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Effects of Exercise and L-arginine on Ventricular Remodeling and Oxidative Stress

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Abstract

Objective—Our aim was to characterize the changes in mRNA abundance, protein, and activity levels of the enzymatic antioxidants, superoxide dismutase (SOD), glutathione peroxidase (GPx), and catalase by exercise training combined with L-arginine after myocardial infarction (MI).

Methods—L-arginine (1 g/kg/day) and N^G-nitro-L-arginine methyl ester (L-NAME, 10 mg/kg/day) were administered in drinking water for 8 weeks. Sprague-Dawley rats were randomized to the following groups: sham-operated control (Sham); MI-sedentary (Sed); MI-exercise (Ex); MI-sedentary+L-arginine (Sed+LA); MI-exercise+L-arginine (Ex+LA); MI-sedentary+L-NAME (Sed+L-NAME); and MI-exercise+L-NAME (Ex+L-NAME).

Results—The GPx, catalase and gp91^{phox} mRNA levels were comparable among all the groups. The SOD mRNA level was significantly increased in the Ex group (5.43±0.87) compared to the Sed group (1.74±0.29), whereas this effect was pronouncedly down-regulated by the L-NAME intervention (2.51±1.17, *P*<0.05). The protein levels of SOD in the Sed and Ex groups were both significantly decreased with the administration of L-NAME. The protein levels of catalase were significantly higher in the Ex and Ex+LA groups than in the Sed, Sed+LA, and L-NAME treated groups. The collagen volume fraction was significantly lowered by the exercise and/or L-arginine treatment when compared to the Sed group. Fractional shortening was significantly preserved in the trained groups compared to their corresponding sedentary groups with or without drug treatments. However, the beneficial effect was not further improved by L-arginine treatment.

Conclusions—Our results suggest that exercise training exerts antioxidative effects, and attenuates myocardial fibrosis in the MI rats. These improvements, in turn, alleviate cardiac stiffness, and preserve post-MI cardiac function. In addition, L-arginine appears to have no additive effect on cardiac function or expression of enzymatic antioxidants.

Keywords

myocardial infarction; free radicals; exercise training; cardiac function

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Conflict of Interest None.

1. Introduction

Living cells have both enzymatic and nonenzymatic defense mechanisms to balance a multitude of oxidative challenges. Superoxide (O_2^-) is one of the main causes of oxidative stress. The three primary antioxidant enzymes are superoxide dismutase (SOD), catalase, and glutathione peroxidase (GPx). SOD promotes the dismutation of O_2^- and forms hydrogen peroxide (H_2O_2) and oxygen, while catalase converts H_2O_2 to water and oxygen. GPx utilizes reduced glutathione (GSH) as a reducing equivalent to reduce H_2O_2 to form oxidized glutathione and water. The nonenzymatic group includes a variety of biologic molecules, such as vitamins E and C. NADPH oxidase, which consists of two subunits, gp91^{phox} and p22^{phox}, is a major source of O_2^- in the heart. Increased NADPH oxidase activity and expression have been found in experimental pressure-overload left ventricular (LV) hypertrophy and myocardial infarction (MI) (10,19).

Nitric oxide (NO), a potent vessel dilator, induces the reduction of vascular resistance and increases blood flow in the heart. It is well-known that exercise increases blood flow and creates shear stress, which, in turn, stimulates NO production. It has been reported that NO protects the heart from myocardial remodeling following MI (28). Augmentation of NO synthesis via administration of L-arginine, a substrate for NO synthase (NOS), markedly attenuates fibrosis formation, whereas inhibition of NO production results in extensive myocardial fibrosis (2, 13). Oxygen free radicals and NO play an important role in the pathogenesis of cardiovascular diseases. The degree of oxidative stress and the severity of subsequent myocardial damage might depend on the imbalance between excess of ROS production and antioxidant defense within the heart (32). Previous studies suggest that oxidative stress plays a major role in the pathogenesis of cardiac remodeling and progression of chronic heart failure (CHF) following MI (11,15,34).

L-arginine treatment enhances exercise-induced endothelial NO synthesis and aerobic capacity in normal mice by augmented NO production (22). Accumulating evidence indicates that exercise training promotes antioxidant capacity and attenuates oxidative stress-mediated tissue damage (7,17,20). In addition, exercise training studies have revealed several positive effects on the improvement of cardiac function (14,25,31); however, it is unknown whether the exercise training-mediated preservation of the cardiac function is associated with restoration of the local enzymatic radical scavenger system.

Therefore, the aim of the present study was to investigate the effect of exercise training on both oxidant and antioxidant systems in rats with MI. Furthermore, we investigated whether post-MI exercise training combined with L-arginine reconstitutes the activity of SOD, catalase, and GPx, thereby reducing oxidative stress in the non-infarcted myocardium in rats.

2. Materials and Methods

2.1. Animals and myocardial infarction

Animals used in these experiments were treated in accordance with both the American College of Sports Medicine (ACSM) animal care standards and the National Institutes of Health *Guide for the Care and Use of Laboratory Animals*. The study protocols were approved by the Institutional Animal Care and Use Committee at University of Texas at San Antonio. Seven-week-old male Sprague-Dawley rats (Harlan Industries, Indianapolis, IN) were housed at constant temperature ($22\pm 2^\circ\text{C}$) on a 12-h light/dark cycle. MI was created by ligation of the left anterior descending coronary artery (LAD) as described elsewhere (42).

2.2. Experimental groups

One week after surgery, echocardiography was performed on the surviving rats. Rats were then matched by cardiac function (Fractional Shortening, FS) and randomly assigned to the following experimental groups: sham-operated control (Sham); MI-sedentary (Sed); MI-exercise (Ex); MI-sedentary+L-arginine (Sed+LA); MI-exercise+L-arginine (Ex+LA); MI-sedentary+ N^{G} -nitro-L-arginine methyl ester (Sed+L-NAME); and MI-exercise+L-NAME (Ex+L-NAME).

2.3. Drug treatment and exercise training

L-arginine (1g/kg body weight /day) and L-NAME (10 mg/kg body weight/day) treatments were initiated 1 wk post-MI (Figure 1). The drugs were administered in drinking water for 8 weeks. The doses of L-arginine and L-NAME were based on previous studies (8,36), which reported significant drug effectiveness in rats, respectively. Rats assigned to the exercise groups started exercising at 1 week post-MI on a motorized rodent treadmill, while the sham and sedentary groups remained sedentary throughout the experiment period. To allow gradual adaptation to exercise stress, exercise training was initiated at 10 m/min, 5° incline for 10 min per session. The speed and duration were gradually increased to 16 m/min and 40 min per session (including a 5 min warm-up at 10 m/min) and maintained constant throughout the experiment. This exercise intensity was about 55% VO_2max (16). Exercise training was performed 5 days per week for 8 weeks. The determination of treadmill speed and exercise duration was based on the previous studies (5,38,42). This exercise regimen was well tolerated by rats with MI. There were no mortalities during the 8 weeks of exercise training.

2.4. Echocardiographic measurements

Echocardiographic measurements were performed the day before the initiation of post-MI exercise training and after 8 weeks of exercise training using an echocardiographic system equipped with a 10-MHz transducer (SonoHeart Elite, SonoSite Bothell, WA). Rats were anesthetized with 2% isoflurane mixed with oxygen, and a two-dimensional short-axis view of the LV was obtained at the level of the papillary muscle to record M-mode tracings. We measured LV anterior wall thickness; LV end-diastolic dimension (LVEDd) and end-systolic LV dimension (LVESd); LV endocardial fractional shortening (FS) was calculated as $(\text{LVEDd} - \text{LVESd}) / \text{LVEDd}$ and was expressed as a percentage (29). All measurements were averaged over three consecutive cardiac cycles.

2.5. In vivo hemodynamic measurements

Following eight weeks of exercise training, all rats were anesthetized as described in the previous section, and the right carotid artery was exposed. A pressure transducer (Model SPR-838, Millar Instruments) was inserted retrograde from the carotid artery to LV cavity. Hemodynamic parameters measured were LV systolic pressure (LVSP), LV end-diastolic pressure (LVEDP), aortic systolic pressure, aortic diastolic pressure, and peak velocities of contraction and relaxation (dP/dt_{max}). After data collection, hearts were harvested, frozen in isopentane with dry ice, and stored at -80°C for later use.

2.6. Determination of infarct size and collagen content

The left ventricles were cut from apex to base into 3 transverse sections. Six-micrometer sections were cut and stained with Masson's trichrome and picrosirius red (PSR), respectively. The total epicardial and endocardial lengths occupied by the infarct as identified by Masson's trichrome staining were measured using computer software (Image Pro Plus, Media Cybernetics, Silver Spring, MD). The infarct size was calculated by dividing the sum of the planimetered endocardial and epicardial circumferences of the infarcted area by the sum of the total epicardial and endocardial circumferences of the LV (23).

Fibrillar collagen content in the non-infarcted myocardium of the left ventricle was measured from PSR stained sections. The PSR stained LV cross-sections were imaged under polarized light which caused clear demarcation of the fibrillar collagens (40). The section images were then digitized using a 20× objective. Collagen volume was measured using Image Pro Plus program.

2.7. Quantitative real-time polymerase chain reaction

Total RNA was extracted from non-infarcted LV with TRIzol. After DNase treatment, total RNA samples were reversed-transcribed with oligo (dT) primers and MMLV reverse transcriptase (Promega, Madison, WI). Quantification of cardiac gene expression was determined by real-time polymerase chain reaction (PCR). The relative expression of catalase, SOD, GPx-1 and gp91^{phox} was normalized to the amount of β -actin in the same cDNA using the standard curve method. The primers and probes used were as follows. 5'-TCACTGTCATCATAAGGCCATCAAA-3' (forward primer), 5'-CCTAACTGCAGTAGAACAGGATTACAG-3' (reverse primer) and 5'-CACAGGCCCCAACACA-3' (TaqMan probe) for SOD (GenBank accession number NM_017051). 5'-TCTTTACCTTCCTGCGGAATGC-3' (forward primer), 5'-TCTCAAAGTTCCAGGAAATGTCTGTT-3' (reverse primer) and 5'-CCCAGTGACGATCCC-3' (TaqMan probe) for GPx (GenBank accession number NM_030826). 5'-GGTCCCATGTTCTGTACCTTTG-3' (forward primer), 5'-GTGATGACCACCTTCTGTTGAGA-3' (reverse primer) and 5'-CCGGACCAACCTCTCG-3' (TaqMan probe) for gp91^{phox} (GenBank accession number AF298656). Other primers and probes used in the study were Assay-on-Demand gene expression products (Applied Biosystems). Because of proprietary issues and the policy of Applied Biosystems, the exact primer sequences used for the real-time PCR experiments are not provided but can be requested from the company based on the information shown in Table 1.

2.8. Western blot

Twenty micrograms of protein was separated by sodium dodecyl sulfate-polyacrylamide gel, and then transferred to PVDF membranes (Bio-Rad, Hercules, CA). The membranes were incubated with the corresponding primary antibodies overnight at 4 °C. The primary antibodies used were anti-SOD (BD Transduction Laboratories), anti-catalase (Sigma), and GAPDH (Santa Cruz). The membranes were then washed and incubated with horseradish peroxidase-conjugated secondary antibodies. Protein bands on the membranes were detected with enhanced chemiluminescence (Amersham, Little Chalfont, Buckinghamshire, UK) followed by exposure to X-ray film. The protein bands on the X-ray film were scanned, and the band density was calculated by Quantity One software (Bio-Rad).

2.9. Antioxidant Activities

The enzymatic activities of GPx, catalase, and SOD were measured in the non-infarcted myocardium of LV using the following commercially available kits (Cayman Chemical, Michigan): SOD assay kit for SOD activity (U/mg protein); catalase assay kit for catalase activity (nmol/min/mg protein); glutathione peroxidase assay kit for GPx activity (nmol/min/mg protein). The assays were performed according to the manufacturer's instructions. Total GPx activity was determined using a kit supplied by Cayman Chemical. In this assay system, the oxidation of glutathione (GSH) was coupled to NADPH oxidation by GSH reductase (GR). Briefly, twenty microliters of heart homogenate were added to the reaction mixture (190 μ l). The reaction was initiated by addition of 20 μ l of 0.2 mM cumene hydroperoxide at room temperature. The decrease in absorbance at 340 nm was recorded at 60 s intervals for 6 min. The rate of decrease in the absorbance is directly proportional to the GPx activity in the sample.

Each assay was performed in duplicates, and enzyme units were recorded as nmol NADPH oxidized/min/mg protein in the tissue sample.

2.10. Oxidative Stress Assessment

Quantification of lipid peroxidation is essential to assess the role of oxidative injury (26). Lipid peroxidation results in the formation of lipid hydroperoxide (LPO) that can then be used to indicate the levels of lipid peroxidation in cardiac tissue. Lipid peroxidation was measured with a LPO assay kit (Cayman Chemical, Michigan) according to the manufacturer's instructions. Briefly, 100 μ l of heart homogenate was added to an equal volume of saturated methanol and 1 ml of cold chloroform. The mixture was mixed thoroughly and then centrifuged at 2000 g for 5 min to extract LPO into chloroform layer. Then, 500 μ l of chloroform extract was mixed with 450 μ l of chloroform-methanol solvent and 50 μ l of freshly prepared chromogen (containing 4.5 mM ferrous sulfate in 0.2 M hydrochloric acid). Absorbance was measured at 500 nm after 5 min incubation at room temperature. The LPO levels were determined from a standard curve of LPO. Each homogenate was assessed in duplicates and LPO was expressed at nmol/g tissue.

2.11. Statistical analyses

One way analyses of variance (ANOVA) were carried out to determine whether there were significant mean differences among the experiment groups and were followed by Student-Newman-Keuls post hoc comparisons. A *P* value of less than 0.05 was considered statistically significant. Values are expressed as mean $\pm$ S.E.M.

Results

3.1. General characteristics and post-infarct survival

Table 2 presents the characteristics of animals included in the studies. Only animals with an infarct size between 30% and 45% were included for further analysis. Five rats were excluded due to small or severe infarction. Thus, the population of the 7 groups was as follows: Sham (n=10), Sed (n=9), Ex (n=10), Sed+LA (n=10), Ex+LA (n=10), Sed+L-NAME (n=8), and Ex+L-NAME (n=8). The infarct size was similar in all MI groups. Body weight was significantly lower in exercise training groups compared with the corresponding sedentary groups with or without drug treatments.

3.2. Echocardiographic data

As shown in Table 3, there were no significant differences among the seven experimental groups in posterior wall thickness at diastole (PWDT) after 8 weeks of exercise training. The anterior wall thickness at systole (AWST) and diastole (AWDT) was significantly decreased by L-NAME intervention when compared with the Sed group. In addition, the posterior wall thickness at systole (PWST) was significantly thinner in Sed+L-NAME group as compared to the Sed group, while PWST was remarkably higher in the Ex+L-NAME group than in the Sed+L-NAME groups. Thus, exercise training may pronouncedly preserve the PWST regardless of L-NAME intervention. Moreover, exercise training significantly reduced LVESd compared to the sedentary infarcted group, while LVESd was significantly higher in the Ex+LA group than in the Ex+L-NAME group. LVESd was significantly higher in the Sed+LA group than in the Sed group. Furthermore, exercise training significantly increased FS compared to the Sed group. FS in the exercise training with L-NAME group was significantly higher than in the sedentary group with L-NAME intervention although FS was significantly lower in Ex+L-NAME group than in both Ex and Ex+LA groups. On the other hand, FS was remarkably lower in the Sed+L-NAME group than in the Sed and Sed+LA groups. Thus, these data suggest that exercise training appreciably preserved cardiac function after MI without causing scar thinning,

while L-NAME intervention aggravated it. The effect of L-arginine appears to be modest when combined with exercise training.

3.3. Hemodynamic data

As shown in Table 2, there were no significant differences among the seven experimental groups in heart rate after 8 weeks of exercise training. LVEDP was significantly decreased by exercise training and/or L-arginine treatment in comparison to the sedentary MI group and the combined exercise training with L-NAME group. Additionally, $dP/dt_{\max}$ was significantly higher in the exercise training with or without L-arginine treatment groups than in the Sed group. Moreover, ejection fraction (EF) was significantly higher in the Ex and Ex+LA groups than the Sed and L-NAME treated groups, whereas EF was comparable between Ex and Ex+LA groups. Animals with L-NAME treatment had significant increases in LVSP, systolic aortic pressure, and diastolic aortic pressure compared with their non L-NAME treated counterparts. In addition, L-NAME intervention resulted in a noticeable elevation of myocardial oxygen demand and workload in the sedentary rats compared with their non-L-NAME treated counterparts as indicated by the rate-pressure product (RPP) data.

3.4. Collagen content in the non-infarcted LV myocardium

Figure 2 showed that MI caused a pronounced increase in cardiac fibrosis in the non-infarcted myocardium of LV compared to the Sham group. The collagen volume fraction in the Sed group was similar to the Sed+L-NAME group, while the collagen volume of the two groups was significantly higher than the rest of the infarcted groups. Nevertheless, the collagen level was significantly decreased by exercise training and/or L-arginine treatment when compared to the Sed group. Furthermore, the fraction in the Ex+L-NAME was significantly lower than in the Sed+L-NAME group. These data revealed that both exercise training and L-arginine treatment attenuated post-MI myocardial fibrosis, although the additive effect of the combined treatment of exercise and L-arginine was mild.

3.5. Gene expressions in the non-infarcted LV myocardium

As seen in Figure 3, we did not find any significant differences in mRNA level of catalase, GPx or gp91^{phox} among the infarcted groups. However, the SOD mRNA level was significantly increased in the Ex group (5.43 ± 0.87) compared to the Sed group (1.74 ± 0.29), whereas this effect was pronouncedly down-regulated by L-NAME intervention (2.51 ± 1.17 , $P < 0.05$). Thus, these data indicate that exercise training promotes SOD expression after 8 weeks of exercise training, while L-NAME attenuates SOD expression.

3.6. Protein expressions and abundance of catalase and SOD in the non-infarcted LV myocardium

As shown in Figure 4, the SOD protein levels in both L-NAME treated groups (Sed+L-NAME and Ex+L-NAME) were significantly decreased compared to the Sed and the Ex groups. The protein levels of catalase were significantly higher in the Ex and Ex+LA groups than in the Sed, Sed+LA and L-NAME treated groups, although there was no significant difference between the Ex and Ex+LA groups.

3.7. Antioxidant enzyme activities

Myocardial catalase, SOD, and GPx activities were examined in the non-infarcted myocardium after 8 weeks of exercise training with and without drug treatments. As shown in Figure 5, Catalase activity was significantly decreased in the Sed group compared with the Sham group, whereas it was significantly increased in the Ex and Ex+LA groups. Nevertheless, the activity was remarkably down-regulated by L-NAME intervention ($P < 0.05$). We did not find any significant differences among all the groups with respect to the SOD and GPx activities.

3.8. Lipid hydroperoxide

Lipid peroxidation was quantified to assess the extent of oxidative stress after 8 weeks of exercise training and/or drug treatments. Figure 6 illustrates that lipid hydroperoxide content was significantly higher in the Sed+L-NAME group ($P<0.001$) compared with the Sham, Sed, Ex, Sed+LA, and Ex+LA groups. However, the increase of lipid hydroperoxide by L-NAME was significantly attenuated by exercise training.

Discussion

In the present study, we demonstrated that exercise training after MI provides beneficial effects on cardiac function and LV remodeling by increasing catalase protein and activity. Data from this study provide further insights into the mechanisms underlying the improvement in morbidity and mortality produced by exercise training in patients with MI. There are five major findings in the current study. First, post-MI exercise training significantly induced SOD gene expression. Second, exercise training with or without L-arginine treatment significantly upregulated both protein production and enzymatic activity of catalase. Third, L-NAME decreased SOD expression and increased lipid peroxidation. Fourth, post-MI exercise training significantly attenuated collagen content and mitigated cardiac fibrosis. Fifth, exercise training significantly increased cardiac function.

ROS, which include superoxide anion ($\cdot\text{O}_2^-$), H_2O_2 , and hydroxyl radical ($\cdot\text{OH}$), serve as signaling molecules in low concentrations (9). However, these agents elicit harmful effects when produced in excess (37). Accumulating evidence suggests that ROS play a major role in the development and progression of LV remodeling and failure, whereas antioxidants exert protective and beneficial effects in the failing heart (6,24,32,33). Hill and Signal (11,12) have shown evidence of a progressive decrease in SOD, catalase, and GPx activity in the infarcted heart, while NAD(P)H oxidase expression dramatically increased in the infarcted myocardium (10,21).

Recent studies have revealed that an antioxidant effect is induced by exercise training via reduction of expression of NAD(P)H oxidase and beneficial augmentation of the activity of radical scavenger enzymes (1,20). On the other hand, the antioxidant SOD has been implicated equivocally in the ischemic tolerance conferred by exercise training. Several studies have reported that exercise training increased cardioprotection without amplifying myocardial SOD content (4,18). Nevertheless, Yamashita *et al* (43) and Brown *et al* (3) reported that exercise training leads to an increase in myocardial SOD content along with improved recovery from ischemia-reperfusion injury. Consistent with their study, our results also showed that exercise training increased gene expression of SOD following MI. GPx is a selenium-containing enzyme that catalyzes the removal of H_2O_2 through oxidation of reduced GSH, which is recycled from oxidized glutathione (GSSG) by glutathione reductase. In this study, we did not find any significant differences in GPx and catalase at gene expression among all the infarcted groups. However, the protein level of catalase and its activity were both significantly augmented after 8 weeks of exercise training with or without L-arginine administration, although GPx and SOD activities remained unchanged. Nevertheless, previous studies also showed that only certain cardiac antioxidant enzyme activities (i.e. SOD) were enhanced in the exercise trained animals (27,35,43). These exercise-induced variations in cardiac antioxidant enzyme may be due to the differences in the intensity and duration of exercise training. Because catalase mRNA expression was not changed by exercise training and/or L-arginine in MI rats, the increase in catalase activity and protein expression may be the result of either enhanced catalase mRNA stability or posttranslational modification of catalase protein. In addition, compared with other non L-NAME treated groups, our data showed that L-NAME caused a decrease in SOD expression at both gene and protein levels, reduction of

catalase activity, and increase in LPO level, suggesting that L-NAME decreases antioxidant abilities and induces lipid peroxidation.

The extracellular matrix forms a structural network between adjoining cardiomyocytes, provides the mechanical supports, and maintains the structural alignment throughout the cardiac cycle. Following MI, however, the accumulation of fibrillar collagen in the extracellular space is not only responsible for cardiac stiffness, but also for the disruption of electrical and mechanical properties of the heart, thus leading to progressive dysfunction and heart failure (39). In the present study, our data demonstrated that MI induced a marked elevation of myocardial collagen as observed in the sedentary rats, whereas both exercise training and L-arginine treatment attenuated this adverse effect. Likewise, L-NAME intervention augmented myocardial collagen level in the infarcted heart, while exercise training counteracted the L-NAME-aggravated myocardial fibrosis. This positive impact of L-arginine along with the negative effect of L-NAME on cardiac fibrosis may verify a beneficial role for NOS in myocardial fibrosis following MI. Conversely, the partial maintenance of the beneficial effect of exercise on fibrosis in the presence of L-NAME suggests a partially NO-independent mechanism. Thus, these results were in accordance with previous studies which demonstrated that chronic exercise attenuated infarct-induced myocardial fibrosis (41,42). This effect may be in part attributable to the stimulation of the NOS system.

Previous studies have demonstrated significant benefits from post-MI exercise training (5, 36). Many studies have shown that exercise increased non-infarct wall thickness, improved cardiac function, attenuated LV cavity and adverse remodeling by reducing ventricular dilation and wall tension (14,26). Our study showed that exercise training with or without L-arginine treatment significantly improved cardiac function by reducing LVESd and decreased LVEDP. Moreover, EF and $dP/dt_{\max}$ were significantly higher in the exercise groups. The anterior and posterior wall thickness in systole and diastole was significantly decreased by L-NAME intervention, whereas the posterior wall thickness in systole was maintained by exercise training regardless of L-NAME intervention. In addition, RPP was significantly increased by L-NAME intervention. The sustained high workload to the infarcted heart may explain, at least in part, the deteriorated cardiac function observed in the L-NAME treated sedentary rats. Accordingly, these results indicate that $dP/dt_{\max}$, EF and FS were well preserved in the exercise training with or without L-arginine group following MI, while L-NAME deteriorated cardiac function. The effect of L-arginine treatment appears to be modest when combined with exercise training. These observations are in agreement with the study by Schulman et al (30) from the "VINTAGE MI" trial which demonstrated that post-MI L-arginine therapy had minimal effects on LV function, but may actually increase risk of heart failure and post-MI mortality. These data together with the current study suggest that L-arginine treatment may not have a beneficial effect on the improvement of cardiac function.

In summary, this study demonstrates that exercise training after MI is likely playing an important role in cardiac remodeling by increased SOD gene expression and enhanced catalase protein and activity. These improvements, in turn, attenuate myocardial fibrosis and cardiac stiffness, and preserve post-MI cardiac function. In addition, L-NAME intervention elevated lipid peroxidation, reduced catalase expression, and augmented myocardial collagen deposition, resulting in deterioration of cardiac function in the infarcted heart, while aerobic exercise training provided significant protection against these adverse effects.

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Figure 1.
Experiment flow chart.

Figure 2.

The results of collagen volume fraction (%) by picrosirius red staining on non-infarcted left ventricle under polarized light. Data are expressed as mean $\pm$ S.E.M. * P <0.05 vs Sham group; ** P <0.001 vs Sham group; # P <0.05 vs the Sed group; + P <0.05 vs the Sed+L-NAME group.

Figure 3.

Real-time PCR for the expression of target genes (catalase, SOD, GPx, and gp91^{phox}) mRNA. The expression was normalized as a ratio using β -actin as a housekeeping gene. Data are expressed as mean $\pm$ S.E.M. [#] $P < 0.05$ vs the Sed and Ex+L-NAME groups.

Figure 4.

The results of western blot for SOD and catalase. Data are expressed as mean $\pm$ S.E.M. * P <0.05 vs the Sed group; ** P <0.05 vs the Sed group; # P <0.05 vs the Ex and Ex+LA groups.

Figure 5. Cardiac catalase, SOD, and GPx activity. A) Catalase activity (nmol/min/mg protein); B) SOD activity (U/mg protein); C) GPx activity (nmol/min/mg protein). * $P < 0.05$ vs the Sham group; ** $P < 0.005$ vs the Sham group; # $P < 0.005$ vs the Sed group; + $P < 0.05$ vs the Ex or the Ex+LA group; ++ $P < 0.005$ vs the Ex or the Ex+LA group.

Figure 6. Lipid hydroperoxide content (nmol/mg protein). * $P < 0.001$ vs the Sham, Sed, Ex, Sed+LA or Ex+LA group; # $P < 0.05$ vs the Sed+L-NAME group.

Table 1

Oligonucleotide primers used for real-time RT-PCR

Gene	Assay ID	Reference Sequence *	Amplicon Length	Exon Boundary
catalase	Rn00560930_m1	NM_012520.1	107	1–2
β-actin	Rn00667869_m1	NM_031144.2	91	4–5

* Accession numbers in GenBank for the sequence used in designing the primers.

In-vivo hemodynamics and general characteristics**Table 2**

Group	Sham (n=10)	Sed (n=9)	Ex (n=10)	Sed+LA (n=10)	Ex+LA (n=10)	Sed+L-NAME (n=8)	Ex+L-NAME (n=8)
Infarct size (%)	-	40.63±3.63	39.54±0.70	39.00±1.00	40.50±0.96	40.71±2.02	40.71±1.30
BW (g)	399.8±6.18	392.5±9.34	357.64±5.96 [*]	397±3.48	352.86±11.52 ^a	367.57±16.16	332.86±7.73 [†]
Heart rate (bpm)	290±4	282±8	283±7	280±6	290±7	281±5	280±7
LVSP (mmHg)	109.96±4.38	97.66±4.46 [†] §	100.94±2.66 [†] §	100.62±4.6 [†]	106.41±4.8	118.33±10.3	117.14±6.48
LVEDP (mmHg)	9.94±1.36	22.38±2.9 ^b	15.12±1.78 ^{*§}	15.33±2.23 [§]	13.14±2.7 ^{*§}	20.02±3.4 ^b	26.83±3.34 ^b
LV +dP/dt _{max} (mmHg/s)	6851±564	4762±498 ^b	5297±236 ^b	5099±388 ^b	5273±494 ^b	4907±391 ^b	4895±141 ^b
LV -dP/dt _{max} (mmHg/s)	6684±304	3624±420 ^b	4760±208 ^{*b}	4373±340 ^b	5030±480 ^{*b}	3834±463 ^b	3767±153 ^b
Systolic aortic pressure (mmHg)	114.06±5.58	97.51±4.65 [†] §	103.34±3.16 [†]	102.14±4.76 [†]	104.54±3.93 [†]	120.32±9.78	116.58±6.31
Diastolic aortic pressure (mmHg)	80.43±3.48	73.01±3.25 [†] §	77.43±1.78 [†]	74.99±3 [†] §	77.78±2.4 [†]	90.28±6.38	86.82±5.15
Pulse pressure (mmHg)	32±1	25±2	26±2	27±2	25±2	30±4	30±2
Rate-pressure product	31088±871	27635±2032	29331±1344	28579±1440	29626±1000	33959±3151 [*]	32647±1927
Ejection fraction (%)	71.49±5.66	44.13±4.13 ^b	57.49±4.34 ^{*†} §	54.4±10.65 [§]	58.11±2.70 ^{*†} §	43.16±6.87 ^b	35.78±4.43 ^b

Values are expressed as mean ± S.E.M. BW, body weight at sacrificed; bpm, beats per minute. LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure; dP/dt_{max}, peak velocities of contraction and relaxation. Ejection fraction (%) was calculated as (Stroke Volume/V@dP/dt_{max})×100.

[#] $P < 0.05$ vs. the Ex group

^{*} $P < 0.05$ vs. the Sed group

[†] $P < 0.05$ vs. the Sed+L-NAME group

[§] $P < 0.05$ vs. the Ex+L-NAME group

^a $P < 0.05$ vs. the Sed+LA group

^b $P < 0.05$ vs. the Sham group.

Table 3

Doppler echocardiographic assessment of left ventricular geometry and function

	Sham (n=10)	Sed (n=9)	Ex (n=10)	Sed+LA (n=10)	Ex+LA (n=10)	Sed+L-NAME (n=8)	Ex+L-NAME (n=8)
AWDT (mm)	1.67±0.14	0.55±0.15	0.44±0.03	0.4±0.02	0.4±0.03	0.33±0.02*	0.36±0.02*
AWST (mm)	2.91±0.15	0.63±0.16	0.5±0.03	0.47±0.04	0.47±0.05	0.38±0.03*	0.41±0.03*
PWDT (mm)	1.82±0.14	1.94±0.29	1.92±0.07	1.85±0.08	1.9±0.08	1.87±0.06	2.17±0.11
PWST (mm)	2.82±0.1	2.46±0.27	2.62±0.11	2.45±0.12	2.5±0.1	2.15±0.08 [#]	2.54±0.09 [†]
LVEDd (mm)	8.08±0.27	11.17±0.24	10.68±0.18	10.94±0.28	10.72±0.31	11.38±0.22 [#]	11.25±0.23
LVESd (mm)	4.42±0.33	9.62±0.31 [#]	8.83±0.23	9.37±0.3 [†]	8.9±0.34 ^{§†}	10.27±0.24 [#]	9.87±0.22 [#]
FS (%)	45.4±2.87	14.03±1.47 ^{††}	17.48±0.92 ^{§†}	14.4±0.74 [†]	17.15±1.01 ^{§#}	9.77±0.9	12.29±0.51 [†]

LVEDd, left ventricular end-diastolic dimension; LVESd, left ventricular end-systolic dimension; FS, left ventricular fractional shortening; AWST, anterior wall systolic thickness; AWDT, anterior wall diastolic thickness; PWST, posterior wall systolic thickness; PWDT, posterior wall diastolic thickness.

* $P<0.05$ vs. the Sed group

[†] $P<0.05$ vs. the Sed+L-NAME group

[#] $P<0.05$ vs. the Ex group

[§] $P<0.05$ vs. the Ex+L-NAME group.