventricular nonprotein thiols, heat shock protein (HSP) 72, and the activities of superoxide dismutase (SOD), phosphofructokinase (PFK), and lactate dehydrogenase. Furthermore, compared with untrained controls, left ventricles from trained animals exhibited lower levels (P < 0.05) of lipid peroxidation after I/R. These data demonstrate that endurance exercise training improves myocardial contractile performance and reduces lipid peroxidation during I/R in the rat in vivo. It appears likely that the improvement in the myocardial responses to I/R was related to training-induced increases in nonprotein thiols, HSP72, and the activities of SOD and PFK in the myocardium.

endurance exercise; heart; free radicals; cardiac hypertrophy; lipid peroxidation; superoxide dismutase

NUMEROUS HUMAN epidemiological studies exist to support the notion that regular exercise is cardioprotective (20, 33, 40). Indeed, Morris et al. (33) have shown that the incidence of myocardial infarctions is reduced in physically active individuals. Furthermore, these investigators reported that the survival rate of heart attack victims is greater in active individuals compared with sedentary ones.

In contrast to the large number of epidemiological studies, experimental studies examining the effects of regular exercise on cardiac responses to ischemia and reperfusion (I/R) are relatively few. Furthermore, studies examining the effects of endurance exercise on myocardial responses to I/R have provided equivocal results, with some investigators reporting that training improves myocardial tolerance to I/R (5, 7, 8, 23, 30), whereas others report no improvement (15, 35) or even a decreased myocardial tolerance to I/R (28). The explanation for the divergent findings is unclear but may be linked to differences in exercise training programs and/or variations in the experimental protocols used to study myocardial responses to I/R. Clearly, additional research is required to clarify this issue.

Many of the aforementioned experimental studies have used rodent swim training as the exercise model. In this regard, Bowles et al. (7) have argued that the swimming model in rats has significant limitations. Their argument centers around two major points. First, swimming results in markedly different cardiovascular, sympathetic, and hormonal responses compared with treadmill exercise (13, 16, 43). Second, the addition of tail weights to increase the swimming workload, and/or large numbers of animals swimming in a single pool, results in animals being submerged periodically. This event can promote a diving reflex and result in intermittent periods of hypoxia (43). Hence, it appears that the swim training exercise model for rodents is not a good model to study human adaptation to exercise in a terrestrial environment.

To date, only four investigations have examined myocardial responses to I/R in treadmill-trained animals. All of these studies employed an in vitro model of global ischemia (7, 8, 30, 35). Although this type of experimental protocol has the advantage of studying the myocardium during carefully controlled preload and afterload conditions, this paradigm does not mimic in vivo conditions associated with myocardial I/R. Furthermore, these studies did not examine whether exercise training reduces I/R-induced myocardial damage (e.g., lipid peroxidation) or alters the incidence of cardiac arrhythmias during I/R. Given the clinical significance of I/R injury and the limited data available concerning the effects of treadmill exercise training on in vivo myocardial responses to I/R, there is a need for additional research in this area. Therefore, this study investigated the effects of 10 wk of rigorous treadmill training on myocardial antioxidant capacity and performance during in vivo myocardial I/R in the rat. The specific goals of this study were to determine 1) whether exercise training results in improved myocardial contractile performance during I/R, 2) whether training results in a reduced incidence of ventricular arrhythmias during I/R, and 3) whether exercise training improves myocardial antioxidant capacity.
antioxidant capacity of the myocardium, and 4) whether exercise training results in a reduction in myocardial lipid peroxidation following I/R.

**METHODS**

**Animals.** 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. Fifty young adult female Sprague-Dawley rats (4 mo old) were randomly assigned to either a sedentary control group (n = 20) or an exercise training group (n = 30). The exercise training group contained more animals than the control group to account for the fact that ~20–30% of exercise-trained animals do not complete a 10-wk training program because of limb injuries associated with rigorous treadmill exercise. During the experimental period, animals were maintained on a 12:12-h light-dark photoperiod and provided rat chow and water ad libitum.

**Exercise training protocol.** The control animals were not exercised but were placed on a nonmoving treadmill three times per week (10–30 min/session) for acclimatization. The exercise-trained animals were exercised 4 days/wk (Monday, Tuesday, Thursday, and Friday) for 10 wk on a motor-driven treadmill. Both the treadmill speed and grade were gradually increased over the course of the 10-wk training period to maintain the relative work rate at ~75–80% of maximal oxygen consumption throughout the training regimen (see Table 1). Electrical shocks were used sparingly to motivate animals to run. The decision to train animals for 10 wk at this work rate is based on work from our laboratory that demonstrates that this training protocol results in both cardiac hypertrophy and biochemical alterations (37).

In vivo protocol for studying I/R-induced myocardial damage. Ten control and 10 trained animals were studied during I/R. Trained animals were studied within 42–48 h after their last training session. Animals were anesthetized with 30 mg/kg pentobarbital sodium and ventilated with room air using a small animal ventilator (Harvard Apparatus model 661). Throughout the experiments body temperatures were monitored via a rectal thermistor probe. Body temperature was maintained at ~37 ± 1°C with a heated operating platform and appropriate heating lamps.

The chest was opened by a left thoracotomy, and a ligature was placed around the left anterior descending coronary artery, close to its origin (ligature ends were exteriorized). At this point, any animals exhibiting significant ventricular arrhythmias were eliminated from the study. Coronary occlusion was achieved by passing both ends of the ligature through a small plastic tube, which was then pressed on the surface of the heart directly above the left anterior descending coronary artery. The resulting arterial occlusion was maintained for 20 min by clamping the plastic tube and ligature with small hemostats. Reperfusion duration was 10 min and was achieved by removing the clamp and the tube.

Validation of coronary occlusion and reperfusion. The aforementioned technique of coronary occlusion has been shown to be effective by others (4, 23). However, to validate that complete coronary occlusion was achieved in our hands, we performed preliminary experiments where small amounts of Evans blue dye was injected directly into the right ventricle cavity (i.e., upstream from the ligature). After injection of the dye, the heart was removed within 10 s and examined for evidence of dye in the ventricular mass supplied by the left anterior descending coronary artery. Failure to observe dye stain in this area of the ventricle was interpreted as achievement of coronary occlusion.

To ensure that reperfusion has been adequately achieved in these studies, we also administered Evans blue dye at the end of the 10-min reperfusion period during pilot experiments. In each case we observed a uniformly stained heart; this was interpreted as evidence that reperfusion was achieved.

**Measurement of arterial blood pressure.** To monitor cardiovascular function during the I/R protocol, an arterial cannula was introduced into the carotid artery and advanced to the arch of the aorta. Peak systolic pressure was measured using a pressure transducer interfaced with a computerized heart performance analysis system (Digi-Med, Louisville, KY). A primary factor in our decision not to measure ventricular pressures directly was that we have observed that catheters placed in the left ventricles of rats results in a significant number of catheter-induced ventricular arrhythmias. This is meaningful because ventricular arrhythmias were considered to be an important dependent variable in this investigation. Furthermore, experiments in our laboratory have shown that peak systolic pressures in rats measured in the arch of the aorta do not differ significantly (P > 0.05) from pressures measured in the ventricle.

**During the I/R protocol, a standard limb lead electrocardiogram (ECG) recording (lead II) was obtained using a high-speed recorder (Grass Instruments Polygraph, model 79), and heart rate was calculated from the ECG tracing. High-speed ECGs were independently analyzed by three investigators to determine 1) the number of ventricular premature beats (VPBs), 2) the incidence and duration of ventricular fibrillation, and 3) the incidence and duration of ventricular tachycardia (defined as 4 or more consecutive VPBs). Classification and analysis of arrhythmias followed the guidelines established by the Lambeth conventions (46).

**Sham operation.** To determine the effects of endurance training on the biochemical properties of ventricular tissue not exposed to I/R, both control and trained animals were studied following a sham operation (i.e., thoracotomy performed) that did not include myocardial I/R. Animals were anesthetized with 30 mg/kg pentobarbital sodium and ventilated with room air using a small animal ventilator (Harvard Apparatus model 661). The hearts and plantaris muscles were rapidly removed and quickly frozen in liquid nitrogen for subsequent biochemical analysis.

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**Table 1. Exercise training protocol**

<table>
<thead>
<tr>
<th>Week of Training</th>
<th>1</th>
<th>2</th>
<th>3</th>
<th>4</th>
<th>5</th>
<th>6</th>
<th>7</th>
<th>8</th>
<th>9</th>
<th>10</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Exercise duration, min/day</strong></td>
<td>30</td>
<td>60</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
<td>90</td>
</tr>
<tr>
<td><strong>Treadmill speed, m/min</strong></td>
<td>25</td>
<td>25</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
<td>30</td>
</tr>
<tr>
<td><strong>%Grade (treadmill inclination)</strong></td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3</td>
<td>6</td>
<td>9</td>
<td>12</td>
<td>15</td>
<td>18</td>
<td>18</td>
</tr>
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</table>

Exercise-trained animals were exercised 4 days/wk on a motorized treadmill for 10 wk.
Tissue preparation. Selected biochemical properties of the ventricular myocardium were studied in both untrained (n = 9) and trained (n = 10) animals following the I/R protocol and from untrained (n = 10) and trained (n = 11) animals who were not exposed to I/R (i.e., sham surgery). Furthermore, as an index of the training-induced increase in oxidative capacity in locomotor muscles, we measured the citrate synthase (CS) activity in the plantaris muscle of sham-operated animals.

To determine the bioenergetic and antioxidant enzyme activities in the hearts of sham-operated animals, small samples of the left ventricle were removed and quickly frozen in liquid nitrogen. These samples were later assayed to determine the activities of superoxide dismutase (SOD), glutathione peroxidase (GPX), catalase (Cat), phosphofructokinase (PFK), lactate dehydrogenase (LDH), and CS.

In animals exposed to the I/R protocol, left ventricular tissue adjacent to the left coronary artery (LCA) was quickly removed, divided into four sections, and frozen in liquid nitrogen for subsequent biochemical analysis of lipid peroxidation and thiol content. Care was taken to harvest only the portion of the left ventricular tissue served by the LCA.

Biochemical assays. To assess the effects of both training and I/R on the myocardium, we measured thiobarbituric acid-reactive substances (TBARS) and lipid hydroperoxide assays are 3 and 4%, respectively.

Because tissue thiols (molecules containing sulfhydryl groups) are important in the regulation of both cellular redox status and antioxidant capacity, we assayed total, protein, and nonprotein thiols in the left ventricle of all experimental animals. Thiol content was determined spectrophotometrically using the DTNB-based technique described by Jocelyn (24).

HSPs are postulated to play a protective role during myocardial I/R. Furthermore, rigorous exercise has been shown to promote HSP expression in heart (31). Therefore, to determine the effects of training on induction of myocardial HSPs, we performed polyacrylamide gel electrophoresis and immunoblotting using the techniques described by Locke et al. (31). Briefly, left ventricular samples from I/R animals were homogenized, and protein content was determined using the Bradford technique (9). One-dimensional SDS (12%)-polyacrylamide gel electrophoresis was performed to separate proteins by molecular weight. After separation, proteins were transferred to nitrocellulose membranes (0.45 μm thick; Bio-Rad, Hercules, CA) using the Bio-Rad mini-protein II gel transfer system at a constant voltage of 10 V for 20 min. After protein transfer, the nitrocellulose membranes were blocked for 2 h using (0.5%) bovine serum albumin. Blots were incubated for 12 h with monoclonal antibodies specific for HSP32, HSP72, and HSP73 (Stressgen, Victoria, BC, Canada). The membranes were then placed in a solution of a secondary antibody [goat anti-rabbit immunoglobulin G (Sigma Chemical, St. Louis, MO)]. Quantification of the bands from the immunoblots was performed using computerized densitometry. Standard curves were constructed during preliminary experiments to assure linearity.

Data analysis. Myocardial contractility measurements were analyzed using a two-way analysis of variance with repeated measures with Fisher’s least-significant difference (LSD) test used post hoc. Myocardial biochemical parameters were analyzed using a two-way analysis of variance with Fisher’s LSD test used post hoc.

Duration of ventricular fibrillation, ventricular tachycardia, and normal sinus rhythm and the number of premature ventricular contractions were subjected to a one-way analysis of variance. For comparisons of the incidence of arrhythmias, a nonparametric test for significance of difference between two proportions was applied. For all statistical analysis, significance was established at P < 0.05.

RESULTS

Body weight and heart weights. Twenty-two animals successfully completed the exercise training program. Table 2 contains the body masses, heart masses, and

<table>
<thead>
<tr>
<th>Body mass (g)</th>
<th>Untrained (n = 9)</th>
<th>Exercise Trained (n = 10)</th>
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<tbody>
<tr>
<td>Initial</td>
<td>257 ± 5</td>
<td>259 ± 6</td>
</tr>
<tr>
<td>Final</td>
<td>296 ± 5</td>
<td>283 ± 7</td>
</tr>
<tr>
<td>Heart mass, g</td>
<td>0.79 ± 0.02</td>
<td>0.90 ± 0.01*</td>
</tr>
<tr>
<td>Heart-to-body mass ratio, mg/g</td>
<td>2.90 ± 0.05</td>
<td>3.30 ± 0.02*</td>
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Values are means ± SE. I/R, ischemia and reperfusion. *Different from control (P < 0.05).
heart-to-body mass ratios in both control and exercise-trained animals. Initial and final body weights did not differ between groups. In contrast, both heart weight and the heart-to-body weight ratio was greater (P < 0.05) in the exercise-trained animals compared with control. The training-induced increase in heart weight and the heart-to-body weight ratio suggests that cardiac hypertrophy occurred.

Myocardial performance during I/R. Successful I/R protocols were performed on 10 trained and 9 untrained animals. Figure 1 contains the mean (±SE) values for peak aortic systolic blood pressure (SBP) during preischemia, ischemia, and reperfusion in both untrained and trained animals. No differences existed (P > 0.05) between groups in SBP before the introduction of ischemia. However, trained animals maintained higher (P < 0.05) SBP throughout the ischemic period and during reperfusion.

Table 3 contains mean (±SE) values for heart rate and rate pressure product (RPP) at selected time intervals during the preischemic period, ischemia, and reperfusion. No group differences existed in any of these variables in the preischemic period. However, trained animals maintained significantly higher RPP during both ischemia and reperfusion.

I/R and cardiac arrhythmias. Table 4 summarizes the cardiac arrhythmias evoked by the I/R protocol. Note that one trained animal and one control animal were eliminated from this analysis because of ventricular arrhythmias that occurred during the surgical procedure before occlusion of the coronary artery.

No ventricular fibrillation was observed in any of the animals; however, 20 percent of both the control and 10 percent of the trained animals exhibited ventricular tachycardia during reperfusion. No group differences existed in the number of VPBs, salvos (2 or 3 consecutive VPBs), and the duration of ventricular tachycardia. The primary type of ventricular arrhythmia observed during I/R was VPBs. In this regard, the primary training-induced effect was that the percent of animals experiencing VPBs during reperfusion was greater in the untrained animals (80%) compared with the trained animals (10%).

Oxidative and antioxidant enzyme activities. Table 5 contains the bioenergetic and antioxidant enzyme activities in the left ventricle and CS activity in the plantaris muscle. Exercise training resulted in a large and significant increase (~80%) in CS activity in the plantaris muscle. Furthermore, exercise training resulted in a significant increase in left ventricular PFK and LDH activity. Also, training resulted in a significant increase in total SOD activity as well as an increase in the activities of both the Mn-SOD and Cu-Zn SOD isoforms. No differences in ventricular CS, Cat, or GPX activities existed between the control and trained animals.

Myocardial thiol content. Figure 2 contains the means (±SE) of all experimental groups for total thiols, protein thiols, and nonprotein thiols. Training resulted in an increase (P < 0.05) in nonprotein thiols in the left ventricle (sham animals); this increase in nonprotein thiols also resulted in an increase in total thiols in trained animals.

Compared with the sham group, animals exposed to I/R contained lower (P < 0.05) total and nonprotein thiol content in the left ventricle. The observed decrease in nonprotein thiols following I/R probably reflects a decrease in both reduced glutathione and other proteins.
nonprotein thiols due to the oxidative stress associated with I/R (22).

Also, compared with control, I/R lowered protein thiol content in untrained animals but did not alter protein thiols in trained animals exposed to I/R. The I/R-mediated decrease in myocardial protein thiols of the untrained animals appears to be a result of reactive oxygen species (ROS)-mediated oxidation of sulfhydryl groups in these molecules (39). Therefore, it seems likely that trained animals were able to resist an I/R-induced reduction in protein thiols due to the fact that trained hearts possess a better antioxidant defense system. The idea will be discussed in detail in DISCUSSION.

Table 5. Bioenergetic and antioxidant enzyme activities in control and exercise-trained animals

<table>
<thead>
<tr>
<th></th>
<th>Control (n = 8)</th>
<th>Exercise Trained (n = 9)</th>
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<tbody>
<tr>
<td>Plantaris</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>0.25 ± 0.01</td>
<td>0.46 ± 0.02*</td>
</tr>
<tr>
<td>Left ventricle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CS</td>
<td>1.17 ± 0.03</td>
<td>1.18 ± 0.003</td>
</tr>
<tr>
<td>PFK</td>
<td>0.47 ± 0.02</td>
<td>0.65 ± 0.05*</td>
</tr>
<tr>
<td>LDH</td>
<td>6.6 ± 0.25</td>
<td>7.8 ± 0.27*</td>
</tr>
<tr>
<td>SOD (total)</td>
<td>160 ± 3.8</td>
<td>202 ± 4.1*</td>
</tr>
<tr>
<td>Mn-SOD</td>
<td>88 ± 2.3</td>
<td>114 ± 3.5*</td>
</tr>
<tr>
<td>Cu-Zn-SOD</td>
<td>71 ± 2.8</td>
<td>88 ± 2.8*</td>
</tr>
<tr>
<td>GPX</td>
<td>1.17 ± 0.03</td>
<td>1.18 ± 0.03</td>
</tr>
<tr>
<td>Cat</td>
<td>3.05 ± 0.24</td>
<td>2.73 ± 0.08</td>
</tr>
</tbody>
</table>

Values are means ± SE. Enzyme activity for citrate synthase (CS), phosphofructokinase (PFK), lactate dehydrogenase (LDH), and glutathione peroxidase (GPX) are expressed in micromolars of substrate converted per minute per milligram protein. Catalase (Cat) activity is expressed as units per minute per milligram protein. Superoxide dismutase (SOD) activity is expressed as units per milligram protein.

Myocardial lipid peroxidation. Figure 4 contains mean (± SE) values for TBARS and lipid hydroperoxides in the left ventricle of all experimental groups. I/R resulted in an increase in TBARS concentration in the left ventricle in both untrained and trained animals; note, however, that the I/R-induced increase in TBARS was significantly lower in the trained animals compared with untrained.

Compared with sham-operated animals, I/R resulted in an increase in lipid hydroperoxides in the left ventricle of untrained animals. However, compared with sham, I/R did not result in significantly elevated hydroperoxides in trained animals.

DISCUSSION

Overview of principal findings. To our knowledge, this is the first experiment to comprehensively examine the effects of treadmill exercise training on cardiac biochemistry, arrhythmias, and contractile performance during I/R in vivo. Our results indicate that endurance exercise training results in an improved myocardial performance during both ischemia and reperfusion. Also, compared with untrained animals, endurance-trained animals experienced lower levels of myocardial lipid peroxidation as a result of I/R. Training promoted an increase in nonprotein thiols, which may act as nonenzymatic antioxidants and thus contribute to the observed reduction in myocardial lipid peroxidation. Furthermore, exercise training was associated

Fig. 2. A: total thiol concentration in left ventricle of experimental groups. B: protein thiol concentration in left ventricle of experimental groups. C: nonprotein thiol levels in left ventricle of experimental animals. Values are means ± SE. Sample sizes from animals exposed to I/R surgery are as follows: untrained (UT), n = 9; trained (TR), n = 10. Sample sizes from sham animals from sham surgery are as follows: untrained, n = 10; trained, n = 11. a Different (P < 0.05) from sham (within same group). b Different (P < 0.05) from untrained sham group.
with an increase in SOD activity as well as the activities of both PFK and LDH. Finally, training resulted in a significant increase in HSP72 in the left ventricle. A brief discussion of each of these points follows.

Training improves myocardial contractile performance during ischemia. As evidenced by measures of SBP, the present data clearly demonstrate that rigorous treadmill training results in improved myocardial performance during ischemia. Furthermore, our in vivo data are in general agreement with studies that have examined in vitro myocardial performance in response to ischemia following both treadmill (7, 8) and swim exercise training in rats (5).

The mechanism responsible for the training-induced improvement in myocardial tolerance to in vivo ischemia is that endurance training could promote an increased myocardial vascularity. In our in vivo model, increased collateral circulation could assist in maintaining ventricular blood flow during ligation of the LCA. This would permit ATP production via oxidative phosphorylation and therefore maintain the energy required to sustain myocardial contractility during ischemia. Although it has been argued that endurance training in young rats results in the development of coronary collaterals, the issue of whether endurance training results in increased myocardial vascularity in adult rats remains controversial. Because myocardial vascularity was not evaluated in the current experiments, we cannot determine whether the observed training-induced improvement in myocardial performance during ischemia occurred because of changes in myocardial glycolytic flux has also been suggested by others (5, 23, 30).

A second possible mechanism to explain the training-induced improvement in myocardial tolerance to in vivo ischemia is that endurance training could promote an increased myocardial vascularity. In our in vivo model, increased collateral circulation could assist in maintaining ventricular blood flow during ligation of the LCA. This would permit ATP production via oxidative phosphorylation and therefore maintain the energy required to sustain myocardial contractility during ischemia. Although it has been argued that endurance training in young rats results in the development of coronary collaterals, the issue of whether endurance training results in increased myocardial vascularity in adult rats remains controversial. Because myocardial vascularity was not evaluated in the current experiments, we cannot determine whether the observed training-induced improvement in myocardial performance during ischemia occurred because of changes in myocardial vascularity.

Fig. 3. A: graphical representation of left ventricular heat shock protein (HSP) values (trained animals) obtained from densitometric scanning of Western blots reacted with antibodies for HSP32, HSP72, and HSP73. Values are means ± SE expressed as a percent of untrained group. Sample sizes from animals exposed to I/R surgery are as follows: untrained, n = 9; trained, n = 10. Sample sizes from sham animals from sham surgery are as follows: untrained, n = 10; trained, n = 11. *Different from untrained at P < 0.05. B: Typical Western blots indicating the responses of HSP32, HSP72, and HSP73 in the left ventricle of exercise-trained and untrained animals.

Fig. 4. A: Levels of thiobarbituric acid-reactive substances (TBARS) in left ventricle of experimental groups. B: levels of cumene hydroperoxide equivalents (CHE) in left ventricle of experimental groups. Values are means ± SE. Sample sizes from animals exposed to I/R surgery are as follows: untrained, n = 9; trained, n = 10. Sample sizes from sham animals from sham surgery are as follows: untrained, n = 10; trained n = 11. a Different (P < 0.05) from sham (within same group). b Different (P < 0.05) from trained I/R group.
coronary vascular beds. This remains an interesting area for future research.

Another potential mechanism to explain the improved myocardial performance during ischemia is that training may promote an increase in end-diastolic volume. A significant increase in end-diastolic volume would result in increased myocardial contractility due to a Starling effect. However, Bersohn and Scheuer (5) reported that training did not result in elevated end-diastolic volume in rats during ischemia in the working heart model.

A final possible explanation for the observation that training improves myocardial performance during ischemia is the possibility that training results in a longer diastolic period. An increase in diastole could allow greater perfusion of the myocardium due to the longer period of blood flow. In this regard, there is evidence that training results in a greater myocardial $-dP/dt$ during both ischemia and reperfusion. This is significant because $-dP/dt$ is a sensitive indicator of calcium reuptake into the sarcoplasmic reticulum (SR) and a greater $-dP/dt$ is correlated with a more rapid ventricular relaxation (36).

Hence, a more rapid ventricular relaxation would result in a longer diastole. The mechanism to explain this training-related change in myocardial calcium handling has been shown to be due to changes in expression of the SR calcium pump, which is highly correlated with calcium ATPase activity. Specifically, Tate et al. (44) have shown that regular endurance exercise training results in a significant increase in myocardial calcium ATPase activity, which is associated with an improved ability for calcium reuptake into the SR. Functionally, this translates into an increase in the diastolic period.

Nevertheless, whether or not small increases in the diastolic period would promote the observed increases in myocardial performance during ischemia cannot be evaluated from the present experiment. Additional experiments to delineate the mechanism(s) to explain the training-induced improvement in myocardial tolerance to ischemia are clearly warranted.

Training reduces I/R-induced myocardial injury and improves postischemic myocardial recovery. Our findings support the notion that endurance training improves in vivo myocardial functional recovery during reperfusion following 20 min of coronary ligation-induced ischemia. Furthermore, similar to what was found in work by Kihlstrom (26), training results in a reduction in reperfusion-induced myocardial injury as evidenced by lower levels of myocardial lipid peroxidation (Fig. 4) and higher levels of protein thiols following I/R (Fig. 2).

What is the mechanism to explain this training-induced reduction in myocardial I/R injury? It is now clear that production of radicals and other ROS are important mediators of myocardial I/R injury (see Ref. 11 for a review). Specifically, several lines of evidence implicate ROS-induced damage as a key mechanism to explain the reversible form of myocardial injury called stunning. Indeed, ROS are produced in the reperfused myocardium and administration of ROS scavengers attenuates stunning (6). The molecular mechanisms to explain myocardial stunning are unknown but may be related to ROS-induced oxidation of both calcium-handling proteins and membrane lipids within the SR because both calcium release and reuptake are altered by ROS in skeletal and cardiac myocytes (see Ref. 38 for a review).

Because ROS play an important role in myocardial stunning, it follows that the principal determinant of the magnitude of heart I/R injury is the antioxidant capacity of the myocardium (23). In this regard, the heart contains both enzymatic and nonenzymatic antioxidant defense systems that collectively act to remove ROS. Principal antioxidant enzymes include SOD, GPX, and Cat (47). Important nonenzymatic antioxidants include glutathione and vitamin E (47). If these enzymatic and nonenzymatic antioxidant systems fail to remove ROS at the rate that they are produced, the myocardium is subjected to oxidative stress and myocyte damage occurs.

Again, exercise training provides myocardial protection against I/R injury, it is probably due to exercise-induced changes in the antioxidant capacity of the heart (23). The effects of exercise training on myocardial antioxidant capacity remain controversial. Some investigators have reported that cardiac hypertrophy, due to hypertension or regular exercise training, increases both antioxidant enzyme activity (18, 37) and glutathione levels (23, 25) in the heart. In contrast, other investigators have failed to observe a training-induced improvement in myocardial antioxidant capacity (29).

In the current experiments, myocardial activities of Cat and GPX were not increased by training. Although we did not measure myocardial glutathione reductase activity, a recent report suggests that endurance training does not elevate this enzyme in the rat myocardium (12). Nonetheless, training was associated with an increase in both the Mn and Cu-Zn isoform of SOD in the left ventricle. Therefore, it appears that training-induced changes in myocardial SOD activity is a potential mechanism to explain the observed training-induced protection. Furthermore, training resulted in large and significant increases in myocardial nonprotein thiols (Fig. 2). Because glutathione is known to be the dominant nonprotein thiol in cells (22), we interpret this finding as evidence that endurance training resulted in elevated levels of myocardial (reduced) glutathione. This is significant because glutathione plays a pivotal role in the maintenance of intracellular redox status, antioxidant vitamin levels (i.e., vitamins E and C), and the function of the antioxidant enzyme GPX (22). Because GPX requires a supply of reduced glutathione to remove organic hydroperoxides and hydrogen peroxide, it follows that a training-induced increase in myocardial glutathione should result in an improved capacity to remove these potentially injurious metabolites. Therefore, it seems that the training-induced increases in both myocardial glutathione and SOD...
activity are potential mechanisms to explain the training-induced reduction in myocardial I/R injury. Similar conclusions have been reached by Ji et al. (23).

Another inherent mechanism that probably contributed to the improved postischemic myocardial recovery is that training resulted in increased left ventricular content of HSP72. In mammalian cells, the inducible form of the HSP70 family, HSP72, has been associated with an improved myocardial postischemic functional recovery and a reduction in infarct size (21, 31). Furthermore, a recent report (21) has demonstrated a high correlation between the amount of HSP72 and the reduction of infarct size following I/R in the rat heart. Collectively, these studies provide evidence that induction of myocardial HSP72 provides protection to the myocyte during I/R stress. The mechanism of how HSP72 provides protection against I/R injury remains unclear. However, there is evidence that HSP72 can stabilize and refold damaged proteins during stress and there is speculation that HSP72 may modulate myocardial antioxidant enzyme activity and therefore provide protection against ROS (see Ref. 27 for a review). Regardless of the mechanism, in the present experiments, it seems likely that exercise training-induced increases in HSP72 and perhaps other HSPs may explain, at least in part, the myocardial protection associated with exercise.

The component of exercise training that is responsible for the upregulation of HSP72 expression is unknown. A variety of stresses associated with endurance exercise could contribute to the elevation of HSP72 in the cardiac myocyte. For example, heat stress, metabolic overload, hypoxia, production of ROS, and stretch of the cardiac myocyte have all been shown to promote HSP72 synthesis (see Ref. 27 for a review). Therefore, it is likely that exercise training results in the accumulation of redundant signals that could act independently or collectively to upregulate the level of HSP72 in the heart.

Exercise training and I/R arrhythmias. The specific mechanisms responsible for I/R arrhythmias are complex and vary depending on the duration of ischemia. Nonetheless, it is clear that myocardial I/R results in cardiac myocyte ionic disturbances due to a reduction in cellular energy levels and increased production of ROS (11). To date, there are no published reports concerning the effects of treadmill exercise training on the incidence of arrhythmias during in vivo I/R. Therefore, one of the objectives in these experiments was to determine whether endurance training alters the incidence of ventricular arrhythmias following I/R in the rat. Our results indicated that training provided only moderate protection against cardiac arrhythmias during this type of I/R protocol. This is evidenced by the fact that the number or severity of VPBs, salvos, and ventricular tachycardia did not differ between the sedentary and trained animals (see Table 4). Nonetheless, exercise training did reduce the incidence of VPBs during reperfusion. Indeed, the incidence of VPBs was greater in the untrained animals during reperfusion compared with the trained animals (80% vs. 10%).

In contrast to these findings, Belichard et al. (2) have demonstrated that 8 wk of swimming exercise in rats is associated with a large reduction in the severity of I/R arrhythmias following 30 min of ischemia. The explanation for the divergent findings between the current study and that of Belichard et al. (2) is unclear. It seems possible that interstudy differences in the experimental protocol (e.g., training program, duration of ischemia, etc.) may have contributed to the deviant findings between the two studies.

Critique of experimental model and limitations of study. The adult female Sprague-Dawley rat was chosen as an experimental model because 1) the nature of these invasive experiments prevents the use of human subjects, 2) this rat is highly inbred and does not display large interanimal variation in coronary collateral circulation (17), and 3) the rat is a widely accepted model for the study of exercise-induced myocardial adaptations (2, 5, 23, 37).

In reference to our choice to study female rats, there is no evidence that sex differences exist in myocardial responses to I/R or to exercise training. Hence, we arbitrarily chose female animals because of our previous experience with female rats in exercise training studies (37). Also, young adult animals were chosen as experimental subjects to avoid confounding experimental variables associated with both development and old age.

We chose treadmill training as our exercise model in an effort to mimic human exercise and to avoid the potential confounding variables associated with swim training in rodents. Furthermore, the duration and intensity of our exercise training protocol was selected on the basis that this training program has been shown to promote myocardial adaptations in the enzymatic antioxidant system (37).

The surgical procedure used in these experiments has been used successfully by other investigators and has been shown to result in both myocardial ischemia and reperfusion (4, 23). However, it seems possible that this type of experimental protocol could result in interanimal differences in the magnitude of both ischemia and reperfusion. This could occur because of experimental difference (i.e., surgical technique) and/or between-animal differences in collateral blood vessels. Nonetheless, we believe that these differences are physiologically relevant and probably reflect the normal variation that occurs during in vivo I/R insults in humans.

In conclusion, these experiments examined the effects of endurance exercise training on myocardial biochemical and physiological responses during in vivo I/R in the rat. The data revealed that exercise-trained animals maintained higher peak SBP throughout I/R. Training promoted an increase in left ventricular HSP72 and nonprotein thios, as well as an elevation in SOD, PFK, and LDH activities. Also, compared with control, trained animals exhibited lower levels of lipid peroxidation following I/R. These data demonstrate that endurance exercise training improves myocardial contractile performance and reduces lipid peroxidation during I/R. We postulate that training-induced increases in myocar-
dial nonprotein thiols (e.g., glutathione), SOD activity, and HSP72 are collectively important in reducing the I/R-induced myocardial damage.

**Perspectives**

These experiments provide strong evidence to support the notion that endurance exercise training provides myocardial protection during moderate-duration I/R. Although it seems likely that training-induced increases in myocardial glutathione, SOD activity, and HSP72 are collectively important in reducing the I/R-induced myocardial damage, it is also possible that exercise induction of other antioxidants (e.g., thioredoxin and glutaredoxin) as well as other stress proteins play a significant role in myocardial protection under these conditions. In this regard, identification of new and important factors that contribute to myocardial protection during I/R insults is an exciting area for future research.

Finally, although our data clearly indicate that exercise training is associated with a reduction in lipid peroxidation and an improvement in myocardial performance during moderate-duration I/R, whether or not exercise training would result in myocardial protection against long-duration ischemia (e.g., 120 min) cannot be determined by these experiments. Indeed, when comparing protective interventions against myocardial I/R injury, direct extrapolation from one duration of ischemia to another is tenuous and should be approached with caution.

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