

Aerobic exercise reduces cardiomyocyte hypertrophy and increases contractility, Ca^{2+} sensitivity and SERCA-2 in rat after myocardial infarction

Ulrik Wisløff^a, Jan P. Loennechen^a, Susan Currie^b, Godfrey L. Smith^b, Øyvind Ellingsen^{a,*}

^aDepartment of Physiology and Biomedical Engineering, Norwegian University of Science and Technology, OLav Kyrres gt. 3., N-7489 Trondheim, Norway

^bInstitute of Biomedical and Life Sciences, University of Glasgow, Glasgow, UK

Received 16 July 2001; accepted 29 November 2001

Abstract

Objective: Although it is generally accepted that endurance training improves cardiac function after myocardial infarction the sub-cellular mechanisms are uncertain. The present study reports the effects of aerobic endurance training on myocardial mass, myocyte dimensions, contractile function, Ca^{2+} handling, and myofilament responsiveness to Ca^{2+} in cardiomyocytes from healthy and failing rat hearts. **Methods:** Adult female Sprague–Dawley rats ran on a treadmill 1.5 h/day, 5 days a week for 8 weeks. Exercise intervals alternated between 8 min at 85–90% of $V_{O_{2\max}}$ and 2 min at 50–60%. Training started 4 weeks after ligation of the left coronary artery (TR-INF, $n=11$) or sham operation (TR-SHAM, $n=6$). Sedentary animals (SED-SHAM, $n=6$; SED-INF, $n=13$) were controls. **Results:** After 6 weeks $V_{O_{2\max}}$ in TR-INF and TR-SHAM leveled off 65% above sedentary controls. In TR-SHAM, left and right ventricle weights were ~25% higher than in SED-SHAM, myocytes were ~13% longer; width remained unchanged. At physiological stimulation frequencies, relative myocyte shortening was markedly higher whereas peak systolic $[\text{Ca}^{2+}]$ and $t_{1/2}$ of Ca^{2+} transient decay were 10–20% lower, indicating higher Ca^{2+} sensitivity in cardiomyocytes from trained rats, compared to respective controls. In TR-INF the left and right ventricular weights, and myocyte length and width were 15, 23, 12, and 20% less than in SED-INF. Endurance training significantly increased the myocardial SR Ca^{2+} pump (SERCA-2) and sarcolemmal $\text{Na}^+/\text{Ca}^{2+}$ -exchanger (NCX) protein levels to the extent that TR-INF did not differ from SED-SHAM. **Conclusion:** This is the first study to show that aerobic endurance training attenuates the ventricular and cellular hypertrophy in failing hearts. Furthermore, training consistently restores contractile function, intracellular Ca^{2+} handling, and Ca^{2+} -sensitivity in cardiomyocytes from rats with myocardial infarction. © 2002 Elsevier Science B.V. All rights reserved.

Keywords: Heart failure; Hypertrophy; Myocytes

1. Introduction

Current treatment of patients after myocardial infarction (MI) often includes exercise training as an element of cardiac rehabilitation. However, the effects of exercise training on left ventricle (LV) remodeling post-MI have remained controversial. In an early study of patients with extended anterior myocardial infarction, Jugdutt et al. [1] found increased LV dilation and decreased regional and global cardiac function following exercise. In contrast, recent studies suggest that physical training increases

endurance capacity, attenuates LV dilation, improves cardiac function, quality of life, and reduces mortality in patients with heart failure [2–6]. Similar disparity exists in data from animal models; exercise after MI in rats has generally caused ventricular enlargement [7,8], with only one study describing decreased LV dilation [9]. The reason for this lack of consensus is unknown; one possible variable is the form of exercise training undertaken. The cellular basis for contractile dysfunction in post-MI myocardium has been extensively studied [10–12]. An important focus of research has been altered intracellular Ca^{2+} transient in post-MI myocytes, which has been

*Corresponding author. Tel.: +47-735-98-822; fax: +47-735-98-613.
E-mail address: Oyvind.Ellingsen@medisin.ntnu.no (Ø. Ellingsen).

Time for primary review 37 days.

attributed to altered expression of the $\text{Na}^+ - \text{Ca}^{2+}$ -exchanger (NCX) activity, sarcoplasmic reticulum Ca^{2+} -ATPase (SERCA-2) and phospholamban (PLB) [13–15]. However, these changes are not uniform across studies, either due to differences in infarct size or species variation. NCX levels can be increased [16], decreased [15] or not changed [13] in post-MI myocardium, similarly reported changes in SERCA-2 are not consistent [16]. In contrast, treadmill running generally increases SERCA-2 activity [17] and NCX [18,19] in conjunction with improvements in myocyte contractility [19]. Interestingly, Zhang et al. reported that in post-MI rats, myocytes were larger and had a depressed Ca^{2+} transient along with decreased SERCA-2 and PLB expression. Intense anaerobic training post-MI significantly restored cell length [20] and systolic $[\text{Ca}^{2+}]$ but further decreased SERCA-2 and PLB levels [21]. It is not known whether a more clinically relevant aerobic exercise regimen affects cellular adaptations similarly [21]. However, in a recent study [19] we showed that aerobic endurance training increased cardiomyocyte contractility, SERCA-2 and PLB protein content, and improved myofilament sensitivity in healthy rats.

Several factors may contribute to post-MI contractile dysfunction. Either DNA-microarray or single gene analysis indicates that several hundred genes are substantially up-regulated, e.g. endothelin-1 (ET-1), atrial natriuretic peptide (ANP) and insulin-like growth factor (IGF-1) [22]. Even though adaptation to physical exercise includes cardiac hypertrophy in healthy individuals [23], the pattern of myocardial gene expression appears to differ from those observed secondary to hypertrophy in cardiovascular disease [24,25]. It is not known whether any beneficial effects of exercise on MI-induced contractile dysfunction is caused by further hypertrophy or reversal of the hypertrophic response. The aim of the present study was to compare the effects of intensity-controlled aerobic interval training [23] on myocardial mass, cardiomyocyte dimensions, contractile function, Ca^{2+} handling, and myofilament responsiveness to Ca^{2+} in myocytes from healthy and failing rat hearts.

2. Methods

2.1. Study population

Female 300–325 g Sprague–Dawley rats (Møllegaards Breeding Center Ltd., Denmark) were housed under a 12 h dark and 12 h light cycle. Temperature was 22.5 ± 1.4 and humidity $55.6 \pm 4.0\%$. Animals were fed a pellet rodent diet ad libitum and had free access to water. The experimental procedures conformed to the European Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes, and the protocol was approved by the Norwegian Council for Animal Research.

2.2. Myocardial infarction procedure

Animals were anesthetized with 3% isoflurane (Forene, Abbott UK) in a 70% O_2 –30% N_2O mixture and intubated and ventilated on a volume-controlled ventilator (model 655, Harvard Apparatus; Ednbridge, UK). Anesthesia was maintained with 1% isoflurane in a 70% O_2 –30% N_2O mixture during the procedure. After a left thoractomy was performed, the pericardium was opened, and the left coronary artery was ligated with a polyester suture (Ethibond 6-0, needle RB-2, Ethicon; Norderstedt, Germany). Sham-operated animals were subjected to the same surgical procedures except the coronary artery ligation. All animals were given 0.05 mg buprenorphin (Temgesic, 0.3 $\text{mg} \cdot \text{ml}^{-1}$, Reckitt and Coleman; Hull, UK) subcutaneously immediately and 10 h after surgery. Echocardiography (2D and M-mode) was performed after 5–6 days to exclude animals with small infarcts according to the procedure described by Litwin et al. [26]. About 40% of the operated rats survived the protocol and had large infarctions ($44.4 \pm 2.5\%$ of the left ventricle, located at the free anterior wall). Thirty-six rats were randomized to the following groups: infarction and training (TR-INF, $n=13$), infarction and sedentary (SED-INF, $n=11$), sham and training (TR-SHAM, $n=6$), and sham and sedentary (SED-SHAM, $n=6$). Three MI rats, one TR-INF, and two SED-INF died before the end of the protocol.

2.3. Training and test procedure

Four weeks after infarction, rats started training on a treadmill (25° inclination $\sim 47\%$) 1.5 h per day, 5 days per week for 8 weeks. At the start of every week, maximal oxygen uptake ($V_{\text{O}_{2\text{max}}}$) was measured and workloads adjusted accordingly as previously described [19,23]. Exercise intervals alternated between 8 min at 85–90% of $V_{\text{O}_{2\text{max}}}$ and 2 min at 50–60%. Before the first interval, each rat performed a 20 min warm-up at 40–50% of $V_{\text{O}_{2\text{max}}}$. At the day of $V_{\text{O}_{2\text{max}}}$ testing, trained rats performed five intervals after the test. In sedentary rats, running skill was maintained by treadmill running for 15 min at 0° inclination at $0.15 \text{ m} \cdot \text{s}^{-1}$ 3 days per week. After each training or test session, each rat was rewarded with 0.5 g chocolate; sedentary rats were given the same amount.

2.4. Experimental procedures

Echocardiography was performed after sedation with ketamine hydrochloride ($40 \text{ mg} \cdot \text{kg}^{-1}$) and xylazine ($8 \text{ mg} \cdot \text{kg}^{-1}$) intraperitoneally, using GE Vingmed Ultrasound System FiVe ultrasound scanner and a 10 MHz linear array probe (GE Vingmed Ultrasound, Horten, Norway) 1–3 days before measuring left ventricular pressures. Diastolic and systolic left ventricular wall thickness and cavity

diameters were calculated as the mean of five consecutive cardiac cycles in M-mode long axis following the recommendations of the American Society of Echocardiography [27]. The left and right atria diameter were measured as the longest systolic diameter perpendicular to aortic wall in 2D long axis recordings of the aortic valve and ascending aorta. Mitral inflow deceleration time, peak velocity of early and late component of mitral inflow, and isovolumetric relaxation time were calculated as the mean of five consecutive cycles of pulsed wave Doppler spectra recordings. A representative echo trace of left ventricular M-mode echocardiographic and mitral flow-Doppler is presented in Fig. 1. As previously described [22], left ventricular pressures were measured with a micro-tip catheter transducer, model SPR 407 2F (Millar Instruments, Houston, TX, USA) introduced through the right carotid artery during ventilation with 0.5% isoflurane in 70% O₂–30% N₂O mixture. End diastolic, peak systolic pressures and peak rate of left ventricular rise ($+dP/dt_{\max}$) and fall ($-dP/dt_{\max}$) were calculated as the mean of five consecutive pressure cycles.

2.5. Myocyte isolation

After 8 weeks of the experimental period, and 1–3 days after LV pressure measurement, the animals were anaesthetized with diethyl ether and heparinized. Hearts were rapidly removed from the animals and kept 1 min in ice-cold perfusion buffer, and connected to an aortic cannula of a standard Langendorff retrograde perfusion

system. To minimize the effect of day to day variation, LV cardiomyocytes were isolated from two of the four groups each day using a balanced design. Myocytes were isolated from septal plus left ventricular free wall portions of the myocardium using collagenase as previously described [23]. The heart was retrogradely perfused via aorta ($7.5 \text{ ml} \cdot \text{min}^{-1}$) for 10 min with a medium (A) consisting of 24 g Jokliks Medium (cat. no. 223000, Life Technologies, Paisley, Scotland) mixed in 2000 ml deionized water added with (mM) 1.2 MgSO₄, 1.0 DL-carnitine (Sigma, St Louis, MO), and 23.8 NaHCO₃. Medium A was equilibrated with 5% CO₂ and 95% O₂ for 15 min (37 °C, pH 7.4). After 10 min, the hearts were perfused for 20 min with medium B ($7.5 \text{ ml} \cdot \text{min}^{-1}$), which consisted of 300 ml of medium A mixed with 150 U·ml⁻¹ collagenase (Worthington, Freehand, NJ) and 0.1% bovine serum albumin (Sigma). After 10 min medium B was collected for later use. After B-perfusion, heart were cut into left and right ventricles, weighed and samples (~300 mg) was cut of, frozen in liquid nitrogen, and stored at -80 °C for later gene analysis. Left ventricular tissue was cut into infarcted, border zone (0–2 mm outside the infarction) and remote areas. During this procedure the hearts were kept in medium C containing 125 ml medium A supplied with 1% bovine serum albumin and 1.5 mM CaCl₂, equilibrated with 5% CO₂ and 95% O₂. Left ventricular tissue was cut into small pieces, in medium C and shaken for 10 min (37 °C, 5% CO₂, 95% O₂, 100 rpm). The supernatant was removed, 20 ml of medium B was added, and the tissue was shaken for 30 min (37 °C, 5% CO₂, 95% O₂, 150 rpm).

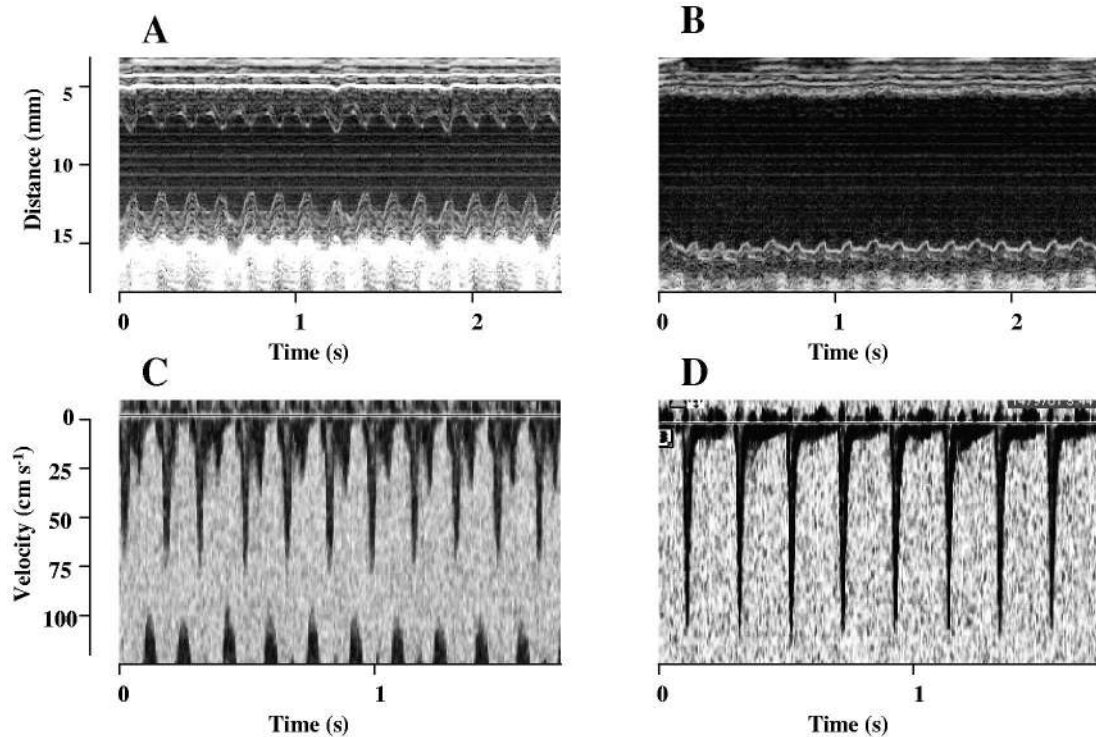


Fig. 1. Left ventricular M-mode echocardiographic (Panels A and B) and mitral inflow Doppler (Panels C and D) recordings 12 weeks after myocardial infarction (Panels B and D) or sham operation (Panels A and C) in sedentary rats.

Thereafter, 10 ml of medium C was added to each cell suspension before centrifugation for 20 s at 600 rpm (37 °C). The supernatant was gently removed and another 10 ml of medium C was added. After centrifugation, the supernatant was gently removed and 5 ml of medium C was added before filtering through a nylon mesh (250 µm). Coverslips were coated with 10 mg·ml⁻¹ laminin (Life Technologies) in medium 199. Myocytes were isolated from septal plus left ventricular free wall portions of the myocardium (mainly septum because of the infarction), attached to coverslips and loaded with Fura-2 [19].

2.6. Intracellular [Ca²⁺] and cell shortening measurements

Cells were studied in a cell chamber on an inverted microscope (Diaphot-TMD, Nikon, Tokyo, Japan), and stimulated electrically by bipolar pulses (5 ms duration, 2–10 Hz, 37 °C). Cells that remained rod shaped, without blebs or other visible morphological alterations, and responded adequately on 5–10 s of 2 Hz electrical stimulation were measured for length and midpoint width. Cellular dimensions were calculated from about 6500 cells, i.e. approximately 200 myocytes from each animal; all measurements were made 1–3 h after cell isolation. Only data from cells that followed the full stimulation protocol were used for analysis (97%). Although stimulus rates of 10 Hz are close to in vivo values they are rarely used in in vitro studies. In pilot studies, the ability of rat myocytes to follow these high rates of stimulation was found to be very temperature dependent, at temperatures below 35 °C myocytes would not follow stimulation rates above at 5 Hz. In about 10% of the cells poor contrast of cellular edges prevented cell length measurements. During stimulation the cells were superfused at 2 ml·min⁻¹ with HEPES buffer at 37 °C. The amplitude of cell shortening and the velocity of contraction and relaxation, and Fura-2 fluorescence were analyzed using a video/edge monitor detector and photomultiplier as previously described [19,28]. Each heart provided measurements from six to 12 cells. Since time to peak cell shortening normally increases with increased fractional shortening [58], we calculated the relative time to peak shortening. Minimum fluorescence ratio ($R_{\min}=0.47\pm0.05$) and maximum fluorescence ratio ($R_{\max}=6.7\pm0.6$) fluorescence ratio, and β (6.2 ± 0.13) were determined using a protocol described by Frampton et al. [29]. R_{\min} , R_{\max} and β were similar among groups. Intracellular [Ca²⁺] was calculated assuming a dissociation constant of 200 nM [30]. Cardiomyocyte shortening and Fura-2 fluorescence data from each cell were calculated from ten consecutive contractions after stabilization at each stimulation frequency.

2.7. Preparation and measurements of permeabilised myocytes

Cells were isolated as previously described [19], with

[Ca²⁺] kept below 100 nM during and after the isolation procedure, and β -escin (Sigma (100 µg/ml) was used to permeabilize cells. To study effects of training on myofibrillar responsiveness to Ca²⁺, cells were perfused with progressively increasing [Ca²⁺] concentrations (pH 7.0, 37 °C) (42, 140, 210 and 315 nM buffered by 10 mM EGTA). The equilibrium concentrations of metal ions in the solutions were calculated using a computer program with the affinity constants for H⁺, Ca²⁺ and Mg²⁺ for EGTA [30]. The affinity constants used for ATP and CrP were those quoted by Fabiato and Fabiato [31]. Corrections for ionic strength, details of pH measurement, allowance for EGTA purity and the principles of the calculations are detailed elsewhere [32]. Free Mg²⁺ concentration was 0.9–1 mM in all solutions.

2.8. Electrophoresis, immunoblotting and densitometry

Samples of intraventricular septum were frozen and stored at -80 °C after animal sacrifice. Before analysis the tissue was defrosted to 0 °C and homogenised in ice cold homogenization buffer. Protein content was determined using the Coomassie Plus protein assay (Pierce) and BSA (0.1–1 mg/ml) as standard. SDS-PAGE was performed as described by Currie and Smith [33] with 6% Tris-glycine gels for SERCA-2 and NCX gels followed by blotting onto a nitro-cellulose membrane (Hybond C, Amersham). Membranes were then exposed to primary antibodies for one of the two specific proteins under study. SERCA-2; the primary antibody used was mouse anti-SERCA-2 monoclonal antibody (IgG₁, Affinity Bioreagents, 1:4000). The secondary antibody was goat anti-mouse IgG-horseradish peroxidase conjugate (Transduction Laboratories, 1:2000). NCX; the primary antibody used was polyclonal antisera raised against canine sarcolemmal NCX (Swant, Switzerland, 1:1000). The secondary antibody was goat anti-rabbit-horseradish peroxidase conjugate (Transduction Laboratories, 1:2000). Protein abundance was quantified by scanning developed immunoblots containing known amounts of total homogenate protein. Measurements were triplicate and average densitometric measurement was taken over the linear range of protein loading (5–25 µg). Protein content was determined using the Coomassie Plus protein assay (Pierce) and BSA (0.1–1 mg·ml⁻¹) as standard. Measurements were performed in triplicate and the average densitometric measurement was taken over the linear range of protein loading (6.26–25 µg). Signals were detected by enhanced chemiluminescence. Maximum light emission is at a wavelength of 428 nm and this is detected by short exposure to blue-light sensitive autoradiography film.

2.9. Competitive reverse transcriptase polymerase chain reaction (RT-PCR)

Myocardial mRNA was isolated with Dynabeads Oligo

(dT)₂₅ (DynaL AS, Oslo, Norway) as previously described [19,22]. Competitive RT-PCR was performed in a Perkin Elmer GeneAmp 2400 PCR system using *rTth* DNA polymerase (Perkin Elmer/Roche Molecular Systems Inc., Branchburg, NJ, USA) according to manufacturers instructions. Reverse transcription and quantification of ET-1, ANP, NHE, and IGF-1 was performed as previously described [19].

2.10. Statistical analysis

Data are presented as mean±S.D. In the final calculations, each animal was represented by the mean of the cells with complete stimulation protocols. Curves representing different treatment groups were compared in a ANOVA model with treatment group as factor and frequency or calcium concentration as repeated measurement variable. The Scheffé statistic was used to calculate post hoc *P*-values of difference between groups. The Friedman test for related observations with appropriate procedures for multiple comparisons was used for testing of differences over time in the weekly measurements of $V_{O_{2max}}$. For comparison of unrelated observations we used the Kruskal–Wallis test including appropriate procedures for multiple comparisons between groups. *P*<0.05 was considered statistically significant.

3. Results

3.1. Heart failure

In the present study, coronary ligation induced infar-

tions that corresponded to 44% of the left ventricle. MI induced marked left ventricular dilation; 55% (*P*<0.001) increase in diastolic and 110% (*P*<0.001) in systolic diameter (Table 1). MI induced substantial myocardial hypertrophy; left and right ventricular weight increased by 80 and 125%, respectively, whereas cardiomyocyte length and width increased by 31 and 41% (Table 2). Left ventricular end-diastolic pressure during anesthesia was about 35 mmHg and confirmed heart failure progression with reduced peak $+dP/dt_{max}$ and $-dP/dt_{max}$, and peak systolic pressure. Atrial dilation was about 30% (*P*<0.001, Table 1).

3.2. Cardiorespiratory performance

Intensity controlled interval training significantly increased $V_{O_{2max}}$ every week until a plateau 65% above control was reached after 5–7 weeks for both TR-INF and TR-SHAM (Fig. 2A). Resting metabolism did not change in any group in the present study. Maximal heart rate was lower in SED-INF compared to the other groups. Training reduced resting heart rate by 30–50 beats·min⁻¹ (Table 2). Work economy, measured as oxygen uptake at a given submaximal running speed, improved by 16% (S.D.=3.5, *P*<0.001) in trained animals. At any given submaximal exercise intensity, heart rate was lowered by 5.5% (S.D.=2.2, *P*<0.01) in trained rats. Training consistently increased oxygen pulse, indicating increased stroke volume both at rest and during submaximal and maximal exercise (Fig. 2B, Table 2).

3.3. Training-induced cardiac hypertrophy

Consistent with previous experiments, the present train-

Table 1
Left ventricular echocardiographic and pressure measurements

	SED-INF	TR-INF	SED-SHAM	TR-SHAM
Left ventricular internal diameter diastole, mm	11.1±0.7**	11.3±0.4**	7.2±0.6	7.2±0.5
Left ventricular internal diameter systole, mm	10.1±1.1**	10.2±0.5**	4.8±0.8	4.9±0.6
Anterior wall thickness diastole, mm	0.5±0.2**	0.5±0.2**	1.9±0.3	2.0±0.2
Anterior wall thickness systole, mm	0.5±0.2**	0.5±0.2**	3.0±0.5	3.0±0.2
Posterior wall thickness diastole, mm	2.3±0.3	2.5±0.5	2.4±0.4	2.5±0.3
Posterior wall thickness systole, mm	3.1±0.9	3.1±0.6	3.0±0.2	3.0±0.1
Fractional shortening, %	9.0±5.9*	9.5±3.6**	33.8±4.1	30.9±5.7
Left atria diameter, mm	5.6±5.8*	5.6±6.1**	4.4±10.1	4.2±3.6
Right atria diameter, mm	4.7±6.5*	4.9±6.0*	3.7±3.7	3.7±2.1
E wave, peak velocity, cm·s ⁻¹	104±19*	112±11*	76±12	68±18
A wave, peak velocity, cm·s ⁻¹	17±8**	15±4**	33±9	33±9
E wave deceleration time, ms	30±5**	29±6**	40±9	46±13
Isovolumetric relaxation time, ms	22±4*	22±4*	27±3	29±5
Left ventricular end-diastolic pressure, mmHg	34.3±5.9*	34.5±4.6*	5.0±2.1	3.9±3.9
Left ventricular peak systolic pressure, mmHg	106.0±7.4*	113.8±7.9*	136.6±18.7	150.7±16.0
Aortic diastolic pressure, mmHg	79.9±7.7*	88.6±7.8*	93.3±20.8	107.3±17.3
Left ventricular $+dP/dt_{max}$, mmHg·ms ⁻¹	5.3±0.5**	5.6±0.7**	12.5±1.5	12.2±2.1
Left ventricular $-dP/dt_{max}$, mmHg·ms ⁻¹	3.4±0.4**	3.7±0.7**	12.1±2.4	12.7±1.3

Values are mean±S.D. * indicates differences from sham rats, *P*<0.01; ** *P*<0.001. dP/dt_{max} , peak rate of left ventricular rise (+) and fall (-); E wave, early left ventricular filling; A wave, filling after atrial contraction. SED-INF, sedentary infarcted rats; TR-INF, trained infarcted rats; SED-SHAM, sedentary sham-rats; TR-SHAM, trained sham rats.

Table 2
In vivo and post mortem data

	SED-INF	TR-INF	SED-SHAM	TR-SHAM
Resting heart rate (beats·min ⁻¹)	412±7.3	344±8.8**	387±6.8	326±6.9**
Maximal heart rate (beats·min ⁻¹)	618±7.0	628±6.4	636±8.3	633±7.1
Oxygen pulse (V _{O₂} ·HR ⁻¹)(ml·kg ⁻¹ ·beat ⁻¹)				
Rest	0.040±0.002	0.052±0.002*	0.046±0.004	0.055±0.002*
Maximal	0.09±0.004	0.15±0.004*	0.12±0.005	0.19±0.006
Body weight (g)				
Pre	263.4±16.7	271.3±11.4	274.5±9.5	281.8±6.7
Post	285.7±21.8	284.5±20.5	292.2±18.2	275.5±15.2
Right ventricular mass (mg)	717.3±76.6	555.2±68.6**	318.8±29.2	405.1±45.3**
Left ventricular mass (mg)	1575.1±67.0	1339.0±90.1**	876.4±74.1	1097.6±84.5**
Left ventricular cell length (μm)	156.0±1.5	138.7±1.2**	119.3±1.8	134.3±1.8**
Left ventricular cell width (μm)	35.96±1.1	28.63±1.6**	25.50±1.0	25.43±1.1
Left ventricular infarction (%)	43.8±2.2	44.9±2.7	–	–

Values are mean±S.D. V_{O₂}, oxygen uptake; HR, heart rate, * indicates differences between groups $P<0.05$; ** $P<0.001$. Cell length and width are calculated from cardiomyocytes from the remote area. SED-INF, sedentary infarcted rats; TR-INF, trained infarcted rats; SED-SHAM, sedentary sham-rats; TR-SHAM, trained sham rats.

ing protocols induced marked adaptive cardiac hypertrophy in non-infarcted rats. LV and RV weights were 25 and 27% higher in TR-SHAM than in SED-SHAM. Myocytes were 13% longer, whereas width was unchanged. In contrast, training reduced cardiac hypertrophy associated with myocardial infarction, as assessed by LV and RV weights (reduced by 15 and 23%, respectively) and myocyte dimensions, (length 12% and width 20% lower, respectively) (Table 2).

3.4. Myocyte contractility and Ca²⁺ handling

Training attenuated the impairment in cardiac function, myocyte contractility, and Ca²⁺-handling substantially. In TR-INF cardiomyocyte shortening was about 60% higher, whereas peak Ca²⁺ transients were 18% lower (S.D. = 3.6%, $P<0.01$) than in SED-INF (Fig. 3), but 10%

(S.D. = 4.1%) higher than in SED-SHAM (Fig. 3C). Surprisingly, the Ca²⁺ amplitude were highest in the SED-INF group, and lowest in the TR-SHAM group ($P<0.05$), with no differences between TR-INF and SED-SHAM (Fig. 3). A Ca²⁺-sensitivity index (myocyte shortening/Ca²⁺-amplitude-ratio) was 5–35% higher in TR-INF than in SED-INF. There were also indications of a lusitropic effect with reduced time to 50% relaxation after training, corresponding with reduced time to 50% decay of intracellular [Ca²⁺], indicating increased Ca²⁺-clearance from cytosol (Table 3). Endurance training reduced time to peak shortening normalized to fractional shortening (Table 3), and no difference were observed between SED-SHAM and TR-INF. In TR-SHAM, cardiomyocyte shortening was 60% higher than in SED-SHAM, whereas corresponding peak Ca²⁺ transients, Ca²⁺ amplitude and $t_{1/2}$ of decay were 10, 30 and 11% lower, indicating higher Ca²⁺

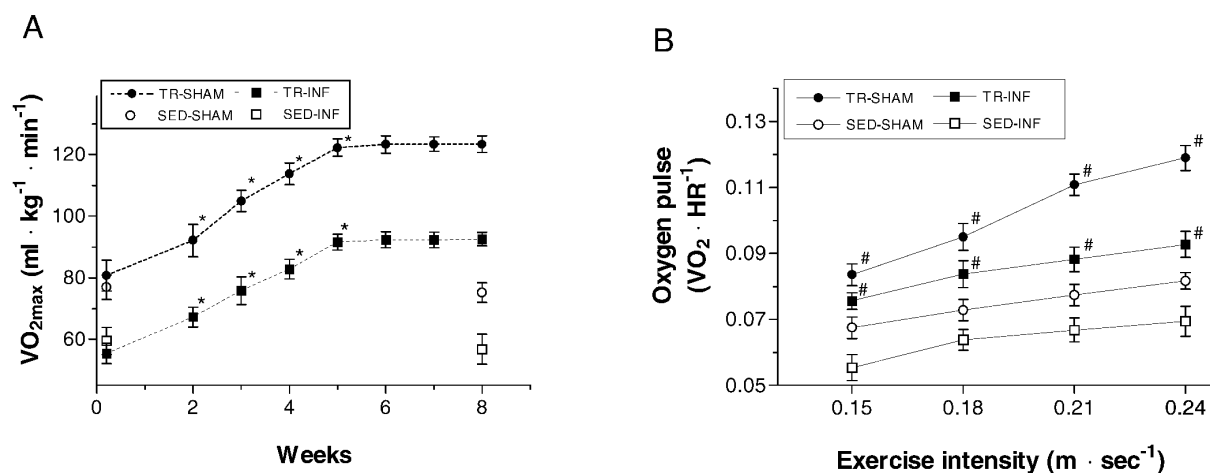


Fig. 2. A: mean±S.D. of maximal oxygen uptake (V_{O_{2max}}) in the experimental period. At pre-test there were differences in V_{O_{2max}} between SHAM and INF rats, $P<0.01$. At post-test there were differences in V_{O_{2max}} between all groups, $P<0.01$. Maximal oxygen uptake in TR-SHAM was significant different from TR-INF at every week, $P<0.01$. * indicates differences from previous week, $P<0.02$. B: oxygen pulse at submaximal exercise intensities after 8 weeks. # indicates differences between groups, $P<0.01$. All curves are significantly different ($P<0.01$).

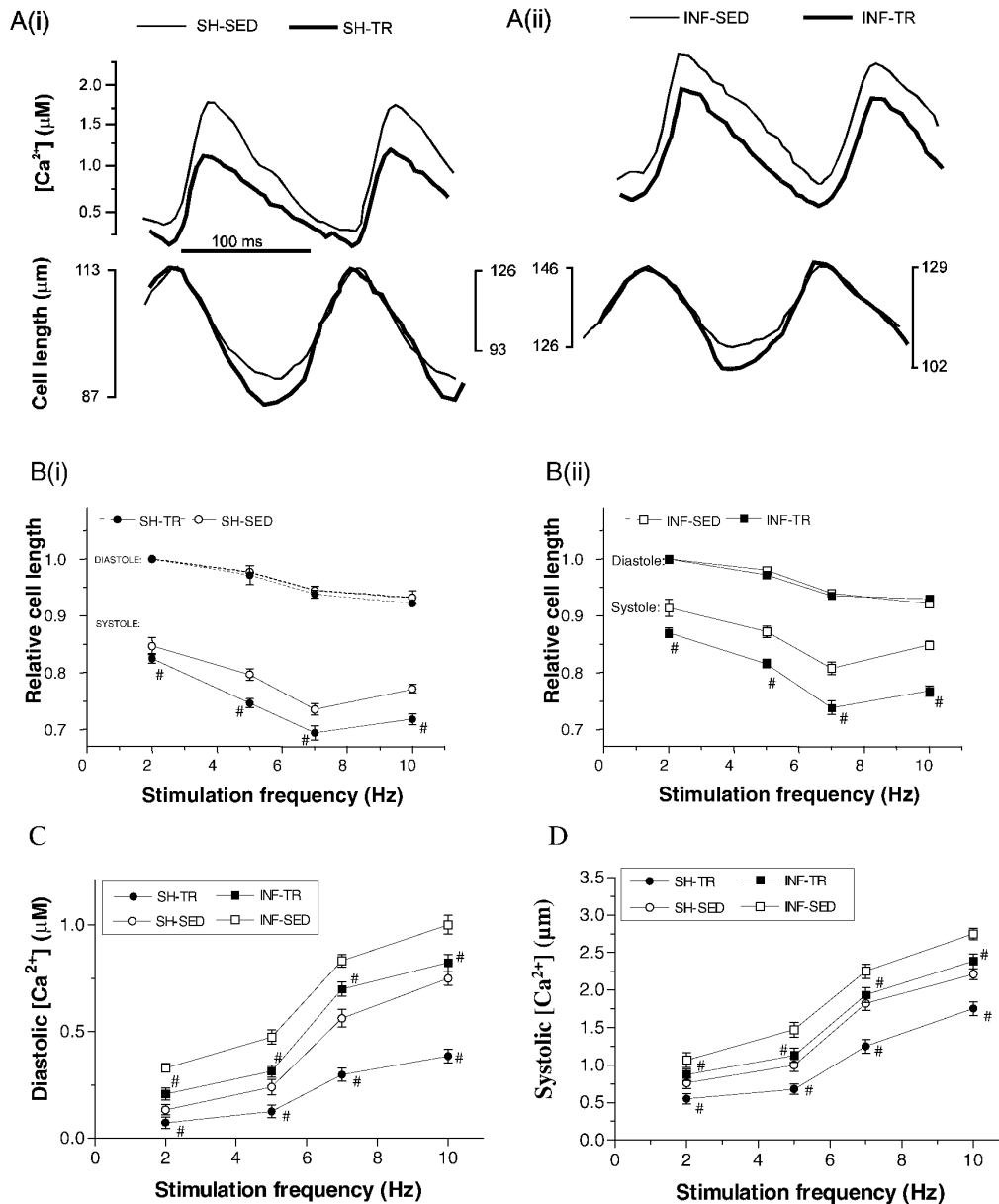


Fig. 3. A: averaged Ca^{2+} transients and cell shortening cardiomyocytes from the remote area recorded at 7 Hz stimulation (Ai and Aii). Data are calculated from a total of 36 cells, three in each rat ($n=6$). Relationship between stimulation frequency and maximal shortening (Bi and Bii), intracellular Ca^{2+} (C and D) at 8 weeks. Each data point represents mean \pm S.D. of 60 cells, 9 ± 3 in each rat ($n=6$). In each cell data were calculated at the mean of ten consecutive contractions after stabilization at each stimulation frequency. # indicates differences between groups ($P < 0.01$). All systolic curves in panel B (i and ii) are significantly different ($P < 0.01$). All curves in panel C are significantly different ($P < 0.03$), except $P = 0.01$ for systolic Ca^{2+} between INF-TR and SHAM-SED (C).

sensitivity and increased Ca^{2+} -clearance from cytosol (Fig. 3B and C and Table 3). Western blot analysis demonstrated about 30% lower protein levels of SERCA-2 and NCX in SED-INF than in SED-SHAM. Training increased the protein level of SERCA-2 by 34 and 82% in TR-INF and TR-SHAM, respectively. The corresponding increases in Na^+ - Ca^{2+} -exchanger were 33 and 26%. The protein level of both SERCA-2 and NCX in TR-INF did not differ from SED-SHAM (Fig. 4).

3.5. Myofilament Ca^{2+} sensitivity in intact and permeabilised myocytes

The relationship between $[Ca^{2+}]$ and cell shortening is difficult to measure in intact cells since steady state $[Ca^{2+}]$'s and cell shortening are not fully achieved during systole. However, at end-diastole the rate of change of $[Ca^{2+}]$ and cell shortening is minimal, thus these values can be used to assess myofilament Ca^{2+} sensitivity in the

Table 3
Cardiomyocyte shortening and Ca²⁺ handling at 7 Hz

	SED-INF	TR-INF	SED-SHAM	TR-SHAM
<i>Contractile function</i>				
Relative time to peak shortening (ms·Fs ⁻¹)	2.9±0.2	2.0±0.1*	1.8±0.1	1.5±0.1* ^a
Time to peak shortening (ms)	39.5±1.2	41.5±1.5	39.2±1.7	39.6±1.3
Time to 50% shortening (ms)	18.0±1.0	18.1±1.2	17.8±0.7	18.0±0.9
Time to 50% relengthening (ms)	45.6±1.5	38.1±1.0*	35.4±1.1 ^a	29.0±1.2* ^a
<i>Calcium regulation</i>				
Time to peak [Ca ²⁺] _i (ms)	31.2±1.2	32.4±2.1	30.1±1.7	30.4±1.6
Time to 50% peak [Ca ²⁺] _i (ms)	18.8±1.1	18.7±0.7	17.9±0.6	17.7±0.6
Time to 1/2 decay [Ca ²⁺] _i (ms)	58.2±1.8	50.4±	46.8±1.0 ^a	41.7±1.0* ^a

Data are mean±S.D. * indicates differences between comparable SED group. ^a different from INF-rats, P<0.001. FS, fractional shortening; SED-INF, sedentary infarcted rats; TR-INF, trained infarcted rats; SED-SHAM, sedentary sham-rats; TR-SHAM, trained rats.

intact cell. Fig. 5A presents this relationship for all groups. Cell length (plotted relative to quiescent cell length) demonstrate a significantly greater cell shortening at comparable [Ca²⁺]_i when the trained groups are compared to respective controls, indicating increased myofilament sensitivity.

The intrinsic sensitivity of the myofilaments was assessed by permeabilising the sarcolemma with β-escin and buffering the cytoplasmic pH and [Ca²⁺]_i. Fig. 5B and C, indicate significantly higher myofilament sensitivity in the TR-SHAM group, with no differences between TR-INF and SED-SHAM. Acid and alkaline shifts in pH at a set

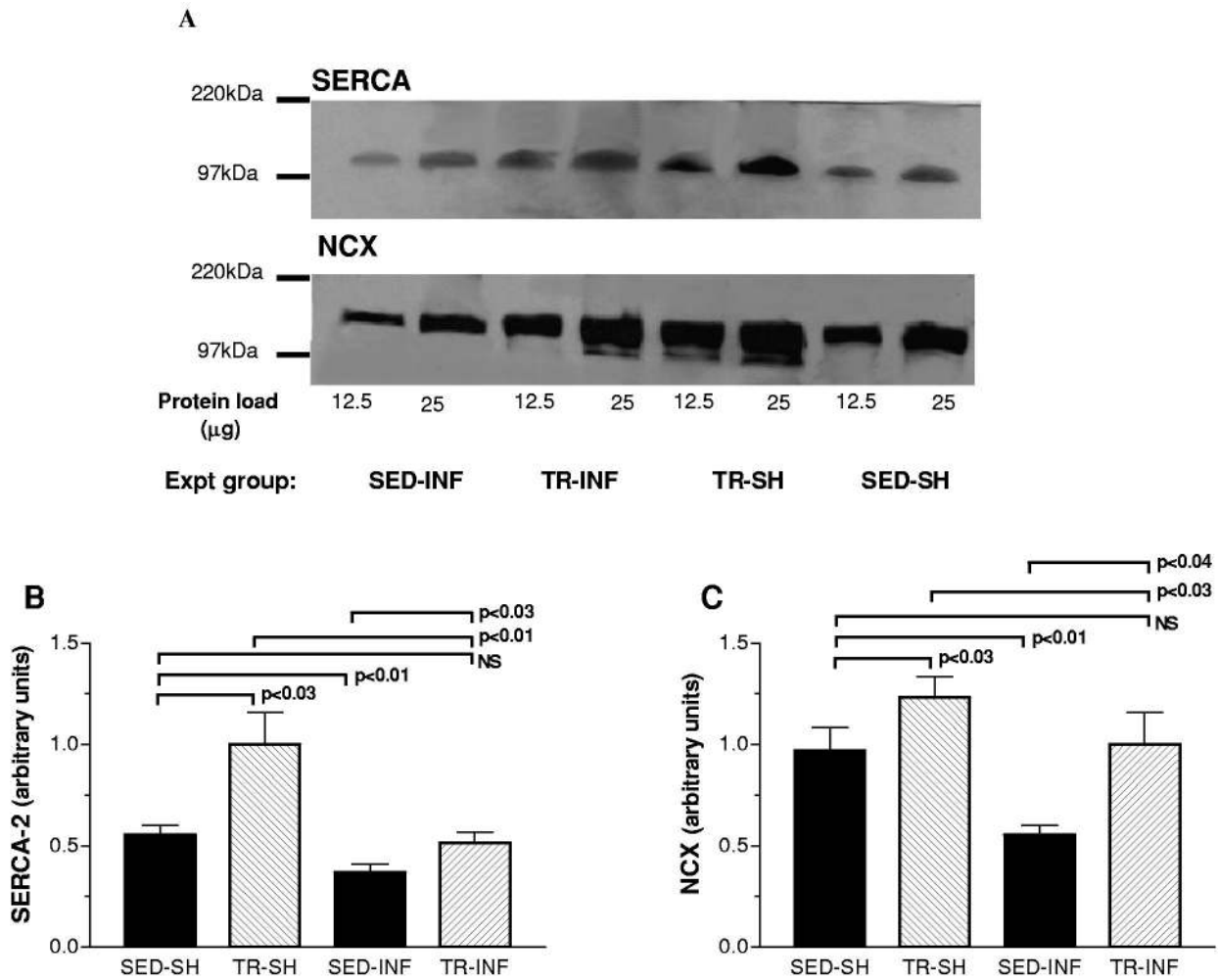


Fig. 4. A: typical Western blot from all experimental groups for SERCA-2 and Na⁺-Ca²⁺-exchanger. B: Western blot for SERCA-2. C: Western blot for Na⁺-Ca²⁺-exchanger (NCX).

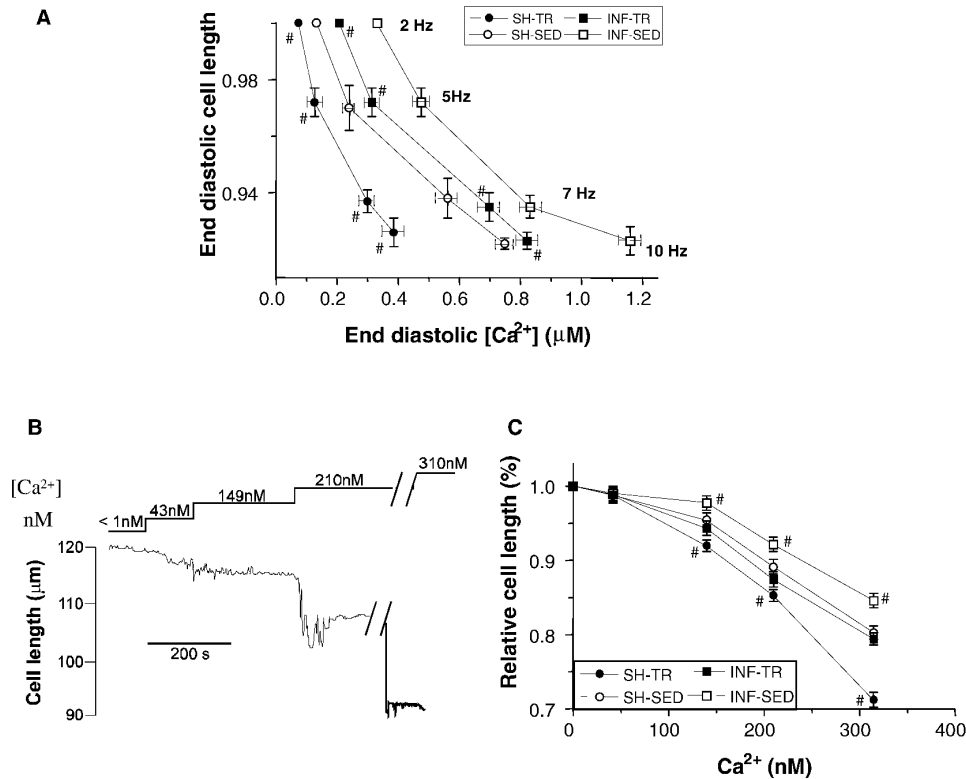


Fig. 5. A: In rats trained for 8 weeks a larger extent of shortening with increasing stimulation frequency indicates increased sensitivity to Ca²⁺. B: typical cell length record from a permeabilised myocyte exposed to a range of [Ca²⁺] (pH 7.0). C: After 8 weeks of training skinned myocytes responded more to changes in Ca²⁺. There was no difference between TR-INF and SED-SHAM. In intact cells each data point represents mean ± SD of 60 cells, 9 ± 3 in each rat (n = 6). In each cell data were calculated as the mean of 10 consecutive contractions after stabilization at each stimulation frequency. In skinned cells data were calculated after stabilization at each [Ca²⁺] in 9 cells from each rat (SED-INF, n = 5; TR-INF, n = 4; SED-SHAM, n = 2; TR-SHAM, n = 2). All curves are significantly different (p < 0.001), except TR-INF and SED-SHAM in panel C.

[Ca²⁺] (210 nM) induced smaller changes in cell shortening in trained groups, suggesting that the myofilaments in TR are less sensitive to altered pH (P < 0.03, data not shown). Myofilament shortening in TR-INF and SED-SHAM groups were similarly sensitive to altered pH.

3.6. ANP, ET-1, IGF-1 and NHE expression

Myocardial infarction markedly induced left ventricular ANP expression with a 20–25-fold increase in ANP mRNA in SED-INF compared to sham. In trained infarcted hearts the ANP mRNA expression was about 40% attenuated compared to SED-INF (Table 4). The mRNA expression of ET-1, IGF-1 and NHE were similar in SED-INF and TR-INF, and 40, 250, and 200% higher than

in SED-SHAM. In sham animals, training did not induce any changes in mRNA expression for any peptide (Table 4), indicating a different growth response in heart failure.

3.7. Echocardiography and pressure measurements

As evident from the internal dimensions, fractional shortening, and pressure measurements, the anesthesia had a negative inotropic effect on the heart. Training did not affect in vivo cardiac function assessed under anesthesia. LV diameter in diastole increased about 8% in both groups during the experimental period (Table 1). LV diastolic diameter at pre test (4 weeks after infarction) was 10.4 ± 0.5 and 10.3 ± 0.4 mm for SED-INF and TR-INF, respectively. Neither left ventricular end-diastolic pressure

Table 4
Left ventricular gene expression

	SED-INF	TR-INF	TR-SHAM	SED-SHAM
<i>Competitive TR-PCR</i>				
ET-1, amol cDNA · mg tissue ⁻¹	4.2 ± 2.0*	4.9 ± 1.9*	3.4 ± 1.9	4.6 ± 3.2
ANP, fmol cDNA · mg tissue ⁻¹	8.0 ± 4.0	4.6 ± 3.7* ^a	0.33 ± 0.2	0.41 ± 0.2
IGF-1, amol cDNA · mg tissue ⁻¹	41.6 ± 17.4*	38.5 ± 23.3*	14.7 ± 7.7	17.5 ± 10.8
Na ⁺ –H ⁺ -exchanger, amol cDNA · g tissue ⁻¹	0.46 ± 0.2*	0.46 ± 0.3*	0.25 ± 0.11	0.24 ± 0.1

Values are mean ± S.D. * indicate differences from SHAM-rats, ^a, different from SED-INF P < 0.01. SED-INF, sedentary infarcted rats; TR-INF, trained infarcted rats; SED-SHAM, sedentary sham-rats; TR-SHAM, trained sham rats.

nor peak systolic and aortic diastolic pressures measured 24–28 h after training were affected (Table 1). Training did not alter the restrictive mitral inflow Doppler pattern or reduced $+ / - dP/dt_{\max}$ after myocardial infarction (Table 1). As noted in the Discussion the negative inotropic effect of anesthesia may have precluded differences between groups.

4. Discussion

The major findings of the present study were that aerobic intensity-controlled interval training attenuates myocardial hypertrophy and increases myocyte contractile function after myocardial infarction. Increased SERCA-2 expression and Ca^{2+} -sensitivity of the myofilaments accompanied these changes. ANP gene expression was attenuated, suggesting reduced loading on the heart.

4.1. Post-mortem data

This is the first study to demonstrate that aerobic interval training attenuates myocardial hypertrophy with corresponding reduction in cellular dimensions in MI rats. These results are similar to those reported in anaerobically trained MI rats [20]. Reduced ventricular hypertrophy post-MI in trained rats is consistent with reduced expression of ANP mRNA, which is associated with wall stress and is a known marker of cardiac hypertrophy. The attenuation of ANP expression and hypertrophy was similar to that observed after chronic treatment with angiotensin-II antagonism with losartan [34,35]. The mechanism behind the different training-adaptation in healthy and failing hearts is not known. In healthy animals, endurance training may temporarily increase loading, and thereby stretch-induced longitudinal cardiomyocyte growth. In MI animals training may improve endothelial function and reduce peripheral resistance, unload the heart, and thereby reduce the expression of ANP. Recently we showed that changes in ANP (in the same order as in this study) correlated strongly with reduced wall stress [35]. It is possible that the negative inotropic effect of anesthesia could have precluded detection of differences in wall stress among groups (see below). However, we cannot exclude that other factors such as hormone and cytokine level, which are known to be changed by exercise [36], could also affect the expression of ANP [35]. Furthermore, training improves cardiomyocyte function, which per se might reduce the stimulus for cardiac hypertrophy within the heart [37].

4.2. Myocyte contractility and myofilament Ca^{2+} sensitivity

The present study shows that aerobic interval training

attenuated the reduced contractility in myocytes associated with myocardial infarction in the rat. A previous study using anaerobically trained post-MI rats [38] showed a similar improvement of myocyte performance thus indicating that the beneficial effects of training can be achieved with both forms of training regimens. The present study provides additional insight into the sub-cellular basis for these effects.

Compared to control values (SED-SHAM), myofilament Ca^{2+} sensitivity was depressed in the SED-INF group and enhanced in TR-INF group. Depressed myofilament sensitivity in the INF group is in agreement with a recent study indicating that a profound depression of Ca^{2+} activated force in trabeculae from failing rat heart is the main cause of depressed contractility [39]. The cellular basis for these changes is not known, but multiple biochemical alterations of the contractile proteins have been described in heart failure, including isoform switching of troponin T [40], and suppression of α -, and increased β -myosin heavy chain expression [41]. In contrast, little is known about changes in the properties of cardiac myofilaments during aerobic training, although stress-induced changes in the expression of troponin I isoforms have been noted [42]. It is also known that treadmill training in rat induces a further increase in the α -myosin heavy chain expression [25]. α -Myosin heavy chain is associated with high ATPase activity and increased contractility. It is clear, however, that changes in ventricular ATPase activity and/or isoform composition are not obligatory for improved ventricular performance secondary to chronic exercise [43]. Altered pH sensitivity may also accompany the altered Ca^{2+} sensitivity of the myofilaments in the trained model, as also observed in a previous study in healthy trained rats [19]. Other factors that may affect the contractile response of the myocyte to Ca^{2+} are the resting sarcomere length and the restoring force within the single myocyte; which were not measured in this study.

4.3. Intracellular $[Ca^{2+}]$ transients, SERCA-2 and NCX

In this study, the increased myocyte shortening in trained groups was associated with a lower peak systolic intracellular $[Ca^{2+}]$. The effect occurred at all stimulation rates. Lower peak systolic $[Ca^{2+}]$ after training has been observed previously [19,44], but not in all studies [21,45]. A complete characterization of mechanism underlying these changes was beyond the scope of the present study. However, some possible mechanisms are discussed below. An increased SERCA-2 expression and increased sensitivity of the myofilaments accompanied the changes of intracellular $[Ca^{2+}]$ [19]. As discussed in a previous study [19], enhanced SERCA-2 may increase Ca^{2+} release from the SR of trained rats, but increased myofilament Ca^{2+} binding may reduce the net effect on the cytosolic Ca^{2+} signal. In line with other studies [18] training induced hypertrophy was associated with increased expression of

NCX. Chronically raised NCX levels are known to reduce systolic $[Ca^{2+}]$ [46] and may contribute to the reduced peak systolic $[Ca^{2+}]$ observed in the trained groups. In a previous study we observed a trend towards increased NCX expression in the TR group, but the effect was not significant [19]. In the present study, the NCX values were consistently higher in the TR group, resulting in a significant increase. Similar increases have been reported in other models of exercise in the rat [15]. The use of newer gel equipment in the present study could also have contributed to less standard deviation in NCX-measures. Peak systolic $[Ca^{2+}]$ was higher in cardiomyocytes from the SED-INF when compared to the sedentary group. This is an unusual result since most studies indicate that reduced peak systolic $[Ca^{2+}]$ is associated with contractile dysfunction in hypertrophic myocardium [13]. There are reports of increased peak systolic $[Ca^{2+}]$ in failing hearts [47–49], in some cases restricted to sub-endocardial cells [14].

Down-regulation of SERCA-2 is a common observation in failing hearts [13] and is normally linked to decreased peak systolic $[Ca^{2+}]$. However, a more unusual observation is the reduction in NCX levels in this model. Increased or unchanged NCX levels have been reported in human and animal models of heart failure [13]. However, there are several reports in the literature of reduced NCX levels in hypertrophy, particularly in the rat model [15]. Reduced extrusion of Ca^{2+} on the NCX would be expected to raise diastolic $[Ca^{2+}]$ and enhance SR Ca^{2+} load. These effects would tend to compensate for decreased SERCA-2 levels and help to increase the amplitude and slow the time course of the Ca^{2+} transient. In a previous study in healthy rats we report that aerobic training increase SERCA-2 and phospholamban similarly, i.e. unchanged SERCA-2/phospholamban ratio. However, in a recent study Zhang et al. [21] reported that phospholamban expression in MI-rats exposed to intense anaerobic training was less affected than the expression of SERCA. The increased systolic and diastolic intracellular Ca^{2+} reported after myocardial infarction might have detrimental effects on cardiac function. Ca^{2+} overload activates several energy-requiring processes, and reduces energy production by uncoupling of oxidative phosphorylation, leading to energy deficit in the cardiomyocyte [50]. Furthermore, increased Ca^{2+} may stimulate cell growth contributing to remodeling and Ca^{2+} activated signals that induce apoptosis [51]. Interestingly, the changes in SERCA-2, NCX, intracellular $[Ca^{2+}]$, myofilament Ca^{2+} sensitivity and cell shortening observed in the TR-INF group are the converse of those seen in SED-INF. Thus the effect of training was to normalize the changes in the infarcted group.

4.4. ET-1, IGF-1, ANP, and NHE

The rat post infarction model is consistent with human studies demonstrating increased ventricular expression of genes coding for the fetal phenotype during remodeling

after myocardial infarction [35,52]. However, endurance training had little effect on the expression of ET-1, ANP, IGF-1 and NHE despite marked hypertrophy both in the infarcted and sham-operated hearts. This observation indicates that other genes regulatory pathways modulate myocardial training induced hypertrophy, compared to hypertrophy in heart failure. However, hypertrophic stimuli after training are temporary, whereas they are likely to be continuous in heart failure. Thus, the time of tissue collection post training might be critical for the results. Maeda et al. [53] found a 1.3-fold increases in ET-1 in tissue collected immediately after 45 min of endurance training with a similar intensity to that in the present study. Tissue sampled at different time points after training and monitoring of several genes using cDNA-microarray will probably give more insight into the gene regulation in response to endurance training.

4.5. Cardiorespiratory performance

Animals trained and tested according to the present procedure [23] displayed most of the changes observed in humans. The increase in $V_{O_{2max}}$, work economy, oxygen pulse and the reduction in heart rate were in line with previous studies in our laboratory [19,23] and significantly larger than other studies [17,24,44,45,53]. It is conceivable that these large effects resulted from a carefully controlled level of exercise intensity throughout the study. Differences in training response reported in the literature are probably due to different regimens used, insufficient control of exercise intensity, or different protocols for measuring $V_{O_{2max}}$. The load required to produce a training effect increases as the performance is improved in the course of training [23]. The training load should therefore be adjusted relative to the level of fitness of the individual throughout the course of training. Even though we managed to reach $V_{O_{2max}}$ with leveling-off of oxygen uptake despite increased exercise intensity with substantial increased resting LVEDP, we did not observe any sign of pulmonary edema. This is in line with other studies (e.g. Ref. [54]) reporting that LVEDP is not a good predictor of severity of dyspnea in CHF, because reactive pulmonary vasoconstriction and obliteration of small pulmonary arteries protects the lungs from overfilling.

4.6. Echocardiography and pressure measurement

Reduced ventricular dilation in trained post-MI rats has been noted by Orenstein et al. [9] and Jain et al. [55]. In the present study training was initiated after scar healing was complete [56], and even though this was associated with a reduced cellular hypertrophy in post-infarction myocytes, we did not observe an attenuation of the LV dilation. However, the large end-diastolic diameter (4.8 mm), low fractional shortening and high LVEDP indicates that the LV was markedly affected by the negative ino-

tropic effect of the anesthesia. Despite this, the method discriminated well between INF and SHAM, where large adaptations occur, whereas it may have masked the differences between TR and SED.

4.7. Heart failure

It is known that not all post-infarction hearts undergo transition to heart failure [36,57,58]. Changes of several measurements confirm that the present MI-model lead to heart failure: (1) left and right ventricular hypertrophy, (2) increased LVEDP, (3) reduced left ventricular dP/dt_{max} , (4) large infarctions, (5) substantial left ventricular dilation, (6) increased atrial dimensions, and (7) reduced fractional shortening. Thus our model is well suitable for determining the effect of endurance training on myocardial function in heart failure.

5. Conclusion

Intensity-controlled interval training attenuated myocardial hypertrophy and rescued cardiomyocyte contractility in MI rats. These beneficial effects on cardiac remodeling and myocyte function were similar to those observed with angiotensin II antagonism inhibitors, indicating that intensity-controlled interval training might be a potent modifier of post-infarction heart failure. This experimental model mimics important aspects of human pathophysiology and could thus help determine cellular and molecular mechanisms of training-induced improvement of cardiac function.

Acknowledgements

U. Wisløff was the recipient of a research fellowship from the National Council on Cardiovascular Diseases. This work was also supported by grants from the Norwegian Research Council, Sintef Unimed, the Langfeldt Fund for Research in Physiology and Medical Biochemistry, the Blix Fund for the Promotion of Medical Science, and the Funds for Cardiovascular and Medical Research at Trondheim University Hospital and the British Heart Foundation (GL Smith and S Currie). The authors want to thank engineers Arnfinn Sira and Ketil Jensen for building the rat training equipment, and research fellow Marianne Berg and bioengineer Sissel Skarra for RT-PCR analysis.

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