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Original Paper

Expression of MicroRNA-29 and Collagen in Cardiac Muscle after Swimming Training in Myocardial-Infarcted Rats

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Key Words
Myocardial Infarction • Aerobic training • microRNA-29 • Collagen

Abstract
Background: Myocardial infarction (MI) is accompanied by cardiac growth, increased collagen deposition, cell death and new vascularization of the cardiac tissue, which results in reduced ventricular compliance. The MiRNA-29 family (29a, 29b, and 29c) targets mRNAs that encode collagens and other proteins involved in fibrosis. In this study we assessed the effects of swimming training (ST) on expression of the cardiac miRNA-29 family and on genes encoding collagen after MI in rats. ***Methods:*** ST consisted of 60 min/day/10 weeks and began four weeks after MI. MiRNA and collagen expression analysis were performed in the infarcted region (IR), border region (BR) of the infarcted region and in the remote myocardium (RM) of the left ventricle. ***Results:*** MiRNA-29a expression increased 32% in BR and 52% in RM in the TR-INF compared with SED-INF. MiRNA-29c increased by 63% in BR and 55% in RM in TR-INF compared with SED-INF group. COL IAI and COL IIIAI decreased by 63% and 62% in TR-INF, respectively, compared with SED-INF. COLIIIAI expression decreased by 16% in TR-INF compared with SED-INF. ***Conclusion:*** Altogether, our results showed that ST restores cardiac miRNA-29 (a and c) levels and prevents COL IAI and COL IIIAI expression in BR and RM, which may contribute to the improvement in ventricular function induced by swimming training, after MI.

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Introduction

Myocardial infarction (MI) represents one of the major causes of morbidity and mortality in adults [1, 2]. After MI, complex molecular and structural modifications occur in both the infarcted and non-infarcted myocardium [3, 4]. The myocardial tissue is composed of different cell types such as myocytes, fibroblasts and vascular cells which play critical roles during the remodeling process [5-7]. Cardiac fibrosis is characterized by an increase in cardiac collagen content and is a dynamic process that plays a essential role in the progression of heart failure after MI. Therapeutic approaches that control fibroblast activity and prevent maladaptive processes represent a potential strategy to attenuate progressive remodeling towards heart failure [8-11].

Exercise training is the most promising lifestyle change that has beneficial effects on cardiac function. The physiological adaptations to aerobic training consist of a set of morphological and functional adaptations to metabolism, circulation, and cardiac function [12-15]. However, the mechanisms responsible for these beneficial effects of exercise training on myocardial remodeling and function after MI remain to be elucidated. Recently, our group (16), showed that swimming training (ST) increases microRNA-29c (miRNA-29) expression, which in turn decreases collagen expression in the heart of healthy rats, and is associated with the improved left ventricular (LV) compliance observed after exercise training.

MiRNAs are currently considered potential therapeutic targets and biomarkers in the cardiovascular research of various physiological and pathological processes [10]. MiRNAs are noncoding single stranded RNA molecules that regulate gene expression. The action of miRNAs occurs at the post-transcriptional level. MiRNAs negatively regulate the expression of their target genes by binding to the 3' untranslated regions (3'-UTR) of mature mRNA expressed by the target gene, to prevent it from being translated, or to induce its degradation. Both mechanisms repress mature mRNA translation into protein [17, 18].

Several miRNAs have been implicated in the expression of genes that are involved in cardiac fibrosis post-MI [19-23]. Among them, miRNA-21 is known to regulate fibroblast proliferation and fibrosis [19] and recently, Wang et al. [23] found that expression of the miRNA-24 cluster was correlated with the degree of fibrosis in hypertrophic hearts. Van Rooij et al. [22] validated target genes of the miRNA-29 family involved in cardiac fibrosis (collagen, fibrillin and elastin) which play an important role in fibrosis during cardiac remodeling after MI.

As regards exercise training, there is a lack of research about its effect on the expression of miRNAs in rats after MI. Thus, the purpose of this study was to investigate the effects of exercise training after MI, on the expression of the cardiac miRNA-29 family and target genes (collagen) both in the infarcted region (IR), and border region (BR) of the infarcted heart and remote myocardium (RM) of the LV.

Materials and Methods

Animal care

Male Wistar rats (body weight between 250-300g and 10 weeks old) were housed in standard cages, with food and water *ad libitum.* The environmental temperature was kept constant at 23 ± 1°C, and 12:12-h dark-light cycle was maintained throughout the experiment. All the protocols and surgical procedures were in accordance with the guidelines of the Brazilian College for Animal Experimentation and were approved by the Ethics Committee of the School of Physical Education and Sport (n° 2010/07) of the University of São Paulo.

Surgical Procedure

The rats were anesthetized intraperitoneally with 0.1mg/kg of a mixture containing 0.67 mg/kg xylazine and 0.33 mg/kg ketamine and intubated via tracheotomy, placed under a rodent respirator apparatus (Harvard model 680). The heart was exposed through left thoracotomy between the fifth and

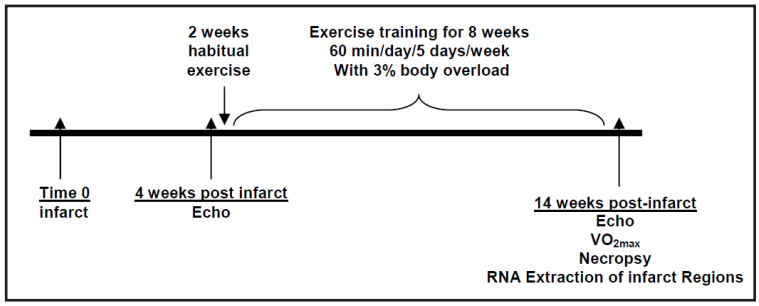

Fig. 1. Experimental design.

sixth ribs (1.5 cm in diameter), and the pericardium was opened. In animals in which the MI was produced, a 9-0 Ethilon suture was placed under the left main coronary artery at a point 1–2 mm distal to the edge of the left atrium, and the artery was ligated [24]. Sham-operated animals underwent the same procedure, except that the suture under the coronary artery was left untied. Muscle and skin incisions were closed with separate purse-string silk sutures (size 0), and the lungs were fully expanded. The heart was then returned to its normal position and the thorax immediately closed. Each animal was allowed a minimum of 4 weeks of recovery.

Experimental design

Four weeks after the infarct surgery, rats were randomly assigned to either a sedentary or training group as followed: Sedentary-Sham (SED-SHAM; n=7), Sedentary Infarct (SED-INF; n=7), Trained Sham (TR-SHAM; n=7) and Trained Infarct (TR-INF; n=7) (Fig. 1).

Echocardiography

Echocardiography was performed in accordance with the recommended guidelines of the American Society of Echocardiography as previously described [25]. Transthoracic echocardiography was performed before and after the training period based on the average of three consecutive cardiac cycles using an HP Sonos-5500 (Hewlett Packard, Andover, MA, USA) echocardiograph with a 12MHz linear transducer. Rats were anesthetized with ketamine (90 mg/kg) and xylazine (10 mg/kg). Two-dimensional parasternal long-axis and short-axis views were recorded, in addition to two-dimensional targeted M-mode traces through the anterior and posterior left ventricular walls. The diastolic LV posterior wall thickness (LVPWd), systolic LV posterior wall thickness (LVPWs), LV end-diastolic (LVEDD) and LV end-systolic (LVESD) diameters and LV end-diastolic transverse area (LVEDA) and LV end-systolic transverse area (LVESA) areas were determined.

LV diastolic and systolic diameters were measured from M mode or 2-dimensional images of the mid-LV chamber. Additionally, both diastolic and systolic transverse areas (SA) of the LV were measured by 2-dimensional echocardiography in the basal, midview, and apical view. The final value was the arithmetic mean of the measures of the 3 views. Left atrium size was determined by the volume of the cavity measured by the Simpson method in only the 4-chamber apical view at end systole.

Systolic function was analyzed by the fractional area change (FAC) (FAC= LVEDA-LVESA/LVEDA) and fractional shortening (FS) (FS=LVEDD-LVESD/LVEDD). Diastolic function was evaluated based on the parameters of mitral inflow and LV outflow tract velocity curves by pulsed wave Doppler and with tissue Doppler imaging of the mitral lateral annulus. From the mitral diastolic flow velocity curve, the maximum velocity of the E and A waves were measured and the E/A ratio calculated. Isovolumetric relaxation time was obtained by measuring the interval between the end of the systolic spectral curve of the LV outflow tract and the beginning of the mitral diastolic spectral curve (E wave). E-wave deceleration time was measured as the interval from the peak of the E wave to the point where the deceleration ramp would reach the baseline. Tissue Doppler imaging was performed to measure the early (E) and late (A) diastolic wave velocities of the lateral mitral annulus in 4-chamber apical view.

Infarction Size

MI size was measured by 2-dimensional echocardiogram and based on LV basal, midtransversal, and apical transverse views. Three measures of the endocardial perimeter (EP) of the LV cavity, and length of

the segment with infarction (ISe) for each transverse view were obtained in diastole. The infarct size for each segment (ISi), expressed as the proportion of the LV perimeter of each transverse view, was calculated by the equation ISi (%) = ISe/EPx100. The total infarct size of each animal was calculated as the mean ISi (%) of the 3 segments. MI by echocardiography was defined as any segment with increased echogenicity and/or change in myocardial thickening or systolic movement (hypokinesia, akinesia, or dyskinesia).

Exercise Training Protocol

Exercise training consisted of swimming sessions of 60-min duration, 5 days/wk, with 3% body overload, for 10 weeks, which were carried out between 11:30 AM and 1:30 PM. This mild-intensity long-period training protocol has been used in our laboratory and is effective for promoting cardiovascular adaptations and increasing muscle oxidative capacity [26].

Oxygen Uptake Measurements

Oxygen uptake (VO_{2max}) was measured by means of expired gas analysis during the graded treadmill exercise. VO_{2max} was determined during a maximal exercise test adapted from Musch et al. [27]. The parameters were measured using the Sable Systems FC-10a Oxyggen Analyzer (Sable Systems, Henderson, NV, USA). The test was carried out after a 1-day recovery period from the last exercise session. The volume of the air supplied was 3.5 l/min. The gas analyzer was calibrated with a reference gas mixture before each test. The VO_{2max} test protocol involved stepwise increase in the treadmill speed as follows: after a 15-min period of acclimation, the treadmill was started at 6 m/min, and the speed was increased by 3 m/min every 3 min until the rat was exhausted. Exhaustion was defined as spending time on the shocker plate without attempting to reengage the treadmill within 10 s.

mRNA and miRNA quantification using real-time PCR

The relative expression of COL IAI, COL IIIAI, miRNA-29a, miRNA-29b, miRNA-29c, cyclophilin A and U6 were analyzed by polymerase chain reaction (PCR), described as follows. MiRNAs and collagen analysis was performed by PCR in the IR, BR and RM of the LV.

Real-time PCR

Frozen LV samples were homogenized in trizol, and ribonucleic acid (RNA) was isolated according to the manufacturer's instructions (Invitrogen Life Technologies, Strathclyde, UK). Samples were quantified by spectrophotometer at 260nm and integrity checked by EtBr-agarose gel electrophoresis.

cDNA for miRNA analysis was synthesized from total RNA using gene-specific primers according to the TaqMan MicroRNA Assay protocol (Applied Biosystems, CA, USA). Reverse transcription (RT) was performed using SuperScript II Reverse Transcriptase (Invitrogen Life Technologies).

The mRNA expression of type I and III collagens were assessed by oligonucleotides primers as follows: COL IA1, 5'-AgA gAg CAT gAC CgA TggA-3' and 5'-gAggTTgCC AgT CTg TTg g-3'; COL IIIA1, 5'-AAg gTC CAC gAggTg ACA A-3' and 5'-Agg gCC TggACT ACC AAC T-3'. Quantification of the target genes expression was performed with a SYBRgreen PCR Master Mix, (Applied Biosystem, PE, Foster City, CA, USA) using ABI PRISM 7700 Sequence Detection System (Applied Biosystem). The expression of cyclophilin A (5'-AAT gCT ggA CCA AAC ACA AA-3' and 5'-CCT TCT TTC ACC TTC CCA AA-3') was measured as an internal control.

In order to accurately detect the expression of miRNAs, real-time PCR quantification was performed using TaqMan MicroRNA Assay protocol (Applied Biosystems, CA, USA). TaqMan MicroRNA Assay protocol for miRNA-29a (ID2222), -29b (ID2223) and -29c (ID415). Samples were normalized by evaluating U6 (#4373381) expression.

Relative quantities of target gene expressions were compared after normalization to the values of reference gene (ΔCT). Fold changes in mRNA and miRNA expression were calculated using the differences in ΔCT values between the two samples (ΔΔCT) and equation $2^{-\Delta\Delta CT}$. The results were expressed as % of control.

Cardiac morphological analysis

The LV was fixed in 6% formaldehyde and embedded in paraffin, cut into 5-μm sections at the level of the papillary muscle. The collagen volumetric fraction (CVF) of interstitial myocardial tissue was determined using direct light of the Picrosirius red prepared tissues, as reported previously [26]. Twenty fields were selected from sections placed in a projection microscope (x200), and interstitial collagen was determined

Table 1. Echocardiography measurements and *in vivo* and post mortem data. Results of the evaluation of morphology (percentage of MI, mass and thickness of the anterior and posterior wall of the LV), ventricular function assessed by % fractional area change and fractional shortening (systolic function) and E/A ratio (diastolic function), performed by echocardiographic evaluation n = 7 each group. All results are presented as mean ± standard deviation. Significant difference * *vs.* SED-SHAM, & *vs*. TR-SHAM # *vs*. SED-INF, + *vs*. pre (P< 0.05)

Parameters	SED-SHAM	TR-SHAM	SED-INF	TR-INF
Body weight				
Pre	353±9.1	354±13.3	348±12.4	346±13.6
Post	388±14.0+	387±2.6+	382±14.6+	387±12.3+
Heart weight to body weight	2.17±0.2	2.4±0.1*	2.4±0.2*	2.5±0.1*
VO_{2max}	46.0±2.25	53.8±2.6*#	43.0±1.9*&	48.9±2.8&#
Echocardiography				
Infarct size (%)			29%	28%
Anterior wall thickness (mm)	1.76±0.002	1.80±0.003#	1.28±0.003*&	1.30±0.003*&
Posterior wall thickness (mm)	1.47±0.01	1.72±0.003*	1.74±0.02*	1.82±0.01*#&
Fractional area change (%)	70%	71%#	33%*&	42%*#&
Fractional shortening (%)	38%	40%	28%*&	33%*&
Ratio Peak E/ Peak A(m\s)	1.40±0.1	1.55±0.4#	2.05±0.1*&	1.74±0.1*#&

by a computer-assisted image analysis system (Image-Pro Plus; Media Cybernetics, Silver Spring, MD) in the IR and in the BR + RM of the left ventricle.

Hydroxyproline determination

LV collagen content was quantified from the hydroxyproline (OH-proline) concentration by a modified method, as described previously [28]. Tissue samples were taken from the same area of the LV wall. The color/dye intensity was measured at 558 nm. OH-proline content was determined from duplicate samples using a calibration curve of 0.5–5 μg of OH-proline. The data are expressed as milligrams per gram of OH-proline.

Statistical Analysis

Statistical analysis was performed using randomized two-way analysis of variance. Bonferroni post hoc test was used for individual comparisons between means when a significant change was observed (Sigma software Stat). A level of significance of $p<0.05$ was adopted for all experiments. All results are presented as mean ± standard deviation.

Results

Body weight

There was no difference in body weight within each group in the pre-training (SED-SHAM 353 ± 9.1; TR-SHAM 354 ± 13.3; SED-INF 348 ± 12.4; TR-INF 346 ± 13.6 g) or post-workout periods (SED-SHAM 388 ± 14.0; TR-SHAM 387 ± 2.6; SED-INF 382 ± 14.6; TR-INF 387 ± 12.3 g). However, all groups showed increased body weight in the post-training compared to the pre-training period (Table 1, $p <0.05$).

Heart weight to body weight

Both ST and surgical procedure induced cardiac hypertrophy in the study measured by LV weight / BW ratio. LV weight / BW ratio increased by 12.3% in TR-SHAM (2.43 ± 0.1 mg/g), 12.5% in SED-INF (2.44 ± 0.2 mg/g) and 15.3% in TR-INF (2.52 ± 0.1 mg/g) in comparison with the SED-SHAM group (2.17 ± 0.2 mg/g) (Table 1, $p <0.05$).

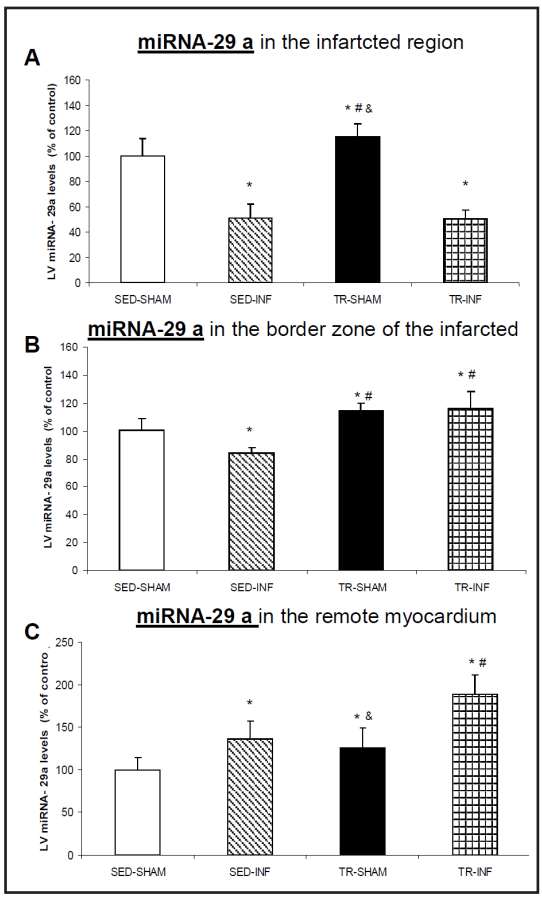

Fig. 2. Cardiac expression of miRNA-29a in different regions of infarcted rats. (A), infarcted region; (B), border region of the infarcted region; and (C) remote myocardium performed by real-time PCR reaction. Percentage related to SED-SHAM group, n = 7 each group. All results are presented as mean ± standard deviation. Significant difference * *vs.* SED-SHAM, # *vs.* SED-INF, & *vs.* TR-SHAM (P< 0.05).

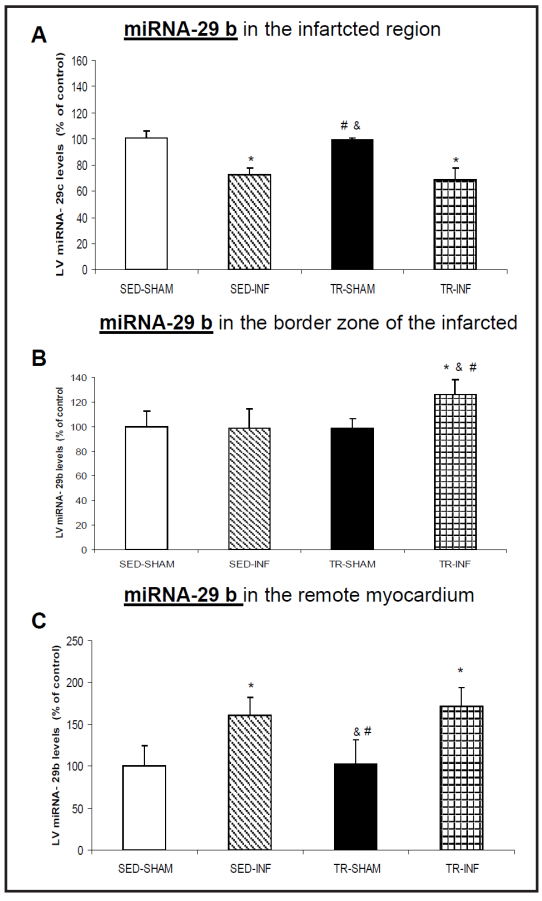

Fig. 3. Cardiac expression of miRNA-29b in different regions of infarcted rats. (A), infarcted region; (B), border region of the infarcted region; and (C) remote myocardium performed by real-time PCR reaction. Percentage related to SED SHAM group, n = 7 each group. All results are presented as mean ± standard deviation. Significant difference * *vs.* SED-SHAM, # *vs.* SED-INF, & *vs.* TR-SHAM (P< 0.05).

Oxygen uptake measurement

The aerobic training protocol effectively induced improvement in cardiorespiratory function showed by increased VO_{2max} in the TR-SHAM group (14%, 53.81 ± 2.6 mL.kg^{-1}.min^{-1}) in comparison with the SED-SHAM group (39.09 ± 1.9 mL.kg^{-1}.min^{-1}), and in TR-INF group (12%, 48.96 ± 2.8 mL.kg^{-1}.min^{-1}) compared with the SED-INF group (43.09 ± 1. 7 mL.kg^{-1}. min^{-1}). The VO_{2max} in the TR-SHAM group was 9% higher (46.08 ± 2.2 mL.kg^{-1}.min^{-1}) than in TR-INF group (39.09 ± 1.9 mL.kg-1.min-1) (Table 1, p <0.05).

Echocardiography measurements

MI area reached 30% in Group SED-INF, and 29% in Group TR-INF before the ST period and remained similar after ST (Table 1, p <0.05). The LV anterior wall thickness was similar between Group SED-INF (1.28 ± 0.003) and TR-INF (1.30 ± 0.003), and between Group SED-SHAM (1.76 ± 0.002) and TR-SHAM (1.80 ± 0.003) after the ST protocol, but both Group SED-INF and TR-INF showed 27% lower values compared with Group SED-SHAM and TR-SHAM, respectively. The LV posterior wall thickness in Group TR-SHAM (1.72 ± 0.003),

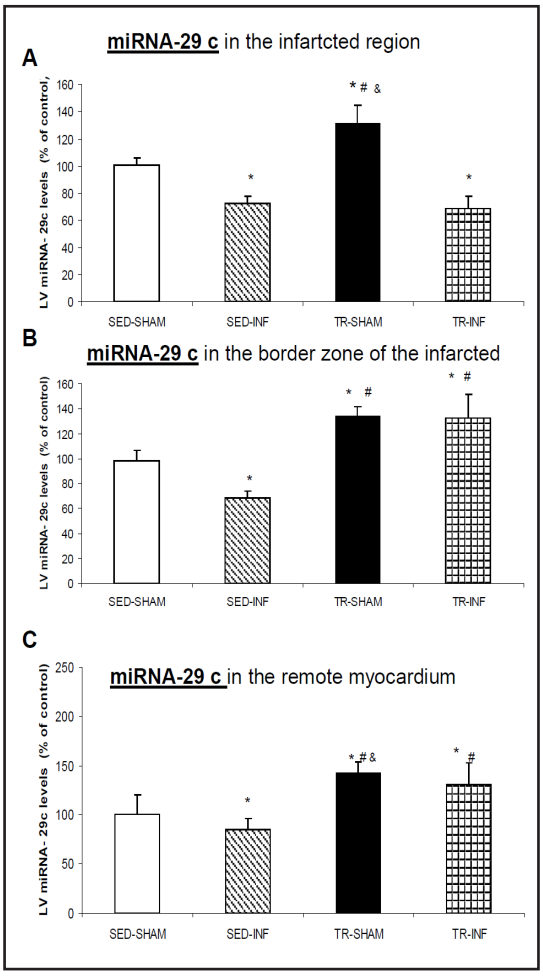

Fig. 4. Cardiac expression of miRNA-29c in different regions of infarcted rats. (A), infarcted region; (B), border region of the infarcted region; and (C) remote myocardium performed by real-time PCR reaction. Percentage related to SED SHAM group, n = 7 each group. All results are presented as mean ± standard deviation. Significant difference * *vs.* SED-SHAM, # *vs.* SED-INF, & *vs.* TR-SHAM (P< 0.05).

SED-INF (1.74 ± 0.02) and TR-INF (1.82 ± 0.01) showed 14.0%, 14.4% and 18.8% higher values, respectively, than those in Group SED-SHAM (1.47 ± 0.01) after ST (Table 1, p <0.05). There was no difference in FAC between Group SED-SHAM (70%) and Group TR-SHAM (71%), however, Group TR-INF (42%) showed an improvement in FAC% compared with Group SED-INF (33%). LV infarction induced a decrease in systolic function measured by fractional shortening, which was not recovered by ST (Table 1, p <0.05). The diastolic function measured by the E/A wave ratio did not differ between Group SED-SHAM (1.40 ± 0.1) and TR-SHAM (1.55 ± 0.4). However, Group TR-INF (1.74 ± 0.1) showed an decrease of 17% in E/A ratio compared with Group SED-INF (2.05 ± 0.1) after the ST protocol. (Table 1, p <0.05).

MiRNA expression

Myocardial Infarction induced down-regulation of MiRNA-29a expression by 16% in the IR and by 49% in the BR in both SED groups while it was increased by 36% in RM. MiRNA-29a expression was increased by 15% in IR, by 16% in BR and by 25% in RM in GroupTR-SHAM compared with SED-SHAM. In Group TR-INF, miRNA-29a expression was further increased by 32% in BR and by 52% in RM compared with SED-INF, but remained decreased in the IR by 17 % (Fig. 2, p <0.05).

With regard to MiRNA-29b expression, myocardial infarction induced its down-regulation by 33% in IR and its up-regulation by 60% in RM in Group SED-INF compared with SED-SHAM. MiRNA-29b expression was not changed with swimming training in any

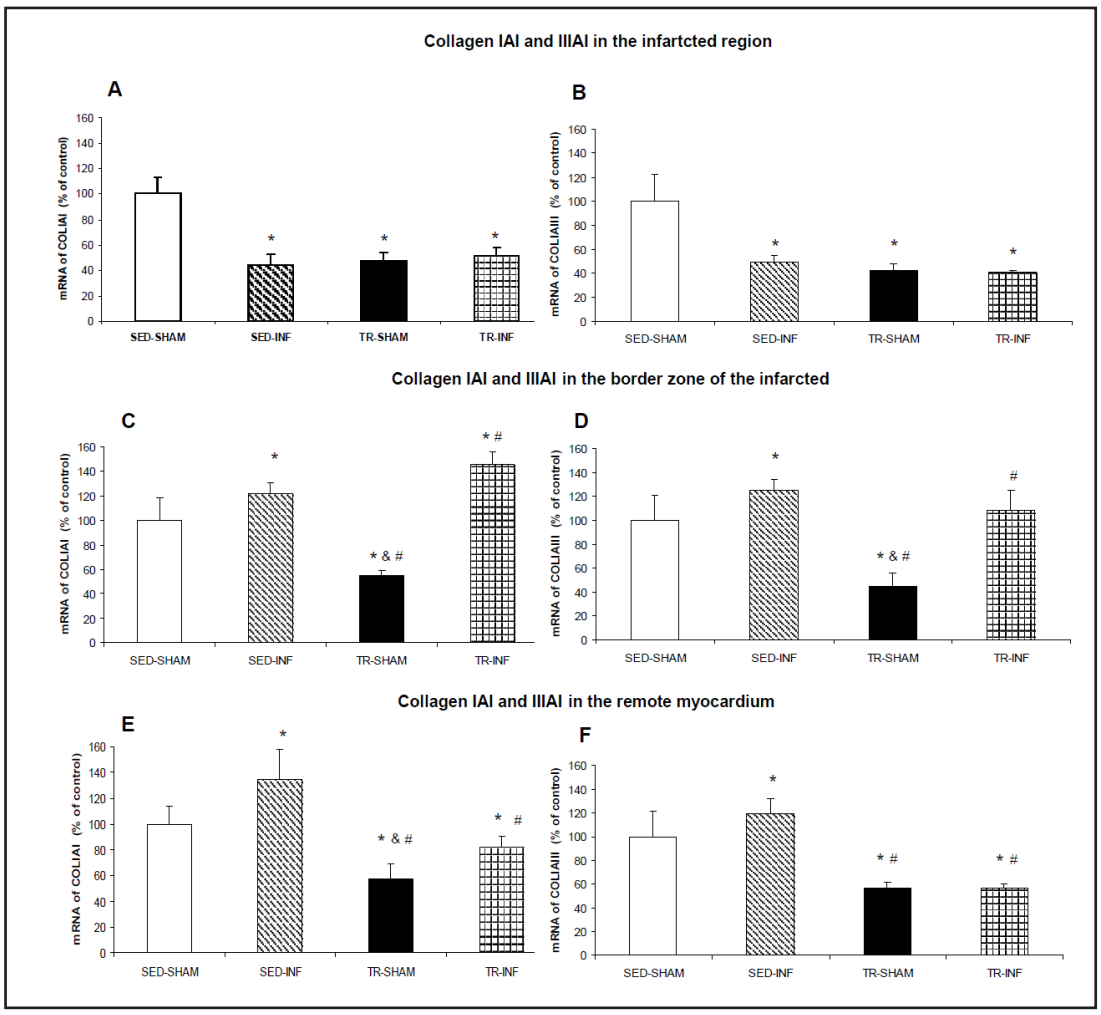

Fig. 5. Cardiac expression of mRNA collagens (A, C, E COL IAI; and B, D, F, COL IIIAI) in the infarcted region (A and B), in the border region (C and D), and in the remote myocardium (E and F) performed by real-time PCR. Percentage related to Group SED-SHAM, n = 7 each group. All results are presented as mean ± standard deviation. Significant difference * *vs.* SED-SHAM, # *vs.* SED-INF, & *vs.* TR-SHAM (P< 0.05).

studied region, however, its expression was increased by 27% in BR and by 65% in RM in Group TR-INF compared with TR-SHAM. In the IR miRNA-29b expression was decreased by 35% in Group TR-INF compared with TR-SHAM (Fig. 3, p <0.05).

MiRNA-29c expression was down-regulated by 28% in IR, by 31% in BR and by 15% in RM in Group SED-INF compared with SED-SHAM. Swimming training increased MiRNA-29c expression in all the regions studied (38% in the IR, 34% in BR and 42% in RM) in GroupTR-SHAM compared with SED-SHAM. Similarly MiRNA-29c was increased by 63% in BR and 55% in RM of Group TR-INF compared with SED-INF, however, miRNA-29c expression was not changed with training in IR after myocardial infarction (TR-INF) (Fig. 4, p <0.05).

Collagen Expression

Myocardial infarction induced collagen expression in the BR and RM while it expression was decreased in the IR. COL IAI and COL IIIAI was decreased by 56% and 51%, respectively in Group SED-INF compared with SED-SHAM in the IR. In contrast, COL IAI and COL IIIAI expression was increased by 22% and 25%, respectively, in Group SED-INF compared with SED-SHAM in the BR and increased by 35% and 19%, respectively, in the RM.

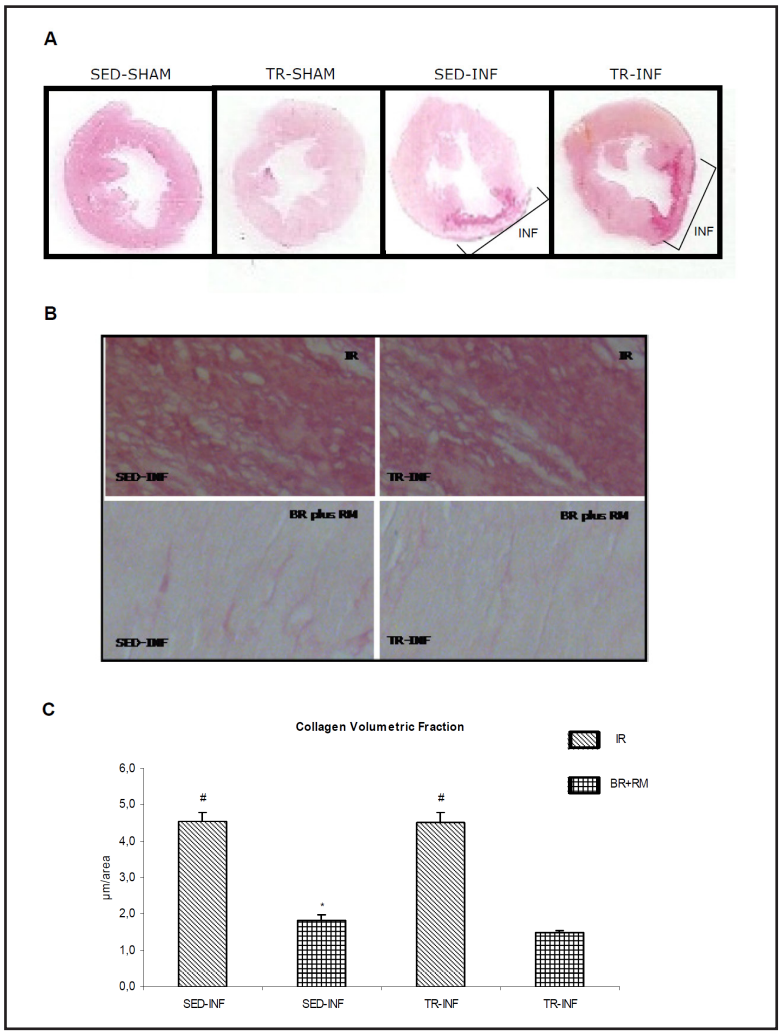

Fig. 6. A) Representative histological sections of the infarcted region in myocardium. B) Representative images of PAS stained collagen fibers in the infarcted region and in the border zone + remote myocardium of the SED-INF and TR-INF groups (n = 7 each group). C) Quantitative analysis of collagen fibers in the myocardium. All results are presented as mean ± standard deviation. Significant difference * *vs.* TR-INF (BR+RM), # *vs*. SED-INF and TR-INF (BR+RM) (P< 0.05).

Swimming training decreased collagen expression in all the studied regions. COL IAI expression was decreased by 53% in IR, 55% in BR and 43% in RM in Group TR-SHAM compared with SED-SHAM. COL IIIAI expression was decreased by 57% in IR, 56% in BR and 43% in RM in Group TR-SHAM compared with SED-SHAM.

COL IAI and COL IIIAI expression remained decreased in Group TR-INF compared with SED-SHAM in the IR. In the BR, COL IAI further increased by 17% in Group TR-INF compared with SED-INF, while COL IIIAI remained similar to Group SED-SHAM. In the RM, swimming training reduced both COL IAI and COL IIIAI expression in Group TR-INF compared with SED-INF. (Fig. 5, p <0.05).

Quantitative analysis of collagen fibers

Image analysis showed the CVF in the BR + RM was decreased by 18.7% in Group TR-INF compared with SED-INF (1.8 ± 0.1 vs. 1.5± 0.1 µm/area), but it remained unchanged in the IR (4.6± 0.2 vs. 4.5± 0.3 µm/area) (Fig. 6, p <0.05).

Quantitative analysis of Hydroxyproline

LV collagen content quantified by hydroxyproline (OH-proline) concentration (mg/g) was decreased by 38% in the BR and by 36% in the RM of Group TR-SHAM compared with SED-SHAM. However, OH-proline concentration was increased by 19% in the BR and 25% in the RM in Group SED-INF compared with SED-SHAM. However, when swimming training

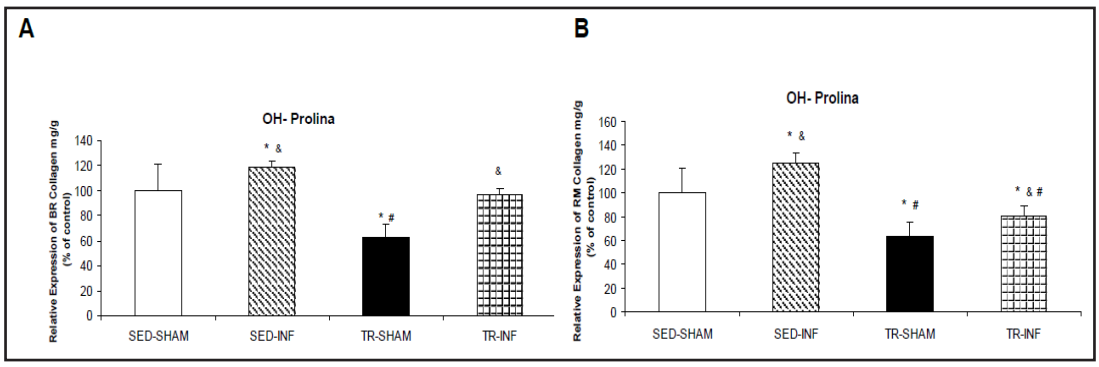

Fig. 7. Collagen content quantified by hydroxyproline (OH-proline) concentration (mg/g) in the border region (A) and in the remote myocardium (B) of the SED-INF and TR-INF groups (n = 7 each group). All results are presented as mean ± standard deviation. Significant difference * *vs.* SED-SHAM, # *vs.* SED-INF, & *vs.* TR-SHAM (P< 0.05).

was associated with the myocardial infarction, the OH-proline concentration decreased by 35.2% in Group TR-INF compared with SED-INF only in the RM region (Fig. 7, p <0.05).

Discussion

The main finding of the present study was that aerobic training induces up-regulation of expression of miRNAs 29a and 29c in the BR and RM, which was correlated with a significant decrease in the gene expression of COL IIIAI in the BR, and COL IAI and COL IIIAI in rats with MI. These molecular adaptations were associated with improved ventricular function assessed by % FAC (systolic function) and E/A ratio (diastolic function).

The miRNA expression profile in the late phase of MI (3 and 14 days after MI) has recently been identified by Van Rooij et al. [22]. They found that the decreased expression of the miRNA-29 family (a, b and c) was most pronounced in the BR 3 days after MI and remained unchanged in RM after 14 days. The investigators also showed down-regulation of miRNA-29 *in vitro* and *in vivo* using a mouse model of MI. Whereas specific overexpression of miRNA-29 in fibroblasts reduced collagen expression during the LV repair process. In the present study miRNA-29c significantly decreased in the RM after IM, but unexpectedly miRNA-29a and 29b increased. On the other hand, increased miRNA-29 (29a and 29c) after training could be associated to the decrease in COL IAI and COL IIIAI expression. This discrepant result as regards miRNA-29c may be due to regulatory factors for gene expression that may alter the stability and half-life of the mRNA for collagens COL IAI and COL IIIAI. These changes are not correlated with similar changes in transcription rates for those mRNAs [5].

In another recent study, Port et al. [29] showed that expression of miRNA-29c was decreased at 2 weeks post-MI, but up-regulated at 2 months post-MI, a finding that correlates temporally with the expression of collagen genes. Here, our results show that miRNA-29 (29a and 29c) were decreased in the BR of the MI which correlated with increased expression of COL IAI and COL IIIAI. Moreover, it should be noted that COL IIIAI decreased after training, which is also associated with decrease in miRNA-29 (29a and 29c), while COL IAI remained increased. An explanation for the effect of exercise training on the expression of different collagen genes is that exercise may exert indirect effects. Hemodynamic changes induced by exercise may lead to the release of neurotransmitters and growth factors, such as norepinephrine and TGF- β that may lead to inverse regulation of collagen type I and III expression in fibroblasts [6, 30-32]. The complete regulatory mechanisms responsible for mRNA expression levels for collagen types I and III in heart of exercised-animals are still unclear.

Our results also demonstrated a reduction in miRNA-29 (29a, 29b and 29c) in IR of rats after myocardial infarction. However, this reduction did not influence the expression of

collagens. We speculate that the gene expression of collagens might be attenuated by some conditions in the infarction zone, such as increased cell death [3, 7]. Furthermore impaired microvasculature must have been responsible for the non significant effect of exercise in this region [3, 33]. The reasons for the discrepancies among these studies as regards miRNA-29 and gene expression in the IR, BR and RM, are unclear. However, the variations in disease state, tissue location, and time points might be responsible for the discrepancies.

Others miRNAs have been implicated in regulating the expression of genes that are involved in multiple biological processes post-MI. Overexpression of miRNA-21 by adenovirus has been shown to reduce MI size and decrease LV dimensions [19]. These results suggest that miRNA-21 overexpression may have a positive effect on the post-MI setting, but that miRNA-21 therapy may also increase myocardial fibrosis. Another study, Wang et al. [23], showed that miRNA-24 was down-regulated after MI, and change in miRNA-24 expression was closely associated with fibrosis remodeling in the cardiac muscle and that miRNA-24 could improve heart function 2 weeks after MI by shortening the infarct segment through regulating the metabolism of fibrosis.

Activated cardiac fibroblasts following MI increases the expression of extracellular matrix which causes mechanical stiffness, contributes to ventricular dysfunction and participates in pathological remodeling [3, 6]. Several studies have shown that the miRNA-29 family is involved in the regulation of fibrosis in diseases such as diabetic nephropathy, systemic sclerosis, chondropathy and MI [22, 34, 35]. These miRNAs already have *in vitro* and *in vivo* validated target genes for extracellular matrix protein, such as collagen IAI, IAII, IAIII, IIIAI, fibrilin and elastin in the heart and several other tissues [30]. Recently, our group (16) showed that rats subjected to swimming training presented physiological cardiac hypertrophy and increased expression of miRNA-29, which were linear with the increase in training volume. The miRNA-29 up-regulation was accompanied by improvement in ventricular compliance and was closely correlated with the decrease in gene and protein expression of collagens IAI and IAIII.

We concluded that ST induced up-regulation of the expression of miRNA-29, which was related to a significant decrease in gene expression of collagen and improvement in ventricular function. The precise mechanisms that lead to repression of miRNA-29 expression after MI in rats submitted aerobic training remain to be determined. The present study reinforces the novel approach of using miRNA therapies to regulate the fibrosis of cardiac muscle, which may consequently improve heart function and reduce scar size in MI patients.

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