Cardiovascular Research (2008) **78**, 385–394
doi:10.1093/cvr/cvm109

Exercise promotes angiogenesis and improves β-adrenergic receptor signalling in the post-ischaemic failing rat heart

Dario Leosco[1*†], Giuseppe Rengo[2,3†], Guido Iaccarino[1], Luca Golino[1], Massimo Marchese[1], Francesca Fortunato[1], Carmela Zincarelli[1,2], Emma Sanzari[1], Michele Ciccarelli[1,2], Gennaro Galasso[1], Giovanna Giuseppina Altobelli[4], Valeria Conti[5], Gianfranco Matrone[1], Vincenzo Cimini[4], Nicola Ferrara[3,6], Amelia Filippelli[5], Walter J. Koch[2], and Franco Rengo[1,3]

[1]*Cattedra di Geriatria, Dipartimento di Medicina Clinica, Scienze Cardiovascolari ed Immunologiche, Università Federico II, Via Pansini 5, 80131 Napoli, Italy;* [2]*Department of Medicine, Thomas Jefferson University, Philadelphia, PA, USA;* [3]*Fondazione S. Maugeri—IRCCS, Telese, Italy;* [4]*Dipartimento di Scienze Biomorfologiche e Funzionali, Università Federico II, Napoli, Italy;* [5]*Dipartimento di Medicina Sperimentale e Centro di Eccellenza delle Malattie Cardiovascolari, 2^ Università di Napoli, Napoli, Italy; and* [6]*Dipartimento di Scienze della Salute, Università del Molise, Campobasso, Italy*

Received 19 September 2007; revised 9 December 2007; accepted 11 December 2007; online publish-ahead-of-print 18 December 2007

Time for primary review: 27 days

KEYWORDS
Heart failure;
Exercise training;
Angiogenesis;
Vascular endothelial growth factor;
β-adrenergic receptor

Aims We investigated whether exercise training could promote angiogenesis and improve blood perfusion and left ventricular (LV) remodelling of the post-myocardial infarction (MI) failing heart. We also explored the contribution of ameliorated β-adrenergic receptor signalling and function on the overall improvement of cardiac contractility reserve induced by exercise.

Methods and results Adult Wistar male rats were randomly assigned to one of four experimental groups. Sham-operated and post-MI heart failure (HF) rats were housed under sedentary conditions or assigned to 10-weeks of a treadmill exercise protocol. At 4 weeks after MI, sedentary HF rats showed LV eccentric hypertrophy, marked increase of LV diameters associated with severely impaired fractional shortening ($14 \pm 5\%$), increased LV end diastolic pressure (20.9 ± 2.6 mmHg), and pulmonary congestion. In addition, cardiac contractile responses to adrenergic stimulation were significantly blunted. In trained HF rats, exercise was able to (i) reactivate the cardiac vascular endothelial growth factor pathway with a concurrent enhancement of myocardial angiogenesis, (ii) significantly increase myocardial perfusion and coronary reserve, (iii) reduce cardiac diameters, and (iv) improve LV contractility in response to adrenergic stimulation. This latter finding was also associated with a significant improvement of cardiac β-adrenergic receptor downregulation and desensitization.

Conclusions Our data indicate that exercise favourably affects angiogenesis and improves LV remodelling and contractility reserve in a rat model of severe chronic HF.

1. Introduction

After myocardial infarction (MI), adequate growth of new capillaries and arterioles represents a crucial phenomenon for the development of compensatory hypertrophy in the surviving portion of myocardium.[1] However, previous studies have demonstrated that angiogenesis may be inadequate in the post-MI hearts, as indicated by the severely reduced coronary conductance and reserve.[2] In this regard, recent evidence demonstrates that impaired angiogenesis may induce maladaptive left ventricular (LV) remodelling and promote the transition from adaptive cardiac hypertrophy to LV dilation and dysfunction.[3]

Dysregulation of cardiac β-adrenergic receptor (β-AR) signalling represents another important factor leading to pathological LV remodelling and heart failure (HF). β_1-AR downregulation and desensitization/uncoupling of both β_1 and β_2-ARs represent the main alterations of β-AR signalling in failing myocardium.[4–6] Increased cardiac G-protein coupled receptor kinase-2 (GRK2) expression/activity has been shown to significantly promote these molecular abnormalities.[7–10] Consistent with its pathogenic role in HF, when GRK2 is inhibited in the heart, there is increased cardiac function[11–13] and improved survival.[14]

Exercise has been proven to increase coronary vascular supply by promoting vascular growth and remodelling in

† Dario Leosco and Giuseppe Rengo were co-first authors of this work.
* Corresponding author. Tel: +39 081 7462267; fax: +39 081 7462339.
E-mail address: dleosco@unina.it

physiological conditions.[15,16] The activation of vascular endothelial growth factor (VEGF) dependent angiogenic pathways represents a crucial molecular mechanism by which exercise triggers angiogenesis.[17–19] In regards to β-AR function, exercise improves receptor signalling in the aged heart[20] and vasculature,[21] in the post-MI myocardium of mice,[22] and in hypertensive rat hearts.[23] To our knowledge, the present study investigates for the first time the effects of chronic exercise on cardiac angiogenic mechanisms and β-AR function in the rat model of post-ischaemic chronic HF. We evaluated whether training was able to (i) stimulate VEGF-dependent angiogenic signalling and promote angiogenesis in failing myocardium, (ii) reduce GRK2 levels and ameliorate cardiac β-AR signalling and adrenergic responsiveness, and (iii) attenuate negative LV remodelling after large MI.

Our results represent the first demonstration that exercise reactivates angiogenesis in severely decompensated rat hearts. Herein, we also extend to a rat model of chronic post-MI HF our previous evidence on the crucial role of GRK2 in regulating β-ARs in the heart.

2. Materials and methods

For detailed description, see Supplementary material online, Materials and Methods.

The study protocol was designed in accordance with *The Guide for Care and Use of Laboratory Animals* of the National Institutes of Health (NIH Publication No. 85–23, Revised 1996), and was approved by the Ethics Committee for the Use of Animals in Research of our institution.

2.1 Experimental groups

One-hundred and sixty-eight Wistar male rats (6 months old) entered the study and were randomly assigned to one of the four experimental groups. Sham-operated and post-MI HF rats were housed sedentary for 14-week (SH_{SED}, $n = 31$; HF_{SED}, $n = 51$) or assigned to 4-week sedentary plus 10-week treadmill exercise protocol (SH_{EX}, $n = 33$; HF_{EX}, $n = 53$).

2.2 Experimental procedures

A large antero-septal MI was obtained by surgical ligation of the proximal tract of the left anterior descending coronary artery. Mortality rate was equally distributed between sedentary and exercise groups both at 4 weeks after MI (27.5 and 28.3%, respectively) and during 10 weeks of exercise and sedentary protocols (16.3 and 15.8%), with an overall mortality rate of ∼44%. Serial M-mode echocardiographic evaluations were performed at 4 weeks after surgery and at the end of the study protocols. Basal and isoproterenol (ISO)-stimulated LV contractility was evaluated by invasive haemodynamic studies at 4 weeks after surgery ($n = 8$ rats per each group) and after 10 weeks of sedentary and exercise protocols ($n = 10$ rats per each group). Infarct size was determined by morphometric studies.

2.3 Exercise training protocol

At 4 weeks after MI, rats assigned to SH_{EX} and HF_{EX} groups underwent a training aerobic program consisting of a 10-weeks treadmill protocol. In the first 2 weeks, exercise relative intensity was set at 40–50% of maximal oxygen uptake (V_{02max}), while, in the following 8 weeks, rats exercised 5 days/week, 45 min/day, with a speed running of 17 m/min (15° inclination), and with an exercise relative intensity calculated at 75–80% of V_{02max}. Due to the poor compliance of rats to run spontaneously, our protocol could not provide a voluntary treadmill running and animals were encouraged to run by means of a shock bar positioned at the bottom of the treadmill lane. In order to limit the stress, when rats failed to stay off the shock bar, the system was switched-off and exercise was stopped for 5 min. After this resting period, animals restarted to run till they reached an overall daily exercising time of 45 min.

2.4 Measurements of single myocyte contractility

Myocytes were isolated from the non-infarcted zone of the LV ($n =$ 10 rats per each group) by a standard enzymatic digestion.[24] Cell contraction was evaluated under baseline and after ascending doses of ISO.

2.5 Myocardial perfusion studies

Myocardial perfusion was determined using 15 μm fluorescent microspheres (Triton Inc.) ($n = 8$ rats per each group). Tissue samples from the heart and blood samples were processed for microsphere determination. Measurements of myocardial blood flow were obtained at basal and after maximal vasodilation.

2.6 Histology

Capillary and arteriolar length density were evaluated in five randomly selected LV sections in either anterior and lateral wall (border zones) at ∼1 mm from the edge of scar tissue, and in the lateral wall, far from the infarcted area (remote). Capillaries (5–10 μm thick) were detected by Lectin Bandeiraea simplicifolia I (BS-I) staining. Arterioles (<50 μm thick) were identified by immunofluorescence using anti-SM α-actin antibody.

2.7 β-Adrenergic receptor signalling

Receptor binding, adenylyl cyclase activity, and cardiac GRK2 protein assays were obtained as previously described.[11]

2.8 VEGF/Akt/eNOS measurement

Time course measurements of cardiac VEGF, Akt, serin473-phospho-Akt, eNOS, and serin1177-phospho-eNOS protein levels were performed by western blot.

2.9 Statistics

Data were analysed by two-way ANOVA, followed by a Bonferroni's *post hoc* analysis, or by unpaired *t*-testing, as appropriate. Significance was set at level of $P < 0.05$ and all data are reported as means ± SEM.

3. Results

3.1 Physical, haemodynamic, and myocardial performance data after myocardial infarction

As illustrated in *Table 1*, at 4 weeks from MI, marked LV dilation and hypertrophy occurred, thus indicating an unfavourable LV remodelling. These changes of cardiac morphometry were associated with severe cardiac dysfunction, haemodynamic deterioration, and marked pulmonary congestion, as expected. Taken together, these findings assured us of examining the effects of exercise in a model of severe HF. Notably, LV + d*P*/d*t* responses to β-AR stimulation were also significantly blunted in MI rats, thus indicating the diminished β-AR responsiveness in the failed hearts.

Table 1 Physical, haemodynamic, and left ventricular contractility and anatomical data in Sham-operated and heart failure rats at 4 weeks after myocardial infarction

	SH	HF
Physical data		
Body wt (Kg)	0.397 ± 0.011	0.366 ± 0.015*
Heart wt (g)	1.22 ± 0.07	2.14 ± 0.15**
Heart wt/body wt (g/Kg)	3.06 ± 0.15	5.84 ± 0.53**
Lung wt (gr)	1.81 ± 0.22	3.23 ± 0.33**
Lung wt/body wt (g/Kg)	4.52 ± 0.54	8.82 ± 0.93***
Tibial length (cm)	3.46 ± 0.17	3.50 ± 0.16
Heart wt/tibial length (g/cm)	0.352 ± 0.03	0.606 ± 0.05**
Haemodynamic and LV data		
Heart rate (bpm)	378 ± 19	355 ± 18
MAP (mmHg)	102 ± 5	80 ± 7**
LV internal diameter (mm)		
Diastolic	6.67 ± 0.36	9.78 ± 0.67**
Systolic	3.55 ± 0.31	8.38 ± 0.78**
LV fractional shortening (%)	46.6 ± 5.0	14.2 ± 4.6***
Interventricular septum (mm)		
Diastolic	1.55 ± 0.10	1.05 ± 0.13**
Systolic	2.56 ± 0.28	1.09 ± 0.12**
LV posterior wall (mm)		
Diastolic	1.57 ± 0.18	1.99 ± 0.22**
Systolic	2.62 ± 0.33	2.57 ± 0.26
LVSP (mmHg)	137 ± 4	111 ± 5*
LVEDP (mmHg)	4.86 ± 0.6	20.9 ± 2.6***
LV+dP/dt (mmHg/s)		
Baseline	8150 ± 420	4150 ± 320***
ISO (μg/Kg/min)	16580 ± 940	6600 ± 730***
Infarct size (%)	–	51.3 ± 4.5
Scar surface (cm^2)	–	1.26 ± 0.09

SH, Sham; HF, heart failure; MAP, mean arterial pressure; LV, left ventricle; LVSP, left ventricular systolic pressure; LVEDP, left ventricular end-diastolic pressure. SH ($n = 8$); HF ($n = 8$).

*$P < 0.05$ HF vs. SH.

**$P < 0.01$ HF vs. SH.

***$P < 0.001$ HF vs. SH.

3.2 Effects of training on exercise resistance, physical, and echocardiographic data

As illustrated in *Table 2*, at 4 weeks after surgery, before training or sedentary protocols were started, exercise resistance was higher in SH than in HF rats. A significant increase of exercise tolerance was evident in both trained groups, although final levels remained lower in HF rats compared with SH. Training reduced body weight and significantly increased LV weight and LV weight to body weight ratio in both HF_{EX} and SH_{EX} rats compared with sedentary groups. A reduced lung weight and enhanced mean arterial pressure highlighted the positive effect of exercise on systemic haemodynamics in HF rats.

Unexpectedly, HF_{SED} rats did not show a progression of cardiac dysfunction at 14 weeks when compared with 4 weeks after MI. This latter finding may be probably ascribed to the large LV remodelling, occurred within 4 weeks from MI, and to the late deaths of those animals ($n = 6$) which presented the highest LV dilation and function deterioration at 4-week echo studies. Training favourably affected LV geometry, as indicated by the reduction of both LV diastolic and systolic diameters in HF_{EX} rats compared with HF_{SED} (*Table 2*, *Figure 1A* and *B*). Nevertheless, LV fractional shortening showed only a modest, but not significant, improvement in HF_{EX} compared to HF_{SED} rats (*Figure 1C*). Of note, LV wall thickness in the non-infarcted area was increased in HF_{EX} animals. These data indicate that, in post-MI failing heart, exercise is able to limit further LV dilation through the activation of hypertrophic responses. In SH_{EX} rats, training induced a slight but not significant increase in cardiac wall thickness without affecting LV dimensions and fractional shortening. Infarct size was not affected by exercise probably because the most of LV remodelling and infarct expansion occurred within 3 weeks after MI. Furthermore, other studies have yet demonstrated that training is not able to affect infarct size, even when it is started early after coronary ligation.[22]

3.3 Effects of exercise on global left ventricular and isolated myocyte contractility

Basal LV + dP/dt values did not differ between HF_{EX} and HF_{SED} rats (*Figure 1D*; Supplementary material online, *Table S1*), thus indicating that training was not able to increase resting LV performance. HF_{SED} hearts displayed significantly impaired contractile responses to β-AR stimulation, as indicated by the lower ISO-induced LV + dP/dt increase compared with SH. HF_{EX} rats showed significantly improved LV contractile responses. Importantly, similar behaviour was observed for single myocyte contractility responses to β-AR stimulation that were significantly increased in HF_{EX} rats, compared with $HF_{SED,}$ at almost all doses of ISO utilized (*Figure 1E*; Supplementary material online, *Table S1*). Also in this case, basal contractility was not significantly affected by training. Collectively, these results indicate that exercise is able to enhance adrenergic responsiveness of the failing heart. SH_{EX} rats displayed unchanged LV + dP/dt responses and slight, but not significant, increases of cell shortening responses to ISO compared to SH_{SED}.

3.4 Effects of exercise on cardiac angiogenesis and perfusion

HF rats showed a marked capillary rarefaction in both LV border and remote zones (*Figure 2*; Supplementary material online, *Table S2*). Exercise induced a significant increase of capillary density in lateral border and remote zones of LV in HF_{EX} hearts, but not in the LV anterior wall which was largely involved in the infarcted area. As with capillary density, arteriolar length density was dramatically reduced in the LV anterior wall (*Figure 3*; Supplementary material online, *Table S2*). Interestingly, a significant growth of small arterioles (<35 μm) was found in the remote LV zones after exercise, thus demonstrating the ability of training to promote the maturation of resistance vessels from pre-existing capillaries in non-infarcted LV myocardium.

Perfusion data are consistent with the exercise induced angiogenesis improvement. In fact, myocardial blood flow and coronary conductance were significantly reduced after maximal vasodilation in HF_{SED} compared with SH rats (*Figure 4*; Supplementary material online, *Table S3*). Exercise improved myocardial perfusion and reduced coronary vascular resistances in HF_{EX} rats compared with HF_{SED}. Accordingly, coronary reserve, which showed a two-fold decrease in HF_{SED} rats compared with SH, significantly

Table 2 Physical, haemodynamic, and echocardiographic data in sham-operated and heart failure rats at 10 weeks sedentary or exercise protocols

	SH_{SED}	SH_{EX}	HF_{SED}	HF_{EX}
Exercise resistance (min)				
Before protocols	25 ± 2	27 ± 3	12 ± 2	11 ± 2
After protocols	26 ± 3	38 ± 4*	10 ± 2	19 ± 3**
Physical data				
Body wt (Kg)	0.402 ± 0.015	0.373 ± 0.011*	0.350 ± 0.02	0.323 ± 0.017**
Heart wt (g)	1.19 ± 0.6	1.32 ± 0.09*	2.22 ± 0.16	2.56 ± 0.14**
Heart wt/body wt (g/Kg)	2.92 ± 0.15	3.51 ± 0.17*	6.31 ± 0.33	7.90 ± 0.37**,***
Lung wt (gr)	1.75 ± 0.18	1.81 ± 0.15	3.48 ± 0.36	2.50 ± 0.21**
Lung wt/body wt (g/Kg)	4.33 ± 0.6	4.85 ± 0.5	9.94 ± 0.92	7.75 ± 0.4**,***
Haemodynamic and echo data				
Heart rate (bpm)	386 ± 14	356 ± 11*	361 ± 12	365 ± 15
Mean arterial pressure (mmHg)	100 ± 4	98 ± 5	78 ± 5	89 ± 4**
LV internal diameter (mm)				
Diastolic	6.64 ± 0.4	6.61 ± 0.6	10.02 ± 0.4	7.82 ± 0.3**
Systolic	3.54 ± 0.3	3.52 ± 0.4	8.29 ± 0.7	6.34 ± 0.6**
LV fractional shortening (%)	48.7 ± 4	47.0 ± 5	16.7 ± 3	18.9 ± 4***
Interventricular septum (mm)				
Diastolic	1.57 ± 0.11	1.63 ± 0.10	1.04 ± 0.13	1.03 ± 0.09***
Systolic	2.61 ± 0.29	2.65 ± 0.31	1.04 ± 0.10	1.03 ± 0.10***
LV posterior wall (mm)				
Diastolic	1.58 ± 0.12	1.66 ± 0.14	1.98 ± 0.20	2.24 ± 0.18**
Systolic	2.60 ± 0.27	2.62 ± 0.18	2.64 ± 0.33	2.68 ± 0.25
Infarct size (%)	–	–	52.3 ± 3.8	51.6 ± 4.3

SH_{SED}, sham sedentary; SH_{EX}, sham exercised; HF_{SED}, HF sedentary; HF_{EX}, heart failure exercised; MAP, mean arterial pressure; LV, left ventricle; SH ($n = 10$); HF ($n = 10$).

*$P < 0.05$ SH_{EX} vs. SH_{SED}.

**$P < 0.05$ HF_{EX} vs. HF_{SED}, SH_{EX} and SH_{SED}.

***$P < 0.01$ HF_{EX} vs. SH_{EX} and SH_{SED}.

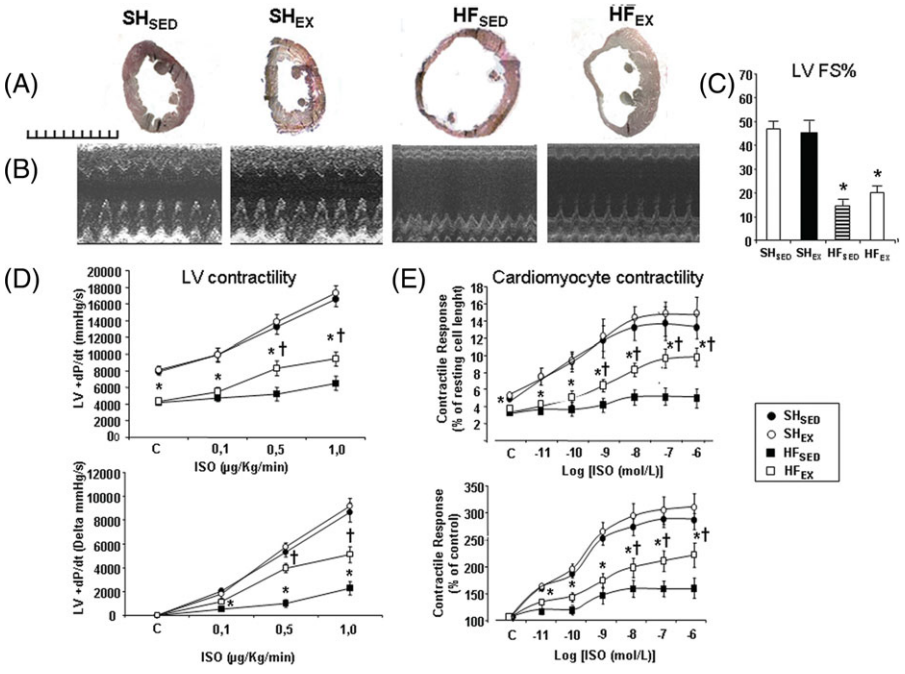

Figure 1 Post-MI heart failure model and effects of exercise on cardiac responses to β-AR stimulation. (*A*) Representative LV cross sections and (*B*) echocardiographic M-mode recordings from sham-operated sedentary and exercised (SH_{SED}, SH_{EX}) rats, and heart failure sedentary and exercised (HF_{SED}, HF_{EX}) animals at 14 weeks after surgery. Scale bar: 10 mm. (*C*) Left ventricular fractional shortening (LVFS). (*D*) *In vivo* global LV contractility responses to isoproterenol (ISO) (0.1, 0.5, and 1.0 µg/Kg/min) expressed as absolute (top) and delta (bottom) values of maximal first derivative of LV pressure rise (LV + d*P*/d*t*). Delta values represent change from baseline. C represents baseline conditions (infusion with vehicle). (*E*) Single myocyte contractility in response to ISO (from 10^{-11} to 10^{-6} mol/L) expressed as % of resting cell length and % of control. All data are expressed as mean ± SEM. *$P < 0.05$ HF_{SED} and HF_{EX} vs. SH_{SED} and SH_{EX}. †$P < 0.05$ HF_{EX} vs. HF_{SED} ($n = 10$ rats per each group).

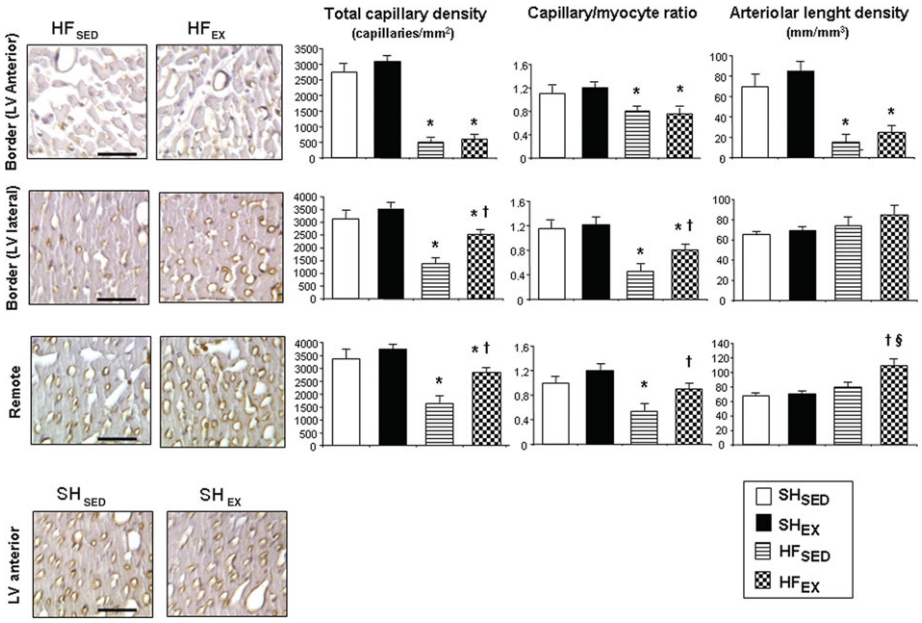

Figure 2 Effects of exercise on cardiac capillary network. (Left) Representative images of Lectin Bandeiraea simplicifolia I (BS-I) staining of capillaries in 5 μm-thick LV sections obtained from heart failure sedentary and exercised (HF_{SED}, HF_{EX}) rats at 14 weeks after MI in either anterior and lateral wall at ~1 mm from the edge of scar tissue (border), and in the lateral wall far from the infarcted area (remote). In sham sedentary and exercised rats (SH_{SED}, SH_{EX}), LV sections were obtained from anterior wall. Brown colour identifies capillary vessels. Magnification ×40. Scale bar: 50 μm. (Right) Bar graphs show data on capillary counts (expressed as total capillary density/mm^2, and capillary to myocyte ratio), and arteriolar length density in either LV border anterior and lateral, and remote zones in SH_{SED}, $SH_{EX,}$ HF_{SED}, HF_{EX} rats at 14 weeks after surgery. In SH animals, border area was identified as the transition zone between anterior and lateral wall at mid LV level. Data are expressed as mean ± SEM. $^{*}P < 0.05$ HF_{SED} and HF_{EX} vs. SH_{SED} and SH_{EX}. $^{\dagger}P < 0.05$ HF_{EX} vs. HF_{SED}, $^{\S}P < 0.05$ HF_{EX} vs. SH_{SED}, and SH_{EX} ($n = 8$ rats per each group).

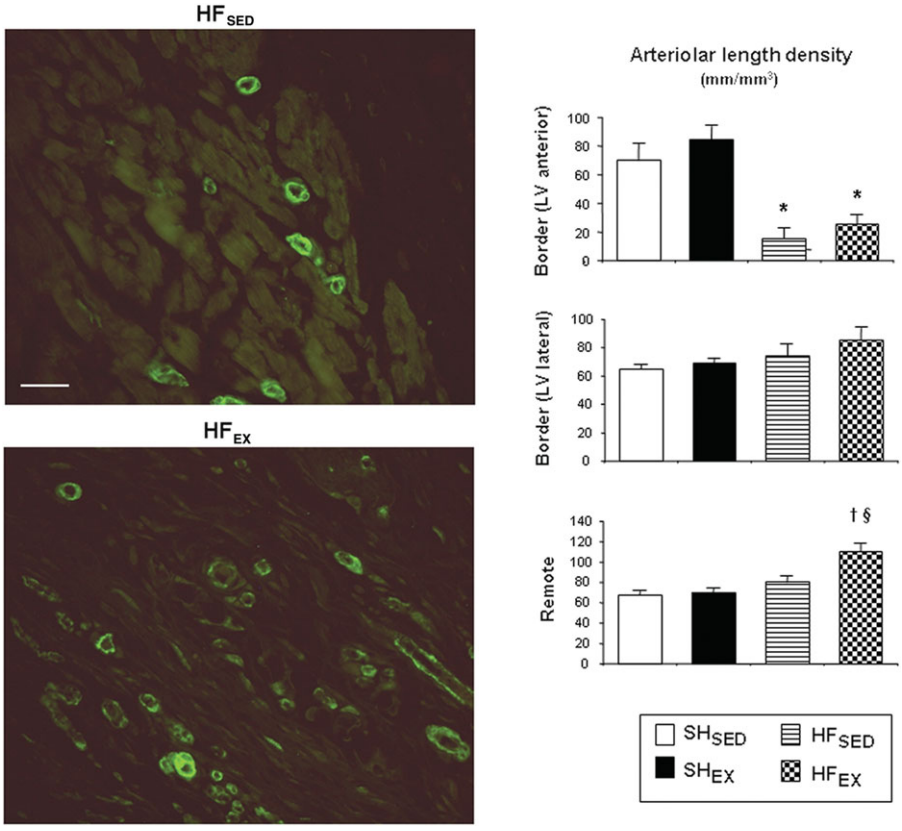

Figure 3 Effects of exercise on cardiac arteriolar growth. (Left) Representative images of arterioles stained with antibodies against smooth muscle (SM) α-actin obtained from heart failure sedentary and exercised (HF_{SED}, HF_{EX}) rats at 14 weeks after MI in the lateral LV wall (remote). Magnification ×40. Scale bar: 100 μm. (Right) Bar graphs show data on arteriolar length density in either LV border anterior and lateral, and remote zones in SH_{SED}, $SH_{EX,}$ HF_{SED}, HF_{EX} rats at 14 weeks after surgery. Data are expressed as mean ± SEM. $^{*}P < 0.05$ HF_{SED} and HF_{EX} vs. SH_{SED} and SH_{EX}. $^{\dagger}P < 0.05$ HF_{EX} vs. HF_{SED}, SH_{SED}, and SH_{EX} ($n = 8$ rats per each group).

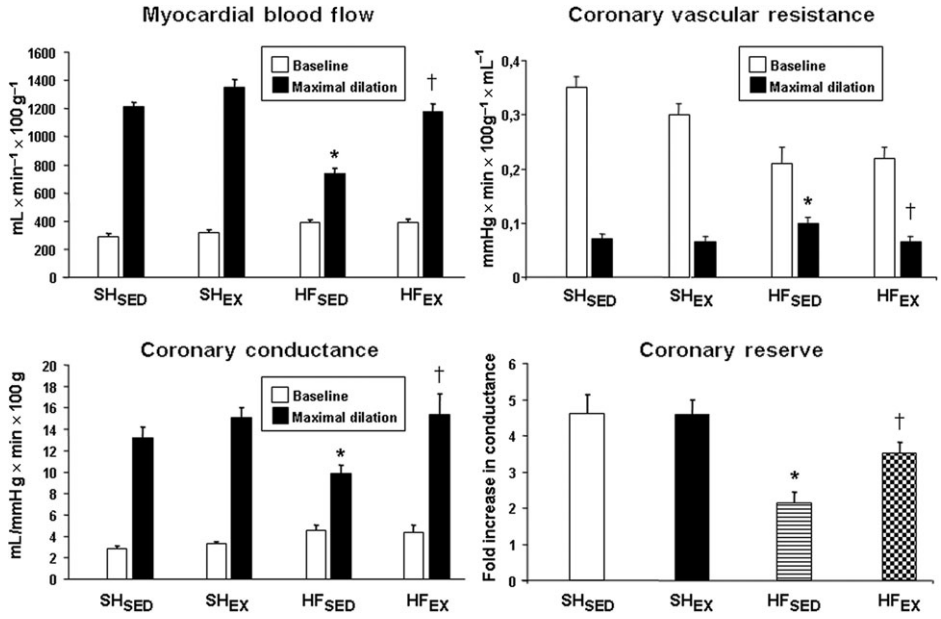

Figure 4 Effects of exercise on myocardial perfusion. Myocardial blood flow was determined by dyed beads assay using 15 μm fluorescent microspheres injected into LV of sham-operated sedentary and exercised (SH_{SED}, SH_{EX}) rats, and heart failure sedentary and exercised (HF_{SED}, HF_{EX}) animals at 14 weeks after surgery. Total myocardial blood flow, coronary vascular resistance, and coronary conductance (coronary blood flow normalized by corresponding perfusion pressure) were measured at basal condition and after maximal coronary dilation by dipyridamole (6 mg kg^{-1} min^{-1} iv). Coronary reserve was calculated as maximal coronary conductance divided by basal coronary conductance. Data are expressed as mean ± SEM. *$P < 0.05$ HF_{SED} vs. SH_{SED} and SH_{EX}. †$P < 0.05$ HF_{EX} vs. HF_{SED} ($n = 8$ rats per each group).

increased after exercise, although final values remained still lower than in SH.

Histological and blood flow data clearly indicate that exercise was associated with improved vascularity and perfusion of the failing myocardium.

3.5 Effects of myocardial infarction and exercise on cardiac VEGF/Akt/eNOS pathway

At 1 week post-MI, cardiac VEGF, Akt activation, and Akt-induced eNOS phosphorylation were increased in HF rats compared with SH (*Figure 5A*), whereas, at 4 weeks after MI, VEGF and phospho-eNOS returned to basal levels, whereas phospho-Akt remained higher in HF hearts (*Figure 5B*). Interestingly, 7 days after training protocol was started, exercise was able to reactivate cardiac pro-angiogenic signalling in HF rats (*Figure 5C*). The observed switch-off of this pathway after 10 weeks of training was probably due to the improved myocardial vascularization and perfusion of HF_{EX} hearts (*Figure 5D*). Notably, at this time point of the study, cardiac phospho-Akt remained high in HF_{SED} rats. This finding strongly supports recent evidence indicating that chronically up-regulated Akt signalling becomes maladaptive in HF.[3]

3.6 Effects of exercise on cardiac β-AR signalling

As expected, HF was associated with cardiac β-AR down-regulation, and the loss of cardiac membrane β-AR density (*Figure 6A*; Supplementary material online, *Table S4*). Importantly, exercise was able to increase β-AR density in HF hearts. Moreover, myocardial samples from HF_{SED} rats displayed reduced basal, and ISO and NaF-stimulated cAMP production compared with SH (*Figure 6B*; Supplementary material online, *Table S4*). Basal cAMP levels were not affected by training, while they increased after stimulation in HF_{EX} hearts compared with HF_{SED}. Consistent with the HF phenotype, GRK2 was significantly up-regulated in HF_{SED} hearts compared with SH. Exercise was able to reduce GRK2 protein overexpression in HF_{EX} hearts (*Figure 6C*; Supplementary material online, *Table S4*). Training did not induce any significant change of β-AR signalling in SH animals, at both receptor and post-receptor levels.

4. Discussion

In this study, we hypothesized that exercise might favourably affect the performance of the failing heart through reactivation of angiogenesis and restoration of cardiac β-AR signalling and function.

In a rat model of severe post-ischaemic HF, we have demonstrated that exercise is able to: (i) reactivate cardiac VEGF-dependent angiogenic pathway, increase coronary vascular network, and enhance myocardial blood perfusion; (ii) reduce cardiac GRK2 levels and ameliorate β-AR signalling at receptor and post-receptor levels; (iii) improve global LV and single myocyte contractility responses to adrenergic stimulation, and (iv) attenuate negative post-MI cardiac remodelling. The significant improvement of systemic haemodynamics and physical performance represented the net balance of the positive effect of exercise in our HF animal population.

Our results confirm previous evidence showing that low to moderate treadmill exercise, started late after a large MI, offers beneficial effect in the failing heart.[25,26] In contrast, training programs using an higher exercise intensity,[27] and started early after MI have reported detrimental effects on LV geometry and survival in rats.[28] In particular, the use of endurance swimming, which is associated with higher

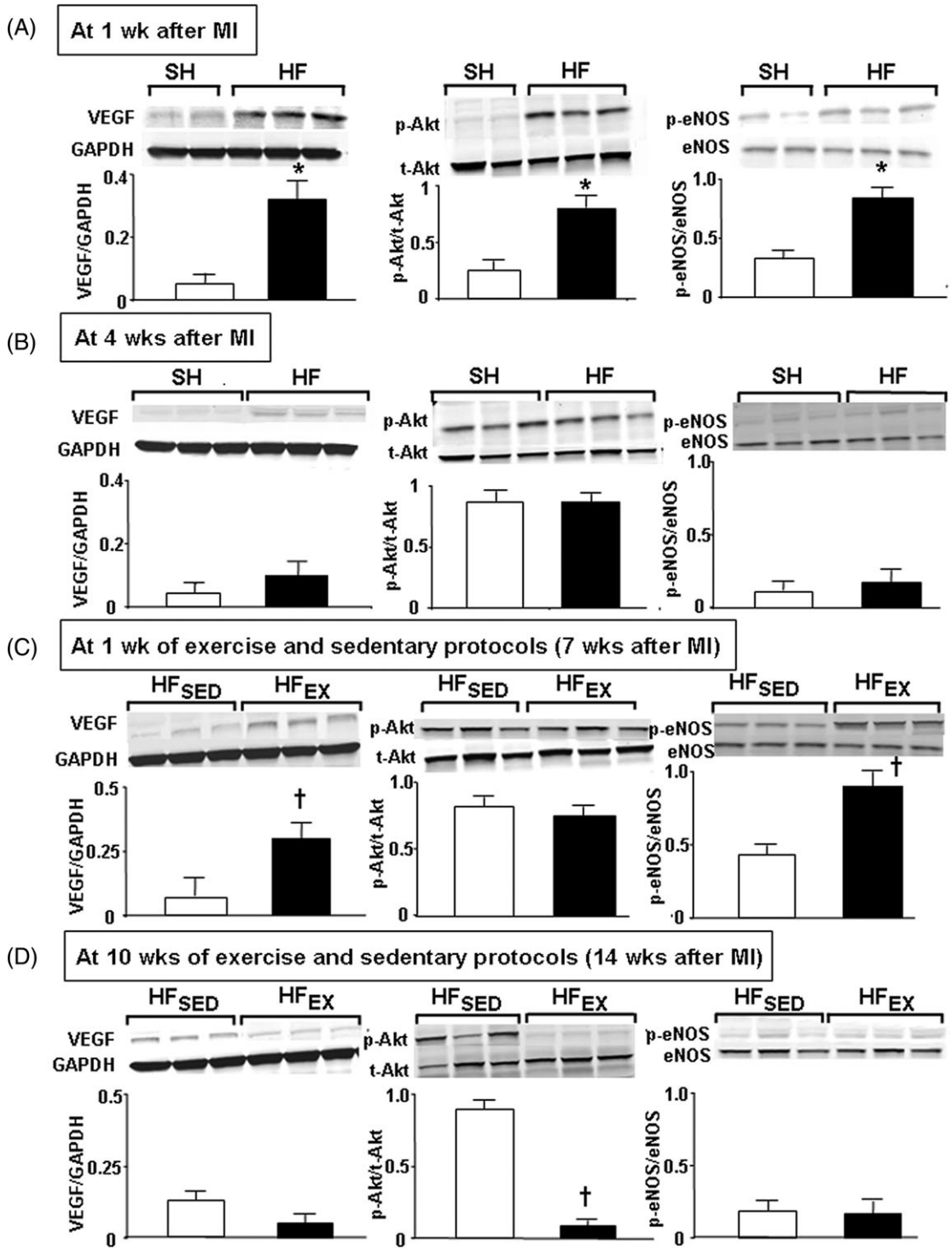

Figure 5 Time course of cardiac VEGF/Akt/eNOS pathway. Shown is cardiac protein expression of VEGF, Akt, Serin473-phospho(p)-Akt, eNOS, and Ser1177-phospho(p)-eNOS in Sham (SH) and heart failure (HF) hearts at 1 (*A*) and 4 (*B*) weeks after surgery, and in HF sedentary (HF_{SED}) and exercised (HF_{EX}) hearts at 1 (*C*) and 10 weeks (*D*) after the start of exercise or sedentary protocol. The expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control to normalize VEGF protein levels. p-Akt to Akt ratio, and p-eNOS to eNOS ratio indicated respectively the levels of Akt and eNOS phosphorilation in the heart at the different steps of the study. Data are expressed as mean ± SEM. $^{*}P < 0.05$ HF vs. SH. $^{\dagger}P < 0.05$ HF_{EX} vs. HF_{SED} ($n = 6$ rats per each group per each time point).

mental and haemodynamic stresses compared with treadmill, and a longer daily/weekly exercising time strongly differentiate these previous training strategies from that used in the present investigation and may account for the negative effects of exercise in HF animals observed by these authors.[27]

4.1 Exercise stimulates angiogenesis in the failing myocardium

The present study offers the first demonstration that exercise training may promote capillaries growth and capillary arteriolarization in non-infarcted areas of severely

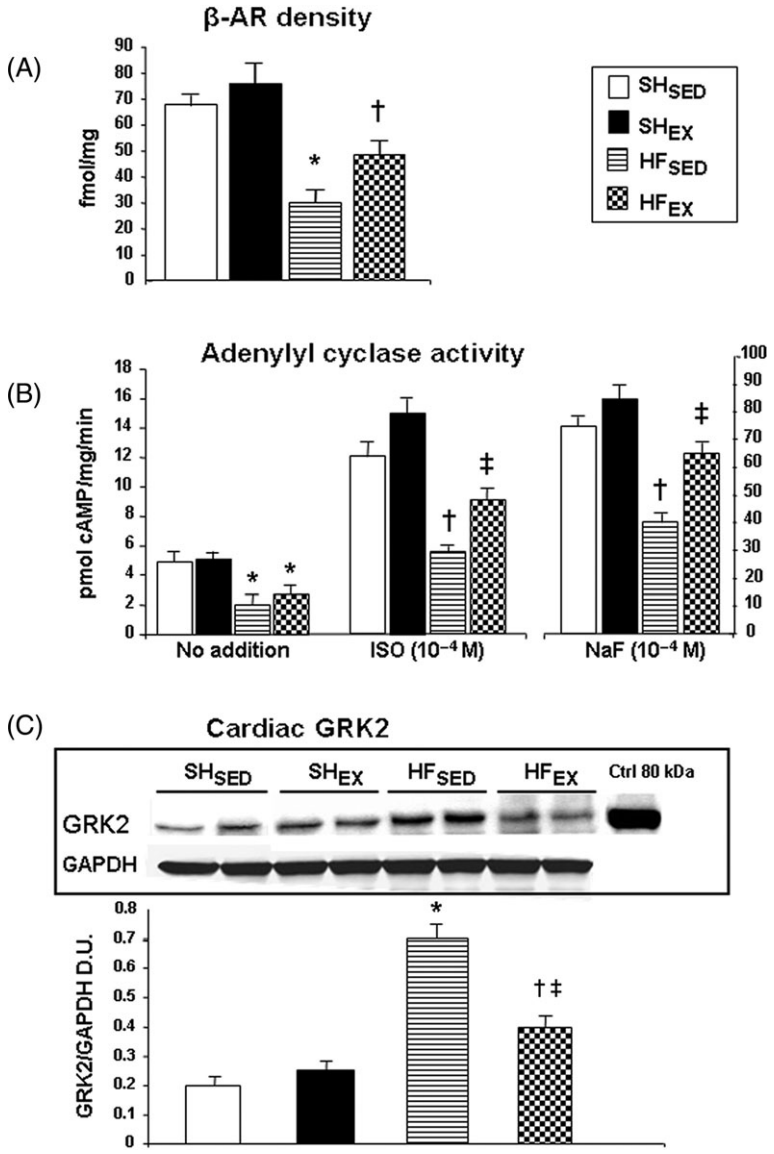

Figure 6 Effects of exercise on cardiac β-AR signalling. (*A*) β-AR radioligand binding in LV membranes isolated from sham-operated sedentary and exercised (SH_{SED}, SH_{EX}) rat hearts, and from failing hearts of sedentary and exercised (HF_{SED}, HF_{EX}) animals at 14 weeks after surgery. $^{*}P < 0.05$ HF_{SED} vs. SH_{SED} and SH_{EX}. $^{\dagger}P < 0.05$ HF_{EX} vs. HF_{SED}, SH_{SED}, and SH_{EX} ($n = 10$ rats per each group). (*B*) Adenylyl cyclase activity expressed as rate of cAMP production in crude cardiac membranes. Shown is cAMP production at basal and after stimulation with Isoproterenol (ISO) (10^{-4} M), and NaF (10^{-4} M). $^{*}P < 0.05$ HF_{SED} and HF_{EX} vs. SH_{SED} and SH_{EX}. $^{\dagger}P < 0.01$ HF_{SED} vs. SH_{SED} and SH_{EX}. $^{\ddagger}P < 0.05$ HF_{EX} vs. HF_{SED}, SH_{SED}, and SH_{EX} ($n = 10$ rats per each group). (*C*) Cardiac GRK2 protein levels. Levels of expression of GRK2 in cardiac membranes were assessed by protein immunoblotting. The expression of glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was used as an internal control. The *Inset* shows a representative protein immunoblot from two preparations per each rat groups. $^{*}P < 0.01$ HF_{SED} vs. SH_{SED} and SH_{EX}. $^{\dagger}P < 0.01$ HF_{EX} vs. HF_{SED}. $^{\ddagger}P < 0.05$ HF_{EX} vs. SH_{SED}, and SH_{EX} ($n = 10$ rats per each group). All data are expressed as mean ± SEM.

decompensated hearts. The pro-angiogenic effect of exercise has been previously demonstrated in healthy swine hearts,[29] but no data were available still now on the ability of training in promoting angiogenesis in chronic failing myocardium. Although previous evidence has indicated that bradycardia stimulates angiogenesis in the post-MI heart,[30] our data of unchanged heart rate in HF_{EX} animals suggest that, in this model of severe chronic HF, exercise-dependent enhanced angiogenesis is not related to a reduced chronotropic state. Several studies have been conducted to explore the mechanisms by which training may stimulate angiogenesis.[16–19,30,31] The role of VEGF dependent angiogenic signalling pathway has been widely studied in both physiologic and pathologic conditions.[32,33] We have previously demonstrated that exercise improves age-dependent VEGF downregulation and angiogenesis responses to hindlimb ischaemia.[17] In the present study, we explored for the first time the effects of exercise on VEGF/Akt pathway in chronic post-MI failing myocardium. Notably, Akt was strongly activated early after MI with a concurrent VEGF overexpression. In the chronic phase, Akt activation was still evident, while VEGF was downregulated. These molecular changes were associated with maladaptive hypertrophic LV remodelling and severe cardiac dysfunction. Surprisingly, exercise restored the angiogenic signalling by increasing VEGF and eNOS phosphorylation by Akt in the heart. We propose that the switch-on of this pathway promotes new vessel growth and the transition from a maladaptive to an adaptive, angiogenesis-dependent LV hypertrophy which attenuates the negative LV remodelling observed in

sedentary animals late after MI. Our evidence seems to confirm previous data indicating as Akt is able to promote adaptive cardiac hypertrophy only when associated with increased VEGF production and concurrent angiogenesis.[3,34] This is the case of 'physiological' cardiac hypertrophy induced by exercise which allows to maintain normal wall stress and is not associated with impaired systolic and diastolic function.[35] This latter phenomenon may be explained by the growth of vascular network and enhanced coronary reserve which accompany the increase in cardiac mass in healthy exercised animals.[15] Interestingly, as we have herein demonstrated, VEGF/Akt-dependent angiogenesis plays a positive role also in the pathophysiology of exercise-induced hypertrophy in the failing myocardium. In fact, although exercise further increases cardiac mass of the post-MI heart, it reverses the process of eccentric hypertrophy and attenuates cavity dilation. In this regard, in a similar model of large transmural infarction leading to severe dysfunction, it has been previously demonstrated that exercise-induced hypertrophy reduces wall stress through inducing a normalized wall thickness to cavity area ratio.[36] The positive LV remodelling induced by exercise in the failing myocardium may contribute to the increased LV contractility reserve observed in this study.

4.2 Exercise ameliorates β-AR function in the failing myocardium

The crucial role of β-AR dysregulation in the pathophysiology of HF is well established.[4–7] GRK2, which plays a key role in the regulation of β-AR,[6] is significantly elevated in human and experimental HF.[6–10] Moreover, molecular manipulations of β-AR utilizing GRK2 inhibitors, such as the peptide known as the βARKct, restore β-AR signalling in the heart and increase cardiac function.[11–14] A significant reduction of cardiac GRK2 expression has been also recognized as a potential mechanism by which selective and non selective β-AR blockade may positively affect β-AR signalling.[20,37] Previous works from our group have shown that exercise is able to decrease GRK2 myocardial levels and improve β-AR signalling and responsiveness in spontaneously hypertensive rats[23] as well as in the aged heart.[20] The current study extends our previous observations demonstrating for the first time that training evokes similar effects on β-AR system also in the post-ischaemic hypertrophied failing myocardium leading to an enhanced cardiac inotropic state at the adrenergic stimulation. A favourable effect of exercise on cardiac β-AR downregulation/desensitization has been previously reported in post-MI exercised mice by de Waard *et al*.[22] These authors found increased cardiac β-AR protein and cAMP levels in the exercise group, but these findings were not associated with significant changes of GRK2 protein levels. There are at least two plausible explanations to reconcile these data with our results on exercise-related GRK2 downregulation in the failing heart: (i) a more advanced HF stage observed in our MI animal population. This is consistent with our previous observations on the relationship between GRK2 increased activity and expression in failing human myocardium and the degree of LV dysfunction and HF stage;[10] (ii) the different animal species utilized in the two studies (rats vs. mice). In this vein, it has been demonstrated that the mechanisms of β-AR desensitization may be GRK2- dependent[23] or GRK2-independent,[24] even in the same animal species. Importantly, the observation of improved β-AR responses in intact cardiomyocytes indicate that training may restore receptor signalling aberrations in remote non-infarcted myocardium contributing to LV dysfunction. It still remains an unresolved issue why exercise does not seem affect basal LV contractility in failing hearts,[25,27] despite the improved β-AR function. Consistent with this is the observation that basal cAMP production, which remained still depressed in exercised HF rats, is unchanged, since adenylyl cyclase activity and cardiac contraction via protein kinase A-mediated downstream effects are closely interlinked.[38] This finding strongly supports the importance of downstream cellular events in the improvement of β-AR signalling and responsiveness in the failing heart.

In conclusions, our observations on exercise-induced improvement of coronary angiogenesis and β-AR signalling in the failing heart raise an important issue which merits explanation in future investigations: is there a link between angiogenesis and β-ARs in HF? In this regard, several lines of evidence seem to answer this question in the affirmative since: (i) a relationship between β_2-ARs and angiogenesis responses has been demonstrated in the ischaemic skeletal muscle;[39] (ii) nitric oxide has been shown to downstream mediate angiogenic signalling pathways but also decrease GRK2-dependent β-AR desensitization in the heart[40]; and (iii) the PI3kinase, which is involved in β-AR pathway, as well as in Akt-dependent cardiac angiogenic signalling.[41] These studies suggest that angiogenesis stimulation combined with GRK2 inhibition in the heart might have a synergistic effect in advanced HF.

Supplementary material

Supplementary Material is available at *Cardiovascular Research* Online.

Funding

Università degli Studi di Napoli 'Federico II', Italy (1/9/01-3464).

Conflict of interest: none declared.

References

1. Anversa P, Beghi C, Kikkawa Y, Olivetti G. Myocardial infarction in rats. Infarct size, myocyte hypertrophy, and capillary growth. *Circ Res* 1986; **58**:26–37.
2. Karam R, Healy BP, Wicker P. Coronary reserve is depressed in postmyocardial infarction reactive cardiac hypertrophy. *Circulation* 1990;**81**: 238–246.
3. Shiojima I, Sato K, Izumiya Y, Schiekofer S, Ito M, Liao R *et al*. Disruption of coordinated cardiac hypertrophy and angiogenesis contributes to the transition to heart failure. *J Clin Invest* 2005;**115**:2108–2118.
4. Bristow MR, Ginsburg R, Minobe W, Cubicciotti RS, Sageman WS, Lurie K *et al*. Decreased catecholamine sensitivity and β-adrenergic-receptor density in failing human hearts. *N Engl J Med* 1982;**307**:205–211.
5. Bristow MR, Ginsburg R, Umans V, Fowler M, Rasmussen R, Zera P *et al*. β_1- and β_2-adrenergic-receptor subpopulations in nonfailing and failing human ventricular myocardium: coupling of both receptor subtypes to muscle contraction and selective β_1-receptor down-regulation in heart failure. *Circ Res* 1986;**59**:297–309.
6. Rockman HA, Koch WJ, Lefkowitz RJ. Seven-transmembrane-spanning and heart function. *Nature* 2002;**415**:206–212.

7. Petrofski JP, Koch WJ. The β-adrenergic receptor kinase (βARK1) in heart failure. *J Mol Cell Cardiol* 2003;**35**:1167–1174.
8. Gros R, Chorazyczewski J, Meek MD, Benovic JL, Ferguson SS, Feldman RD. G-Protein-coupled receptor kinase activity in hypertension: increased vascular and lymphocyte G-protein receptor kinase-2 protein expression. *Hypertension* 2000;**35**:38–42.
9. Ungerer M, Bohm M, Elce JS, Erdmann E, Lohse MJ. Altered expression of β-adrenergic receptor kinase and β1-adrenergic receptors in the failing human heart. *Circulation* 1993;**87**:454–463.
10. Iaccarino G, Barbato E, Cipolletta E, De Amicis V, Margulies KB, Leosco D *et al*. Elevated myocardial and lymphocyte GRK2 expression and activity in human heart failure. *Eur Heart J* 2005;**26**:1752–1758.
11. Koch WJ, Rockman HA, Samama P, Hamilton RA, Bond RA, Milano CA *et al*. Cardiac function in mice overexpressing the β-adrenergic receptor kinase or a βARK inhibitor. *Science* 1995;**268**:1350–1353.
12. Rockman HA, Chien KR, Choi D-J, Iaccarino G, Hunter JJ, Ross J Jr *et al*. Expression of a β-adrenergic receptor kinase 1 inhibitor prevents the development of heart failure in gene targeted mice. *Proc Natl Acad Sci USA* 1998;**95**:7000–7005.
13. Shah AS, White DC, Emani S, Kypson AP, Lilly RE, Wilson K *et al*. In vivo ventricular gene delivery of a β-adrenergic receptor kinase inhibitor to the failing heart reverses cardiac dysfunction. *Circulation* 2001;**103**: 1311–1316.
14. Harding V, Jones L, Lefkowitz RJ, Koch WJ, Rockman HA. Cardiac βARK1 inhibition prolongs survival and augments β blocker therapy in a mouse model of severe heart failure. *Proc Natl Acad Sci USA* 2001;**98**: 5809–5814.
15. Hudlicka O, Brown M, Egginton S. Angiogenesis in skeletal and cardiac muscle. *Physiol Rev* 1992;**72**:369–417.
16. Brown MD. Exercise and coronary vascular remodelling in the healthy heart. *Exp Physiol* 2003;**88**:645–658.
17. Leosco D, Rengo G, Iaccarino G, Sanzari E, Golino L, De Lisa G *et al*. Prior exercise improves age-dependent vascular endothelial growth factor downregulation and angiogenesis responses to hind-limb ischemia in old rats. *J Gerontol A Biol Sci Med Sci* 2007;**62**:471–480.
18. Iemitsu M, Maeda S, Jesmin S, Otsuki T, Miyauchi T. Exercise training improves aging-induced downregulation of VEGF angiogenic signaling cascade in hearts. *Am J Physiol Heart Circ Physiol* 2006;**291**: H1290–H1298.
19. Gustafsson T, Puntschart A, Kaijser L, Jansson E, Sundberg CJ. Exercise-induced expression of angiogenesis-related transcription and growth factors in human skeletal muscle. *Am J Physiol Heart Circ Physiol* 1999;**276**:H679–H685.
20. Leosco D, Rengo G, Iaccarino G, Filippelli A, Lymperopoulos A, Zincarelli C *et al*. Exercise and β-blocker treatment ameliorate age-impaired β-adrenergic receptor signaling and enhance cardiac responsiveness to adrenergic stimulation. *Am J Physiol Heart Circ Physiol* 2007;**293**:H1596–H1603.
21. Leosco D, Iaccarino G, Cipolletta E, De Santis D, Pisani E, Trimarco V *et al*. Exercise restores β-adrenergic vasorelaxation in aged rat carotid arteries. *Am J Physiol Heart Circ Physiol* 2003;**285**:H369–H374.
22. de Waard MC, van der Velden J, Bito V, Ozdemir S, Biesmans L, Boontje NM *et al*. Early exercise training normalizes myofilament function and attenuates left ventricular pump dysfunction in mice with a large myocardial infarction. *Circ Res* 2007;**100**:1079–1088.
23. MacDonnell SM, Kubo H, Crabbe DL, Renna BF, Reger PO, Mohara J *et al*. Improved myocardial beta-adrenergic responsiveness and signaling with exercise training in hypertension. *Circulation* 2005;**111**:3420–3428.
24. Xiao RP, Spurgeon HA, O'Connor F, Lakatta EG. Age-associated changes in beta-adrenergic modulation on rat cardiac excitation–contraction coupling. *J Clin Invest* 1994;**94**:2051–2059.
25. Musch TI, Moore RL, Leathers DJ, Bruno A, Zelis R. Endurance training in rats with chronic heart failure induced by myocardial infarction. *Circulation* 1986;**74**:431–441.
26. Wisloff U, Loennechen JP, Currie S, Smith GL, Ellingsen O. Aerobic exercise reduces cardiomyocyte hypertrophy and increases contractility, Ca^{2+}-sensitivity and SERCA-2 in rat after myocardial infarction. *Cardiovasc Res* 2002;**54**:162–174.
27. Gaudron P, Hu K, Schamberger R, Budin M, Walter B, Ertl G. Effect of endurance training early or late after coronary artery occlusion on left ventricular remodeling, hemodynamics, and survival in rats with chronic transmural myocardial infarction. *Circulation* 1994;**89**:402–412.
28. Kloner RA, Kloner JA. The effect of early exercise on myocardial infarct scar formation. *Am Heart J* 1983;**106**:1009–1013.
29. White FC, Bloor CM, McKirnan MD, Carroll SM. Exercise training in swine promotes growth of arteriolar bed and capillary angiogenesis in heart. *J Appl Physiol* 1998;**85**:1160–1168.
30. Lei L, Zhou R, Zheng W, Christensen LP, Weiss RM, Tomanek RJ. Bradycardia induces Angiogenesis, Increases Coronary Reserve, and Preserves Function of the Postinfarcted Heart. *Circulation* 2004;**110**:796–802.
31. Rehman J, Li J, Parvathaneni L, Karlsson G, Panchal VR, Temm CJ *et al*. Exercise acutely increases circulating endothelial progenitor cells and monocyte-/macrophage-derived angiogenic cells. *J Am Coll Cardiol* 2004;**43**:2314–2318.
32. Koller A, Huang A, Sun D, Kaley G. Exercise training augments flow-dependent dilation in rat skeletal muscle arterioles. Role of endothelial nitric oxide and prostaglandins. *Circ Res* 1995;**76**:544–550.
33. Carmeliet P. Mechanisms of angiogenesis and arteriogenesis. *Nat Med* 2006;**6**:389–395.
34. Nagoshi T, Matsui T, Aoyama T, Leri A, Anversa P, Li L *et al*. PI3K rescues the detrimental effects of chronic Akt activation in the heart during ischemia/reperfusion injury. *J Clin Invest* 2005;**115**:2128–2138.
35. Shapiro LM, Smith RG. Effect of training of left ventricular structure and function—an echocardiographic study. *Br Heart J* 1983;**50**:534–539.
36. Orenstein TL, Parker TG, Butany JW, Goodman JM, Dawood F, Wen WH *et al*. Favorable left ventricular remodeling following large myocardial infarction by exercise training. Effect on ventricular morphology and gene expression. *J Clin Invest* 1995;**96**:858–866.
37. Iaccarino G, Tomhave ED, Lefkowitz RJ, Koch WJ. Reciprocal in vivo regulation of myocardial G protein-coupled receptor kinase expression by β-adrenergic receptor stimulation and blockade. *Circulation* 1998;**98**: 1783–1789.
38. Georget M, Mateo P, Vandecasteele G, Jurevicius J, Lipskaia L, Defer N *et al*. Augmentation of cardiac contractility with no change in L-type Ca2+ current in transgenic mice with a cardiac-directed expression of the human adenylyl cyclase type 8 (AC8). *FASEB J* 2002;**16**:1636–1648.
39. Iaccarino G, Ciccarelli M, Sorriento D, Galasso G, Campanile A, Santulli G *et al*. Ischemic neoangiogenesis enhanced by beta-2 adrenergic receptor overexpression: a novel role for the endothelial adrenergic system. *Circ Res* 2005;**97**:1182–1189.
40. Whalen EJ, Foster MW, Matsumoto A, Ozawa K, Violin JD, Que LG *et al*. Regulation of beta-adrenergic receptor signaling by S-nitrosylation of G-protein-coupled receptor kinase 2. *Cell* 2007;**129**:511–522.
41. Perrino C, Naga Prasad SV, Mao L, Noma T, Yan Z, Kim HS *et al*. Intermittent pressure overload triggers hypertrophy-independent cardiac dysfunction and vascular rarefaction. *J Clin Invest* 2006;**116**:1547–1560.