Effects of body temperature during exercise training on myocardial adaptations

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Harris, M. Brennan, and Joseph W. Starnes. Effects of body temperature during exercise training on myocardial adaptations. Am J Physiol Heart Circ Physiol 280: H2271–H2280, 2001.—This study determined the role of body temperature during chronic exercise on myocardial adaptations.

HEAT STRESS AND EXERCISE are two interventions reported to provide intrinsic myocardial protection against ischemia-reperfusion injury (5, 6, 9, 18, 26, 33, 38). Earlier studies from this laboratory (5, 6) reported that participation in a chronic exercise program results in an attenuation of functional and metabolic damage in the isolated perfused rat heart after ischemia and subsequent reperfusion. Subsequent studies have confirmed greater tolerance to ischemia-reperfusion stress in in vivo models (9, 33). Although the mechanism is unclear, many investigations have focused on increased expression of various cardioprotective proteins. Because an important factor in ischemia-reperfusion injury is oxidative stress, several investigators have explored the potential cardioprotective properties of antioxidant enzymes (see Ref. 4 for a review). Also, a positive relationship between postischemic recovery and myocardial heat shock protein 70 (HSP 70) has been reported after heart stress (18), acute exercise (26), and more recently, chronic exercise (9, 33). Other stress proteins such as glucose-regulated protein 75 (GRP 75), heme oxygenase-1 (HO-1), HSP 90, and αB-crystallin have also been reported to increase after heat stress, exercise, and/or contractile activity (10, 15, 31); however, the role of these proteins in chronic exercise-induced cardioprotection has not been evaluated.

Because exercise causes an increase in core temperature, it is important to evaluate the role of exercise on changes in myocardial stress-protein expression and postischemic functional recovery during a chronic exercise program. Acute exercise studies that examined the effect of core temperature on myocardial HSP 70 expression have yielded conflicting results (36, 38). Skidmore and colleagues (36) reported a 50% increase in myocardial HSP 70 content by enzyme-linked immunosorbant assay immediately postexercise; however, using Western blot analysis, Taylor and co-workers (38) found no increase in myocardial HSP 70 by 24 h after exercise. The different time points evaluated and methods of analysis may explain the reason for this discrepancy. However, heat stress alone or when induced by exercise normally results in a severalfold increase in HSP 70 within 24 h. Thus the physiological significance of the relatively small change observed by Skidmore and colleagues (36) is unknown.

Adherence to a chronic exercise program is widely recommended to aid cardiovascular health. Taylor and colleagues (38) recently demonstrated that acute exercise independent of increased core temperature can improve postischemic recovery. However, the role of core temperature on cardioprotection after chronic exercise has not been tested. It is not appropriate to infer about cardioprotection from acute studies alone be-
cause chronic exercise may result in considerable differences in overall protein expression and the stress response. Therefore, a purpose of this study was to determine the expression of several potential cardioprotective proteins during adaptation to a chronic training program. Specifically, we determined changes in five myocardial stress proteins (HSP 70, GRP 75, HSP 90, HO-1, and αB-crystallin) as well as key antioxidant enzymes [catalase (CAT), glutathione peroxidase (GPx), total superoxide dismutase (SODtot), CuZn-SOD, and Mn-SOD] after the last exercise bout. This investigation was approved from the University of Texas Animal Resource Center. Animals and training protocols. Male 10-wk-old Sprague-Dawley rats were obtained from the breeding colony maintained by the University of Texas Animal Resource Center. The animals were on a 12-h:12-h light-dark cycle and fed ad libitum. Rats were randomly divided into seven treatment groups: sedentary control (SED; n = 20), exercised for 3 wk at room temperature (3WK; n = 7) and in the cold (3WKc; n = 8), exercised for 6 wk at room temperature (6WK; n = 7) and in the cold (6WKc; n = 8), and exercised for 9 wk at room temperature (9WK; n = 22) and in the cold (9WKc; n = 22). All training groups were initially habituated to the treadmill by running for 1 wk at 20 m/min with 6% grade for 10 min/day. After habituation to the treadmill, the duration of exercise was gradually increased to 60 min/day for 5 days/wk by the end of 3 wk. The training protocol was then maintained at this duration and frequency for an additional 6 wk. Training was carried out either at room temperature (23°C) or at cold temperatures (8°C with wetted fur for the first 3 wk and then at 4°C with wetted fur and a fan to maintain basal core temperature). The animals that ran in the cold were returned to a 23°C environment when not exercising. Preliminary studies were performed to establish an environmental condition that would maintain basal core temperature during exercise. Throughout the exercise program in the cold, core temperature was monitored periodically to assure that it did not vary from resting temperature. Core temperature was measured by inserting a probe 5 cm into the rectum during brief rest periods at 15-min intervals. Trained animals were killed 24 h after the last exercise bout. This investigation was approved by the University of Texas Animal Care and Use Committee and conforms with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication 85-23, Revised 1985). Tissue preparation. Animals were anesthetized with rodent anesthesia cocktail (obtained from University of Texas Animal Resource Center) at a dosage of 0.7 ml/kg ip. The composition of the cocktail was (in mg/ml): 100 ketamine, 20 xylazine, and 10 acepromazine. Heparin (100 IU) was injected into the inferior vena cava, and hearts were rapidly excised and placed in ice-cold saline on a tared electronic balance for determination of gross wet weight. The aortas were secured on a stainless steel cannula and retrograde (Langendorff) perfusion was started at 80 cmH2O perfusion pressure with a modified Krebs-Henseleit buffer containing (in mM) 118.5 NaCl, 4.7 KCl, 24.7 NaHCO3, 1.0 MgSO4, 10 glucose, 1.75 CaCl2, and 0.5 EDTA, with 12 IU/ml of insulin. The buffer was maintained at 37°C and gassed with 95% O2-5% CO2. Some hearts were freeze-clamped with aluminum tongs precooled in liquid N2 after 5 min of perfusion and stored at −80°C until being analyzed for antioxidant enzymes and stress proteins. Other hearts were perfused longer for evaluation of cardiac function before and after ischemia-reperfusion stress. METHODS Animals and training protocols. Male 10-wk-old Sprague-Dawley rats were obtained from the University of Texas Animal Resource Center. 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The animals that ran in the cold were returned to a 23°C environment immediately after exercise and dried with a towel. All animals were housed in a 23°C environment when not exercising. Preliminary studies were performed to establish an environmental condition that would maintain basal core temperature during exercise. Throughout the exercise program in the cold, core temperature was monitored periodically to assure that it did not vary from resting temperature. Core temperature was measured by inserting a probe 5 cm into the rectum during brief rest periods at 15-min intervals. Trained animals were killed 24 h after the last exercise bout. This investigation was approved by the University of Texas Animal Care and Use Committee and conforms with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH publication 85-23, Revised 1985). Tissue preparation. 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Other hearts were perfused longer for evaluation of cardiac function before and after ischemia-reperfusion stress. Stress-protein determinations. A piece of left ventricle (130–140 mg) was homogenized (1:20 wt/vol ratio) in HEPES buffer (N-2-hydroxyethylpiperazine-N’-2-ethanesulfonic acid and 1 mM of EDTA at pH 7.4) using a Teflon and glass Potter-Elvehjem homogenizer for myocardial stress-protein determination. Entire gastrocnemius muscles were homogenized (1:20 wt/vol) in 50 mM of K2HPO4 and 1 mM of EDTA at pH 7.4. Homogenates were diluted 1:1 with Laemmli sample buffer containing 125 mM of Tris·HCl (pH 6.8), 20% vol/vol of glycerol, 2% wt/vol of SDS, 0.008% wt/vol of bromophenol blue, and 200 mM of dithiothreitol. The protein concentration of each sample was determined by the method of Lowry and colleagues (27) and 80 μg of protein was subjected to SDS-PAGE electrophoresis on a 10% (HSP 70 and GRP 75) or 12.5% (HO-1 and αB-crystallin) resolving gel using the Mini-Protean II system (Bio-Rad; Richmond, CA). The proteins were then transferred to a polyvinylidene difluoride (PVDF) sheet (Bio-Rad) with a Bio-Rad semidry transfer unit as has been previously described (38). The PVDF membranes were blotted with either HSP 70 mouse monoclonal IgG (sc-024, Santa Cruz Biotechnology), GRP 75 mouse polyclonal (SPA-825, StressGen Biotechnologies), HO-1 mouse monoclonal IgG2b (OSA-111, StressGen Biotechnologies), or αB-crystallin rabbit polyclonal antibodies (SPA-223, StressGen Biotechnologies). The membranes were then blotted with anti-mouse Ig (NXA-931, horseradish peroxidase (HRP)-linked whole antibody from sheep; Amersham Life Science) or anti-rabbit IgG-HRP (SAB-300, HRP-linked whole antibody from goat; StressGen Biotechnologies) and detected with Super Signal chemiluminescent substrate lumino/enhancer (Pierce; Rockford, IL). The resulting labeled bands were quantified using a Macintosh IIsi computer (Apple Computer; Cupertino, CA). The scans were then digitized and imported into an image-analysis software program (Image Beta 2, Scion; Frederick, MD) and the density of each individual band sample was calculated. A standard sample created from the myocardium or spleen of a heat-shocked rat was loaded on each gel along with samples of the treatment groups. Gels for HSP 70 and GRP 75 blots were loaded with half of the amount (40 μg) of protein for groups trained at room temperature to ensure that the signals fell within the linearity of the film. The content of each sample was reported as a percentage of the standard loaded on each gel and was adjusted for the concentration of total protein loaded in the respective sample. Each sample was run in duplicate or triplicate, if necessary, to ensure that results were not influenced by loading errors. CAT. Portions of the left ventricle were homogenized (1:20 wt/vol) in 10 mM of KH2PO4 with 0.1 mM of EDTA (pH 7.4) using a Teflon and glass Potter-Elvehjem homogenizer. The homogenate was then centrifuged at 4°C for 10 min at 13,500 g. The supernatant was used to evaluate CAT activity at 25°C by the method of Aebl (1). GPx. Myocardial GPx activity was measured at 37°C using the method of Gunzler and Flohé (13). A portion of the left ventricle was pulverized under liquid N2 and then homoge-
nized (1:20 wt/vol) in 25 mM of HEPES with 0.1 mM of EDTA (pH 7.0) with a Potter-Elvehjem homogenizer. The sample was then centrifuged at 4°C for 10 min at 5,000 g. The supernatant was collected and stored on ice until being analyzed.

SOD. SODtot was measured at 30°C using the method of epinephrine autoxidation inhibition (29). Samples were prepared by homogenizing a portion of the left ventricle in 25 mM of HEPES (pH 7.4). The sample was then centrifuged at 4°C for 10 min at 700 g and the supernatant was used for analysis. To determine the respective contributions of the Cu,Zn-SOD and Mn-SOD isoforms to the total activity, 2 mM of KCN was added to the reaction mixture to inhibit Cu,Zn-SOD. Protein content for the antioxidant assays was determined by the biuret method (12) with bovine serum albumin used as a standard.

Cytochrome oxidase. Tissues were homogenized (1:20 wt/vol) in phosphate buffer (50 mM of K2HPO4 with 0.1 mM of EDTA at pH 7.4). Cytochrome oxidase levels were then measured at 24°C as previously described (35).

Cardiac function. Myocardial function was evaluated using an isolated working-heart preparation as previously described (5). After 10 min of Langendorff perfusion, hearts were switched to the working-heart mode for an additional 10 min at 4°C for 10 min at 700 g and the supernatant was used for analysis. To determine the respective contributions of the Cu,Zn-SOD and Mn-SOD isoforms to the total activity, 2 mM of KCN was added to the reaction mixture to inhibit Cu,Zn-SOD. Protein content for the antioxidant assays was determined by the biuret method (12) with bovine serum albumin used as a standard.

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Cardiac function. Myocardial function was evaluated using an isolated working-heart preparation as previously described (5). After 10 min of Langendorff perfusion, hearts were switched to the working-heart mode for an additional 10 min at 13 cmH2O preload and 80 cmH2O afterload before ischemia-reperfusion. After ischemia, the hearts were initially reperfused in the Langendorff mode at a perfusion pressure of 80 cmH2O for 15 min and then returned to the working mode for an additional 15 min before final evaluation of the posts ischemic hemodynamics. In each heart, the cardiac function after 30 min of reperfusion was compared with its own preischemic function to determine the amount of cardiac dysfunction caused by ischemia-reperfusion.

Statistical analyses. Descriptive data (means ± SD and SE) were calculated for each dependent variable. Overall differences among groups were analyzed using a one-way ANOVA with post hoc analysis using a Fisher protected least-significant difference test for determining differences between the means when comparing more than two groups. In all tests, a probability level of <0.05 was used as the decision rule for significance testing.

RESULTS

Animal characteristics. Animal body weights, heart weights, and heart weight-to-body weight ratios are summarized in Table 1. Body weights from any of the exercising groups were not significantly different (P > 0.05) from SED controls, except for the 9WKC group which was higher (P < 0.05) than all other groups. Heart weights did not change from SED levels in groups run at room temperature but were significantly greater (P < 0.05) in groups run in the cold. Heart weight-to-body weight ratios were significantly higher in SED animals run in the cold compared with SED controls. As a result of cardiac hypertrophy in all exercised groups compared with SED controls.

Core temperature. Core temperatures at the end of the last exercise bout are displayed in Table 1. There was an elevation in core temperature of ~2.5°C in animals run at room temperature for 3, 6, and 9 wk (P < 0.05). The elevation in temperature was attained (P < 0.05) in all exercised groups compared with SED controls. As a result of cardiac hypertrophy in all groups run in the cold, the heart weight-to-body weight ratios were greater (P < 0.05) than the corresponding groups run at normal room temperature.

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by 15 min of exercise and remained constant throughout (data not shown). Core temperatures in animals run in the cold for 3, 6, or 9 wk did not vary from SED values ($P > 0.05$).

Myocardial HSP 90 content. The results for left ventricular HSP 90 content are illustrated in Fig. 1. Myocardial HSP 90 was increased by $\sim 50\%$ ($P < 0.05$) in the cold runners at 3 and 6 wk, but returned to SED levels after 9 wk. No significant differences were observed at any time during the exercise program for animals run at room temperature.

Myocardial HSP 70 content. The results for left ventricular HSP 70 content are illustrated in Fig. 2A. Compared with SED controls, myocardial HSP 70 was significantly ($P < 0.05$) elevated in rats run at room temperature for 3 (8.5-fold), 6 (10.7-fold), and 9 (12.3-fold) wk. In addition, 6WK and 9WK groups had significantly ($P < 0.05$) higher HSP 70 levels compared with 3WK rats. Myocardial HSP 70 content of the groups trained in the cold environment was not different ($P > 0.05$) from SED controls at any time during the exercise program.

Skeletal muscle HSP 70 content. The results for gastrocnemius HSP 70 content are illustrated in Fig. 2B. Skeletal muscle HSP 70 was elevated ($P < 0.05$) in rats run at room temperature for 3 (8.5-fold), 6 (10.7-fold), and 9 (12.3-fold) wk compared with SED controls and animals run in the cold. The magnitude of the increases was similar to those observed in the heart. There was no increase in the content of this stress protein in muscles of animals run in the cold environment compared with SED controls ($P > 0.05$).

Myocardial GRP 75, HO-1, and GPx. No differences were found among any of the groups ($P > 0.05$) at any time point in the exercise program for the left ventricular content of GRP 75, HO-1, or GPx (data not shown).

Myocardial αB-crystallin content. The results for left ventricular αB-crystallin content are illustrated in Fig.
The content of this stress protein did not differ from SED in any group exercised at room temperature ($P > 0.05$). However, after 9 wk of running in the cold environment, αB-crystallin content was increased 90% above SED levels ($P < 0.05$) and was significantly greater ($P < 0.05$) than all other groups except 6WKC animals.

**CAT.** The results for left ventricular CAT activity are illustrated in Fig. 4. Animals run for 3 wk at room temperature were found to have a 23% increase ($P < 0.05$) in activity compared with SED animals and were not different from 3WKC rats. Beyond 3 wk, CAT activity tended to return toward SED values as there were no differences among groups at 6 or 9 wk.

**SOD.** Figure 5 illustrates the results for left ventricular SODtot, Cu,Zn-SOD, and Mn-SOD activity. No differences in left ventricular SODtot were observed among the groups at any time in the exercise program ($P > 0.05$). Cu,Zn-SOD activity was significantly elevated ($P < 0.05$) in 3WK compared with 3WKC and 6WKC. In addition, left ventricular Mn-SOD activity was significantly lower ($P < 0.05$) in 3WK compared with SED, 3WKC, 6WKC, and 9WKC rats. Mn-SOD activity was also significantly lower ($P < 0.05$) in 6WK compared with 6WKC and 9WKC rats.

**Cytochrome oxidase.** Cytochrome oxidase was measured as a marker of mitochondrial changes with training and the expected results were found for both skeletal muscle and heart. Cytochrome oxidase activity in the gastrocnemius muscle increased from $48.2 \pm 2.8$ to $59.0 \pm 1.9$ μatoms oxygen/g wet wt ($P < 0.05$) after 9 wk of exercise training at room temperature. However, the left ventricular content of this mitochondrial enzyme was not changed from that in the SED group in any exercise group ($P > 0.05$) (data not shown).

**Cardiac function.** Absolute values during both preischemia and postischemia for several functional parameters are reported in Table 2. A more complete analysis of the effects of exercise training on intrinsic cardiac function after ischemia and subsequent reperfusion can be found in our earlier studies (5, 6). No
differences in preischemic functional characteristics were observed between animals run for 9 wk at room temperature compared with the SED controls. Preischemic systolic pressure was significantly higher ($P < 0.05$) in animals run in the cold environment compared with the SED control group. This was likely the result of the 23% increase in cardiac mass in the cold runners compared with the SED controls (Table 1). No other significant differences in preischemic function were observed. Postischemic recovery of cardiac mechanical function as indicated by percentage recovery of preischemic cardiac output $\times$ systolic pressure was significantly enhanced after training for 9 wk at room temperature. However, the same exercise training program carried out in the cold environment did not improve postischemic recovery compared with SED animals.

**DISCUSSION**

Several studies have reported that chronic exercise training can attenuate the amount of myocardial dysfunction associated with ischemia-reperfusion stress (5, 6, 9, 33), but the intrinsic adaptation providing the benefit has not been determined. In the present study, we report that myocardial HSP 70 increased severalfold after 9 wk of exercise carried out at normal room temperature but was unchanged from SED levels when the identical exercise program was carried out in a cold environment that prevented an increase in body temperature. No other stress protein or antioxidant enzyme measured was increased after completing the exercise program in either environment except $\alpha_B$-crystallin in the cold runners. However, transient changes in certain myocardial stress proteins and antioxidant enzymes were observed during the initial stage of adapting to the exercise program. Exercise training for 9 wk at room temperature also resulted in better recovery of myocardial pump function after an in vitro ischemia-reperfusion stress, but training in the cold environment did not result in better recovery. Because HSP 70 was the only protein elevated in the exercising group that developed improved cardioprotection and HSP 70 was not elevated in the exercising group that did not, HSP70 stood out as being the most important protein of those measured in providing the protection. The mechanisms by which HSP 70 is cardioprotective have not been fully elucidated. It is known to act as a molecular chaperone that may facilitate the restoration of enzymes and other key proteins denatured during ischemic stress (see Ref. 3 for a review). Also, Kawana and colleagues (19) have recently provided evidence that HSP 70 can protect the heart by attenuating the activity of nuclear poly(ADP-ribose) synthetase, which consumes excessive amounts of energy during reoxygenation thereby making an insufficient amount available for pump function.

**HSP 70.** Myocardial and skeletal muscle HSP 70 content were increased 12.3-fold and 10.2-fold, respectively, after 9 wk of exercise at room temperature (Fig. 2). This dramatic increase in protein expression was completely blocked in both tissues by maintaining basal core temperature during exercise. This observation provides clear evidence that core temperature is the primary factor in determining HSP 70 expression after exercise (at least up to the intensity used herein).

It is interesting to note that myocardial HSP 70 continued to increase throughout the 9-wk program even though the exercise load was not increased beyond three wk when the HSP 70 content had already increased 8.5-fold. The reason for the continued increase in HSP 70 is unclear. Although no studies have examined the time course of HSP 70 expression and degradation after acute or chronic exercise, Karmazyn and colleagues (18) reported that after a single bout of heat stress myocardial HSP 70 expression peaked at 48 h and “was slowly degraded” thereafter. Perhaps continuously repeating exercise bouts at 24-h intervals results in chronic attenuation of protein degradation relative to synthesis.

**GRP 75.** Despite finding large changes in myocardial HSP 70, no change in myocardial GRP 75 (also known as mitochondrial HSP 70) was observed in any exercise

### Table 2. Functional characteristics of isolated perfused hearts

<table>
<thead>
<tr>
<th></th>
<th>PSED (n = 8)</th>
<th>9WK (n = 8)</th>
<th>PSEDC (n = 6)</th>
<th>9WKC (n = 6)</th>
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<tbody>
<tr>
<td><strong>Preischemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF, ml·min$^{-1}$·g wet wt$^{-1}$</td>
<td>15.7 ± 0.8</td>
<td>14.6 ± 0.9</td>
<td>14.9 ± 0.8</td>
<td>13.9 ± 1.1</td>
</tr>
<tr>
<td>CO, ml·min$^{-1}$·g wet wt$^{-1}$</td>
<td>50.1 ± 3.1</td>
<td>49.7 ± 3.3</td>
<td>50.1 ± 2.2</td>
<td>45.5 ± 3.0</td>
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<tr>
<td>SP, mmHg</td>
<td>113 ± 11</td>
<td>114 ± 6</td>
<td>120 ± 5</td>
<td>138 ± 6*</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>301 ± 9</td>
<td>305 ± 8</td>
<td>298 ± 21</td>
<td>266 ± 17</td>
</tr>
<tr>
<td>CO $\times$ SP, ml·min$^{-1}$·g wet wt$^{-1}$ $\times$ mmHg</td>
<td>5,701 ± 455</td>
<td>5,767 ± 552</td>
<td>6,043 ± 365</td>
<td>6,305 ± 437</td>
</tr>
<tr>
<td><strong>30 min postischemia</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CF, ml·min$^{-1}$·g wet wt$^{-1}$</td>
<td>12.5 ± 0.8</td>
<td>12.3 ± 0.8</td>
<td>12.4 ± 1.2</td>
<td>13.0 ± 2.0</td>
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<tr>
<td>CO, ml·min$^{-1}$·g wet wt$^{-1}$</td>
<td>26.4 ± 2.9</td>
<td>31.8 ± 2.9</td>
<td>28.4 ± 3.5</td>
<td>23.4 ± 5.4</td>
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<tr>
<td>SP, mmHg</td>
<td>85 ± 3</td>
<td>91 ± 4</td>
<td>89 ± 2</td>
<td>94.3 ± 10.8</td>
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<tr>
<td>HR, beats/min</td>
<td>316 ± 18</td>
<td>325 ± 17</td>
<td>305 ± 18</td>
<td>251 ± 21</td>
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<tr>
<td>CO $\times$ SP, ml·min$^{-1}$·g wet wt$^{-1}$ $\times$ mmHg</td>
<td>2,261 ± 297</td>
<td>2,909 ± 312</td>
<td>2,583 ± 369</td>
<td>2423 ± 74</td>
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<tr>
<td>%REC</td>
<td>39.4 ± 2.9</td>
<td>50.5 ± 2.5*</td>
<td>43.7 ± 6.4</td>
<td>36.1 ± 9.2</td>
</tr>
</tbody>
</table>

Values are means ± SE; $n$, no. of hearts. PSED, perfused SED; PSEDC, perfused SED for cold group; CF, coronary flow; CO, cardiac output; SP, systolic pressure; HR, heart rate; CO $\times$ SP, cardiac output $\times$ systolic pressure; %REC, percent recovery. *Significantly different from PSEDC ($P < 0.05$). †Significantly different from PSED.
group. GRP 75 has been suggested to play a role in mitochondrial biogenesis and has been found to increase along with mitochondrial content and protein import into mitochondria after chronic electrical stimulation of skeletal muscle (see Ref. 15 for review). Our findings are consistent with this role because mitochondrial content as indicated by cytochrome oxidase activity was also not changed in our study. The lack of an exercise-related change in myocardial GRP 75 also indicates that this heat shock protein is not regulated by temperature changes in the range of 37° to 40.5°C.

HSP 90. Locke and colleagues (25) reported increased synthesis of a protein with the apparent molecular mass of 90,000 kDa in skeletal muscle and spleen immediately after one bout of exercise. It is very likely that this protein was actually HSP 90, but it is unknown how long the increased synthesis persists. Whether exercise affects HSP 90 expression in the heart had not been evaluated before the present study. The results herein indicate that no increase in myocardial HSP 90 levels occurs 24 h after the last exercise bout in chronically trained rats (Fig. 1).

Although myocardial HSP 90 levels were not elevated after chronic training at room temperature, we observed a significant increase of ~50% in animals run in the cold for 3 or 6 wk. The reason for the increase in HSP 90 with exercise in the cold is unknown but may be due to its interaction with steroid hormone receptors (3). Because animals trained in the cold environment had significantly greater cardiac hypertrophy compared with those trained at room temperature, HSP 90 may have played a role in signaling the growth. At the end of 9 wk, however, HSP 90 levels were no longer significantly elevated, which may indicate that a certain degree of adaptation to this type of stress had occurred.

αB-Crystallin. Myocardial αB-crystallin was elevated only in animals exercised for 9 wk without increases in core temperature. αB-crystallin can be induced in other tissues at high temperatures but already exists in high concentrations in the myocardium (3). Therefore, the increase in core temperature in the animals run at normal room temperature may not have been sufficient to cause any further increase in this protein. In studies carried out on skeletal muscle, Neufer and co-workers (31) proposed that αB-crystallin may play a role in cellular remodeling. Our data appear to be consistent with this proposal because both cardiac hypertrophy and an increase in αB-crystallin content were observed in the animals run in the cold environment. Importantly, our findings that postischemic recovery of cardiac function was improved without an increase in αB-crystallin expression (room-temperature runners) and recovery was not improved when αB-crystallin increased (cold-environment runners) provides compelling evidence that αB-crystallin is not involved in protection against the ischemia-reperfusion stress imposed herein.

HO-1. Our results for myocardial HO-1 content are consistent with those of Powers and colleagues (33) who reported no change after chronic treadmill training in rats. However, these results appear to be in contrast with reports that heat induces an increase in myocardial HO-1 mRNA (34) and that contractile activity in skeletal muscle results in concomitant HO-1 and HSP 70 mRNA expression (10). It is possible that myocardial HO-1 expression may not have been altered in the present study because core temperatures were not elevated as high as in the study by Raju and Maines (34) (40.4 ± 0.6°C vs. 42°C, respectively). Furthermore, unlike skeletal muscle the myocardium experiences continuous contractile activity and the increased activity due to exercise may not be sufficient to induce changes in HO-1 expression. Maulik and co-workers (28) demonstrated that HO-1 expression can also be induced by reperfusion of ischemic myocardium and the expression is blocked by prior treatment with antioxidant enzymes suggesting that the signal for HO-1 expression might be oxygen-derived free radicals. If this is the case, then the amount of free radicals generated in the exercise protocols employed by Powers and colleagues (33) and by us were below the threshold required to stimulate increased HO-1 expression.

Antioxidants. An important factor in ischemia-reperfusion injury is oxidative stress, and increased antioxidant levels are reported to enhance ischemia-reperfusion protection (4). Several investigations have been carried out on the effects of chronic exercise on antioxidant enzymes but the results are equivocal. Myocardial antioxidant enzyme activities have been reported to increase, decrease, and remain the same after exercise (16). In an attempt to clarify these discrepancies, we examined myocardial antioxidant enzyme activity at three time points during a chronic treadmill training program in rats with and without increases in core temperature during the exercise bouts. CAT activity increased during the early adaptive phase in animals whose core temperature increased during exercise (Fig. 4). In addition, the activities of Cu,Zn-SOD increased and those of Mn-SOD decreased in these animals compared with animals whose core temperature did not change during exercise (Fig. 5). However, at the end of 9 wk of training in which the exercise intensity and duration had been constant for the final 6 wk, no differences in myocardial antioxidants were observed. Thus our data indicate that myocardial antioxidant levels are related to the duration of the training program, the state of adaptation to the exercise stress, as well as core temperature during exercise. In this context we can now evaluate some of the earlier studies.

The results of our study are consistent with the findings of two studies by Leeuwenburgh and colleagues (23, 24). In both studies rats were trained on a treadmill for 10 wk with increasing duration and intensity up to 4 wk and maintained for an additional 6 wk. The authors found no changes in myocardial CAT, GPx, or SOD as a result of training for 10 wk. Additional studies utilizing a progressive increase in duration and intensity throughout the exercise program have also found no changes in myocardial antioxidant
enzyme activities (9, 14). This would appear to be in contrast to our findings that CAT and SOD isoforms undergo alterations during the early phases of adaptation. However, this response may be blunted as animals become trained. In this regard Somani and co-workers (37) found a greater increase in CAT and Mn-SOD activities after an acute bout of exercise in untrained animals compared with animals previously treadmill trained for 10 wk.

Changes in mitochondrial (Mn-SOD) and cytosolic (Cu,Zn-SOD) SOD isoforms were observed at 3 and 6 wk of training at room temperature, but they gradually returned to control levels by the end of 9 wk of training (Fig. 5). Although differential changes in the activities of these enzymes in response to various stressors have been reported previously (8, 37), the reason for the observed shift is unclear. Studies have shown increases in both (33, 37) or none (14) of the two isoforms in the myocardium after chronic exercise. Recently, Powers and colleagues (33) found increases in both Cu,Zn-SOD (24%) and Mn-SOD (30%) after 10 wk of treadmill training that was constantly increased to maintain a relative work rate of ~75–80% of maximum oxygen consumption. During the last week of the program, the rats were running 90 min/day at 30 m/min up an 18% grade. It is therefore possible that the milder exercise protocol used herein induced only transient changes in the SOD isoforms because the animals adapted to the exercise and there was no longer sufficient stress to induce alterations. It should also be mentioned that Demirel and colleagues (9) did not find increased myocardial SOD activity using the same animals and exercise protocol as Powers and co-workers (33). Perhaps an explanation for this apparent discrepancy is that the small increases observed by Powers and co-workers (33) requires more sensitive measures to detect a change.

In addition to the transient changes in SOD isoforms, we observed an increase in CAT activity at 3 wk and a return toward basal levels at 6 and 9 wk of training. Furthermore, it appears that both CAT and SOD may be affected by temperature as the changes were considerably blunted in the animals that were run in the cold. Myocardial CAT activity has been shown to increase after heat shock (18); thus repeated bouts of exercise in which core temperature is elevated may provide sufficient heat stress to increase CAT activity. Although several studies indicate that myocardial CAT activity is not increased after chronic treadmill training (9, 14, 33), Somani and colleagues (37) reported increased activity when the exercise load was continuously increased throughout the training period. The findings of Somani and colleagues (37) would be consistent with our results during the first 3 wk of training when the exercise load was increased daily and the activity of CAT increased.

A number of investigations have utilized swim training to evaluate the effect of exercise on antioxidant enzymes in the heart. In contrast to treadmill training, a majority of the studies using swim training have reported decreases in myocardial antioxidant enzymes (17, 20, 21). Ji (16) suggested that the reason for the reduced activity could be that swimming-induced cardiac hypertrophy causes decreased enzyme levels per unit of mass. Another factor that can now be considered is temperature. The swimming studies by Kihlström and co-workers (20, 21) were carried out in 32°C water, which results in an exercising core temperature that actually decreases compared with resting core temperatures in air in a 23°C room (personal observation). Our data indicate that preventing the associated increase in core temperature blunts exercise-induced changes in myocardial antioxidant enzymes. Furthermore, exercise-induced cardiac hypertrophy does not appear to necessarily decrease antioxidant enzymes; we found that antioxidant enzymes after 9 wk of exercise were similar in the larger hearts of the cold runners compared with the hearts of the room-temperature runners. Additional support for this point comes from the study by Kirshenbaum and colleagues (22), who reported no changes in CAT activity and significantly higher GPx and SOD activities in rat hearts hypertrophied by pressure overload compared with controls.

**Acute versus chronic exercise.** Previously we reported that 1 day of treadmill exercise in which core temperatures were maintained at basal levels could provide improvement in postischemic myocardial function (38). In addition, the observed exercise-induced cardioprotection was independent of myocardial HSP 70 content, which led us to conclude that other factors may also be involved in exercise-induced cardioprotection. These results appear to be in partial conflict with our current study in which postischemic myocardial function was not improved in hearts that did not have an increase in HSP 70 content. A potential reason for the difference between acute and chronic exercise is that repeated bouts of exercise in the cold result in myocardial adaptations that are detrimental to improved cardioprotection such as increased cardiac hypertrophy (as discussed in Cardiac hypertrophy). Another plausible explanation is that some cardioprotective proteins such as CAT may have been elevated after acute exercise because we found that it and other proteins were increased only during the early stages of the training program used herein.

**Cardiac hypertrophy.** Absolute heart weight and ratio of heart weight-to-body weight were both significantly greater in animals run in the cold environment compared with SED controls and compared with animals run at room temperature. A possibility to be considered for increased heart size in these animals is hyperthyroidism caused by repeated exposure to the cold (11). It must be pointed out, however, that in this study no significant decreases in core temperature occurred in the animals run in the cold because of the heat produced by the forced exercise. Evidence that thyroid hormone was not elevated is that cytochrome oxidase did not increase in the cold runners. Moreno and co-workers (30) demonstrated that treatment with triiodothyronine causes both significant cardiac hypertrophy and increased myocardial cytochrome oxidase.
activity. In addition, Nishiki and co-workers (32) reported that hyperthyroidism results in significantly higher contents of cytochromes in the heart. Therefore, it does not appear that the cardiac hypertrophy observed in the animals run in the cold environment was due to increased thyroid hormone.

A more likely explanation for the cardiac hypertrophy is greater pressure overload in animals run in the cold environment. Exposure to this environment may have resulted in peripheral vasoconstriction and increased total peripheral resistance relative to the animals with higher core temperatures. Support for this hypothesis comes from an early study by Thompson and Stevenson (39), who studied male Sprague-Dawley rats that were exercised on a motorized treadmill at room temperature (22°C) or in the cold (4°C). During exercise at room temperature the core temperatures began to increase, which suggests an increase in peripheral vasodilation. However, during exercise in the cold, tail/skin temperature either decreased or remained the same, which indicates peripheral vasoconstriction.

Pressure-overload hypertrophy has been found to cause significant alterations in postischemic myocardial recovery (2, 7, 17). Cardiac hypertrophy induced by injections of isoproterenol resulted in reduction in preischemic functional capacity as well as diminished recovery from cardioplegia-induced arrest compared with controls (7). In addition, Auffermann and colleagues (2) compared the responses of thyroxine-treated and aortic-banded rats to 30 min of myocardial ischemia and 30 min of reperfusion. The results of their study showed that although both treatments resulted in significant cardiac hypertrophy, treatment by aortic banding decreased postischemic myocardial recovery and increased intracellular calcium levels, whereas thyroxine treatment did not. Therefore we cannot rule out the possibility that the protective effect of exercise was masked by pressure overload-induced cardiac hypertrophy caused by training in a cold environment.

In conclusion, the results of this study support our earlier conclusion that the exercise-induced increase in myocardial HSP 70 content is primarily related to elevation in core temperature. Furthermore, these results continue to support the important role of myocardial HSP 70 in exercise-induced cardioprotection but do not rule out the involvement of other unexplored cardioprotective proteins. Our results do, however, appear to rule out the notion that increased myocardial antioxidant activity is responsible for improved postischemic myocardial recovery after chronic low-intensity treadmill exercise in rats. In addition, because no changes in the content of GRP 75, HO-1, HSP 90, and α-crystallin were observed after the exercise program was completed, these proteins also do not appear to be important factors in cardioprotection resulting from the training program used herein. Finally, the results suggest that periodic elevation of body temperature may protect against in vivo ischemia-reperfusion injury by raising endogenous HSP 70 levels.

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