Effects of exercise training on cardiac function, gene expression, and apoptosis in rats

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Jin, Hongkui, Renhui Yang, Wei Li, Hsienwie Lu, Anne M. Ryan, Annie K. Ogasawara, John Van Peborgh, and Nicholas F. Paoni. Effects of exercise training on cardiac function, gene expression, and apoptosis in rats. Am J Physiol Heart Circ Physiol 279: H2994–H3002, 2000.—This study determined the effects of exercise training on cardiac function, gene expression, and apoptosis. Rats exposed to a regimen of treadmill exercise for 13 wk had a significant increase in cardiac index and stroke volume index and a concomitant decrease in systemic vascular resistance compared with both age-matched and body weight-matched sedentary controls in the conscious state at rest. In exercise-trained animals, there was no change in the expression of several marker genes known to be associated with pathological cardiac adaptation, including atrial natriuretic factor, β-myosin heavy chain, α-skeletal and smooth muscle actins, and collagens I and III. Exercise training, however, produced a significant induction of α-myosin heavy chain, which was not observed in rats with myocardial infarction. No histological features of cardiac apoptosis were observed in the treadmill-trained rats. In contrast, apoptotic myocytes were detected in animals with myocardial infarction. In summary, exercise training improves cardiac function without evidence of cardiac apoptosis and produces a pattern of cardiac gene expression distinct from pathological cardiac adaptation.

The laboratory rat has been used by many investigators to study the adaptation of cardiac function to chronic exercise (3, 4, 6, 8, 14, 15, 17, 21, 24, 25, 29–31, 33, 41, 45, 47, 52, 57, 61, 62), and much useful information has emerged from these studies. The purpose of this investigation was to extend previous findings in several important ways. First, exercise training in this model system can have a significant impact on rodent body weight (BW), and there is a direct relationship between BW and hemodynamic parameters, including blood volume, cardiac output, stroke volume, and peripheral vascular resistance in rats (10). There are no observations of cardiac function, however, where exercise-trained rats were compared with both BW-matched and age-matched sedentary controls. Furthermore, systematic studies on the effects of exercise on hemodynamics and cardiac function assessed in conscious rats are limited. In this study, the effects of treadmill training (for 13 wk) on cardiac function and hemodynamics were assessed by comparison of two sets of control animals: sedentary rats of the same age and others of the same BW as the exercised cohort. Hemodynamic and cardiac function measurements were made while the animals were conscious and unrestrained.

Second, to evaluate the molecular effects of exercise on the heart, real-time RT-PCR was used to study the relative expression of several cardiac muscle and extracellular matrix genes in the left ventricle (LV) of the exercised rats compared with sedentary controls. These results were compared and contrasted to changes in gene expression induced by adaptation to the pathological stimulus of myocardial infarction.

Finally, exercise has been reported to produce apoptosis in the thymocytes of rats (12) and in the skeletal muscle of mice (40). It is also known that cardiac adaptation to myocardial infarction and chronic pressure overload is accompanied by programmed cell death (27, 50). The effect of exercise training on cardiac apoptosis, however, has not been investigated. The hearts of exercised-trained rats were examined for evidence of apoptotic cell death at 4 days, 10 days, and 13 wk after exercise training was initiated, and the results were compared with what was observed at similar time points after myocardial infarction.

MATERIALS AND METHODS

All experimental procedures conformed to the guiding principles of the American Physiology Society and were approved by the Institutional Animal Care and Use Committee of Genentech. The animals used in this study were male Sprague-Dawley rats (6–8 wk of age, Charles River Breeding Laboratories). The animals were acclimated to the facility for at least 1 wk before the initiation of the study, fed a pelleted rat chow and water ad libitum, and housed in a light- and temperature-controlled room.

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Exercise Training

Rats of approximately the same age were randomly divided into two groups: the exercise group (n = 31) and the age-matched sedentary controls (n = 19). These groups were age-matched in the sense that the average ages of the two groups were almost identical. The rats in the exercise group trained on a rodent treadmill (model CT-2, Columbus Instruments International) according to the training protocol described previously (32, 39). An electric grid at the rear of the belt was used as the running stimulus. The animals trained 5 days/wk for 13 wk, with speed, grade, and duration progressively increased. The rats began training at 10 min/min and 5% grade for 15 min/day. The speed and grade were gradually increased such that by the end of the second week, the animals ran at 15 min/min, 15% grade, for 60 min/day. Thereafter, the grade and duration were maintained but speed was increased 2–3 m/min each wk. By 10 wk, the rats ran at 36 m/min and 15% grade for 60 min/day, and this exercise program was maintained until the end of the study. Because the exercise training significantly decreased the poststudy BW, the age-matched sedentary controls could not serve as BW controls. Thus a younger group of sedentary rats (n = 12) was established to serve as BW controls. With the use of the knowledge of the BW-versus-age relationship for both sedentary and exercise-trained rats, we determined that rats ~2.5 wk younger than the exercise group should emerge from the study with average BW roughly the same as that of the exercise group. Note that the average initial BW will necessarily be smaller in this BW-matched group than in the older, exercise group.

Assessments of Cardiac Growth and Cardiac Function

Catheterization. At the end of 13 wk of exercise training, rats in the three experimental groups were anesthetized with ketamine hydrochloride (100 mg/kg ip) and xylazine (10 mg/kg ip). A catheter [polyethylene (PE)-10 fused with PE-50] filled with heparin-saline solution (50 U/ml) was implanted into the abdominal aorta to allow comparison with cardiac output by the thermodilution method (10, 13, 26, 63). The catheter was used to measure arterial pressure and heart rate. A second catheter (PE-50) was implanted into the right atrium, through the right jugular vein, for measurement of left atrial pressure and for saline injection. A thermistor catheter (Lyons Medical Instrument, Sylmar, CA) was inserted into the aortic arch from the right femoral artery for measurement of cardiac output by the thermodilution method (10, 13, 26, 63). The catheters were exteriorized at the back of the neck with the aid of a stainless steel wire. After the catheters were implanted, all rats were housed individually.

Hemodynamic measurements. Mean arterial pressure and heart rate were measured in conscious, unrestrained rats 1 day after catheterization by connecting the catheters to a pressure transducer (model P23 XL, Viggo-Spectramed, Oxnard, CA) coupled to a polygraph (model 7, Grass Instruments, West Warwick, RI). For measurement of cardiac output, the thermistor catheter was connected to a microcomputer system (Lyons Medical Instrument) (26, 63). Isotonic saline (0.1 ml) at room temperature was injected as a bolus via the jugular vein catheter. The thermodilution curve was monitored by VR-16 Simultrace recorders (Honeywell, NY), and cardiac output was digitally obtained by the microcomputer. Cardiac indexes were calculated as follows: stroke volume = cardiac output/heart rate; cardiac index = cardiac output/BW; stroke volume index = stroke volume/BW; and systemic vascular resistance = mean arterial pressure/cardiac index. Hemodynamic measurements were performed in 11 exercise-trained, 10 age-matched, and 6 BW-matched rats, and cardiac output was not successfully measured in 2 rats (1 in the exercise group and 1 in the age-matched group) because the thermodilution curve was not reliable.

At the conclusion of the experiments, the rats were anesthetized with pentobarbital sodium (60 mg/kg). The hearts were removed, dissected, and weighed in 14 exercise-trained, 14 age-matched, and 12 BW-matched rats.

Echocardiography

Echocardiograms were performed in eight exercise-trained rats and eight age-matched controls before catheterization. The rats were anesthetized with ketamine and xylazine as described above and examined in the lateral decubitus position. An annular array echocardiographic system (Apogee CX, ATR Interspec, Bothell, WA) with a 7.5-MHz transducer was used for two-dimensional and M-mode imaging. With the use of the two-dimensional parasternal short-axis imaging plane as a guide to the level of the papillary muscles, a M-mode tracing of the LV was obtained. The LV anterior and posterior wall thickness at end diastole, LV end-diastolic internal diameter, and LV end-systolic internal diameter were measured according to standard procedures. The LV mass was calculated with the standard cube formula as follows: LVM = 1.04[(AWT + PWT + EDD)² – EDD³], where LVM is LV mass, AWT and PWT are anterior and posterior wall thickness, respectively, and EDD is LV end-diastolic internal diameter. Relative wall thickness was calculated as the ratio of 2PWT to 1EDD.

Studies on Cardiac Gene Expression

Animal model and sample preparation. The hearts from the exercise-trained rats (n = 5) and age-matched controls (n = 5) were removed and dissected, and the LV were fast-frozen in liquid nitrogen and stored at −70°C for subsequent RNA analysis. Cardiac gene expression analysis was also performed in four rats 13 wk after myocardial infarction induced by ligation of the left coronary artery and four sham-operated control rats to allow comparison with cardiac adaptation to a pathological load. The procedure used for left coronary ligation has been described in detail elsewhere (18, 26, 38, 63). In brief, the rats were anesthetized with ketamine hydrochloride and xylazine as described above, intubated via tracheotomy, and ventilated by a respirator (model 683, Harvard Apparatus). After a left-sided thoracotomy, we ligated the left coronary artery ~2 mm from its origin with a 7–0 silk suture. Electrocardiograms were obtained under light metofane anesthesia 1 wk after surgery to document the development of infarcts (26, 63). The rats without evident pathological Q waves across the precardial leads were excluded. Our previous studies (26, 63) have shown that rats selected by electrocardiogram have myocardial infarcts averaging 32–35% of the LV, which led to ventricular hypertrophy and cardiac dysfunction 6–14 wk after ligation.

Cardiac RNA analysis. Total RNA was isolated from the ventricular samples using the RNeasy Maxi Kit (Qiagen) according to the manufacturer’s instructions. Gene expression analysis was performed using real-time RT-PCR (TaqMan) technology. RT-PCR was performed on 1 ng of total RNA per reaction using the TaqMan sequence detector (model 7700, ABI-Perkin Elmer) (19). Amplification reaction conditions (for 50 μl) were 1× TaqMan buffer A, 300 μM dATP, 300 μM dCTP, 300 μM dGTP, 600 μM dUTP, 10% glycerol, 5.5 mM MgCl₂, 50 U murine leukemia virus reverse transcriptase, 0.33 μM of each primer (50 μM each), 0.2 μM of each reporter TaqMan probe (MGB), and 0.025 U Platinum Taq polymerase (Platinum Taq DNA Polymerase, Life Technologies, Inc.). The PCR was performed in a reaction volume of 50 μl containing 5 μl of total RNA. Following reverse transcription and the first denaturation step at 95°C, cDNA was amplified for 40 cycles comprised of 95°C for 15 s, and 60°C for 1 min. The resulting cDNA was analyzed using the ABI PRISM 7700 Sequence Detector (Applied Biosystems). The gene expression analysis was performed on the following cardiac genes: (1) α-myosin heavy chain (αMHC), (2) β-myosin heavy chain (βMHC), (3) cardiac troponin T (cTnT), (4) cardiac troponin I (cTnI), (5) β-actin (ACTB), and (6) glyceraldehyde 3-phosphate dehydrogenase (GAPDH). The expression of each gene was normalized to the expression of GAPDH. A Student’s t-test was used to determine the statistical significance of the differences in gene expression between the groups.
transcriptase, 20 U RNase Inhibitor, 1.25 U AmpliTaq Gold, 100 nM forward and reverse primers, and 100 nM fluorescent probe. RT-PCR reagents and glycerol were purchased from Perkin Elmer and Sigma, respectively. Reactions were performed in MicroAmp optical tubes and caps (ABI-Perkin Elmer). TaqMan primers and probes were designed according to guidelines determined by Perkin Elmer and synthesized at Genentech except for those for rodent GAPDH, which were a generous gift from Perkin Elmer. Reverse transcription was performed at 48°C for 30 min followed by heat activation of AmpliTaq Gold at 95°C for 10 min. Thermal cycling was at 95°C for 30 s and 60°C for 1.5 min for 40 cycles.

Quantitation of the TaqMan results was performed as described by Heid et al. (23) with modifications. Briefly, standard curves (1.5 serial dilution) for each target gene of interest were run in duplicate. The threshold cycle (CT) was plotted on the y-axis versus the log of the total RNA concentration (x-axis), and the equation describing the line was determined. Experimental samples were analyzed using 3–5 replicates each, and the quantity of the mRNA for each target gene was determined from the appropriate standard curve by entering the CT (y value) and solving for the input mRNA (x value). The value for the target gene was then normalized to GAPDH by solving the following equation: $10^{y_1/y_2}$, where $x_1$ is the target gene and $x_2$ is GAPDH.

Studies on Cardiac Myocyte Apoptosis

Programmed cell death in the heart has been demonstrated during the first 1–2 wk, with a peak at several days, after the onset of pressure overload or myocardial infarction in rats (27, 50). Cardiac apoptosis was evaluated by examination of morphological features under light microscopy and by terminal deoxynucleotidyl transferase-mediated dUTP-biotin nick end-labeling reaction (TUNEL) labeling of the 3’ OH ends of DNA in myocardial tissue sections after 4 days, 10 days, or 13 wk of exercise training (n = 4 for each time point) or after myocardial infarction induced by coronary ligation (n = 5 for each time point) as described above. The hearts were removed from the rats under anesthesia, fixed in 10% neutral-buffered formalin, processed routinely, embedded in paraffin, and sectioned at 5 µm. Replicate sections were stained with hematoxylin and eosin for light microscopic analysis; apoptotic cells were identified by positive staining with the digoxigenin-dUTP terminal deoxytransferase method (Apotag kit, Oncor, Gaithersburg, MD). Twelve sections were evaluated on each heart. Formalin-fixed thymus from 4-wk-old C57BL/6 mice treated with 50 µg of cortisone acetate for 12 h (to induce thymic involution) and embryonic day 14 (E14) mouse embryos were used as positive controls for apoptotic staining. With this method, apoptotic cells were identified in the thymic cortex and in the embryonic heart of the control tissues.

Statistical Analysis

Results are expressed as means ± SE. One-way analysis of variance (ANOVA) was performed to assess differences in parameters between groups. Significant differences were then subjected to post hoc analysis using the Newman-Keuls method. For analysis of gene expression, parameters between the exercise or infarct group and the respective control group were compared by an unpaired Student’s t-test. P < 0.05 was considered significant.

RESULTS

Effects of Exercise on BW and Cardiac Growth

Because chronic exercise generally induces a significant reduction in BW, we compared the exercised animals to not only age-matched but also BW-matched sedentary controls. After 13 wk of treadmill training, the BW of the exercised group was ~17% lower than the age-matched sedentary controls (P < 0.01) and the same as the BW-matched group, which contained animals that were ~2.5 wk younger (Table 1). The ratios of heart and ventricular weights to BW were the same in the two sedentary groups despite the difference in BW and age, indicating that the heart and body grew proportionally in these animals. The BW-normalized heart and ventricular weights of the exercised group were significantly greater than the two sedentary control groups, however (Table 1).

LV Geometry Measured by Echocardiography

There was a close correlation between echocardiogram-derived LV mass and actual LV wet weight in a combined group of exercise-trained rats and age-matched controls (r = 0.84, P < 0.0001, n = 16) indicating the accuracy of echocardiographic measurements.

Table 1. Effects of exercise training on BW, HW, MAP, and HR

<table>
<thead>
<tr>
<th></th>
<th>Exercise</th>
<th>Age Matched</th>
<th>BW Matched</th>
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<tbody>
<tr>
<td>BW, g</td>
<td>307.8 ± 4.6(14)</td>
<td>306.2 ± 5.8(14)</td>
<td>252.7 ± 4.7†(12)</td>
</tr>
<tr>
<td>BW, g/6</td>
<td>508.8 ± 11.1(14)</td>
<td>612.6 ± 15.1(14)</td>
<td>502.3 ± 7.6(12)</td>
</tr>
<tr>
<td>HW, g</td>
<td>1.351 ± 0.041(14)</td>
<td>1.374 ± 0.043(14)</td>
<td>1.082 ± 0.086†(12)</td>
</tr>
<tr>
<td>HW, g/6</td>
<td>1.281 ± 0.040(14)</td>
<td>1.277 ± 0.039(14)</td>
<td>1.015 ± 0.086†(12)</td>
</tr>
<tr>
<td>LVW, g</td>
<td>0.019 ± 0.034(11)</td>
<td>0.001 ± 0.037(11)</td>
<td>0.080 ± 0.013†(12)</td>
</tr>
<tr>
<td>LVW, g/6</td>
<td>0.241 ± 0.021(12)</td>
<td>0.244 ± 0.010(11)</td>
<td>0.213 ± 0.008†(12)</td>
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<tr>
<td>HW/BW, g/6</td>
<td>2.668 ± 0.096†(14)</td>
<td>2.247 ± 0.056(14)</td>
<td>2.151 ± 0.167(12)</td>
</tr>
<tr>
<td>HW/BW, g/kg</td>
<td>2.531 ± 0.095†(14)</td>
<td>2.107 ± 0.055(14)</td>
<td>2.017 ± 0.166(12)</td>
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<td>LVW/BW, g/kg</td>
<td>2.037 ± 0.079†(11)</td>
<td>1.636 ± 0.050(11)</td>
<td>1.601 ± 0.021(12)</td>
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<td>RVW/BW, g/kg</td>
<td>0.482 ± 0.024†(11)</td>
<td>0.400 ± 0.014(11)</td>
<td>0.408 ± 0.014(12)</td>
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<td>AWT, mm</td>
<td>1.69 ± 0.07(8)</td>
<td>1.68 ± 0.08(8)</td>
<td>1.67 ± 0.07(8)</td>
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<tr>
<td>ESD, mm</td>
<td>3.84 ± 0.20(8)</td>
<td>4.40 ± 0.22(8)</td>
<td>4.45 ± 0.27(8)</td>
</tr>
<tr>
<td>EDD, mm</td>
<td>7.85 ± 0.18(8)</td>
<td>8.45 ± 0.16(8)</td>
<td>8.75 ± 0.16(8)</td>
</tr>
<tr>
<td>RWT</td>
<td>0.445 ± 0.016‡(8)</td>
<td>0.396 ± 0.016(8)</td>
<td>0.427 ± 0.016(8)</td>
</tr>
<tr>
<td>MAP, mmHg</td>
<td>112.6 ± 1.9(11)</td>
<td>109.9 ± 3.8(10)</td>
<td>117.1 ± 4.0(6)</td>
</tr>
<tr>
<td>HR, beats/min</td>
<td>360.0 ± 7.1(11)</td>
<td>370.1 ± 9.2(10)</td>
<td>368.3 ± 12.5(6)</td>
</tr>
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</table>

Data are expressed as means ± SE; the number in parentheses represents the number of rats. BW, body weight before the initiation of exercise training; BW, body weight 13 weeks after exercise training; HW, heart weight; VW, ventricular weight; LVW, left ventricular (LV) weight; RVW, right ventricular weight; AWT, anterior wall thickness; PWT, posterior wall thickness; ESD, LV end-systolic internal diameter; EDD, LV end-diastolic internal diameter; RWT, relative wall thickness; MAP, mean arterial pressure; HR, heart rate. *P < 0.05 and †P < 0.01 compared to other two groups. ‡P < 0.05 compared with the age-matched group.
No significant difference in LV anterior and posterior wall thickness was observed between the exercise group and age-matched group (Table 1). LV end-systolic and end-diastolic internal diameters tended to be decreased in the exercise-trained animals compared with the age-matched sedentary controls, but the difference was not statistically significant. However, there was a significant increase in relative wall thickness, an index of cardiac geometry, in the exercise group compared with the age-matched sedentary controls (Table 1), indicating that treadmill running was associated with alterations in cardiac morphology.

**Effects of Exercise on Cardiac Function**

Mean arterial pressure and heart rate at rest were similar in the three experimental groups (Table 1). The cardiac index and stroke volume index of the treadmill-trained rats were significantly higher ($P < 0.01$) than those of either sedentary control group (Fig. 1). Exercise also significantly reduced systemic vascular resistance ($P < 0.01$). No differences in cardiac index, stroke volume index, and systemic vascular resistance were found between the two sedentary control groups.

**Effects of Exercise and Myocardial Infarction on Cardiac Gene Expression**

LV expression levels of 11 genes were used to compare the molecular phenotypes of cardiac adaptation to stress induced by exercise training versus myocardial infarction (Table 2). Treadmill training for 13 wk resulted in a significant increase in the expression of only one measured gene, $\alpha$-myosin heavy chain ($P < 0.05$), which was unchanged in animals after myocardial infarction. In contrast, the mRNA abundance of six genes were significantly increased 13 wk after myocardial infarction. mRNA levels of atrial natriuretic factor, $\alpha$-skeletal actin, and $\alpha$-smooth muscle actin were increased by 5.7-, 2.9-, and 2.2-fold, respectively (Fig. 2). The $\beta$-myosin heavy chain isoform was induced, and mRNA levels of the extracellular matrix proteins collagen I and III increased by 2.3- and 2.6-fold, respectively (Fig. 3).

**Effects of Exercise and Myocardial Infarction on Cardiac Myocyte Apoptosis**

Apoptotic myocytes, 3–5 apoptotic cells/high-power field, were detected adjacent to the myocardial infarct 4 days after left coronary artery ligation (Fig. 4). In contrast, no apoptotic cells were detected in the hearts of exercise-trained animals. No histological features of apoptosis (nuclear pyknosis and karyorrhexis) were observed in either hematoxylin and eosin-stained or ApoTag-stained myocardial sections after 4 days, 10 days, or 13 wk of treadmill training.

**DISCUSSION**

There are three major findings in the present study. First, rats subjected to chronic treadmill exercise for 13 wk exhibited a significant increase in cardiac index and stroke volume index at rest in the conscious state compared with both age-matched and BW-matched sedentary controls, indicating that exercise training enhances cardiac function. Second, mRNA levels for atrial natriuretic factor, $\beta$-myosin heavy chain, $\alpha$-skeletal actin, $\alpha$-smooth muscle actin, collagen I, and collagen III in the LV were significantly elevated in rats 13 wk after myocardial infarction but not in the exercise-trained animals. In contrast, there was a significant induction of $\alpha$-myosin heavy chain in the exercise group but not in the infarct group. This suggests a distinct pattern of cardiac gene expression induced by exercise.
the physiological load versus pathological load. Third, myocardial apoptosis was detected in rats 4 days after myocardial infarction but not in the exercise-trained animals at 4 days, 10 days, and 13 wk. This is the first demonstration that myocardial adaptation to exercise training was not associated with cardiac apoptosis.

In the present study, animals receiving exercise training exhibited a significant enhancement in cardiac index and stroke volume index at rest in the conscious state compared with both age-matched and BW-matched sedentary controls. The improvement in cardiac function was associated with a reduction in systemic vascular resistance. The decrease in afterload may contribute to the enhanced cardiac function by reducing the impedance of LV ejection. Recent studies (46, 54) in dogs suggest that exercise training is associated with an increase in nitric oxide formation that may mediate endothelium-dependent peripheral vasodilation. In addition, another mechanism for enhanced cardiac function observed in exercise-trained rats might relate to an increase in myocardial contractility. It has been reported that exercise training increases contractile performance of rat hearts in vitro (5, 20, 42, 43, 51). A study (51) on the effect of exercise training on excitation-contraction coupling in the rat myocardium demonstrated that treadmill exercise enhances myocardial performance by increasing Ca\(^{2+}\) availability to the contractile element. A further study is needed to determine the effect of exercise training on myocardial contractility in conscious rats, because it was not feasible for us to use a high-fidelity Millar catheter with a pressure transducer at the tip to obtain these measurements in the conscious state.

The present study demonstrated a significant increase in stroke volume index at rest in conscious rats by exercise training. This is consistent with the finding that exercise training significantly augments resting stroke volume in healthy humans and pigs (7, 48, 59). However, our finding of increased resting cardiac index in exercise-trained rats is not in agreement with the observation that there is no significant increase in resting cardiac index after exercise training in humans and pigs. This discrepancy may be due mainly to the change in resting heart rate after exercise training. Humans receiving exercise training exhibit bradycardia at rest that offsets the increase in resting stroke volume, leading to an insignificant change in cardiac output or cardiac index at rest (7, 48). In pigs, exercise training tends to reduce resting heart rate, which is associated with a tendency to increase resting cardiac output (59). In the present study, however, resting heart rate did not change after exercise training in conscious rats, which is consistent with previous reports (8, 11, 24, 31, 33–35, 62) by the majority of other investigators who observed little or no change in rest-

<table>
<thead>
<tr>
<th>Genes</th>
<th>Exercise</th>
<th>MI</th>
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<tr>
<td>Natriuretic factors</td>
<td></td>
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<tr>
<td>Atrial natriuretic factor</td>
<td>NS</td>
<td>↑ †</td>
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<tr>
<td>Actins</td>
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<tr>
<td>α-Cardiac actin</td>
<td>NS</td>
<td>NS</td>
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<tr>
<td>α-Skeletal actin</td>
<td>NS</td>
<td>↑ †</td>
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<tr>
<td>α-Smooth muscle actin</td>
<td>NS</td>
<td></td>
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<tr>
<td>Myosins</td>
<td></td>
<td></td>
</tr>
<tr>
<td>α-Myosin heavy chain</td>
<td>↑ NS</td>
<td></td>
</tr>
<tr>
<td>β-Myosin heavy chain</td>
<td>NS</td>
<td>↑ †</td>
</tr>
<tr>
<td>Myosin light chain 2</td>
<td>NS</td>
<td>NS</td>
</tr>
<tr>
<td>Calcium handling</td>
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<td></td>
</tr>
<tr>
<td>Sarco(endo)plasm reticulum Ca(^{2+})-ATPase</td>
<td>NS</td>
<td>NS</td>
</tr>
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<td>Phospholamban</td>
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<tr>
<td>Extracellular matrix</td>
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<tr>
<td>Collagen I</td>
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<td>Collagen III</td>
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\(n = 5\) rats in each exercise and age-matched group, and \(n = 4\) rats in each myocardial infarction (MI) and sham group. Results are as compared with the respective control: NS, no significant change; ↑, significant increase (\(P < 0.05\)); and ↑ † , very significant increase (\(P < 0.01\)).

Table 2. Effects of exercise training vs. MI on cardiac gene expression

![Graph](http://example.com/graph.png)

Fig. 2. Effects of exercise training versus myocardial infarction (MI) on cardiac gene expression of atrial natriuretic factor (ANF), α-skeletal actin (SKA), and α-smooth muscle actin (SMA). Expression data were first normalized by dividing all values in a particular comparison by the average expression in the corresponding control group, thus forcing the control average to be 1. Means ± SE of these normalized data are displayed. *\(P < 0.05\) and **\(P < 0.01\) compared with the respective control; \(n = 5\) rats in each exercise and age-matched group, and \(n = 4\) rats in each MI and sham group.
ing heart in conscious and anesthetized normal rats after exercise training. With unchanged heart rate, the increased stroke volume index would elevate cardiac index in exercise-trained rats.

The incremental load on the heart after myocardial infarction reflects a blend of pressure and volume overloading. The adaptation to this pathological load has been shown to be associated with a unique molecular phenotype of altered myocardial gene expression. The present study showed that LV expression of several genes, including atrial natriuretic factor, β-myosin heavy chain, α-skeletal actin, and α-smooth muscle actin, were increased 13 wk after myocardial infarction. This is consistent with recent studies (22, 36, 64, 65) that demonstrate the increased ventricular expression of these genes coding for the fetal phenotype during ventricular remodeling after myocardial infarction in rats. Less is known, however, about ventricular expression of the fetal genes after chronic physiological loads. It has been reported that atrial natriuretic factor gene expression in the rat ventricle is unchanged after treadmill training (2) and minimally increased after swimming training compared with a profound increase after chronic pathological loads in rats (9). The present study is the first to demonstrate that cardiac adaptation to exercise training was not associated with the LV induction of mRNA encoding the fetal contractile proteins (β-myosin heavy chain, α-skeletal actin, and α-smooth muscle actin) in addition to atrial natriuretic factor.

In the present study, the LV mRNA level of α-myosin heavy chain was significantly increased after exercise training but not after myocardial infarction, whereas there was an induction of LV β-myosin heavy chain gene in the infarct group but not in the exercise group. It is known that the mature adult rat expresses mainly α-myosin heavy chain as the major contractile protein in the LV. Our findings are consistent with the previous observations that a physiological load (exercise training) results in a further increase in the V1 myosin isoenzyme (α-myosin heavy chain) and a pathological load induces a shift in the isoenzyme pattern from the V1 to V3 isoenzyme (β-myosin heavy chain) in rats (37, 44). α-Myosin heavy chain is associated with high ATPase activity and increased contractility, which might contribute, in part, to the enhanced cardiac index and stroke volume index observed in the exercise-trained rats. In contrast, β-myosin heavy chain
has a fivefold lower ATPase activity, conferring decreased velocity of shortening, and its expression in the heart after myocardial infarction may be teleologically attributable to the more efficient utilization of decreased energy reserves (37, 44).

Experimental and clinical studies have demonstrated an increase in interstitial collagens of the LV or nonischemic myocardium at a chronic or late stage after myocardial infarction, which may enhance cardiac stiffness and result in diastolic dysfunction, finally leading to heart failure (16, 22, 53, 55, 56). In contrast to myocardial infarction, we found exercise training did not affect the cardiac mRNA of collagen I and III. Consistent with this finding, Burgess et al. (8) showed that total collagen content in the LV is not altered in exercise-trained rats compared with control rats but is significantly greater in the heart subjected to chronic hypertension. In addition, we show that the LV gene expression of α-smooth muscle actin is substantially increased after myocardial infarction but is unchanged after exercise training. Recent studies (1, 16) suggest that α-smooth muscle actin expression by fibroblasts and myofibroblasts contributes to collagen remodeling and may play a role in mediating wound healing in the heart after myocardial infarction.

The pathological adaptation to pressure overload has also been shown to be associated with an increase in the expression of several marker genes, including atrial natriuretic factor, β-myosin heavy chain, α-skeletal actin, and collagens (28, 49, 58, 60), whereas cardiac expression of these marker genes were not changed in exercise-trained animals in the present study. In addition, cardiac expression of α-myosin heavy chain is decreased in pressure overload (49). In contrast, this gene expression was upregulated after exercise training. Furthermore, mRNA levels of sarcoplasmic reticulum Ca2+-ATPase and phospholamban have been reported to be depressed in rats with pressure overload (28, 49, 58, 60), but exercise training did not alter cardiac expression of these two calcium handling genes. Thus compared with pathological adaptation to pressure overload, physiological adaptation to exercise training is also associated with distinct alterations in cardiac molecular phenotype.

Recent studies have shown that apoptosis may be involved in the pathogenesis of heart remodeling after pathological loads. With the use of an in situ assay, Teiger et al. (50) found a phase of apoptosis during the first 7 days after pressure overload, with a peak at 4 days, whereas cardiac growth continued for over 30 days (50). The apoptosis was mainly observed in cardiomyocytes. Their findings suggest that cardiac adaptation to pressure overload is initiated by a wave of apoptosis of cardiomyocytes. Furthermore, Kajstura et al. (27) demonstrated that programmed cardiomyocyte death is the major form of myocardial damage at 2–6 h after coronary artery ligation in rats. The apoptosis is continuously observed for at least 7 days with gradually decreasing values. Consistent with these findings, we showed apoptosis in cardiomyocytes 4 days after myocardial infarction in a similar experimental model.

In contrast, there was no evidence of myocardial apoptosis 4 days, 10 days, and 13 wk after chronic running exercise. These data suggest that cardiomyocyte apoptosis may play an important role in the early stage of cardiac adaptation to pathological loads but not to physiological loads. Accordingly, myocardial apoptosis appears to be a new index for distinction between cardiac adaptation to physiological loads and pathological loads at the early period.

It is known that the effect of exercise training on heart and body growth varies because of differences in species, age, sex, the mode or regimen of exercise training, the disease model, etc. In rats, for example, treadmill running is often associated with “relative hypertrophy” (an increase in heart-to-BW ratio with unchanged absolute heart weight), whereas swimming training may cause “true cardiac hypertrophy” (an increase in both heart-to-BW ratio and absolute heart weight). The data on rats that have been exercise trained by swimming are confounded, however, by experimental evidence that swimming produces additional stress in the animals that may also contribute to the induction of cardiac hypertrophy independent of the exercise (38). Furthermore, a recent study (66) in rats after myocardial infarction showed that high-intensity sprint training improved cardiac function and increased cardiac expression of α-myosin heavy chain but was associated with reduced myocyte hypertrophy. Perrault and Turcotte (38) reviewed animal and human studies on exercise training over the past three decades and found that using cardiac hypertrophy as an expected adaptation to regular exercise may not be totally warranted. Although we did not find true cardiac hypertrophy in the treadmill-trained rats compared with age-matched sedentary controls, it is clear from our data that myocardial adaptation to treadmill training is characterized by improved function (cardiac index and stroke volume index) and altered cardiac gene expression (induction of α-myosin heavy chain) and geometry (increased relative wall thickness).

In summary, treadmill-trained rats displayed improved cardiac function in association with a profile of cardiac gene expression distinct from pathological cardiac adaptation. The cardiac adaptation to exercise training was not associated with myocyte apoptosis, which also contrasted to cardiac remodeling in the early phase after pathological loads.

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REFERENCES


