



Short-term exercise improves myocardial tolerance to in vivo ischemia-reperfusion in the rat

Authors:

Demirel, Haydar A., Scott K. Powers, Murat A. Zergeroglu, **R. Andrew Shanely**, Karyn Hamilton, Jeff Coombes, and Hisashi Naito

Abstract

These experiments examined the independent effects of short-term exercise and heat stress on myocardial responses during in vivo ischemia-reperfusion (I/R). Female Sprague-Dawley rats (4 mo old) were randomly assigned to one of four experimental groups: 1) control, 2) 3 consecutive days of treadmill exercise [60 min/day at 60–70% maximal O₂ uptake ($\dot{V} \text{O}_2 \text{ max}$)], 3) 5 consecutive days of treadmill exercise (60 min/day at 60–70% $\dot{V} \text{O}_2 \text{ max}$), and 4) whole body heat stress (15 min at 42°C). Twenty-four hours after heat stress or exercise, animals were anesthetized and mechanically ventilated, and the chest was opened by thoracotomy. Coronary occlusion was maintained for 30-min followed by a 30-min period of reperfusion. Compared with control, both heat-stressed animals and exercised animals (3 and 5 days) maintained higher ($P < 0.05$) left ventricular developed pressure (LVDP), maximum rate of left ventricular pressure development (dP/dt), and maximum rate of left ventricular pressure decline (dP/dt) at all measurement periods during both ischemia and reperfusion. No differences existed between heat-stressed and exercise groups in LVDP, dP/dt, and dP/dt at any time during ischemia or reperfusion. Both heat stress and exercise resulted in an increase ($P < 0.05$) in the relative levels of left ventricular heat shock protein 72 (HSP72). Furthermore, exercise (3 and 5 days) increased ($P < 0.05$) myocardial glutathione levels and manganese superoxide dismutase activity. These data indicate that 3–5 consecutive days of exercise improves myocardial contractile performance during in vivo I/R and that this exercise-induced myocardial protection is associated with an increase in both myocardial HSP72 and cardiac antioxidant defenses.

Short-term exercise improves myocardial tolerance to in vivo ischemia-reperfusion in the rat

HAYDAR A. DEMIREL, SCOTT K. POWERS, MURAT A. ZERGEROGLU,
R. ANDREW SHANELY, KARYN HAMILTON, JEFF COOMBES, AND HISASHI NAITO

These experiments examined the independent effects of short-term exercise and heat stress on myocardial responses during in vivo ischemia-reperfusion (I/R). Female Sprague-Dawley rats (4 mo old) were randomly assigned to one of four experimental groups: 1) control, 2) 3 consecutive days of treadmill exercise [60 min/day at 60–70% maximal $\dot{V}O_2$ uptake ($\dot{V}O_{2\max}$)], 3) 5 consecutive days of treadmill exercise (60 min/day at 60–70% $\dot{V}O_{2\max}$), and 4) whole body heat stress (15 min at 42°C). Twenty-four hours after heat stress or exercise, animals were anesthetized and mechanically ventilated, and the chest was opened by thoracotomy. Coronary occlusion was maintained for 30-min followed by a 30-min period of reperfusion. Compared with control, both heat-stressed animals and exercised animals (3 and 5 days) maintained higher ($P < 0.05$) left ventricular developed pressure (LVDP), maximum rate of left ventricular pressure development (+dP/dt), and maximum rate of left ventricular pressure decline (-dP/dt) at all measurement periods during both ischemia and reperfusion. No differences existed between heat-stressed and exercise groups in LVDP, +dP/dt, and -dP/dt at any time during ischemia or reperfusion. Both heat stress and exercise resulted in an increase ($P < 0.05$) in the relative levels of left ventricular heat shock protein 72 (HSP72). Furthermore, exercise (3 and 5 days) increased ($P < 0.05$) myocardial glutathione levels and manganese superoxide dismutase activity. These data indicate that 3–5 consecutive days of exercise improves myocardial contractile performance during in vivo I/R and that this exercise-induced myocardial protection is associated with an increase in both myocardial HSP72 and cardiac antioxidant defenses.

endurance exercise; heart; reactive oxygen species; heat shock proteins; lipid peroxidation; antioxidant enzymes

MANY HUMAN EPIDEMIOLOGICAL studies suggest that regular exercise is associated with cardiovascular benefits (13, 25, 31). Indeed, the incidence of myocardial infarctions is reduced in physically active individuals, and the survival rate of heart attack victims is greater in

active individuals compared with sedentary people (13, 25).

Recent experimental studies also indicate that regular endurance exercise provides myocardial protection against ischemia-reperfusion (I/R) injury (3, 6, 7, 9, 15, 19, 21, 29). For example, 10 wk of endurance exercise training has been shown to enhance myocardial recovery from an I/R insult, as evidenced by an enhanced recovery of left ventricle developed pressures (LVDPs) and reduced oxidative damage to the myocardium (9, 29). Although the exact biochemical mechanism(s) responsible for this protection is not known, it is believed that the training-induced changes in the myocardium are the cumulative result of consecutive exercise bouts over a long period (i.e., several months). This type of long-duration exercise training has been shown to elevate myocardial levels of heat shock protein (HSP) 72 and improve myocardial antioxidant defenses (9, 28, 29); collectively, these changes could lead to improved myocardial protection during I/R (9, 29).

To date, the minimum duration of exercise required to protect the heart during an I/R insult is unknown. Nonetheless, two recent studies suggest that as few as 1–3 consecutive days of exercise elevates myocardial levels of HSP72 and can improve myocardial contractile performance after exposure to an in vitro model of ischemia and reoxygenation (21, 34). In contrast, Yamashita et al. (37) reported that one bout of exercise did not improve myocardial contractility (i.e., rate-pressure product) during reperfusion after 20 min of in vivo coronary ischemia. The explanations for these divergent findings are unclear and warrant further investigation.

Although it is clear that prolonged exercise training (i.e., weeks to months) results in improvements in ventricular antioxidants (9, 28, 29), limited information exists regarding the effects of short-duration exercise on myocardial levels of important enzymatic and nonenzymatic antioxidants. Given the clinical importance of I/R injury and the paucity of data available on both the effects of short-term exercise on cardiac anti-

Address for reprint requests and other correspondence: S. K. Powers, Dept. of Exercise and Sport Sciences, Center for Exercise Science, Univ. of Florida, Gainesville, FL 32611 (E-mail: spowers@hhp.ufl.edu).

The costs of publication of this article were defrayed in part by the payment of page charges. The article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. Section 1734 solely to indicate this fact.

oxidants and the *in vivo* myocardial response to I/R, there is a need for additional research in this area. Therefore, the primary purpose of this study was to determine whether short-term endurance exercise (3–5 consecutive days) results in enhanced myocardial recovery after *in vivo* I/R stress. We also investigated the effects of short-term exercise on myocardial levels of HSP72 and important components of the antioxidant enzyme defense system. On the basis of preliminary studies in our laboratory (unpublished observations) and the work of Locke et al. (21) and Taylor et al. (34), we hypothesized that short-term exercise would enhance myocardial recovery from I/R and that this improvement would be associated with an increase in myocardial levels of both HSP72 and antioxidants.

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. Female Sprague-Dawley rats (4 mo old; 250–280 g body wt) were randomly assigned to one of four experimental groups ($n = 14/\text{group}$): 1) control group (no experimental treatment), 2) heat stress, 3) 3 consecutive days of treadmill exercise, and 4) 5 consecutive days of treadmill exercise. Each of these three experimental groups were then randomly subdivided into two additional groups: 1) sham surgery group ($n = 7/\text{group}$) or 2) myocardial I/R group ($n = 7/\text{group}$). During the study, all experimental groups were maintained on a 12:12-h light-dark photoperiod and provided rat chow and water *ad libitum*. The control animals were not exercised and remained in their cages during the experimental period. The exercised animals performed either 3 or 5 days of consecutive exercise (60 min/day).

Exercise Protocol

Before beginning the formal exercise protocol, animals were habituated to treadmill running (5–20 min/day) for 5 consecutive days. After this period of habituation, the exercised animals performed either 3 or 5 days of consecutive treadmill exercise (60 min/day) at an estimated work rate of 60–70% maximal O_2 uptake. The maximal O_2 uptake of our animals was estimated using published data from our laboratory for untrained rats (18). Furthermore, the treadmill grade and speed required to produce this relative workload were estimated from oxygen cost of running studies performed in our laboratory (18). Note that, during the 60 min of daily exercise, the animals were provided up to three separate 3-min rest periods during the exercise bout. Mild electrical shocks were used sparingly to motivate animals to run. Control animals did not perform treadmill exercise but were placed on a nonmoving treadmill for 60 min/day for 5 days. Exercised animals were studied 24 h after their last exercise session.

Heat Stress Protocol

Whole body heat stress has been shown to provide myocardial protection against an I/R insult (reviewed in Refs. 16 and 20). Therefore, we incorporated one bout of heat stress into our experimental design as a “positive” control to compare with 3–5 days of exercise. Animals subjected to a bout of

heat stress were anesthetized with an intraperitoneal injection of pentobarbital sodium (30 mg/kg) and placed on a heating pad set at 45°C until the animal's colonic temperature reached 42°C; this temperature was maintained for an additional 15 min. To aid in the recovery from heat stress, 10–15 ml of water were then administered via an esophageal tube; our experience indicates that this practice reduces the morbidity associated with whole body heat stress in rats. The animals were allowed to recover for 24 h before being exposed to either sham or I/R surgery.

Sham Surgery

To determine the effects of short-term exercise on selected biochemical properties of ventricular tissue not exposed to I/R, each experimental group (i.e., control, heat-stressed, and exercised animals) was studied after a sham operation that did not include myocardial I/R. This protocol was identical to the I/R experimental protocol with the exception that animals were not catheterized and exposed to myocardial I/R. At the completion of the sham surgery, the heart was rapidly removed and quickly frozen in liquid nitrogen for subsequent biochemical analyses.

In Vivo Protocol for Studying I/R-Induced Myocardial Damage

All experimental groups were studied during *in vivo* I/R. Animals were anesthetized with pentobarbital sodium (50 mg/kg ip) and ventilated with room air using a small-animal ventilator (Columbus Instruments). Throughout the surgery, body temperature was monitored via a rectal thermistor probe. Body temperature was maintained at $37 \pm 0.2^\circ\text{C}$ with a heated operating platform and appropriate heating lamps. Cardiac rhythm was monitored continuously via standard electrocardiogram (lead II).

The chest was opened by a left thoracotomy and a ligature placed around a prominent branch of the left anterior descending coronary artery (LCA). Evans blue dye infusion studies indicate that this artery serves the interventricular septum and a portion of the left ventricular free wall. After placement of the ligature around the vessel, any animal exhibiting significant ventricular arrhythmias (i.e., >3 bouts of ventricular fibrillation) was eliminated from the study. Coronary occlusion was achieved by passing both ends of the ligature through one end of a small plastic tube, which was then pressed against the surface of the heart directly above the artery. The resulting arterial occlusion was maintained for 30 min by clamping the plastic tube and ligature with a small hemostat. Reperfusion duration was 30 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 (3, 9, 15, 29). However, to validate that complete coronary occlusion was achieved in our hands, we performed preliminary experiments where Evans blue dye was injected directly into the right ventricle (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 branch of the left anterior descending coronary artery. Failure to observe dye stain in this area of the ventricle was interpreted as achievement of coronary occlusion (29).

To ensure that reperfusion had been adequately achieved in these studies, we also administered Evans blue dye at the end of the 10-min reperfusion period during pilot experi-

ments. In each case, we observed a uniformly stained heart; this was interpreted as evidence that reperfusion was achieved.

Measurement of LVDP and Rates of Left Ventricular Pressure Development and Decline

To monitor cardiovascular function during the I/R protocol, a fluid-filled cannula was introduced via the carotid artery into the left ventricle. LVDP, heart rate, maximum rate of left ventricular pressure development ($+dP/dt$), and maximum rate of left ventricular pressure decline ($-dP/dt$) were measured using a pressure transducer interfaced with a computerized heart performance analysis system (Digi-Med, Louisville, KY).

Tissue Preparation

Selected biochemical properties of the ventricular myocardium were studied in control, heat-stressed, and exercised animals after the I/R protocol and from these same groups not exposed to I/R (i.e., sham surgery). To determine the antioxidant capacity in the hearts of both I/R and 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 concentration of GSH along with the activities of superoxide dismutase (SOD), glutathione peroxidase (GPX), glutathione reductase (GR), thioredoxin reductase (TR), and catalase (Cat).

In animals exposed to the sham or I/R protocol, left ventricular tissue distal to the LCA was quickly removed, divided into four sections, and frozen in liquid nitrogen for subsequent biochemical analysis. Care was taken to consistently harvest the same region of the left ventricle from all animals.

Biochemical Assays

Measurement of myocardial antioxidant capacity. To assess the effects of exercise on the antioxidant capacity of the myocardium, the activities of several important antioxidant enzymes were assayed along with the level of reduced glutathione. The antioxidants selected were chosen on the basis that each of these elements is important in providing myocardial protection against oxidative injury. Before homogenization, the epicardium and endocardium were rapidly (~60 s) removed from each sample using a dissecting microscope; the remaining ventricular myocardium was then homogenized. Sections of the left ventricular myocardium were individually minced and homogenized in cold 100 mM phosphate buffer with 0.05% bovine serum albumin (1:20 wt/vol; pH 7.4). Homogenization included a 15-s treatment with a tissue homogenizer (Ultra-Turrax T25, IKA Works, Cincinnati, OH) followed by 10 passes of the homogenate in a tight-fitting Potter-Elvehjem homogenizer. Homogenates were then centrifuged (3°C) for 10 min at 400 g. The supernatant was decanted and assayed to determine total protein content along with the activities of SOD (EC 1.15.1.1), Cat (EC 1.11.1.6), GPX (EC 1.11.1.9), GR (EC 1.6.4.2), and TR (EC 1.6.4.5). These antioxidant enzymes were selected because of their importance in the regulation of myocardial redox status. Protein content was determined using methods described by Bradford (8). SOD and GPX activities were determined in the left ventricle using a modification of the procedures described by Oyanagui (26) and Flohe and Gunzler (11), respectively. Exercise-induced changes in SOD isoforms (i.e., MnSOD and CuZnSOD) were determined by KCN inhibition of the CuZnSOD isoform. Cat activity was

determined using techniques described by Aebi (1). Finally, GR and TR activities were assayed using techniques reported by Luthman and Holmgren (22). The coefficients of variation for SOD, GPX, GR, TR, Cat, GR, and TR assays ranged between 2 and 5%. These and all other biochemical assays were performed in duplicate at 22°C, and samples from all experimental groups were assayed on the same day to avoid interassay variation.

GSH levels are important in the regulation of both cellular redox status and antioxidant capacity; hence, we assayed GSH in the left ventricles of sham animals (12).

Measurement of myocardial HSPs. To determine the effects of exercise on induction of myocardial HSPs, we performed polyacrylamide gel electrophoresis and immunoblotting using the techniques described by Locke et al. (21) and modified by Powers et al. (29). Briefly, left ventricular samples from I/R animals were homogenized, and one-dimensional sodium dodecyl sulfate (12%)-polyacrylamide gel electrophoresis was performed to separate proteins by molecular mass. After separation, proteins were transferred to nitrocellulose membranes (0.45 mm thick, Bio-Rad, Hercules, CA) using the Bio-Rad semidry 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 2 h with alkaline phosphatase-conjugated monoclonal antibodies specific for HSP72 and HSP73 (SPA-810 and SPA-815, StressGen, Victoria, BC, Canada). The membranes were then reacted with 5-bromo-4-chloro-3-indolyl phosphate-nitro blue tetrazolium substrate (Sigma Chemical, St. Louis, MO). Quantification of the bands from the immunoblots was performed using computerized densitometry and the NIH Image analysis program. Standard curves were constructed during preliminary experiments to ensure linearity.

Data Analysis

Myocardial contractility measurements were analyzed using a two-way analysis of variance with repeated measures. When appropriate, a Fisher's least significant difference test was used post hoc. Myocardial biochemical parameters were analyzed using analysis of variance with a Fisher's least significant difference test used post hoc. For all statistical analysis, significance was established at $P < 0.05$.

RESULTS

Myocardial Performance During I/R

Successful I/R protocols were performed on seven animals from each experimental group. Ten animals were eliminated from these experiments because of greater than three repeated bouts of ventricular fibrillation during ischemia. The number of animals eliminated per experimental group was as follows: control, $n = 6$; heat stress, $n = 1$; 3 days exercise, $n = 2$; and 5 days exercise, $n = 1$. Table 1 contains the absolute mean values (means \pm SE) for the rate-pressure product, LVDP, $+dP/dt$, and $-dP/dt$ during preischemia and at selected periods during I/R in animals from all experimental groups. No group differences existed ($P > 0.05$) in any of these variables before the initiation of ischemia. However, compared with control, exercised animals (both 3 and 5 days) and heat-stressed animals maintained higher ($P < 0.05$) rate-pressure product, LVDP, $+dP/dt$, and $-dP/dt$ at *minute 30* of I/R. No

Table 1. Myocardial performance during preischemia, ischemia, and reperfusion

Group	HR, beats/min	LVDP, mmHg	RPP, mmHg/min	+dP/dt, mmHg/s	-dP/dt, mmHg/s
<i>Preischemia</i>					
Control	389±9	135±3	52,515±1,215	2,657±186	2,205±153
3 Days Exercise	366±10	141±2	51,606±1,410	2,611±178	2,141±146
5 Days Exercise	379±12	137±2	51,923±1,644	2,625±186	2,153±152
Heat Stress	375±14	131±3	49,125±1,834	2,481±161	2,034±122
<i>Minute 30 Ischemia</i>					
Control	320±21	66±5	21,120±1,386	1,068±75	876±60
3 Days exercise	345±13	118±7*	40,710±1,534*	2,056±144*	1,686±106*
5 Days exercise	359±14	119±7*	42,721±1,666*	2,159±151*	1,770±104*
Heat stress	325±18	113±5*	36,725±2,034*	1,856±149*	1,522±127*
<i>Minute 30 Reperfusion</i>					
Control	349±14	77±8	26,873±1,078	1,332±87	1,092±66
3 Days exercise	391±17	125±8*	48,875±2,125*	2,470±157*	2,025±119*
5 Days exercise	380±15	128±7*	48,640±1,920*	2,457±147*	2,020±133*
Heat stress	350±13	119±6*	41,650±1,547*	2,062±164*	1,691±142*

Values are means ± SE. HR, heart rate; LVDP, left ventricular developed pressure; +dP/dt, maximum rate of pressure development; -dP/dt, maximum rate of pressure decline. *Different from control, $P < 0.05$.

differences existed in rate-pressure product, LVDP, +dP/dt, and -dP/dt between the exercise groups (both 3 and 5 days) and heat-stressed animals during ischemia or reperfusion.

Figure 1 contains the mean values for LVDP, +dP/dt, and -dP/dt (expressed as a percentage of the initial value) during preischemia, ischemia, and reperfusion in animals from all experimental groups. No group differences existed ($P > 0.05$) in any of these measures before the introduction of ischemia. However, compared with control, exercised animals (both 3 and 5 days) and heat-stressed animals maintained higher ($P < 0.05$) LVDP, +dP/dt, and -dP/dt throughout I/R. No differences existed in LVDP, +dP/dt, and -dP/dt between the exercised groups (both 3 and 5 days) and heat-stressed animals during ischemia and reperfusion.

Heart rate was compared at 10-min time periods during the preischemic period, ischemia, and reperfusion. No group differences existed in heart rate during the preischemic period or during ischemia or reperfusion (Table 1).

Biochemical Measurements

Myocardial antioxidants. Table 2 contains GSH concentrations and antioxidant enzyme activities in the left ventricle of both sham- and I/R-operated animals. No group differences existed in ventricular GSH concentration and antioxidant enzyme activities between the 3- and 5-day exercise groups; hence, we pooled these data into one exercise group. Compared with untrained control (sham), both exercise (sham) and heat stress (sham) resulted in a significant increase in MnSOD activity with no changes in myocardial levels of CuZnSOD activity. Furthermore, compared with control, exercise resulted in a significant increase in myocardial levels of GSH.

To determine whether cardiac performance at the conclusion of reperfusion was highly correlated with

cardiac antioxidant levels, we examined the relationship between ventricular MnSOD activity, GSH levels, and myocardial contractile performance (+dP/dt) after 30 min of reperfusion. The correlation between ventricular MnSOD activity and +dP/dt was $r = 0.95$ ($P < 0.05$), whereas the correlation between ventricular GSH and +dP/dt was $r = 0.75$ ($P < 0.05$).

Myocardial HSP72 and HSP73 content. To determine whether HSP72 and HSP73 content was elevated in the hearts from both exercised and heat-stressed animals, portions of the left ventricle from control, heat-stressed, and exercised animals were analyzed for HSP content using Western blotting. The results revealed that heat stress and exercise did not alter ventricular HSP73 content (data not shown). However, compared with control, HSP72 content was significantly greater in the left ventricle of both heat-stressed and exercised animals (Fig. 2). Although myocardial HSP72 levels tended to be higher in the 5-day exercise group compared with the 3-day group, these group differences were not significant. Finally, note that heat stress resulted in a greater increase ($P < 0.05$) in HSP72 compared with control and both exercise groups.

It has been argued that there is an inverse relationship between the amount of cardiac HSP72 and infarct size after I/R (13); therefore, we examined the relationship between HSP72 and myocardial contractile performance after 30 min of reperfusion. Note that, whereas the cardiac levels of HSP72 and myocardial contractile performance are significantly correlated ($r = 0.83$; $P < 0.05$), the improvement in myocardial contractile performance appears to plateau with increasing cardiac HSP72 (Fig. 3).

DISCUSSION

Overview of Principal Findings

This experiment examined the effects of both heat stress and short-duration exercise on cardiac antiox-

Exercise Improves Myocardial Contractile Performance During Ischemia

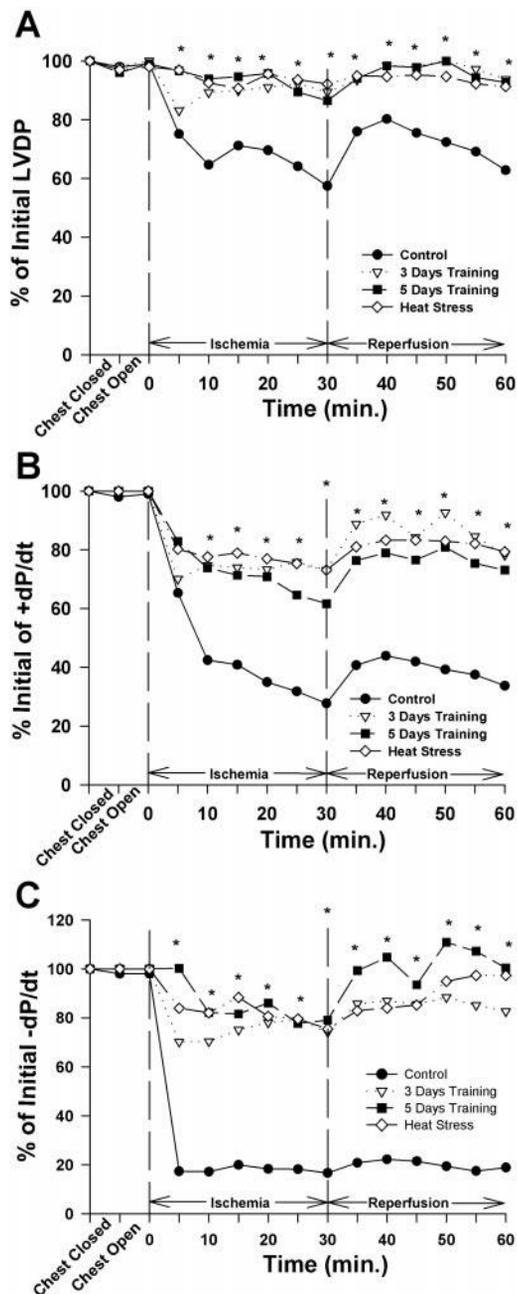


Fig. 1. Changes in left ventricular developed pressure (LVDP; A), maximum rate of left ventricular pressure development (+dP/dt; B), and maximum rate of left ventricular pressure decline (-dP/dt; C) during the ischemia-reperfusion protocol. Values are means and are expressed as percentage of initial (preischemia) values. Error bars are not included because of the overlap of symbols in the exercise and heat-stress groups. *3 days Exercise, 5 days exercise, and heat-stress groups were greater than control, $P < 0.05$.

idant capacity and contractile performance during in vivo I/R. Our results indicate that both heat stress and short-duration endurance exercise resulted in improved myocardial performance during I/R. This heat- and exercise-induced improvement in cardiac performance during I/R was associated with an increase in myocardial levels of both HSP72 and anti-oxidants.

As evidenced by measures of cardiac performance, the present data clearly demonstrate that both short-duration exercise and heat stress result in improved myocardial performance during ischemia (Fig. 1, A–C). The mechanism responsible for the exercise-induced improvement in myocardial performance during ischemia is not clear from previous investigations or the present study. Theoretically, exercise results in several myocardial changes that could improve contractile performance during ischemia. For example, exercise could have altered either preload or afterload of the hearts from exercised animals during ischemia. Because arterial blood pressure was not measured in our experiments, we cannot determine the role of afterload on ventricular performance. In reference to preload, our data indicate that short-term exercise promoted a greater myocardial -dP/dt during both ischemia and reperfusion. This is significant because -dP/dt is an indicator of calcium reuptake into the sarcoplasmic reticulum (SR), and a greater -dP/dt is correlated with higher SR calcium-ATPase activity and a more rapid ventricular relaxation (3, 27, 33, 35, 36). Hence, a more rapid ventricular relaxation could result in a greater end-diastolic volume and an increase in ventricular force production due to Frank-Starling mechanisms.

Because it seems likely that a portion of the ischemic region within the left ventricle of both trained and untrained hearts was not contracting during the final minutes of ischemia (32), this raises the question of whether the exercise-induced improvement in LVDP during ischemia was due to elevated force production in nonischemic regions of the left ventricle. This is an interesting possibility that warrants additional study.

Another explanation for the exercise-induced improvement in cardiac contractile performance during ischemia could be the exercise-induced improvements in myocardial antioxidant capacity (Table 2). In this regard, it is clear that oxidants are produced in cardiomyocytes during both ischemia (2) and reperfusion (4, 5). Furthermore, there is unequivocal evidence that oxidative stress can impair contractile performance of both cardiac and skeletal muscle myocytes (reviewed in Refs. 4 and 30). Therefore, in the present experiments, the exercise-induced improvement in myocardial antioxidant capacity could reduce oxidative stress during ischemia and minimize the oxidant-mediated impairment in ventricular contractile performance. Although this possibility could contribute to the improved ventricular performance during the early minutes of ischemia, this explanation would not seem plausible for the cardioprotection during late ischemia.

In summary, whether alterations in preload or afterload, contractile differences in nonischemic regions of the ventricle, and/or improved antioxidant capacity is responsible for the improved myocardial performance during ischemia cannot be evaluated from the present experiment. Additional experiments to delineate the mechanism(s) to explain the exercise-induced improve-

Table 2. Antioxidant enzyme activities in control and exercise-trained animals

	MnSOD U/mg protein	CuZnSOD U/mg protein	Cat. U·mg protein ⁻¹ ·min ⁻¹	GPX, μmol substrate·mg protein ⁻¹ ·min ⁻¹	GR. U·mg protein ⁻¹ ·min ⁻¹	TR, μmol substrate·mg protein ⁻¹ ·min ⁻¹	GSH, μmol/g
Control-ischemia-reperfusion	42.32±1.44	20.09±1.73	2.34±0.07	1.31±0.04	11.48±0.4	7.5±0.27	1.76±0.11
Exercise-ischemia-reperfusion	40.92±1.63	15.97±1.04	2.5±0.06	1.31±0.03	11.50±0.25	6.16±0.09	1.86±0.07
Heat stress-ischemia-reperfusion	41.47±4.11	18.94±2.01	2.65±0.1	1.37±0.04	12.42±0.66	6.9±0.27	1.77±0.14
Control-sham	42.26±2.9	24.24±1.39	2.52±0.16	1.36±0.03	12.22±0.52	7.94±0.41	1.86±0.08
Exercise-sham	49.84±1.8*	26.70±1.0	2.28±0.08	1.32±0.04	12.06±0.15	6.76±0.19	2.07±0.05*
Heat stress-sham	54.83±2.1*	21.03±2.61	2.49±0.29	1.43±0.04	13.0±1.24	7.36±0.59	1.96±0.1

Values are means ± SE. Exercise-trained animals include pooled data for both the 3- and 5-day trained groups. GPX, glutathione peroxidase; TR, thioredoxin reductase; Cat, catalase; GR, glutathione reductase; MnSOD, manganese superoxide dismutase; CuZnSOD, cytosolic superoxide dismutase. * Different from control-sham; $P < 0.05$.

ment in myocardial tolerance to ischemia are clearly warranted.

Short-Duration Exercise Attenuates Postischemic Myocardial Injury

Our findings support the hypothesis that short-duration endurance exercise attenuates post-ischemic myocardial injury after 30 min of coronary ligation-induced ischemia. These results closely agree with the in vitro work by Locke et al. (21) and Taylor et al. (34), which indicate that 1–3 consecutive days of exercise result in enhanced postischemic myocardial recovery. In contrast, the present findings differ from an investigation by Yamashita et al. (37). These investigators reported that 1 day of exercise resulted in myocardial protection against I/R-induced infarction but that exercise did not alter the rate-pressure product at the completion of 20 min of in vivo ischemia or after 30 min of reperfusion. Two major differences between the present study and the work by Yamashita et al. are the

strain of rats investigated (i.e., Wistar vs. Sprague-Dawley) and the fact that our animals exercised for 3 or 5 consecutive days (60 min/day), whereas Yamashita et al. exercised animals for 1 day only (30-min exercise bout). Unfortunately, on the basis of the present data alone, we cannot provide a definitive explanation as to why the present results differ from the data of Yamashita et al.

What factors contribute to the exercise-enhanced myocardial recovery from an I/R insult? It seems likely that improved cardiac function after I/R in exercise-trained animals indicates a decrease in myocardial injury during I/R. This reduced myocardial injury could result from a variety of physiological factors, including reduced oxidant injury, improved high-energy phosphate profile, refined calcium handling (i.e., SR release and uptake), a greater inotropic response to external calcium, and lower diastolic stiffness (5–7). Although several theories to explain exercise-induced cardioprotection have been advanced, two of the most promising current theories are linked to the exercise-induced improvements in myocardial antioxidant capacity and/or exercise-induced elevation in myocardial HSP72.

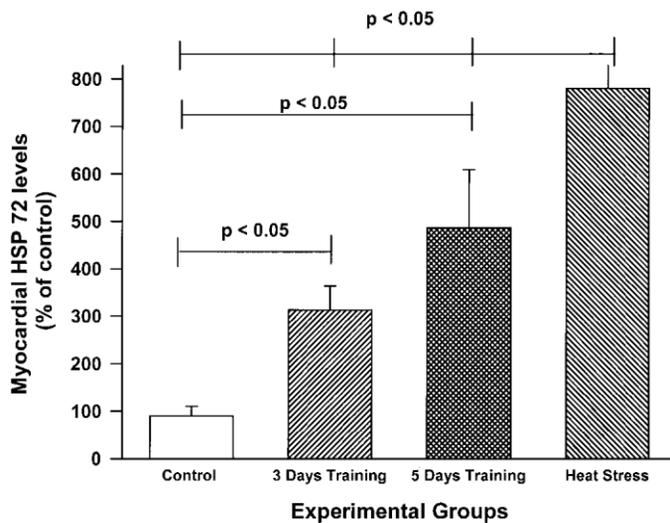


Fig. 2. Left ventricular heat shock protein 72 (HSP72) levels in control, heat-stressed, and exercised animals obtained from densitometric scanning of Western blots reacted with an antibody for HSP72. Values are means ± SE expressed as a percentage of the control group.

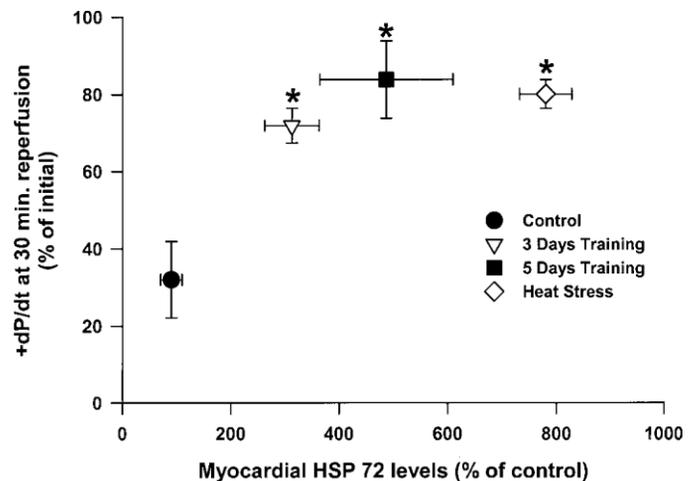


Fig. 3. Relationship between the rate of +dP/dt at the conclusion of reperfusion (i.e., *minute 30*) and ventricular HSP72 content. Values are means ± SD. *Different from control, $P < 0.05$.

Ji et al. (15) have suggested that the exercise-induced myocardial protection against I/R injury is likely due to exercise-induced changes in the magnitude of oxidant damage to the heart. This proposition is based on the fact that radicals and other reactive oxygen species (ROS) are important mediators of myocardial I/R injury (reviewed in Refs. 5 and 10). Indeed, several lines of evidence implicate ROS-induced injury as an important mechanism to explain a reversible form of I/R-induced myocardial injury (i.e., myocardial stunning). For example, ROS are produced in the reperfused myocardium, and administration of ROS scavengers attenuates stunning (4, 5). The molecular mechanism to explain myocardial stunning is unknown but may be related to ROS-induced oxidation of calcium-handling proteins within the SR because both calcium release and reuptake are altered by ROS in skeletal and cardiac myocytes (reviewed in Ref. 30).

In the present study, we investigated the effects of 3–5 days of exercise on the upregulation of several important myocardial antioxidants (Table 2). These antioxidants were chosen because of their varied roles in regulating redox status in cells. Of the eight antioxidants studied, we observed an exercise-induced increase in only two antioxidants (MnSOD activity and GSH concentration). It seems likely that the exercise-induced increases in both myocardial MnSOD activity and GSH concentrations are important adaptations that could provide myocardial protection against I/R-induced oxidative injury. Indeed, these two antioxidants work as a unit to remove oxidant precursors [i.e., O_2^- , hydrogen peroxide (H_2O_2)] before they can interact to form more reactive cytotoxic oxidants (i.e., OH^\cdot). The interaction between SOD and GSH begins when superoxide radicals are dismutated by MnSOD to yield H_2O_2 and O_2 . H_2O_2 can then be removed by the action of GPX at the expense of GSH. Although exercise does not increase myocardial GPX activity, an increased total ability to remove H_2O_2 can be achieved by increasing cellular levels of GSH. Hence, it is possible that an increase in both myocardial MnSOD activity and GSH concentration is required to provide cardiac antioxidant protection against I/R-mediated oxidative injury. This is a testable hypothesis and is worthy of future research.

It is well established that a correlation between two biological variables does not prove cause and effect. Nonetheless, it is expected that, if exercise-induced changes in myocardial antioxidant levels contribute to cardiac protection during I/R, the level of protection would be closely correlated with antioxidant levels. In this regard, both MnSOD activity ($r = 0.95$) and GSH concentration ($r = 0.75$) were significantly correlated with myocardial $+dP/dt$ at *minute 30* of reperfusion. Therefore, it seems plausible that exercise-induced improvements in myocardial antioxidant defenses could explain, at least in part, the enhanced myocardial contractile recovery after reperfusion observed in our experiments.

Another potentially important mechanism that could have contributed to the improved postischemic

myocardial recovery is that exercise increased the left ventricular content of HSP72. Although a recent report indicates that short-term exercise can improve myocardial protection without increasing HSP content (34), there is strong evidence to indicate that an elevated level of HSP72 provides cellular protection against a variety of stresses (reviewed in Ref. 21). Indeed, the inducible form of the HSP70 family, HSP72, has been associated with an improved myocardial postischemic functional recovery and a reduction in infarct size (14, 16, 21, 29). Furthermore, a previous report has demonstrated a high linear correlation between the amount of HSP72 and the reduction of infarct size after I/R in the rat heart (14). Our data also indicate that myocardial contractile recovery is associated with increases in ventricular HSP72. Nonetheless, our findings do not verify a strong linear relationship between myocardial HSP72 levels and the improvement in cardiac contractile performance after I/R (Fig. 3).

The precise 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. HSP72 facilitates these types of chemical reactions via an ATP-dependent mechanism that involves the binding and release of proteins (23, 24). Specifically, HSP72 is an ATP-binding protein and has ATPase activity; the ATP-bound form of the protein has a low affinity for substrate, whereas the ADP-bound form can bind substrate. Therefore, regulation of the ATPase activity can affect the ability of HSP72 to bind and refold proteins. Recent studies indicate that HSP72 ATPase activity is regulated by a 40-kDa HSP (HSP40) and overexpression of HSP40 accelerates the recovery of damaged proteins when HSP72 is also expressed (23). Therefore, it seems possible that exercise-induced increases in HSP72 and perhaps other HSPs (e.g., HSP40) may explain, at least in part, the myocardial protection associated with exercise.

Summary and Conclusions

This is the first experiment to examine the effects of 3–5 consecutive days of exercise on myocardial levels of HSP72, cardiac antioxidant adaptations, and myocardial performance during in vivo I/R in the rat. The data revealed that compared with untrained controls, both heat-stressed and exercised animals maintained higher LVDP and $\pm dP/dt$ throughout I/R. Thus these experiments provide strong evidence to support the hypothesis that as few as 3–5 consecutive days of exercise provide myocardial protection during moderate duration I/R. Regarding the mechanism behind this protection, exercise elevated both myocardial antioxidant defenses and ventricular levels of HSP72. Hence, it seems possible that exercise-induced increases in both myocardial antioxidants and HSP72 were important in protecting myocardial performance after I/R.

This work was supported by a grant-in-aid from the American Heart Association, Florida Affiliate (to S. K. Powers).

REFERENCES

1. Aebi H. Catalase in vitro. *Methods Enzymol* 105: 121–126, 1984.
2. Becker LB, van Hoek T, Shao Z, LCQ, and Schumacker P. Generation of superoxide in cardiomyocytes during ischemia before reperfusion. *Am J Physiol Heart Circ Physiol* 277: H2240–H2246, 1999.
3. Bersohn M and Scheuer J. Effect of ischemia on the performance of hearts from physically trained rats. *Am J Physiol Heart Circ Physiol* 234: H215–H218, 1978.
4. Bolli R and Marban E. Molecular and cellular mechanisms of myocardial stunning. *Physiol Rev* 79: 609–634, 1999.
5. Bolli R, Zhu W, Michael L, Repine J, Hess M, Kukeia R, and Roberts R. Attenuation of dysfunction in the postischemia “stunned” myocardium by dimethylthiourea. *Circulation* 76: 458–468, 1987.
6. Bowles D, Farrar R, and Starnes J. Exercise training improves metabolic response after ischemia in isolated working rat heart. *J Appl Physiol* 76: 1608–1614, 1994.
7. Bowles D and Starnes J. Exercise training improves cardiac function after ischemia in the isolated, working rat heart. *Am J Physiol Heart Circ Physiol* 263: H804–H809, 1992.
8. Bradford M. A refined sensitive method for the quantitation of microgram quantities of protein utilizing the principle of protein-dye binding. *Anal Biochem* 72: 248–254, 1976.
9. Demirel H, Powers S, Caillaud C, Coombes J, Naito H, Fletcher L, Vrabas I, Jessup J, and Ji L. Exercise training reduces myocardial lipid peroxidation following short-term ischemia-reperfusion. *Med Sci Sports Exerc* 30: 1211–1216, 1998.
10. Downey J. Free radicals and their involvement during long-term myocardial ischemia and reperfusion. *Annu Rev Physiol* 52: 487–504, 1990.
11. Flohe L and Gunzler W. Assays of glutathione peroxidase. *Methods Enzymol* 105: 114–121, 1984.
12. Griffith OW. Determination of glutathione and glutathione disulfide using glutathione reductase and 2-vinylpyridine. *Anal Biochem* 106: 207–212, 1980.
13. Hull S, Vanoli E, Adamson P, Verrier R, Foreman R, and Schwartz P. Exercise training confers anticipatory protection from sudden death during acute myocardial ischemia. *Circulation* 89: 548–552, 1994.
14. Hutter M, Sievers R, Barbosa V, and Wolfe C. Heat-shock protein induction in rat hearts: a direct correlation between the amount of heat-shock protein induced and the degree of myocardial protection. *Circulation* 89: 355–360, 1994.
15. Ji L, Fu R, Mitchell E, Griffiths M, Waldrop T, and Swartz H. Cardiac hypertrophy alters myocardial response to I/R in vivo. *Acta Physiol Scand* 151: 270–290, 1994.
16. Knowlton A. (Editor). *Heat Shock Proteins and the Cardiovascular System*. Boston, MA: Kluwer Academic, 1997.
17. Lankford E, Korzick D, Palmer B, Stauffer B, Cheung J, and Moore R. Endurance exercise alters the contractile responsiveness of rat heart to extracellular Na⁺ and Ca²⁺. *Med Sci Sports Exerc* 30: 1502–1509, 1998.
18. Lawler J, Powers S, Hammeren J, and Martin A. Oxygen cost of treadmill running in 24-month-old Fischer-344 rats. *Med Sci Sports Exerc* 25: 1259–1264, 1993.
19. Libonati J, Gaughan J, Hefner C, Gow A, Paolone A, and Houser S. Reduced ischemia and reperfusion injury following exercise training. *Med Sci Sports Exerc* 29: 509–516, 1997.
20. Locke M. The cellular response to exercise: role of stress proteins. *Exerc Sport Sci Rev* 25: 105–136, 1997.
21. Locke M, Tanguay R, Klabunde R, and Ianuzzo D. Enhanced postischemic myocardial recovery following induction of HSP 72. *Am J Physiol Heart Circ Physiol* 269: H320–H325, 1995.
22. Luthman M and Holmgren A. Rat liver thioredoxin and thioredoxin reductase: purification and characterization. *Biochemistry* 21: 628–6633, 1982.
23. Michels A, Kanon B, Konings A, Ohtsuka K, Bensaude O, and Kampinga H. Hsp70 and Hsp40 chaperone activities in the cytoplasm and the nucleus of mammalian cells. *J Biol Chem* 272: 33283–33289, 1997.
24. Minami Y, Hohfeld J, Ohtsuka K, and Hart F. Regulation of the heat shock protein 70 reaction cycle by the mammalian DnaJ homolog, Hsp-40. *J Biol Chem* 271: 19617–19624, 1996.
25. Morris J, Everitt M, Pollard R, and Chave S, and Semmence A. Vigorous exercise in leisure time: protection against coronary heart disease. *Lancet* 2: 1207–1212, 1980.
26. Oyanagui Y. Reevaluation of assay methods and establishment of kit for superoxide dismutase activity. *Anal Biochem* 142: 290–296, 1984.
27. Penpargkul S, Repke D, Katz A, and Scheuer J. Effect of physical training on calcium transport by cardiac sarcoplasmic reticulum. *Circ Res* 40: 134–138, 1977.
28. Powers S, Criswell D, Lawler J, Martin D, Lieu F, Ji L, and Herb R. Rigorous exercise training increases superoxide dismutase activity in the ventricular myocardium. *Am J Physiol Heart Circ Physiol* 265: H2094–H2098, 1993.
29. Powers S, Demirel H, Vincent H, Coombes J, Naito H, Hamilton K, Shanely RA, and Jessup J. Exercise training improves myocardial tolerance to in vivo ischemia-reperfusion in the rat. *Am J Physiol Regulatory Integrative Comp Physiol* 275: R1468–R1477, 1998.
30. Reid R. Reactive oxygen species and nitric oxide in skeletal muscle. *News Physiol Sci* 11: 114–119, 1996.
31. Siscovick D, Weiss N, Fletcher R, and Lasky T. The incidence of primary cardiac arrest during vigorous exercise. *N Engl J Med* 311: 874–877, 1984.
32. Symons JD, Rendig S, Stebbins C, and Longhurst J. Microvascular and myocardial contractile responses to ischemia: influence of exercise training. *J Appl Physiol* 88: 433–442, 2000.
33. Tate C, Taffet G, Hudson E, Blaylock S, and McBride M. Enhanced calcium uptake of cardiac sarcoplasmic reticulum in exercise trained old rats. *Am J Physiol Heart Circ Physiol* 258: H431–H435, 1990.
34. Taylor R, Harris M, and Starnes J. Acute exercise can improve cardioprotection without increasing heat shock protein content. *Am J Physiol Heart Circ Physiol* 276: H1098–H1102, 1999.
35. Tibbits G, Kashihara H, and O'Reilly K. Na⁺-Ca²⁺ exchange in cardiac sarcolemma: modulation of Ca²⁺ affinity by exercise. *Am J Physiol Cell Physiol* 256: C638–C643, 1989.
36. Tibbits G, Nagamoto T, Sasaki M, and Barnard R. Cardiac sarcolemma: compositional adaptation to exercise. *Science* 213: 1271–1273, 1981.
37. Yamashita N, Hoshida S, Otsu K, Asahi M, Kuzuya T, and Hori M. Exercise provides direct biphasic cardioprotection via manganese superoxide dismutase activation. *J Exp Med* 189: 1699–1706, 1999.