Favorable Left Ventricular Remodeling following Large Myocardial Infarction by Exercise Training

Effect on Ventricular Morphology and Gene Expression

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Abstract

Continued adverse remodeling of myocardium after infarction may lead to progressive ventricular dilation and heart failure. We tested the hypothesis that exercise training in a healed myocardial infarction–dysfunction rat model can favorably modify the adverse effects of ventricular remodeling including attenuation of abnormal myosin gene expression.

Sprague-Dawley rats were subjected to either proximal LAD ligation or sham operation. At 5 wk after the operation, animals were randomly assigned to sedentary conditions or 6 wk of graduated swim training, creating four experimental groups: infarct sedentary (IS), infarct exercise (IE), sham sedentary (SS), and sham exercise (SE). At 11 wk all rats were sacrificed and analyzed.

Compared to sedentary infarct controls, exercise training attenuated left ventricular (LV) dilation and allowed more hypertrophy of the non infarct wall. The exercise-trained hearts also showed a reduction in the estimated peak wall tension. Northern blot analysis showed an increase in β-myosin heavy chain expression in the hearts of the sedentary infarction group soon after infarction when compared to sham controls. However, with exercise training, there was a significant attenuation of the β-myosin heavy chain expression in the myocardium.

Exercise training in a model of left ventricular dysfunction after healed myocardial infarction can improve the adverse remodeling process by attenuating ventricular dilation and reducing wall tension. The abnormal β-myosin expression was also attenuated in the exercise trained group. This is evidence that abnormal gene expression following severe myocardial infarction dysfunction can be favorably modified by an intervention. (*J. Clin. Invest.* 1995. 96:858–866.)

Key words: myocardial infarction • remodeling • exercise • left ventricular hypertrophy • myosin gene expression

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Introduction

Left ventricular remodeling following myocardial infarction is a dynamic process that can lead to the development of heart failure with a significant impact on patient survival (1, 2). Left ventricular remodeling early post myocardial infarction attempts to provide hemodynamic compensation at the expense of ventricular dilation (3, 4). Chronically, continued hemodynamic stress leads to pathologic hypertrophy and progressive dilation. Continued hypertrophy that is inadequate to normalize increased wall tension activates an initially fetal program of gene expression exemplified by switching of MHC from α to β isoform predominance, accompanied by an increase in expression of atrial natriuretic factor, skeletal form of α-actin, and a subsequent decrease in sarcoplasmic reticulum calcium ATPase. The β form of MHC is associated with a low ATPase activity and slower speeds of contraction, and is often a marker of pathological hypertrophy (5–9). Continued late ventricular remodeling along this molecular pathway beyond the period of infarct expansion and scar formation accompanies the transition from ventricular dilation and dysfunction to frank heart failure (2, 10–14).

It is known that aerobic exercise training creates a physiologic form of hypertrophy in the myocardium without systolic or diastolic dysfunction (15–18) or abnormal MHC gene expression in animal models (19). It follows that an intriguing possibility exists that suitably timed exercise training post myocardial infarction may overcome the abnormal fetal gene expression and morphological remodeling, and alter the natural course towards heart failure.

Exercise training acutely postmyocardial infarction is controversial, as it may aggravate infarct expansion (20–22). Exercise training in chronic stages of heart failure on the other hand has been shown to improve peripheral metabolism (23, 24) and oxygen consumption (25–27), but its effects on ventricular remodeling are unknown (28).

To investigate the effects of exercise training in postmyocardial infarction remodeling, we compared exercise by swim training with sedentary controls in a rat healed infarction model with severe left ventricular dysfunction. We hypothesized that suitably timed exercise training can increase noninfarct wall thickness, limit wall tension and attenuate left ventricular cavity area. In addition there will be a reduction in β-MHC expression. These constellation of changes should provide evidence for a more favorable ventricular remodeling and lead to more adaptive hypertrophy.

Methods

Creation of a healed infarction rat model. The study protocol is summarized in Fig. 1. Male Sprague Dawley rats aged 12–14 wk (n = 112,

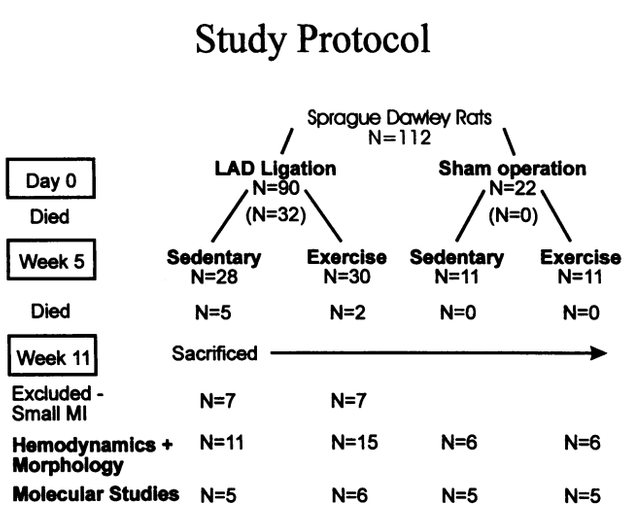

Figure 1. Outline of the research protocol with randomization scheme.

average weight 349 g) were randomized a priori to proximal left anterior descending (LAD)[1] coronary artery occlusion (n = 90) or sham operated (n = 22) groups.

To create the model, animals were anesthetized with ketamine hydrochloride (90 mg/kg) and xylazine (10 mg/kg) intraperitoneally. After adequate anesthesia they were intubated with an 14-gauge polyethylene catheter and ventilated with room air using a small animal ventilator (model 683; Harvard Apparatus, Boston, MA). A left thoracotomy was performed in the fifth intercostal space and the pericardium was opened. The proximal left coronary artery under the tip of the left atrial appendage was encircled and ligated using a 6-0 silk suture. Proximal left anterior descending artery ligation in a rat model creates a reproducibly large lateral wall infarction. The muscle and skin were closed in layers. In sham operated animals, the left coronary artery was encircled but not ligated and the muscle layers and skin were closed similarly.

To ensure complete healing of the infarct zone, all rats recovered in their cages for 5 wk after the operation before beginning the exercise program (29). Rats were given water and standard rat chow ad libitum. They were housed individually in a climate controlled environment at ambient temperature of 21°C, exposed to regular 12 h light and dark cycles. During this time period, 32 rats in the LAD occlusion group died before randomization to exercise training, and were excluded form subsequent analysis.

At week five all rats in both the LAD occlusion and sham groups were randomized in blocks to an additional 6 wk of exercise swim training or to sedentary controls. This resulted in four final groups: infarction sedentary (IS; n = 28), infarction exercise (IE; n = 30), sham sedentary (SS; n = 11), and sham exercise (SE; n = 11). During these 6 wk of intervention, five rats died in the infarction sedentary group, and two rats died in the infarction exercise group.

To determine the natural progression of molecular changes within the myocardium in the infarct and noninfarction regions of the heart following coronary occlusion, we performed coronary ligation without exercise in an additional 50 rats of similar weight and age as the above experimental group. The rats were randomized to be sacrificed on days 1, 2, 7, 14, 21, 28, and 35 after ligation, and their hearts were processed for molecular studies. Of these, five animals were also randomized to receive sham ligation and were sacrificed on day 0 to act as controls.

Exercise protocol. The swimming protocol began on day 35 for 5 min/d initially, and increased by an additional 5 min/d until the rats were swimming continuously for 40 min/d. Swim frequency was 5 d/wk for a total duration of 6 wk. The rats swam in a 60-cm-deep tub with water temperature maintained at 35°C in groups of four animals, and were toweled dry after each session. The rats randomized to sedentary conditions were dipped in the water for 30 s and toweled dry 5 d/wk.

All rats were then sacrificed at 11 wk and randomized to hemodynamic/morphometry analysis or molecular studies. To ensure that we investigated a model of severe LV dysfunction, all hearts with infarction involving < 45% of left ventrical (LV) perimeter were excluded after pathologic and morphometric analysis. Thus seven infarct sedentary and seven infarct exercise hearts were excluded from their respective groups. In total, 51 rats completed the study protocol in the infarction group and were eligible for final analysis, and the individual groups are illustrated in Fig. 1.

Assessment of LV function in isolated heart preparation. 11 wk after the operation, all rats randomized to hemodynamic analysis were anesthetized with ketamine, weighed, and the beating heart was removed through a rapid mid-sternal thoracotomy. The heart was immediately placed in Krebs-Henseleit buffer at 4°C, cleared of excess tissue, and connected through the aorta to the Langendorff perfusion apparatus. The Krebs-Henseleit buffer was prepared in the standard manner, consisting of (in mmol/l): 1.2 KH_2PO_4, 1.2 $MgSO_4$, 1.75 $CaCl_2$, 4.7 KCl, 0.5 EDTA, 118.0 NaCl, 25.0 $NaHCO_3$, and 11.0 dextrose. This solution was continuously bubbled with 95% O_2:5% CO_2 at a rate of 2 l/min and a pressure of 40 Kpa to maintain pH at 7.40±0.05. The buffer was infused at a constant flow rate of 12 ml/min, controlled by a digital roller pump (Masterflex; Parmer Instruments, Burlington, VT), with the effluent volume checked at regular intervals. Constant temperature was maintained at 37°C by a coil condenser heat-exchanger circulator connected to an MGW 20 water bath (Lauda, Westbury, NY).

A custom made oversized intraventricular latex balloon containing warm saline was inserted through the left atrial appendage and attached to a "Y" connector. One arm of the catheter was connected to a transducer (model P231D; Gould Inc., Cleveland, OH) to continuously record left ventricular pressure on a physiological recorder (model 2800S; Gould Inc.). Using a microinjector syringe containing warmed saline, volume in the balloon was adjusted to achieve a steady baseline end diastolic filling pressure (LVEDP) of 5 mmHg. Volume increments at 0.1-cc intervals were infused into the intraventricular balloon, and simultaneous LV pressure recorded at the different LVEDP's attained. The second arm of the Y connector joined a high fidelity microtip catheter (Millar 5F, Houston, TX), which was connected to a differentiator (Gould Inc.) and recorded positive and negative dP/dt on the Gould physiological recorder.

Pathological assessment. At the conclusion of hemodynamic measurements, with the intraventricular balloons still in situ maintaining an internal distention pressure of 5 mmHg, potassium chloride (2 mEq/ml) was infused retrogradely via the aortic cannula to achieve uniform diastolic cardiac arrest. Tissue fixation was then achieved by perfusing 1:1 mixture of paraformaldehyde/glutaraldehyde at a constant pressure of 100 mmHg retrogradely down the aortic cannula for 30 min, while maintaining the intraventricular pressure at the same level.

To determine the potential contributions of intraventricular balloon inflation to the changes observed, we have perfusion fixed an additional five infarcted hearts and five noninfarcted hearts without exercise, where the intraventricular balloon was inflated with an intracavitary effect pressure of 10 mmHg. The hearts were then analyzed in a similar manner to determine the potential contribution of an increase in balloon inflation pressure to the parameters observed.

After perfusion fixation the intraventricular balloon was removed, and the hearts were immersed in universal fixative (4:1 ratio of formaldehyde/glutaraldehyde) for a minimum of 48 h. Four to five 3-mm-thick cross-sectional perpendicular to the long axis cuts of heart were obtained from apex to base. The block sections were dehydrated in increasing concentrations of ethyl alcohol and embedded in paraffin. A minimum of one 5-μm sections from each block was obtained including

1. *Abbreviations used in this paper:* IE, infarction exercise; IS, infarction sedentary; LAD, left anterior descending; LV, left ventrical; SE, sham exercise; SS, sham sedentary.

one section at the mid papillary level. Each section was stained with hematoxylin and eosin, and trichrome.

The processed pathological sections were examined thoroughly by an experienced cardiac pathologist who was not aware of the treatment allocation of each specimen. The stage of infarction, degree of transmurality, and involvement of infarct from apex to base was carefully recorded for each heart.

For morphometric analyses, photomicrographs of the sections were taken through low power field of a light microscope at 6×. After standardizing for magnification, morphometric measurements were obtained from the same mid papillary slice of each heart using a quantitative digital analysis system (Bioquant system E; R + M Biometrics, Inc., Nashville, TN). Septal (noninfarcted) wall thickness was measured and averaged from three equidistant points on an axis which cut the endocardial surface at 90°. Lateral (infarcted) wall thickness was measured in a similar fashion. Left ventricular endocardium was traced and LV cavity area planimetered. LV epicardial surface was also planimetered. Specific parameters of infarct size and peak wall tension were derived based on morphometric and hemodynamic variables.

Relative infarct size. The relative infarct size was determined according to method of Pfeffer et al. (11): the lengths of scar for the endocardial and epicardial surfaces were numerically summed as were the endocardial and epicardial circumferences. The ratio of the lengths of scar and of surface circumferences defined the infarct size for each of the myocardial surfaces.

Peak wall tension. The average peak wall tension for each heart was determined using the following formula:

$$\frac{\text{peak systolic pressure (mmHg)} \cdot \text{LV cavity area (mm}^2\text{)}}{2 \cdot \text{septal thickness (noninfarct wall, mm)}}$$

Peak systolic pressure was obtained from Langendorff preparation, and the cavity area was used instead of radius to account for the noncircular LV cavity shape.

Analysis of β-MHC expression. All rats which were randomized to molecular studies were weighed and anesthetized with ketamine 90 mg/kg and xylazine 10 mg/kg intraperitoneally. This included animals which were sacrificed serially after coronary ligation to determine the natural history of serial gene expression changes, and animals undergoing exercise or rest at 11-wk after ligation. The hearts were immediately removed asepticaly using a mid-sternal approach. After weighing, the hearts were cut in cross-section. A sample for each of the mid-infarcted lateral wall and the mid-non-infarcted septal wall were taken from the mid-papillary slice and placed immediately in liquid nitrogen. An adjacent slice was also taken for pathological analysis using the same method as outlined in the morphological study, and the inclusion criteria were identical for both the morphological and molecular portions of the study. Only tissue sections fitting the criteria were included in the subsequent molecular analysis.

Each frozen tissue sample was first pulverized and then RNA was extracted by acid guanidinium thiocyanate-phenol-chloroform method as described by Chromczynski and Sacchi (30). For each sample the total RNA (15 μg) was denatured in formaldehyde, run in 1.2% agarose-formaldehyde gel (31) and transferred overnight onto a positively charged nylon membrane (Gene Screen plus; Dupont, Wilmington, DE). The transfer was done by an adsorption process using 10× SSPE (1.5 M NaCl, 0.1 M $NaH_2PO_4 \times H_2O$, 0.01 M EDTA) as a transferring buffer. The filters were incubated in prehybridization buffer (5× SSPE, 50% formamide, 5× Denhardt's solution, 1% SDS, 100 g/ml denatured salmon sperm DNA [Pharmacia Fine Chemicals, Piscataway, NJ]) for 4 h at 42°C. The filters were then hybridized overnight at 42°C in fresh prehybridization buffer containing the denatured ^{32}P-labeled rat β-MHC-specific probe (sp. act. 2×10^7 cpm/g). The filter was sequentially washed for 30 min with 2× SSPE at room temperature, and then 1× SSPE at 52°C, followed by 0.1× SSPE and 0.1% SDS at 52°C until the radioactive background was negligible. The filter was then autoradiographed at −70°C with intensifying screens. The steady state expression of β-MHC was quantitated by scanning densitometry (Zeineth Soft Laser Scanning Densitometer model SLR-2D/ID-DNA; Biomed. Instruments Inc., Fullerton, CA).

To confirm equality of loading of the extracted RNA from the heart samples, the same blots were further washed with 0.1× SSPE and 0.1% SDS at the boiling temperature for 15 min and rehybridized with rat GAPDH and β-actin probes, which are housekeeping genes to act as controls, kindly provided by Dr. C. C. Liew from our center. β-MHC expression was normalized to the expression of BAPDH, also analyzed by scanning densitometry.

Preparation of the rat β-MHC-specific probe. The β-MHC-specific probe of 102 bp was derived by PCR gene amplification of rat β-MHC RNA target using specific primers with in the 3′ terminus of rat β-MHC: TP2 (5800-5′GGC CTG AAT GAA GAG TAG-3′-5820) and TP3 (5880-5′CAC AAA CAG AGG CTT CAC CAG-3′-5900). Prior to PCR, the total RNA was first converted into first-strand cDNA by using random hexamers, TP2 and reverse transcriptase—a modification of Wee et al. (32). Samples were precipitated by adding 4 μl of 3 M sodium acetate, pH 6.5, and 70% ethanol overnight at −70°C, centrifuged at 12,000 *g*, washed in 70% ethanol, dried, resuspended in 45 μl of sterile distilled water, and then stored at −20°C. 15 μl of the first strand cDNA from each sample was used as the template for β-MHC specific primers TP2 and TP3 and added to 50 μl of master mix. After 39 cycles of denaturation at 94°C for 1 min, annealing at 55°C for 2 min, and extension at 72°C for 2 min/cycle, 10 μl of the PCR product was analyzed by gel electrophoresis on a 1.6% agarose-ethidium bromide gel using 1× TAE (0.04 M tris acetate and 1 mM EDTA, pH 8.0) as the running buffer. The amplified β-MHC band (102 bp) was excised and further purified with Sephaglas (Pharmacia Fine Chemicals) and labeled with (α-^{32}P)dATP (sp. act. 3,000 Ci/mmol; Boehringer-Mannheim Biochemicals, Indianapolis, IN).

Ethics. The study protocol was approved by the Committee on Animal Research at the Toronto General Hospital and was performed in accordance with the "Position of the American Heart Association on Research Animal Use," adopted on November 11, 1984, by the American Heart Association.

Statistics. All values are expressed as mean±standard deviation, except where stated otherwise. The statistical significance of differences in mean values between the four groups (IS, IE, SS, SE) was assessed by analysis of variance. Specific differences of individual pairs were tested with Student-Newman-Keul post hoc subgroup testing if overall significance was demonstrated by the preceding analysis of variance. A P value < 0.05 was considered statistically significant.

Results

Pathology and morphometry. Representative whole mount mid-papillary muscle sections from each of the infarct experimental groups are illustrated in Fig. 2. The infarct zone is large encompassing approximately half the endocardial perimeter, but is similar in size in both of the infarction groups. There is extensive transmural scarring of the infarct zones in both IE and IS animals creating a markedly thinned lateral wall when compared to sham operated animals. The LV cavities of both IE and IS animals are enlarged compared to sham groups. It is striking that the IE group animals had less cavity area dilation with greater hypertrophy of the noninfarct wall when compared to sedentary post infarction animals.

All cardiac sections were thoroughly examined by a cardiac pathologist. As a criteria for entry into analysis the infarct zone displayed transmural fibrosis in all sections from apex to base. All cardiac sections confirmed the complete healing of the infarct zones in all animals. One animal heart was excluded, however, because the infarct zone did not display transmural fibrosis involving the lateral wall in all sections from apex to base. Other hearts were excluded ($n = 13$) because the mid-

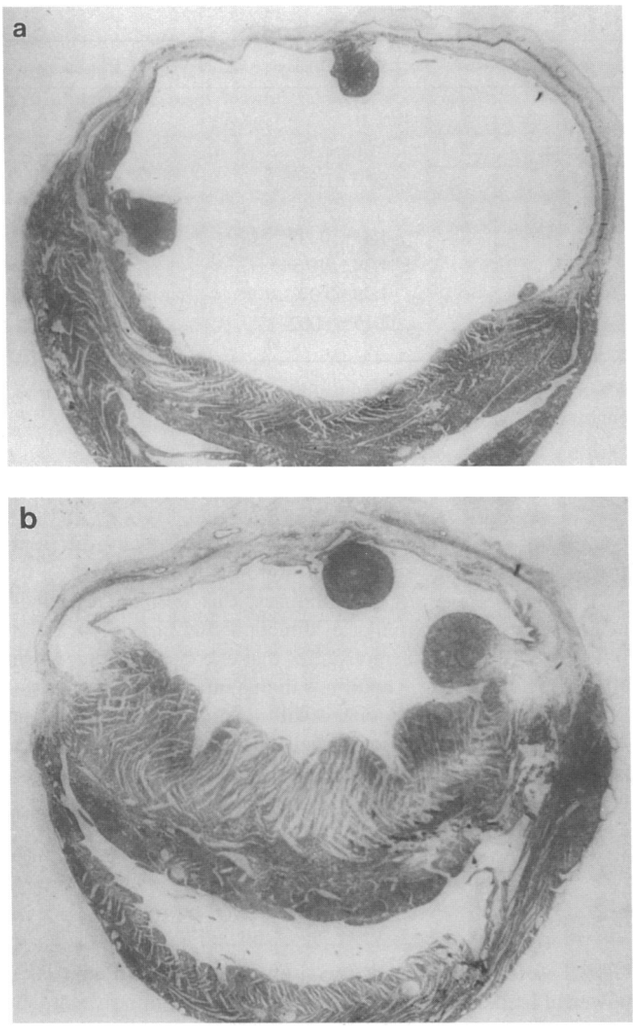

Figure 2. Photomicrograph depicting a representative mid left ventricular transverse slice of each of the infarct groups of rats. (*a*) Infarct sedentary; (*b*) Infarct exercise. Trichrome stain. 6× actual size.

papillary slice infarct size was obviously too small or was confirmed by planimetry as less than 45%. In all sections there was no evidence of rupture of the free wall, septum, or papillary muscles. Although in all LAD occlusion group animals included in analysis the fibrosis was transmural, all cardiac sections did also had evidence of preserved subendocardial cardiac muscle fibers. Quantitative morphometry confirmed the above qualitative observations of pathological sections as detailed in Table I and summarized in Fig. 3.

Both LAD occlusion groups (IE and IS) showed very large infarctions of similar size (54±5% vs. 53±5% respectively, P = NS), conforming to the goal of achieving large infarct size in this study. As further evidence of a model of healed infarction, the infarcted lateral walls were markedly thinned when compared to respective sham controls: (IS vs. SS: 0.32 mm vs. 1.44 mm, $P < .001$; and IE vs. SE: 0.34 mm vs. 1.85 mm, $P < .001$).

When compared to respective sham controls both infarction groups displayed larger mid cavity areas: (IS vs. SS: 63.3 mm^2 vs. 27 mm^2, $P < .01$; and IE vs. SE: 45.2 mm^2 vs. 21.1 mm^2, $P < .05$). In comparison to the infarct sedentary group the exercise trained infarcted rats significantly attenuated LV cavity area and LV cavity perimeter: (IE vs. IS: 45.2 mm^2 vs. 63.3 mm^2, $P < .05$; and 31.1 mm vs. 35.6 mm, $P < .05$), respectively.

The infarct exercise rats also displayed increased septal wall thickness when compared to infarct sedentary rats and to their respective sham controls: (IE vs. IS septal thickness: 2.13 mm vs. 1.56 mm, $P < .05$; and IE vs. SE septal thickness: 2.13 mm vs. 1.78 mm, $P < .05$).

The only significant differences between sham groups were an increase in lateral wall thickness in the exercise trained group SE vs. SS: 1.78 mm vs. 1.34 mm ($P < .001$) and a reduction in peak wall tension SE vs. SS: 737 mmHg · mm vs. 1178 mmHg · mm ($P < .05$, Table II).

Measurements of the left ventricular weight and left ventricle/body weight ratios demonstrated similar changes as that found with wall thickness, confirming the change in cardiac mass to be related to the process of hypertrophy.

To address the potential differences of intracavitary balloon inflation pressure during fixation, the sedentary infarcted hearts inflated at 5 mmHg (n = 11) during fixation were compared with an additional group of hearts inflated at 10 mmHg (n = 5). Overall the infarct size (53% vs. 52%, 5 vs. 10 mmHg inflation) were comparable between the two groups. The cavity area (63.3±19.1 mm^2 vs. 56.1±14.3 mm^2), cavity circumference (35.6±5.9 mm vs. 30.4±4.7 mm), and septal wall thickness (1.56±.30 mm vs. 1.57±.20 mm) were all not significantly different. Similarly for sham-ligated sedentary hearts (n = 5), the cavity area (27.0±4.3 mm^2 vs. 29.6±4.3 mm^2), cavity circumference (20.3±1.8 mm vs. 21.2±.8 mm), and septal wall thickness (1.34±.26 mm vs. 1.25±.20 mm) were also not significantly different.

Assessment of LV function. The results of the ventricular functional assessments for the various groups are detailed in Table II.

Both infarction groups displayed severe depression in parameters of systolic contractility and diastolic relaxation when compared to their respective controls. Although there was a trend toward improved hemodynamic function in the IE vs. IS group animals, this did not reach statistical significance (Table II). Peak positive dP/dt and peak negative dP/dt measured corresponding to LVEDP of 10 mmHg were as follows: (IS vs. SS: +dP/dt: 758 vs. 2,279 mmHg/s, $P < .001$; −dP/dt: 669 vs. 1,852, $P < .001$, IE vs. SE: +dP/dt: 848 vs. 1,763 mmHg/s, $P < .05$; −dP/dt: 796 vs. 1,397 mmHg/s, $P < .05$).

Wall tension was estimated as per previously stated formula, which was directly proportional to the peak systolic LV pressure and cavity area and inversely proportional to the non infarct wall thickness. Wall tension was markedly increased in infarct sedentary group animals when compared to respective sham controls: (IS vs. SS group: 1,294 vs. 1,178 mmHg · mm, $P < .01$). Exercise training after healed myocardial infarction was associated with normalization of wall tension as there was no difference in values between IE vs. SE: (834 vs. 737 mmHg · mm respectively, P = NS). The abnormally increased wall tension seen in sedentary conditions after infarction was significantly reduced with exercise training (IS vs. IE: 1,294 vs. 834 mmHg · mm; $P < .01$) and was normalized when compared to sham controls.

Serial changes in β-MHC expression following coronary ligation. The natural history of serial changes in β-MHC gene expression in the noninfarct regions following coronary ligation

Table I. Morphometry of Hearts in Experimental Groups

	Infarct sedentary	Infarct exercise	Sham sedentary	Sham exercise
	n = 11	*n = 15*	*n = 6*	*n = 6*
LV cavity area (mm^2)	63.3±19.1*	45.2±16.4	27.0±4.3‡‡‡	21.2±6.1§§
LV cavity perimeter (mm)	35.6±5.9*	31.1±4.1	20.3±1.8‡‡‡	18.5±3.5§§§
Percent infarct (%)	53±5	54±5		
Infarcted lateral wall (mm)	0.32±.0.08	0.34±0.13	1.44±.0.20‡‡‡	1.85±.0.07§§§
Noninfarct septum (mm)	1.56±0.30*	2.13±0.31	1.34±.0.26	1.78±0.52§
LV Weight (gm)	1.12±0.22*	1.39±0.29	1.08±0.07	1.13±0.09
LV/body weight ratio	.0020±.0002**	.0028±.0006	.0019±.0002	.0021±.0003

All results are expressed as mean±standard deviation. Significant differences between groups are expressed as follows: Infarct sedentary group vs. infarct exercise group: * $P < .05$, ** $P < .01$, *** $P < .001$. Infarct sedentary group vs. sham sedentary group: ‡ $P < .05$, ‡‡ $P < .01$, ‡‡‡ $P < .001$. Infarct exercise group vs. sham exercise group: § $P < .05$, §§ $P < .01$, §§§ $P < .001$.

was analyzed using β-MHC specific probe on Northern blots, as depicted in Fig. 4 *A*. Probes of GAPDH and β-actin were also included to act as internal controls. Quantitative analysis of β-myosin expression, as a ratio over GAPDH, is illustrated in Fig. 4 *B*. The β-MHC expression was significantly increased in the contralateral noninfarct region within one day following coronary ligation. This elevated level of β-MHC gene expression persisted during the subsequent 35 d of follow-up observation period in this model. This suggested that the noninfarct wall underwent a pathological hypertrophy process with a significantly increased β-MHC expression.

Analysis of β-MHC expression changes following intervention. The β-MHC gene expression from the noninfarct zone was analyzed in the experimental groups using β-MHC specific probe on Northern blots, as depicted in Fig. 5 *A*. The β-MHC gene expression from all the samples were quantitated from the Northern blots by densitometry scanning and normalized to GAPDH. There was a significant increase in the β-myosin/GAPDH ratio in the infarct sedentary group, when compared to sham sedentary or sham exercise groups, which normally showed only minimal expression (19) (Fig. 5 *B*). The exercise-trained infarction group however was able to attenuate the β-MHC expression significantly when compared to their sedentary counterparts, towards a level that is not statistically different from that seen in the sham control groups in this model.

Summary of results. In summary, this is a rat model of healed severe left ventricular dysfunction after large myocardial infarction. This study demonstrates that exercise training, when compared to sedentary conditions in this infarction–severe dysfunction model, was associated with a significant reduction in LV cavity area and increased wall thickness of the noninfarct zone with normalization of wall tension. Swim training of these infarction rats also significantly reduced the abnormal β-MHC expression seen in sedentary rats after large infarction, in the contralateral noninfarct myocardium.

Discussion

Global ventricular remodeling. Left ventricular volume is a powerful predictor of mortality after recovery from myocardial infarction (1). In the early convalescent period after infarction there is global remodeling of the left ventricle including expansion of the infarct zone, volume-overload hypertrophy and myocyte slippage of noninfarcted myocardium (3, 4, 33).

It is now understood that in large myocardial infarctions beyond the period of scar formation and infarct expansion con-

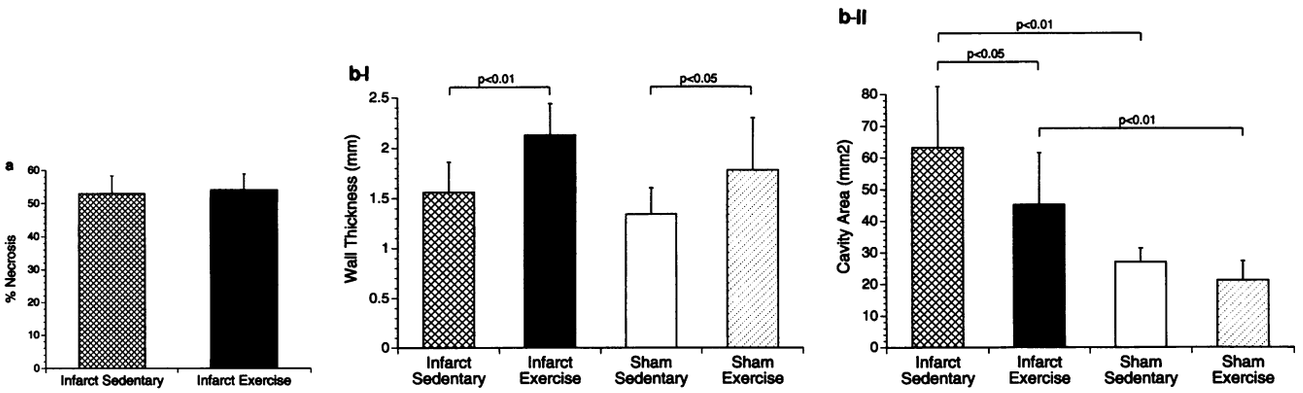

Figure 3. (*a*) The percent left ventricular (LV) necrosis in the infarct sedentary and infarct exercise groups in this severe infarct–dysfunction model. (*b*) The significant differences in function and morphology in the sedentary versus exercise trained animals. (*b I*) Noninfarct (septal) wall thickness. (*b II*) LV cavity area. The results are expressed as mean±SD. Comparisons are made between each of the four groups: infarct exercise, infarct sedentary, sham exercise, sham sedentary.

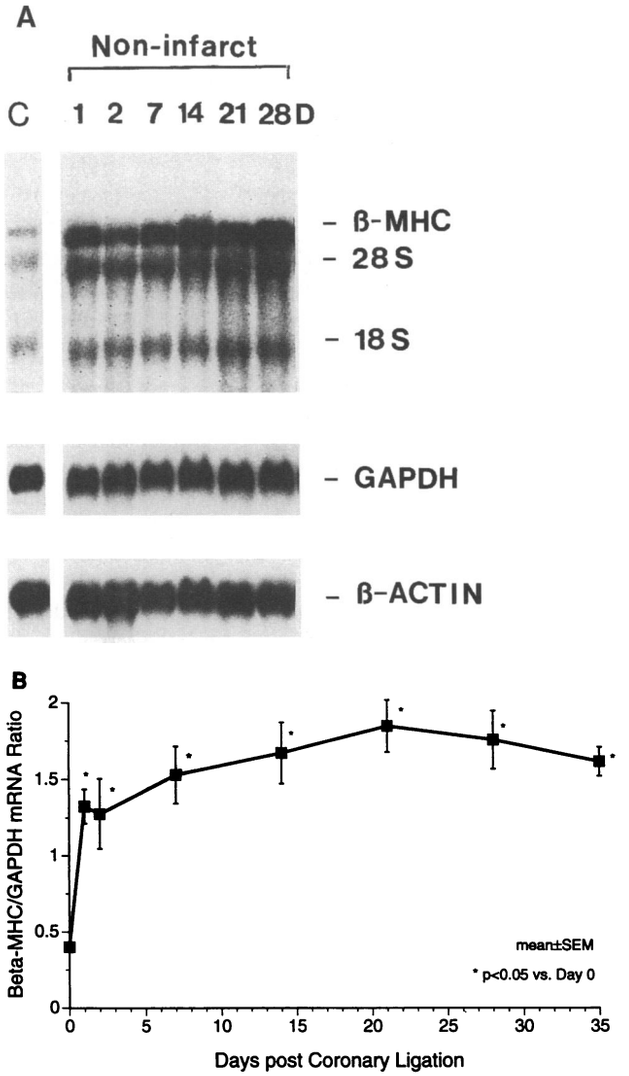

Figure 4. (A) Northern Blot of serial changes in mRNA expression of β-myosin in the infarct–dysfunction model from sedentary animals. Representative RNA samples from the contralateral noninfarct zones serially on days 1, 2, 7, 14, 21, and 28 following coronary ligation in the sedentary animals. The RNA blots are probed for β-myosin gene expression, together with control gene expression of GAPDH and β-actin. There were high levels of β-myosin expression in the noninfarct myocardium soon after coronary ligation. (*C,* control; *MHC,* myosin heavy chain). (*B*) Quantitative changes in mRNA expression of β-myosin in the contralateral noninfarct zone from the sedentary infarct animals. The β-myosin gene expression from the noninfarct zone was quantitated from the the Northern blots by densitometry scanning and normalized to GAPDH (n = 5 for each time point). There was a significant increase in β-myosin/GAPDH mRNA ratio from the noninfarct myocardium on day 1 postinfarct. This increase was sustained throughout the observation period (up to 35 d) following initial infarct injury. (*MHC,* myosin heavy chain; *M.I.,* myocardial infarction; *P < 0.05 compared to day 0 baseline values).

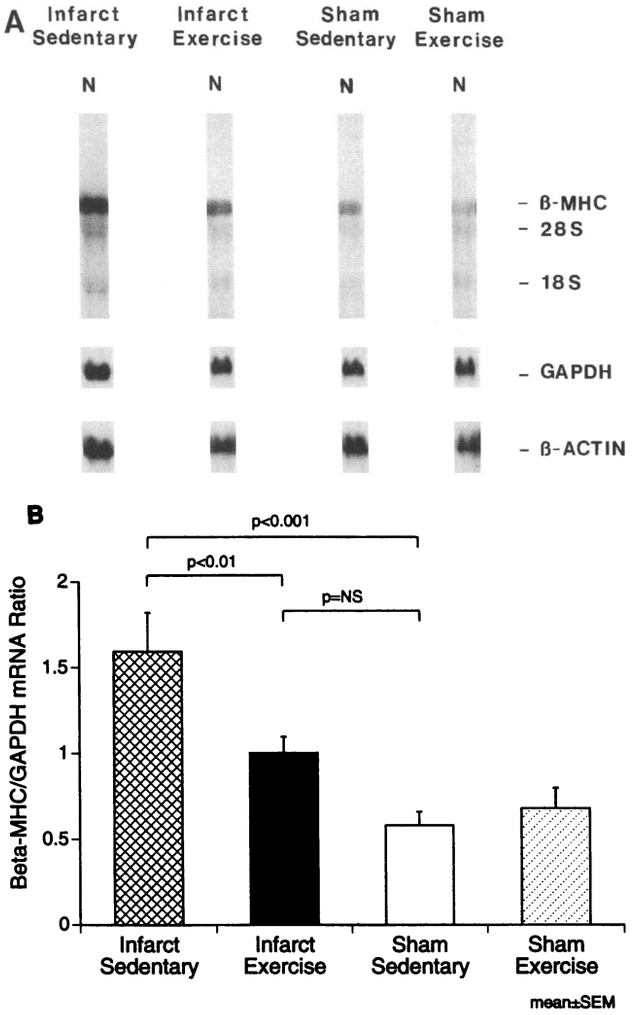

Figure 5. (A) Northern blot of representative RNA samples of each of the four experimental groups. A sample from the noninfarct zones (*N*) of the infarct sedentary (*IS*) and infarct exercise (*IE*) groups are represented. Sham sedentary (*SS*) and sham exercise (*SE*) groups are represented by samples of normal myocardium. Normal RNA bands 18s and 28s are labeled. The level of expression of control genes such as GAPDH and β-actin are also included to act as internal standards. Note the increase in β-MHC expression in the IS sample when compared SS and SE control samples, however this increase is attenuated in the IE sample. (*B*) Quantitative changes in β-MHC expression in the each of the four experimental groups at the end of intervention. The β-myosin gene expression of all the samples was quantitated from the the Northern blots by densitometry scanning and normalized to GAPDH. There was a significant increase in the β-myosin/GAPDH ratio in the infarct sedentary group, when compared to sham sedentary or sham exercise groups ($P < 0.001$, n = 5 per group). However this increase in β-myosin/GAPDH ratio was significantly attenuated in the infarct exercise group when compared to infarct sedentary group ($P < 0.01$). (*NS,* not significant)

tinued adverse remodeling leads to progressive ventricular dilation and dysfunction (2, 10–12). Myocyte hypertrophy associated with myocardial infarction appears to be the result of both pressure and volume overload creating elevation of both systolic and diastolic wall stress and resulting in increased in myocyte diameter and length. Through the operation of Laplace's law, ventricular dilation augments diastolic and systolic wall stress which stimulate a vicious cycle of progressive ventricular enlargement (34, 35). In the rat model, induction of myocardial hypertrophy after LAD ligation reduced ventricular dilation supporting the hypothesis that hypertrophy of residual myocardium

Table II. Assessment of LV Function

	Infarct sedentary	Infarct exercise	Sham sedentary	Sham exercise
	n = 11	*n = 15*	*n = 6*	*n = 6*
Peak LV pressure (mmHg)	64.1±7.8	79.6±9.8	117±4.6	125±8.7
Peak wall tension (mmHg·mm)	1294±208**	834±94	1178±45	737±41
Peak +dP/dt @ LVEDP = 10 mmHg	758±372	848±198	2279±380‡‡‡	1763±327§
Peak −dP/dt @ LVEDP = 10 mmHg	669±359	796±176	1852±193‡‡‡	1397±208§

All results are expressed as mean±standard deviation. Significant differences between groups are expressed as follows: Infarct sedentary group vs. infarct exercise group: * $P < .05$, ** $P < .01$, *** $P < .001$. Infarct sedentary group vs. sham sedentary group: ‡ $P < .05$, ‡‡ $P < .01$, ‡‡‡ $P < .001$. Infarct exercise group vs. sham exercise group: § $P < .05$, §§ $P < .01$, §§§ $P < .001$.

is inadequate in extent to limit wall tension post infarction and thus can not break the cycle leading to progressive left ventricular dilation (36). The hypertrophy also displays abnormal molecular features. There is a reversion to the fetal form of myosin heavy chain expression as demonstrated in our study. With chronic hemodynamic overload, the initial compensatory hypertrophy becomes inadequate in extent to limit wall tension, and in concert with the abnormal molecular features, may contribute to progressive ventricular decompensation (14).

The effects of exercise on ventricular remodeling. Exercise training early after myocardial infarction has been controversial as some studies have raised fears that it may aggravate infarct expansion (20, 21). The only clinical data on remodeling after acute infarction is reported by Jugdutt et al. which depicts a deleterious effect of exercise training in six high risk patients when assessed by echocardiography (37). However, this finding has not been replicated by other investigators and the timing and severity of exercise has not been investigated. The effects of exercise training in severe left ventricular dysfunction has been investigated by Coates et al. (26) and Sullivan et al. (25) and they have demonstrated significant peripheral benefits from exercise training. However the effect of exercise training on ventricular function and remodeling have not been previously investigated.

Our model of healed infarction–severe dysfunction. The rat has minimal to no coronary collaterals and thus proximal LAD occlusion creates a large transmural infarction (38). After three weeks the scar formation is complete and the infarct zone markedly thinned (29). Both the exercise trained and sedentary infarction rats had comparably thinned infarct zones which were fully healed. Only infarctions encompassing > 45% of LV perimeter were included in the analysis.

When compared to sham operated animals, both infarction groups had marked reduction in parameters of systolic contractility (+dP/dt) and diastolic relaxation (−dP/dt) in keeping with severe LV dysfunction. Thus, we were assured of examining mainly the effects of exercise training on late ventricular remodeling after scar formation in a model of severe infarction–dysfunction.

Changes in morphometry. Swim training was the training of choice in this model due to the ability to achieve relatively uniform exercise in the animals and the previously denoted evidence of achieving physiological hypertrophy without abnormal β-MHC gene expression (19). Stress factors were addressed by dipping the control animals in water during each exercise session and toweling all animals dry. Swimming and running training classically create an increase in cardiac mass by volume overload hypertrophy with a proportional increase in cavity radius and wall thickness (17, 18). In this study swim training of infarction rats was associated with increases in septal wall thickness and an attenuation of cavity dilation when compared to sedentary infarction rats. This was also reflected by the increases in overall LV mass and LV/body weight ratio, confirming an overall hypertrophy process. In this model of large healed infarction–severe dysfunction, the left ventricle was already exposed to both pressure and volume overload, and in these rats exercise training created a more normalized wall thickness to cavity area ratio.

In the noninfarct sham operated animals, there is also significant ventricular hypertrophy and a trend towards smaller cavity area with exercise training. However, these changes are much more subtle when compared to the infarcted animals in the experimental groups.

Effect of normalizing the wall tension. One of factors accounting for the more favorable ventricular remodeling following exercise training is the potential normalization of the wall tension through a reduction in cavity size and an increase in wall thickness. The wall tension equation presented above does make an overly simplified assumption that wall tension is uniform around the circumference of the heart, which is not likely true in the infarct area due to significant wall thinning. Furthermore, the cardiac dimensions were measured in diastole at postmortem, rather than systole. However it does serve as a crude estimate for the direction of changes in the wall tension parameter, and may help to identify a potential underlying theme for the changes observed.

This effect is seen in the exercised animals as well as previous studies of hypertensive models. The exact mechanisms that result in these changes are not clear, but may involve cell membrane signaling, local growth factor production, tissue renin-angiotensin, and sympathetic system modulation, or hemodynamic afterload matching during exercise. We hypothesize that the increased wall thickness is mainly due to myocyte hypertrophy even though myocyte diameter and length were not directly measured. Since myocyte cell number is typically fixed soon after birth and only minor changes in the noncellular constituents are seen with exercise training, the increase in wall thickness most likely reflects actual myocyte hypertrophy.

Ventricular function. In both endurance athletes and non athletic individuals, aerobic training induces physiologic ventricular hypertrophy which maintains normal wall stress and is not associated with impaired systolic and diastolic function (15, 16). In heart failure exercise training protocols have uniformly improved peripheral metabolism and oxygen consumption yet

there have been controversial effects on resting central hemodynamics with no direct measurements of contractility reported (24–26). As expected, the infarction exercise group did not demonstrate an improvement in resting positive or negative dP/dt compared with sedentary infarction rats. It is possible that the exercise group may have had a greater contractile reserve that was not demonstrable during resting conditions.

The physiologically hypertrophied heart is able to augment its performance under conditions of extreme demand. At rest a swim trained rat may have no increase in dP/dt but when subjected to aortic constriction it can maintain contractility whereas controls cannot (39, 40).

There was no significant difference in cavity area or perimeter and only marginal increase in lateral wall thickness in the exercise-trained sham group. This study was designed primarily to compare the effects of exercise in an infarction–dysfunction model. With the increased stress of a large infarction, this moderate level of swim training was judged to be safe yet aggressive enough to detect differences in morphometry and molecular gene expression between infarction groups. More intense swim training protocols have produced hypertrophy even in normal rats (41, 42). Given small numbers in the sham group and a moderate exercise program, there were no significant differences in hypertrophy of the septal wall and lateral wall hypertrophy reached marginal significance in the sham exercise vs. sham sedentary groups.

Serial changes of cardiac myosin gene expression after coronary ligation. The mature adult rat expresses mainly α-myosin as the major contractile protein in the left ventricle. α-MHC is associated with high ATPase activity and increased contractility. However under injury or stress conditions such as pressure overload, the rat heart myocytes will preferentially express β-MHC (5–9). β-MHC has a fivefold lower ATPase activity, conferring decreased velocity of shortening, and its increased expression in the failing heart is teleologically attributable to the more efficient utilization of decreased energy reserves. We have observed in our serial monitoring of gene expression changes following coronary ligation, that within 1 d of coronary ligation, there is an isoform switch to the β form in the contralateral noninfarcted myocardium. This is likely due to local wall stress changes and production of growth factors as part of the remodeling process following myocardial ischemic injury. This isoform switch appears to persist in the time frame that we have observed.

Modulation of cardiac myosin gene expression by exercise. Thus far there has been no previous description of maneuvers that can effectively reprogram the gene expression back towards an adult form once major cardiac injury and heart failure have occurred. Exercise conditioning in our rat infarction–dysfunction model significantly modulated the fetal β-MHC expression program back towards the adult phenotype. This is in agreement with previous studies of hypertensive rats with pathologic hypertrophy, where swim training reduced β-myosin while further augmenting hypertrophy providing evidence that the heart under adverse conditions can alter its gene expression, perhaps in keeping with a more physiologic adaptation (19).

Myosin heavy chain expression is one of the molecular features that distinguishes physiologic hypertrophy from pathologic hypertrophy which is thought to be a precursor to ultimate heart failure. However, factors that determine MHC gene expression and type of hypertrophy are not clear. Chronic hemodynamic overload and increased wall tension of the non infarcted myocardium are likely important contributors towards the progressive ventricular dysfunction and heart failure (43, 44).

Summary and conclusions. In summary, in a model of severe left ventricular dysfunction after healed myocardial infarction, hypertrophy of viable myocardium is inadequate to normalize wall stress and thus may contributes to progressive dysfunction and failure. Furthermore, the myocyte switch rapidly to a fetal expression program including high levels of β-myosin, which may contribute to further ventricular dysfunction. Exercise training in this model can improve the adverse remodeling process and significantly attenuate the abnormal expression of β-MHC.

In the future, therapies aimed at prevention or treatment of heart failure should address whether the intervention is able to preserve the differentiated functional gene program in the myocyte. This preservation may be beneficial via both hemodynamic and cellular mechanisms in limiting ventricular remodeling.

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References

1. White, H. D., R. M. Norris, M. A. Brown, P. W. T. Brandt, R. M. L. Whitelock, and C. Wild. 1987. Left ventricular end-systolic volume as the major determinant of survival after recovery from myocardial infarction. *Circulation.* 76:44–51.

2. Jeremy, R. W., K. C. Allman, G. Bautovitch, and P. J. Harris. 1989. Patterns of left ventricular dilation during the six months after myocardial infarction. *J. Am. Coll. Cardiol.* 13:304–310.

3. Hutchins, G. M., and B. H. Bulkley. 1978. Infarct expansion versus extension: two different complications of acute myocardial infarction. *Am. J. Cardiol.* 41:1127–1132.

4. McKay, R. G., M. A. Pfeffer, R. C. Passternak, J. E. Markis, C. P. Come, S. Nakao, J. D. Alderman, J. J. Ferguson, R. D. Safian, and W. Grossman. 1986. Left ventricular remodeling after myocardial infarction: a corollary to infarct expansion. *Circulation.* 74:693–702.

5. Mercadier, J. J., A. M. Lompre, C. Wisnewsky, J. L. Samuel, J. Bercovici, B. Swynghedauw, and K. Schwartz. 1981. Myosin isoenzymic changes in several models of rat cardiac hypertrophy. *Circ. Res.* 49:525–532.

6. Izumo, S., A. M. Lompre, R. Matsuoka, G. Koren, K. Schwartz, B. Nadal-Ginard, and V. Mahdavi. 1987. Myosin heavy chain messenger RNA and protein isoform transitions during cardiac hypertrophy. *J. Clin. Invest.* 79:970–977.

7. Takashashi, T., H. Schunkert, S. Isoyama, J. Y. Wei, B. Nadal-Ginard, W. Grossman, and S. Izumo. 1992. Age-related differences in the expression of proto-oncogene and contractile protein genes in response to pressure overload in the rat myocardium. *J. Clin. Invest.* 89:939–946.

8. Morkin, E. 1993. Regulation of myosin heavy chain genes in the heart. *Circulation.* 87:1451–1460.

9. Morgan, H. E. 1993. Cellular aspects of cardiac failure. *Circulation.* 87 (suppl):IV4–6.

10. Pfeffer, M. A., G. A. Lamas, D. E. Vaughan, A. F. Parisi, and E. Braunwald. 1988. Effect of captopril on progressive ventricular dilation after anterior myocardial infarction. *N. Engl. J. Med.* 319:80–86.

11. Pfeffer, J. M., M. A. Pfeffer, P. J. Fletcher, and E. Braunwald. 1991. Progressive ventricular remodeling in rat with myocardial infarction. *Am. J. Physiol.* 260:H1406–H1414.

12. Pfeffer, M. A., E. Braunwald, L. A. Moye, L. Basta, E. J. J. Brown, T. E. Cuddy, B. R. Davis, E. M. Geltman, S. Goldman, G. C. Flaker, and The SAVE Investigators. 1992. Effect of captopril on mortality and morbidity in patients with

left ventricular dysfunction after myocardial infarction. Results of the survival and ventricular enlargement trial. *N. Engl. J. Med.* 327:669–677.

13. Pouleur, H., M. F. Rousseau, C. Eyll, J. Melin, M. Youngblood, S. Yusuf, and The SOLVD Investigators. 1993. Cardiac mechanics during development of heart failure. *Circulation.* 87(suppl):IV83–89.

14. Gaudron, P., C. Eilles, G. Ertl, and K. Kochsiek. 1993. Adaptations to cardiac dysfunction after myocardial infarction. *Circulation.* 87(suppl):IV83–89.

15. Sharpiro, L. M., and R. G. Smith. 1983. Effect of training of left ventricular structure and function - an echocardiographic study. *Br. Heart. J.* 50:534–539.

16. Granger, C. B., M. K. Karimeddini, V. E. Smith, H. R. Shapiro, A. M. Katz, and A. L. Riba. 1985. Rapic ventricular filling in left ventricular hypertrophy: 1. Physiologic hypertrophy. *J. Am. Coll. Cardiol.* 5:862–867.

17. Snoeckx, L. H. E. H., H. F. M. Abeling, J. A. C. Lambregts, J. J. F. Schmitz, F. T. T. J. Verstappen, and R. S. Reneman. 1982. Echocardiographic dimensions in athletes in relation to their training programs. *Med. Sci. Sports. Ex.* 14:428–434.

18. Morganroth, J., B. J. Maron, W. L. Henry, and S. E. Epstein. 1975. Comparative left ventricular dimensions in trained athletes. *Ann. Int. Med.* 82:521–524.

19. Scheuer, J., A. Malhorta, C. Hirsch, J. Capasso, and T. F. Schaible. 1982. Physiologic cardiac hypertrophy corrects contractile protein abnormalities associated with pathologic hypertrophy in rats. *J. Clin. Invest.* 70:1300–1305.

20. Hochman, J. S., and B. Healy. 1986. Effect of exercise on acute myocardial infarction in rats. *J. Am. Coll. Cardiol.* 7:126–132.

21. Kloner, R. A., and J. A. Kloner. 1983. The effect of early exercise on myocardial infarct scar formation. *Am. Heart. J.* 106:1009–1013.

22. Oh, B. H., S. Ono, H. A. Rockman, and J. Ross Jr. 1993. Myocardial hypertrophy in the ischemic zone induced by exercise in rats after coronary reperfusion. *Circulation.* 87:598–607.

23. Minotti, J. R., E. C. Johnson, T. L. Hudson, G. Zuroske, G. Murata, E. Fukushima, T. G. Cagle, T. W. Chick, B. M. Massie, and M. V. Icenogle. 1990. Skeletal muscle response to exercise training in congestive heart failure. *J. Clin. Invest.* 86:751–758.

24. Musch, T. I., R. L. Moore, D. J. Leathers, A. Bruno, and R. Zelis. 1986. Endurance training in rats with chronic heart failure induced by myocardial infarction. *Circulation.* 74:431–441.

25. Sullivan, M. J., M. B. Higginbotham, and F. R. Cobb. 1988. Exercise Training in patients with severe left ventricular dysfunction: hemodynamic and metabolic effects. *Circulation.* 78:506–515.

26. Coats, A. J. S., S. Adamopoulos, A. Radelli, A. McCance, T. E. Meyer, L. Bernardi, P. L. Solda, P. Davey, O. Ormerod, C. Forfar, J. Conway, and P. Sleight. 1992. Controlled trial of physical training in chronic heart failure—exercise performance; hemodynamics; ventilation; and autonomic function. *Circulation.* 85:2119–2131.

27. Conn, E. H., R. S. Williams, and A. G. Wallace. 1982. Exercise responses before and after physical conditioning in patients with severely depressed left ventricular function. *Am. J. Cardiol.* 49:296–300.

28. Minotti, J. R., and B. M. Massie. 1992. Exercise training in heart failure patients—does reversing the peripheral abnormalities protect the heart? *Circulation.* 85:2323–2325.

29. Fishbein, M. C., M. B. Maclean, and P. R. Maroko. 1978. Experimental myocardial infarction in the rat. *Am. J. Pathol.* 90:57–70.

30. Chomczynski, P., and N. Sacchi. 1987. Single-step method of RNA isolation by acid guanidinium thiocyanate-phenol chloroform extraction. *Anal. Biochem.* 162:156–159.

31. Rosen, K. M., and L. Villa-Komaroff. 1990. An alteration method for the visualization of RNA in formaldehyde agarose gels. *Focus.* 12:23–24.

32. Wee, L., P. Liu, L. Penn, J. W. Butany, P. R. McLaughlin, M. J. Sole, and C. C. Liew. 1992. Persistence of viral genome into late stages of murine myocarditis detected by polymerase chain reaction. *Circulation.* 86:1605–1614.

33. Weisman, H. F., D. E. Bush, J. A. Mannisis, M. L. Wiesfeldt, and B. Healy. 1988. Cellular mechanisms of myocardial infarct expansion. *Circulation.* 78:186–201.

34. Pfeffer, M. A., and E. Braunwald. 1990. Ventricular remodeling after myocardial infarction: experimental observations and clinical implications. *Circulation.* 81:1161–1172.

35. Anversa, P., A. V. Loud, V. Levicky, and G. Guideri. 1985. Left ventricular failure induced by myocardial infarction: myocyte hypertrophy. *Am. J. Physiol.* 248:H876–H882.

36. Litwin, S. E., T. E. Raya, P. G. Anderson, C. M. Litwin, R. Bressler, and S. Goldman. 1991. Induction of myocardial hypertrophy after coronary ligation in rats decreases ventricular dialtion and improves systolic function. *Circulation.* 84:1819–1827.

37. Jugdutt, B. I., B. L. Michorowski, and C. T. Kappagoda. 1988. Exercise training after anterior Q wave myocardial infarction: importance of regional left ventricular function and topography. *J. Am. Coll. Cardiol.* 12:362–372.

38. Hochman, J. S., and B. H. Bulkley. 1982. Expansion of acute myocardial infarction: an experimental study. *Circulation.* 65:1446–1450.

39. Dowell, R. T., A. F. Cutilletta, M. A. Rudnik, and P. C. Sodt. 1976. Heart functional responses to pressure overload in exercised and sedentary rats. *J. Appl. Physiol.* 230:199–204.

40. Schaible, T. F., and J. Scheruer. 1979. Effects of physical conditioning by running or swimming on ventricular performance of rat hearts. *J. Appl. Physiol.* 46:854–860.

41. Schaible, T. F., and J. Scheuer. 1981. Cardiac function in hypertrophied hearts from chronically exercised female rats. *J. Appl. Physiol.* 50:1140–1145.

42. Pfeffer, M. A., B. A. Ferrell, J. M. Pfeffer, K. A. Weiss, M. C. Fishbein, and E. D. Frohlich. 1978. Ventricular morphology and pumping ability of exercised spontaneously hypertensive rats. *Am. J. Physiol.* 235:H193–H199.

43. Katz, A. M. 1990. Cardiomyopathy of overload: a major determinant of prognosis in congestive heart failure. *N. Engl. J. Med.* 322:100–110.

44. Anversa, P., and E. H. Sonnenblick. 1990. Ischemic Cardiomyopathy: pathophysiologic mechanisms. *Prog. Cardiovasc. Dis.* 33:49–70.