Exercise improves postischemic function in aging hearts

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Starnes, Joseph W., Ryan P. Taylor, and Yoonjung Park. Exercise improves postischemic function in aging hearts. Am J Physiol Heart Circ Physiol 285: H347–H351, 2003.—Exercise improves cardioprotection against ischemia-reperfusion in young animals but has not been investigated in older animals, which represent the population most likely to suffer an ischemic event. Therefore, we sought to determine the effects of aging on exercise-induced cardioprotection. Young, middle-aged, and old (4, 12, and 21 mo old) male Fischer 344 rats ran 60 min at 70–75% of maximum oxygen consumption. Twenty-four hours postexercise, isolated perfused working hearts underwent 22.5 min of global ischemia and then 30 min of recovery (reperfusion). Compared with sedentary rats (n = 8–9 rats/group), recovery of function (cardiac output × systolic pressure) improved after exercise (n = 9 rats/group) by 40% at 4 mo, 78% at 12 mo, and 59% at 21 mo. Exercise increased inducible heat shock protein 70 expression 105% at 4 mo but only 27% at 12 mo and 24% at 21 mo. Catalase activity progressively increased with age (P < 0.05) and was increased by exercise at 4 mo (26%) and 21 mo (19%). Manganese superoxide dismutase activity was increased by exercise only at 21 mo (45%). No exercise-related change in any antioxidant enzyme was observed at 12 mo. We conclude that exercise can enhance cardioprotection regardless of age, but the cardioprotective protein phenotype changes with age.

heat shock protein 70; antioxidant enzymes; free radicals; reperfusion; physical conditioning

ENHANCED CARDIOPROTECTION against ischemia-reperfusion (I/R) injury has been observed after one to three exercise bouts (4, 11, 13, 15, 23, 27, 34, 36, 38). Exercise, along with brief heat shock or ischemia, is among a group of the manipulations that have been reported to induce the late phase of preconditioning against I/R (for reviews, see Refs. 5, 19, 30, and 31). The late phase of protection is due to de novo synthesis of cardioprotective proteins, requires many hours to develop, and lasts for 72 ± h (for reviews, see Refs. 5 and 19). These manipulations may share some common protective pathways, especially exercise and heat shock, because exercise can significantly increase core temperature. Proteins reported to have prominent roles in exercise-induced preconditioning against I/R include inducible heat shock protein (HSP70), superoxide dismutase (SOD), and catalase (11, 13, 15, 23, 27, 31, 34, 38). The antioxidant enzymes function to prevent protein denaturation during the oxidative stress associated with both exercise and I/R, whereas HSP70 aids in the subsequent recovery by promoting restoration of dysfunctional enzymes and preventing aggregation of severely denatured proteins (for reviews, see Refs. 18 and 30). Together, the complimentary protective mechanisms of HSP70 and antioxidants are considered to form a strong defense against I/R injury.

Importantly, eight of the nine exercise-induced cardioprotection studies mentioned above were carried out on rats <6 mo old, which represents a very early stage of life (39). The other study investigated cardioprotection 24 h after a single exercise bout in dogs of uncertain age (4). Individuals who would most benefit from improved cardioprotection are the middle-aged and elderly because most cardiac complications occur in late adulthood. It may not be appropriate to extrapolate results from exercised young animals to older animals. For example, several studies conclude that HSP70 expression after heat stress is impaired in the heart of aged animals (for a review, see Ref. 30), and the cardioprotective benefits induced by ischemic preconditioning are attenuated or abolished with age (2, 12, 20, 24, 29). Thus the purpose of this study was to determine the effect of age on exercise-induced protection against I/R and on changes in cardioprotective proteins.

METHODS

Animals and exercise protocol. Male Fischer 344 rats aged 4, 12, and 21 mo old (young, middle-aged, and old) were purchased from the National Institute on Aging colony maintained by Harlan Sprague Dawley. The 12-mo-old group is represented as middle aged because the median life span of ad libitum-fed male Fischer 344 rats is ∼24 mo (39). A total of 60 animals were divided into sedentary (n = 10 at each age) and exercised (n = 10 at each age) groups. Some animals were eliminated from the study during the heart perfusion protocol (see Isolated heart perfusions), yielding a final n of 8–9 rats/group. All animals were initially familiarized with a motor-driven treadmill three times during 1 wk by exercising at very low intensity (10 m/min, 0% grade) for 5 min. The exercise protocol consisted of running 60 min up a 6° grade at 14 m/min for the 12- and 21-mo-old animals and 20 m/min for the 4-mo-old animals. These speeds correspond to 70–75% of maximum oxygen consumption (9) and provide similar total calculated work (body weight × distance run) by all animals. Body core temperature during exercise was measured by inserting a probe 5 cm into the rectum during brief rest

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periods at 20-min intervals. An additional brief rest was granted to 21-mo-old animals as required. This investigation was approved by the University's Animal Care and Use Committee and conforms with the National Institutes of Health Guide for the Care and Use of Laboratory Animals (NIH Pub. No. 85-23, Revised 1985).

Isolated heart perfusions. Myocardial function was evaluated 24 h postexercise using an isolated, working heart as previously described (7, 34). Animals were anesthetized with 40 mg/kg pentobarbital sodium administered intraperitoneally. The perfusion buffer was gassed with 95% O2-5% CO2 and contained (in mmol/l) 118.5 NaCl, 4.7 KCl, 24.7 NaHCO3, 1.0 MgSO4, 10 glucose, 1.75 CaCl2, and 0.5 EDTA, with 12 IU/l insulin. Hearts were maintained at 37°C at all times. Atrial filling pressure was maintained at 10 mmHg, and afterload was set by raising the aortic column 80 cm above the heart. Hearts were electrically paced at 295 beats/min during preischemia and the final 5 min of postischemia recovery. Cardiac external work in the working mode is defined as cardiac output (CO) × peak systolic pressure (SP).

Hearts were initially perfused under normoxic conditions for 25–30 min. Hemodynamic measurements made at 5-min intervals beginning at 10 min to establish a stable preischemic baseline. Global ischemia was then induced by cross-clamping the atrial inflow and aortic outflow lines for 22.5 min, which produced the desired posts ischemic dysfunction (50% of preischemic external work) in young animals. After ischemia, hearts were reperfused in the retrograde (Langendorff) mode for 15 min at a perfusion pressure of 62 mmHg and then returned to the working mode. Function after 30 min of reperfusion was compared with preischemic function to determine the amount of I/R dysfunction. Any heart displaying persistent ventricular fibrillations throughout the first 25 min of reperfusion was eliminated from the study. This occurred in seven hearts, two hearts each at 4 and 21 mo and three hearts at 12 mo. An additional 4-mo-old heart was eliminated because stable hemodynamic performance was not achieved during the preischemia perfusion. At the end of the perfusion period, hearts were freeze clamped with aluminum clamps precooled in liquid nitrogen and stored at ~80°C until being analyzed for antioxidant enzymes and stress proteins. The custom-built clamps were designed to produce an ~1-mm-thick frozen wafer between two aluminum disks that measured 80 mm in diameter and 30 mm in thickness.

Tissue preparation and assay methods. Inducible HSP70 was identified by Western blotting and quantified by densitometry as described previously (34). For enzyme assays, left ventricular tissue was homogenized in 20 volumes of 50 mM KH2PO4, 0.1 mM EDTA, and 0.1% (vol/vol) Triton X-100 (pH 7.4) and centrifuged at 1,500 g for 10 min. The supernatant was analyzed for catalase activity according to del Rio et al. (10) and for SOD [total, mitochondrial (MnSOD), and cytosolic (CuZnSOD)] according to McCord and Fridovich (26). All measurements made at 5-min intervals were analyzed for catalase activity according to del Rio et al. (10) and for SOD [total, mitochondrial (MnSOD), and cytosolic (CuZnSOD)] according to McCord and Fridovich (26). All measurements made at 5-min intervals were analyzed for catalase activity according to del Rio et al. (10) and for SOD [total, mitochondrial (MnSOD), and cytosolic (CuZnSOD)] according to McCord and Fridovich (26). All measurements made at 5-min intervals were analyzed for catalase activity according to del Rio et al. (10) and for SOD [total, mitochondrial (MnSOD), and cytosolic (CuZnSOD)] according to McCord and Fridovich (26).

Statistical analyses. All data are expressed as means ± SE. Differences among groups were analyzed using a one-way ANOVA with post hoc analysis utilizing a Fisher protected least-significant-difference test.

RESULTS

Animal characteristics. Animal body weights, heart weights, and heart weight-to-body weight ratios are summarized in Table 1. Body and heart weights of the 4-mo-old animals were considerably smaller than in either older group (P < 0.05), whereas no differences were observed between the 12- and 21-mo-old animals. Heart weight-to-body weight ratios were similar in all age groups (P > 0.05). Exercise elevated core temperature 2.5–3.0°C in all groups (Table 1), and most of the increase was attained by 20 min (data not shown). Exercise core temperature of the old animals did not differ from either younger group, indicating that they were exercising at similar relative intensities. Although statistical analysis indicated that the middle-aged rats had a lower exercising core temperature than the young rats, the mean values differed by only 1.5%.

Cardiac function. Preischemic CO and external work (CO × SP) normalized to heart weight progressively declined with age (Table 2). The decline in function from 4 to 12 mo was proportional to the increase in heart weight, i.e., cardiac function per heart was similar at 4 and 12 mo (data not shown). By the age of 21 mo, function was decreased regardless of how it was expressed (P < 0.05 vs. younger ages). Preischemic cardiac function of the runners was similar to the age-matched sedentary groups in the 4- and 21-mo-old animals; however, in the 12-mo-old runners, decreases of 10–15% (P < 0.05) were observed for coronary flow, CO, and external work.

Recovery of all hemodynamic parameters after 22.5 min of ischemia is displayed in Table 2, and the percentage of preischemic CO × SP recovered is illustrated in Fig. 1. In sedentary animals, recovery of aortic flow, CO, and external work after 30 min of reperfusion relative to preischemic values in the same hearts were similar among all ages (P > 0.05). However, there was a trend for an age-related decrease in percent recovery of aortic flow, a sensitive measure of pump function, as mean values decreased 6–7% at each increase in age. After exercise, recovery of hemodynamics after global ischemia was considerably improved at all ages. As a result, the posts ischemic runners had higher absolute values for most hemodynamic parameters compared with age-matched sedentary groups, which was not the case before ischemia (Table 2).

Tissue analysis. As illustrated in Fig. 2, HSP70 content was similar in all sedentary animals (P > 0.05) and increased 24 h postexercise in all ages (P < 0.05).
Animals and, by 21 mo, was 40% above the 4-mo value progressively increased with age in the sedentary animals. Exercise resulted in a 45% increase in MnSOD at 21 mo (P<0.05) to the age-matched sedentary group. No age-related change occurred at any age for CuZnSOD. The 21-mo-old runners had higher catalase and MnSOD activities than any other group (P<0.05).

### DISCUSSION

The present study clearly indicates an improvement in myocardial tolerance to I/R 24 h after appropriate exercise regardless of age. Furthermore, because cardiac function was evaluated using an isolated perfused...
working heart with equal preload, afterload, and heart rate, the enhanced cardioprotection after exercise preconditioning was due to adaptations within the ventricle. Although several reports indicate that aging attenuates or abolishes the improved tolerance to I/R after ischemic preconditioning (1, 2, 12, 20, 24, 29), those studies focused on the “early” stage of preconditioning, which does not require protein synthesis and lasts only 2–3 h after the preconditioning stimulus (5, 19). In this regard, Abete et al. (1) reported that a 6-wk swimming program restored the effectiveness of ischemic preconditioning in aged Wistar rats.

Age-related declines in baseline (preischemia) cardiac performance in the sedentary rats in the present study are consistent with changes observed by others (3, 8, 17, 33). When isolated hearts were forced to work under conditions where performance is entirely dependent on intrinsic factors, significant declines were observed from adulthood to old age in several hemodynamic variables (Table 2). However, aging did not attenuate the amount of recovery of cardiac function after a bout of I/R in the sedentary animals (Fig. 1 and Table 2). This lack of an age-related decline in ischemic tolerance is at odds with many studies (1, 14, 22, 25) but is in agreement with several others (12, 20, 21, 24, 29, 35). In addition, two studies (6, 32) have reported that aging from immaturity to mature adulthood results in greater I/R injury, but then tolerance improves from adulthood to senescence. Interestingly, the primary focus of many of the studies mentioned above was on the potential benefits of early phase preconditioning (12, 20, 24, 29, 35). Although the consensus that aging necessarily results in greater I/R injury may be changing, research still needs to be focused on preconditioning of the aged heart because most cardiac events occur in mature adulthood and beyond.

The nature of the preconditioning adaptation that we observed 24 h after the exercise stimulus appears to differ with age. The youngest group, which is approximately the same age as rats used in all prior exercise studies, responded in a manner consistent with these prior studies. In the youngest group, there was an exercise-induced increase in HSP70 (105%) and catalase (26%) accompanying the improved cardioprotection. It could be concluded that HSP70 is the primary mediator of protection in this age group because the magnitude of the increase was similar to that reported by other studies supporting this chaperone as the primary mechanism of exercise-induced cardioprotection (23, 27), and there is considerable data indicating that an elevation of HSP70 is cardioprotective against I/R (for reviews, see Refs. 18, 30, and 31). However, there is also evidence that its increased expression is not required for improved myocardial tolerance to I/R in young rats after one to three bouts of exercise (13, 34) and that increased expression of HSP70 after heat shock does not necessarily result in cardioprotection (16, 28, 37). Whereas HSP70 could have played a major role in exercise-induced cardioprotection in the youngest group, this is unlikely to be the case in the older groups because the expression of the chaperone was only modestly elevated after exercise. The finding that HSP70 expression is attenuated in the oldest group is consistent with several earlier studies reporting impaired myocardial HSP70 synthesis in old rats after heat stress (for a review, see Ref. 30). It appears that the oldest animals in the present study are relying less on HSP70 and more on MnSOD and catalase to achieve exercise-induced cardioprotection. The magnitude of the increase in MnSOD by the old animals was similar to that reported by Yamashita et al. (38), who proposed that this antioxidant enzyme is responsible for the exercise-induced decrease in infarct size after I/R. Also, the oldest runners had higher catalase and MnSOD activities than any other group (Table 3).

Although exercise did not result in an obvious cardioprotective phenotype in the middle-aged group, postischemic recovery was improved dramatically over the age-matched sedentary group. Therefore, it is likely that a mediator other than HSP70 or antioxidant enzymes had a role in the exercise-induced improvement noted at middle age. We focused exclusively on HSP70 and antioxidant enzymes because the vast majority of reports on exercise-induced cardioprotection point to these proteins as having major roles in the protective adaptation (11, 13, 15, 23, 27, 31, 34, 38). However, all of these studies were carried out on young rats, and the list of proteins reported to be mediators of exercise-induced cardioprotection is growing. For example, Babai et al. (4) recently reported that inducible nitric oxide synthase was increased threefold 24 h after a single exercise bout in dogs and that exercise-induced cardioprotection against I/R was blocked when the enzyme was inhibited. The search for the key biochemical mediators of exercise-induced cardioprotection in middle-aged and old animals has important therapeutic clinical implications because most of the cardiac events occur in late adulthood. In summary, the most important finding of the present study is that exercise can improve myocardial tolerance to I/R regardless of age. We also provided evidence that the intrinsic cardioprotective benefits in the older animals cannot be fully explained by changes in HSP70 or antioxidant enzymes, currently the two leading candidates for mediators of exercise-induced cardioprotection.

Table 3. Antioxidant enzyme activity

<table>
<thead>
<tr>
<th>Group</th>
<th>Catalase</th>
<th>Total SOD</th>
<th>MnSOD</th>
<th>CuZnSOD</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 mo</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sedentary</td>
<td>7.5 ± 0.4</td>
<td>15.5 ± 0.4</td>
<td>11.1 ± 0.8</td>
<td>4.5 ± 0.9</td>
</tr>
<tr>
<td>Runner</td>
<td>9.5 ± 0.6</td>
<td>16.4 ± 1.3</td>
<td>11.3 ± 0.7</td>
<td>5.1 ± 1.1</td>
</tr>
<tr>
<td>12 mo</td>
<td></td>
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<tr>
<td>Sedentary</td>
<td>8.5 ± 0.4</td>
<td>18.1 ± 0.5</td>
<td>11.7 ± 1.4</td>
<td>6.3 ± 1.1</td>
</tr>
<tr>
<td>Runner</td>
<td>9.5 ± 0.4</td>
<td>18.6 ± 0.8</td>
<td>11.6 ± 0.8</td>
<td>7.0 ± 1.1</td>
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<tr>
<td>21 mo</td>
<td></td>
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</tr>
<tr>
<td>Sedentary</td>
<td>10.5 ± 0.4</td>
<td>15.9 ± 1.1</td>
<td>9.9 ± 0.9</td>
<td>6.0 ± 0.9</td>
</tr>
<tr>
<td>Runner</td>
<td>12.5 ± 0.4</td>
<td>19.5 ± 1.0</td>
<td>14.3 ± 0.6</td>
<td>5.2 ± 1.1</td>
</tr>
</tbody>
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Values are means ± SE (in U/mg protein); n = 6–9 rats. SOD, superoxide dismutase. Catalase unit = 1 μmol H2O2/min; SOD unit = 50% inhibition of cytochrome c reduction. *P < 0.05 vs. sedentary at same age; †P < 0.05 vs. sedentary at older ages; ‡ P < 0.05 vs. all groups.

Antioxidant enzyme activity

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<td>11.3 ± 0.7</td>
<td>5.1 ± 1.1</td>
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<tr>
<td>12 mo</td>
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<tr>
<td>Sedentary</td>
<td>8.5 ± 0.4</td>
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<td>21 mo</td>
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<tr>
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<td>10.5 ± 0.4</td>
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Antioxidant enzyme activity

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REFERENCES


