Moderate exercise training improves survival and ventricular remodeling in an animal model of left ventricular volume overload.

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Abstract

Background: Exercise training has beneficial effects in patients with heart failure although there is still no clear evidence that it may impact on their survival. There are no data regarding the effects of exercise in subjects with chronic left ventricular (LV) volume overload. Using a rat model of severe aortic valve regurgitation (AR), we studied the effects of long term exercise training on survival, development of heart failure and LV myocardial remodeling.

Methods and Results: One hundred sixty male adult rats were divided in three groups: sham sedentary (n=40), AR sedentary (n=80) and 3) AR trained (n=40). Training consisted in treadmill running for up to 30 minutes, five times / week for 9 months, at a maximal speed of 20 m/min. All sham-operated animals survived the entire course of the protocol. After 9 months, 65% of trained animals were alive compared to 46% of sedentary ones (p=0.05). Ejection fractions remained in the normal range (all above 60%) and LV mass between AR groups were similar. There was significantly less LV fibrosis in the trained group as well as lower LV filling pressures and improved echocardiographic diastolic parameters. Heart rate variability was also improved by exercise.

Conclusion: Our data show that moderate endurance training is safe, does not increase the rate of developing heart failure and, most importantly, improves survival in this animal model of chronic LV volume overload. Exercise improved LV diastolic function, heart rate variability and reduced myocardial fibrosis.

Key words: survival, exercise, hypertrophy, valves, collagen

Introduction:

Regular exercise seems to be beneficial in most patients with stable cardiac diseases. In patients with heart failure, current evidence suggests that exercise can improve functional capacity and quality of life. However, the impact of exercise on mortality in patients with heart failure has not yet been clearly established [1]. The ability of exercise to prevent or delay the occurrence of heart failure in high risk subjects also remains unclear. Chronic left ventricular (LV) volume overload diseases such as aortic valve regurgitation are well tolerated for many years before heart failure occurs. There is currently no treatment proven effective to decrease morbidity, mortality, delay evolution towards heart failure or the need of surgery in this category of patients [2]. The potential benefits of exercise have never been evaluated in subjects with chronic left ventricular volume overload, dilated ventricles but preserved LV systolic function. The body of evidence suggests that aerobic exercise can improve cardiac performance by several mechanisms such as improvement of contractility, increased myocardial perfusion and angiogenesis, normalization of the sympathetic-parasympathetic balance, improvement of myocardial energetic metabolism, decreasing oxidative stress, better myocardial calcium handling and improvement of peripheral arterial compliance [3-8]. We have previously shown in a pilot study that exercise can be well tolerated in female rats with volume overload and that it may improve left ventricular remodeling. However, that study was not designed to assess survival and was clearly underpowered to evaluate the impact of exercise on cardiac tissue [9]. Considering these encouraging preliminary data and the potential benefits of exercise on cardiac physiology, we designed the

current study to assess the effects of a regular exercise program on cardiac remodeling, occurrence of systolic and diastolic dysfunction and survival in male rats suffering from chronic left ventricular volume overload caused by severe aortic valve regurgitation.

Methods

Animals: One hundred sixty adult male Wistar rats were purchased from Charles River (Saint-Constant QC, Canada) and divided in 3 groups as follows: 1) Sham-sedentary (SS; n=40); 2) AR sedentary (ARS; n=80) and 3) AR trained (ART n=40). Six additional groups of male rats (n=8-10 per group) underwent the same protocol but for shorter periods to obtain intermediate tissue data after 3 months (SS3, ARS3, ART3) and 6 months (SS6, ARS6 and ART6).

Exercise protocol: Animals in the trained groups were exercised 5 days week^{-1} for 9 months on a motorized treadmill with a slope of 10º. The duration and the intensity increased progressively during the first 8 weeks until the animals were running for 30 min at 20m min^{-1}. This protocol was approved by the Université Laval's animal protection committee and was consistent with the recommendations of the Canadian Council on animal care.

Aortic regurgitation: Severe AR induced by retrograde puncture of the aortic valve leaflets under 1.5% inhaled isoflurane anesthesia as previously described [10-12]. Sham animals had their right carotid artery cannulated under anesthesia without puncture of the aortic valve. Animals were clinically evaluated daily by experienced animal laboratory technicians for the presence of signs of heart failure (increased respiratory rate/distress and/or peripheral edema) and were weighed weekly. At the end of the protocols (3, 6 or 9 months), surviving animals were sacrificed, hearts were quickly

dissected and all cardiac chambers were weighed. LV were snap-frozen in liquid nitrogen and kept at -80^{o} Celsius for further analysis.

Echocardiography

A complete M-Mode, 2D and Doppler echocardiogram was performed on the animals under 1.5% inhaled isoflurane anesthesia using a 12 MHz probe with a Sonos 5500 echograph (Philips Medical Imaging, Andover, MA) immediately before and during surgery, after 2 weeks, 3 months, 6 months and 9 months. The echocardiogram after 2 weeks was performed to quantify AR before starting the training program to ensure all animals still met the entry criteria. Left ventricular dimensions, wall thickness, ejection fraction, diastolic function, cardiac output, myocardial performance index were evaluated as previously reported [13-15].

Heart rate and heart rate variability in conscious animals

Heart rate, heart rate variability as well as low to high frequency in conscious animals were measured using the ECGenie system and eMouse ECG analyses software from Mouse Specifics. Inc. (Quincy, MA). A 20 minute adaptation period was allowed before recordings were made. The animals were freely moving on the recording platform and the ECG was recorded via the contact of their paws with the electrodes. Several ECG stretches (mean of 10) of at least 30 seconds were then recorded for analysis.

Hemodynamic measurements

Left ventricular and aortic pressures and dP/dt (positive and negative) were measured invasively in 15 animals per group using a Millar 2F PV catheter under 1.5% isoflurane anesthesia at the end of the protocol [15].

Cardiomyocyte cross-sectional area (CSA) and evaluation of LV fibrosis

Sections from paraffin-embedded mid-LV portions were stained using Trichrome-Masson coloration. Three sub-endocardial sections/slide from all surviving animals were analyzed for the evaluation of cross sectional area (CSA) of the cardiomyocytes as previously described [11;13;16] as well as for the evaluation of the proportion of LV sub-endocardial fibrosis as the blue (fibrosis)/red (myocytes) ratio using a computerized image analysis system (Image-Pro Plus, Version 4.5, Media Cybernetics, Silver Springs, MD). The sub-endocardial sections were defined as the inner third of the LV wall facing the LV cavity [15;16]. Determination of LV peri-vascular fibrosis was performed as previously described [17].

LV collagen content

LV hydroxyproline content was determined by using a modified Stegemann procedure [18]. LV midsection pieces were homogenized and then hydrolyzed in a solution of 6N HCl for 24 hours at 100°C. Samples were then dried overnight under vacuum and resuspended in 1ml of water. Hydroxyproline standards were also made in water. Five hundred µl of standard solutions or LV samples were used for the determination. One ml of isopropanol was then added to the tubes before being vortexed. To this solution, 500 µl of oxidant (0.35g of chloramine-T dissolved in a solution of 5 ml of water plus 20

ml citrate buffer) was added. After 4 minutes of incubation at room temperature, 3.25 ml of Ehrlich`s reagent were added and the mix was let to incubate at 25°C overnight. The intensity of red coloration was measured using a spectrophotometer (550 nm). The amount of hydroxyproline per mg of LV tissue was calculated and the collagen content was estimated by multiplying the hydroxyproline content by a factor of 8.2.

Analysis of mRNA accumulation by quantitative RT-PCR

The analysis of LV mRNA levels by quantitative RT-PCR has been described in details elsewhere [15]. Briefly, one µl RNA (500 ng) was converted to cDNA using the QuantiTect® Reverse Transcription kit (Qiagen, Valencia, CA). The cDNA obtained was further diluted 11-fold with water prior to amplification. Five µl of diluted cDNA were amplified in duplicate by Q-PCR in a Rotor-Gene™ thermal cycler (Corbett Life Science. Sydney, Australia), using QuantiTect® Primer Assays (pre-optimized specific primer pairs from Qiagen) and QuantiFast® SYBR Green PCR kits (Qiagen).The quantification of gene expression was based on the $-2\Delta\Delta Ct$ method [19] using the cyclophilin A as an housekeeping gene as a control.

Statistical analysis

Results are presented as mean ± SEM unless specified otherwise. Inter-group comparisons were done using one-way ANOVA and Tukey's post-test or Student's t-test. Survival was analyzed by standard Kaplan-Meier analysis with log-rank test. Statistical significance was set at a $p<0.05$. Data and statistical analysis were performed using Graph Pad Prism version 5.01 for Windows, Graph Pad Software (San Diego

CA). Sample sizes were calculated based on a prior survival study [15] to ensure a 0.80 power to detect a mean of 30 days benefit of survival in trained animals with an α value of 0.05 using a 2:1 ratio of untreated/treated animals.

Statement of Responsibility

The authors had full access to the data and take responsibility for its integrity. All authors have read and agree to the manuscript as written.

Results

Clinical data and animal characteristics:

Exercise training was well tolerated. Figure 1 shows the survival curves of sedentary (sed) or trained (tr) AR animals over a period of 270 days. All sham-operated animals were alive at the end of the protocol (not shown). Ninety percent of trained animals were alive after 6 months compared to only 74% in the sedentary group. After nine months, the survival of trained animals was significantly better with a survival rate of 65% compared to 46% for the untreated group ($p=0.05$). No animals developed signs of overt heart failure defined as excessive weight gain, labored breathing, peripheral edema or decrease of ejection fraction below 50%. Most deaths were sudden, unexpected and un-witnessed (occurring overnight). One death occurred suddenly during the exercise on the treadmill.

Tissue weights:

After 9 months (3 and 6 months for the shorter protocols), surviving animals were sacrificed and results for tissue weights are summarized in Figure 2. Total heart weight was smaller in trained animals. This difference in total heart weight was mainly due to a reduction in right ventricular and left atrial hypertrophy in the trained animals whereas left ventricular weight was not statistically different. Lung weight was similar in both groups. After nine months, surviving trained animals were leaner than the sedentary ones as shown in Figure 2 by their smaller weight, weight gain and retroperitoneal fat content. Overall growth however was similar between groups (tibial length; not shown).

Hemodynamic measurements:

Hemodynamic measurements were made at the end of the 9 month protocol in surviving animals and the results are summarized in Table 1. All measurements were made under isoflurane anesthesia. There were no differences between groups in resting heart rate, stroke volume, systolic and diastolic pressures. The dP/dt_{max} was also similar in both groups. However, left ventricular end-diastolic pressures were significantly lower in the trained animals as well as the values of dP/dt_{min}. Heart rate and heart rate variability were evaluated by telemetry in conscious animals and results are shown in Figure 3. Conscious resting heart rates were similar between groups. However, heart rate variability' decreased in sedentary AR animals was normalized by exercise in trained animals.

Echocardiographic data:

As shown in Figure 4, exercise training reduced left ventricular dilatation both in diastole and in systole. This protective effect was detectable as early as 12 weeks after the beginning of the protocol. Left ventricular wall thickness remained similar between groups. Despite a slow steady decrease in ejection fraction throughout the protocol calculated ejection fraction remained above 60% in all the animals of both groups. Trained animals had higher ejection fractions after 12 and 26 weeks than sedentary animals. However, after 40 weeks, only a trend for a better ejection fraction was present in trained animals but that difference did not reach statistical significance.

Diastolic echocardiographic parameters were also measured throughout the protocol (Figure 4). Trained animals had lower E and A wave velocities and E wave slopes than

sedentary ones. The E/Ea ratio was significantly lower in trained animals correlating well with the invasive LVEDP measurements and left atrial weights. Figure 4 also depicts the longitudinal measurements of diastole-related parameters during the protocol. Marked differences in E wave and A wave velocities were detectable as early as 12 weeks after the beginning of the training protocol. Similar differences were noted after 12 weeks for the E wave slope, left atrial diameter and E/Ea ratios. E/A ratios remained similar between groups.

Sub-analysis of the characteristics of survivors versus deceased animals:

Tables 2 and 3 summarize comparative data obtained in the animals who survived the entire protocol (n=58) and animals who died during the protocol (n=62). Table 2 compares the necropsic data in those animals. These data clearly show that animals who would not survive the entire protocol had significantly larger hearts than late survivors. The left and right ventricles, left atria, lungs and livers were all significantly larger in those animals compared to the survivors. Table 3 summarizes the 6-month echocardiographic data in the survivors and in the animals which would later die before the end of the protocol (death occurring between 6 and 9 months). These data clearly show that after 6 months, animals who would die prematurely had larger left ventricles, more left ventricular hypertrophy (LV mass), larger left atria and some abnormal diastolic filling parameters (E wave slope and E/Ea ratio). Left ventricular ejection fraction however was still normal in both groups after 6 months (above 60%) even though animals that would die prematurely had a slightly lower LVEF that can be considered clinically non significant (65% vs. 67%).

Tissue analysis:

Results obtained from tissue analysis of explanted hearts at the end of the 9 month protocol are shown in figures 5 and 6. Data for total collagen content from 3 and 6 months trained animals are shown as comparative data in Figure 5. Trained animals had significantly less myocardial fibrosis than sedentary animals. Fibrosis content evaluated by Trichrome-Masson staining was significantly lower in trained animals compared to sedentary ones (Figure 5). This finding was confirmed by total collagen content measurement. Peri-vascular fibrosis was also normalized in trained animals compared to sedentary ones. Myocyte cross-sectional area was increased compared to healthy rats in both AR groups but this parameter was unaffected by exercise. Gene expression of various extracellular matrix components related to fibrosis was measured and results are summarized in the top panel of Figure 6. Collagen I, collagen 3 and fibronectin mRNA levels were all increased in both AR groups compared to normal sham animals. Both collagen 1 and 3 expressions were significantly reduced by training whereas fibronectin expression was unaffected. Lysyl oxydase mRNA expression was also unaffected by training. Matrix metalloproteinase 2 (MMP2) and tissue inhibitor of metalloproteinase 1 (TIMP-1) mRNA expressions were significantly increased in AR animals compared to sham animals and exercise training successfully reduced both over-expressions. The mRNA expression of pro-fibrotic TGFβ1, TGFβ2 and CTGF were also assessed. Again, AR animals displayed a significant over-expression of all 3 components with a reduction of that expression in trained animals. The expression status of genes encoding for three secreted cardio-protective factors namely the atrial and the brain natriuretic peptides (ANP and BNP) as well as follistatin-like 1 (Fstl1)[20]

were measured. Gene expression was increased for all three of them in AR animals but only the levels of Fstl were reduced by exercise training. β1-adrenergic receptor gene expression was reduced by AR while the β2-adrenergic receptor mRNA levels remained stable. β1/β2 receptor ratio was improved by exercise. Finally, we also evaluated the regulation of three genes implicated in myocyte calcium handling namely SERCA2a, phospholamban (PLB) and S100a1 protein. Messenger RNA levels encoding for PLB and S100a1 in AR were decreased compared to normal animals while those of SERCA2a were unchanged. Exercise had no effect on the mRNA levels of these genes.

Discussion

The main and most important finding of this study is that a moderate-intensity aerobic exercise program improves survival in the setting of experimental left ventricular volume overload. Using this model of chronic aortic valve regurgitation, we have also demonstrated that exercise is well tolerated and helps reduce myocardial fibrosis. Diastolic filling parameters were improved by exercise as well as heart rate variability. To our knowledge, these data are the first to be reported in the setting of chronic volume overload. The impact of regular exercise on subjects with chronic left ventricular overload has never been evaluated before.

The benefits of exercise in animal models of pre-heart failure and heart failure have been demonstrated in the context of systemic hypertension, post myocardial infarction and transgenic dilated or hypertrophic cardiomyopathies [4;21-25]. Few of them assessed the impact of exercise on survival [23]. None of these studies were related to chronic volume overload. There are no human clinical trials evaluating exercise and survival in chronic volume overload or any other type of valvular disease.

In contrast to other models of volume overload such as aorto-caval fistulae which result in rapid heart failure involving right and left ventricles [26], our model of severe aortic valve regurgitation is well tolerated for a long period of time during which the ventricle progressively dilates and hypertrophies but still retains a normal left ventricular ejection fraction [15]. This model is therefore more relevant and closer to human disease than more aggressive models such as aorto-caval fistula which are virtually never encountered in real life. Although the aorto-caval fistula model is very useful for the study of heart failure and the acute response to volume overload, it cannot be used for

longer chronic studies since reported mortality rates are as high as 50% after just 12 weeks and close to 80% after 20 weeks [27]. Rats with severe aortic valve regurgitation can survive up to one year and few will develop clinical heart failure [15]. This model is therefore more appropriate for chronic studies and evaluation of long-term therapies.

The exercise protocol we chose in this study is of moderate intensity. Considering the severe volume overload imposed on the animals and the severe left ventricular dilatation to be expected, we voluntarily excluded an intense training program in fear that it would not be tolerated by the animals. Based on published data by other groups, it could also be suspected that intense exercise could increase mortality [24]. On the other hand, we did not want to test a low intensity exercise protocol that might have little if any physiological impact. The moderate intensity exercise protocol we chose was therefore selected based on preliminary data and on our pilot study that confirmed a significant physiological impact of the protocol in normal animals and also that this exercise intensity was accepted and tolerated by the AR animals [9]. Oxygen consumption was not measured in our animals but based on other publications, it can be estimated that our protocol corresponded roughly to 50% of maximal VO_2 consumption [28].

Exercise improved survival in our animals. The survival curves started to diverge between 120 and 150 days after the training program started and remained different until the end of the protocol. Interestingly, most deaths were sudden and unexpected. Clinical heart failure was of rare occurrence and we suppose as in previous studies that deaths must have been arrhythmic in nature. Since the animals were not constantly monitored, arrhythmias were unfortunately not documented. However, considering that the animals did not develop clinical signs of heart failure and that ejection fractions

remained in the normal range after 6 months in the animals which would decease prematurely, it would be very surprising that heart failure was the cause of death.

The risk of sudden death and arrhythmia in heart failure [29;30] and aortic valve regurgitation [2;31] has been correlated to the severity of left ventricular dilatation and hypertrophy. The sub-group analysis of survivors versus deceased animal in our study definitively showed that animals that would eventually die had significantly larger left ventricles after 6 months than survivors. Exercise attenuated left ventricular dilatation. It is therefore logical to suggest that less left ventricular dilatation in exercising animals protected against sudden death.

Myocardial fibrosis is also known to be pro-arrhythmic. Fibrosis promotes electrical heterogeneity and arrhythmias by various electrophysiological mechanisms [32-36]. In our protocol, trained animals had significantly less myocardial fibrosis. This could be another factor influencing the risk of sudden death. Finally, we have also shown that heart rate variability was improved in the trained animals. A decrease in heart rate variability is another risk factor for sudden death that may have been favorably influenced by exercise training in our animals [37;38].

Exercise was clearly beneficial in trained animals despite a lack of difference in any of the hemodynamic parameters that were measured. Indeed, both trained and sedentary animals had similar resting heart rates, systolic pressures, diastolic pressures and pulse pressure. Stroke volume, cardiac output and AR severity were also similar in both groups. The benefits induced by exercise cannot therefore be attributed to significant hemodynamic improvements or reduction in volume overload. Peripheral arterial compliance does not seem to be affected either. We have to acknowledge however that

these hemodynamic parameters were measured under general anesthesia and that significant differences might have been present in awaken animals. Improved heart rate variability in the trained animals suggests that there might have been such a difference. Systolic function was similar in both groups as shown by the similar values of dP/dt_{max} and normal ejection fraction. However, most diastole-related echocardiographic parameters were improved by exercise and these improvements are corroborated by lower LF filling pressures (LVEDP), higher dP/dt_{min}, smaller left atria, lungs and right ventricles all suggesting lower diastolic filling pressures and improved left ventricular compliance. Diastolic properties may be influenced by numerous factors, one of these being the amount of myocardial fibrosis. Our results clearly show that exercise reduced myocardial fibrosis in AR rats and that pro-fibrotic gene expressions were clearly decreased by exercise. This decrease in interstitial fibrosis must have helped improve diastolic function.

Intense exercise can result in physiologic volume overload and consequent physiologic hypertrophy. Even a moderate exercise program such as the one used in our study can impose some volume overload on the heart and induce mild LV dilatation and hypertrophy [9]. Therefore, there was a theoretical risk of increasing LV dilatation and hypertrophy by combining exercise and AR (physiologic + pathologic volume overload). The fear remained that the combination of both volume overloads may have additive or even synergistic effects, cause worse LV dilatation or even accelerate the heart failure process. Not only did we find that exercise did not worsen LV remodeling and function but instead it had protective effects against dilatation and fibrosis.

Considering all these data, it seems reasonable to think that moderate aerobic exercise is safe and probably beneficial in subjects with chronic LV volume overload. We however need to keep in mind that high-intensity exercise training might not have given similar results and that we must remain cautious in that regard.

Limitations:

Our animal model has limitations like any animal models and we must keep those limitations in mind before transposing the current data to humans. Rodent cardiovascular physiology differs from humans in many aspects. Oxygen consumption was not measured during our exercise protocol and therefore exercise intensity was not precisely quantified. Exercise was started rather early in the course of the disease and it is not known if the beneficial effects would have been found if training had been started later or in older animals with co-morbidities. However, we have previously reported in our model that significant LV dilatation is already established in the first month of the protocol and that most acute adaptive mechanisms have receded after 2 weeks [17]. The effects of long term training in males versus females were not evaluated although we have shown in our pilot study that exercise seems to be beneficial in females also ([9]).

Despite these limitations, we found no deleterious effects of exercise on any of the measured clinical, hemodynamic, and echocardiographic or tissue analysis parameters in our animals after 9 months. Survival was improved by exercise. Diastolic parameters and filling pressures were also improved and myocardial fibrosis decreased. Therefore, we can reasonably conclude that a moderate supervised aerobic exercise training program is beneficial in rats with chronic volume overload.

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Conflict of interest disclosures

Eric Plante PhD: Fellowship from the Canadian Institutes for Health Research

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Serge Champetier PhD: none

Élise Roussel MSc: none

Marie-Claude Drolet : none

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Marie Arsenault MD: Senior scholar from the Fonds de la recherche en santé du Québec.

Figure legends:

Figure 1: Survival Kaplan-Meier curves of rats with severe chronic aortic valve regurgitation trained (tr: black circles) or not (sed: white circles) over a period of 40 weeks.

Figure 2: Body and tissue weights at sacrifice in sedentary (sed; n= 37)) and trained (tr; n=26) AR rats after 12 weeks (3 month protocol), 24 weeks (6 month protocol) or 40 weeks (9 month protocol). HW/TL: heart weight/tibial length. Results are reported in as mean ± SEM. *: $p<0.05$, **: $p<0.01$ and ***: $p<0.001$ between sedentary and trained groups.

Figure 3: Heart rate (HR) and heart rate (RR) variability in conscious animals (survivors). ECG was recorded 6 months after the beginning of the protocol in 15 conscious animals per group. Results are reported in as mean ± SEM. Sed: sedentary animals; Tr: trained animals.

Figure 4: Evolution of LV dimensions, ejection fraction and diastolic function parameters throughout the course of the protocol as assessed by echocardiography in sedentary (sed: white circles) and trained (tr: black circles) AR rats at the beginning of the protocol, after 12 weeks, 26 weeks and 40 weeks. EDD: end-diastolic diameter, ESD: end-systolic diameter, Septum: septal wall thickness LAD: left atrial diameter. Results are reported in as mean ± SEM of the number of living animals at the time of

each echocardiographic exam. *: p<0.05, **: p<0.01 and ***: p<0.001 between sedentary and trained groups.

Figure 5: Left ventricular fibrosis, extra-cellular matrix (ECM) remodeling and myocyte hypertrophy after 9 months in sedentary and trained AR rats. Top left: Quantification of sub-endocardial fibrosis by blue/red ratio from trichrome-Masson stained LV sections. Top right: Peri-vascular fibrosis. Middle left: LV collagen content; data from animals sacrificed after 12 weeks or 24 weeks are included for comparison with results obtained after 40 weeks. Middle right: Myocyte cross-sectional area. Results are reported in as mean ± SEM (n=10-15). *: p<0.05 and **: p<0.01 vs. sedentary group. Bottom panels: Typical examples of trichrome-Masson stained sub-endocardial LV sections. Collagen fibers (blue); cardiomyocytes (red), magnification: X200. Sed: sedentary animals; Tr: trained animals; Sham: normal sham controls.

Figure 6: Evaluation by real-time quantitative RT-PCR of the LV mRNA levels of various genes related to extracellular matrix remodeling. Results are reported in arbitrary units (AU) as mean ± SEM (n=15/gr.). Indicated p values are for the comparison of trained AR animals with sedentary AR ones for each gene studied. Sham (sham-operated animals) group mRNA levels were normalized to 1 and are represented as a black line. Col1: collagen I; Col3: collagen III; Fn: fibronectin; LOX1: lysyl oxidase 1; MMP2: matrix metalloprotease 2; TIMP-1: tissue inhibitor of metalloprotease 1; TGF: transforming growth factor; CTGF: connective tissue growth factor; ANP, atrial natriuretic peptide; BNP, brain natriuretic peptide; Fstl1, follistatin-like 1; β1AR, β1

adrenergic receptor; β2AR, β2 adrenergic receptor; β1/β2 ratio of mRNA expression; SERCA2a, sarcoplasmic reticulum Ca2+-ATPase and PLB, phospholamban.

Table 1. Hemodynamic data.

Parameters	sed (n=37)	tr (n=26)	P value
Resting HR, bpm	347 ± 3.3	348 ± 3.9	0.94
SV, µl	387 ± 5.7	393 ± 6.3	0.66
SBP, mm Hg	120 ± 3.2	117 ± 2.6	0.71
DBP, mm Hg	64 ± 2.0	62 ± 2.6	0.65
$dP/dt_{min,}$ mmHg/sec	-3908 ± 133.6	-4651 ± 190.0	0.01
$dP/dt_{max,}$ mmHg/sec	5935 ± 233.1	6597 ± 239.2	0.11
LVEDP, mm Hg	14.2 ± 1.33	10.8 ± 0.52	0.014

Measurements obtained under inhaled 1.5% isoflurane anesthesia in surviving animals. Sed: AR sedentary group; tr: AR trained group. HR: heart rate; SV: stroke volume in left ventricular outflow tract by pulsed Doppler; AR trained group. SBP: systolic blood pressure; DBP: diastolic blood pressure; dP/dt_{min}; minimal derivative of pressure/time; dP/dt_{max}: maximal derivative of pressure/time; LVEDP: left ventricular end-diastolic pressure. Values are mean ± SEM of the indicated number of animals with the exception of for the dP/dt and LVEDP values (n=15).

Table 2. Surviving vs. deceased animals. Necropsic data.

	Surviving (n=58)	Deceased (n=62)	P value
Survival (days)	270	206 ± 8.8	---
Heart, mg	2542 ± 48.8	3151 ± 77.7	<0.0001
Left ventricle, mg	1809 ± 29.8	2065 ± 43.4	<0.0001
Right ventricle, mg	427 ± 11.3	568 ± 25.9	<0.0001
Left atria, mg	95 ± 5.0	135 ± 9.8	0.0004
Lungs, mg	3321 ± 135.4	4439 ± 131.5	<0.0001
Liver, g	19.2 ± 0.42	27.9 ± 0.65	<0.0001

Measurements obtained at necropsy at the time of death (deceased) or at the end of the 9 months protocol (surviving). Values are expressed as mean ± SEM of the indicated number of animals per group.

Table 3. Surviving vs. deceased animals: echocardiographic data at 6 months.

	Alive (n=62)	Deceased (n=29)	P value
EDD ,mm	11.7 ± 0.10	12.5 ± 0.13	<0.0001
ESD ,mm	6.7 ± 0.09	7.4 ± 0.12	<0.0001
LV mass, mg	1834 ± 42.0	2189 ± 68.0	<0.0001
EF, %	66.8 ± 0.42	65.2± 0.5	0.030
LAD, mm	7.1 ± 0.08	7.9 ± 0.20	<0.0001
E slope	4666 ± 76.0	5053 ± 150.3	0.012
E/Ea	12.0 ± 0.30	13.1 ± 0.43	0.044

Measurements obtained under inhaled 1.5% isoflurane anesthesia after 6 months. EDD: end-diastolic diameter, ESD: end-systolic diameter, EF: ejection fraction, LAD: left atrial diameter. Values are expressed as mean ± SEM of the indicated number of animals.

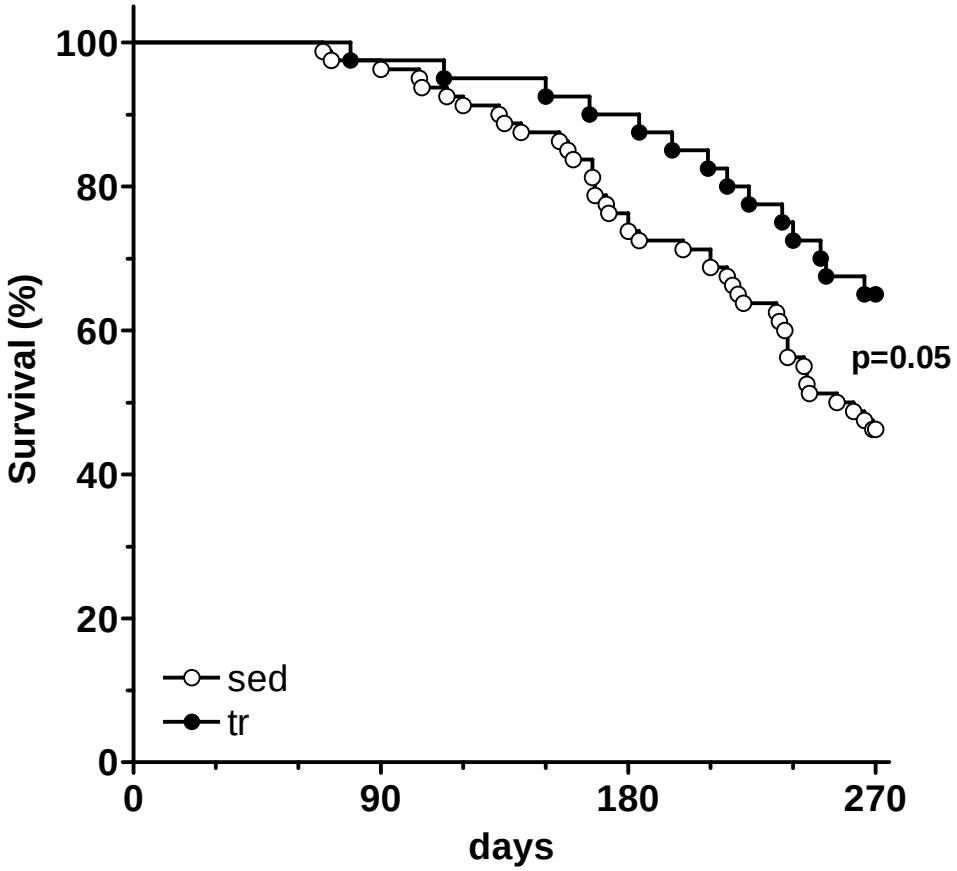

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Fig. 1

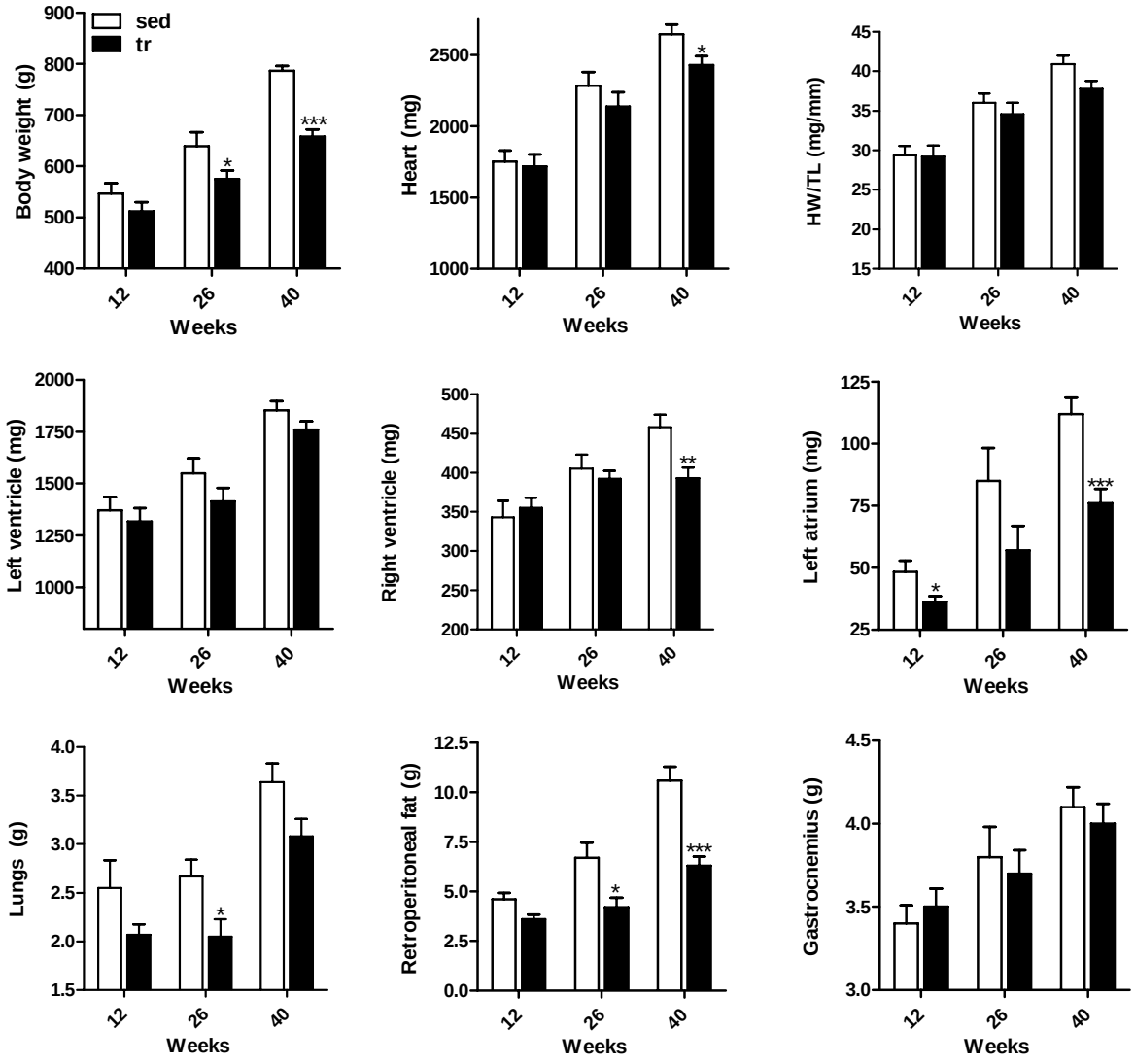

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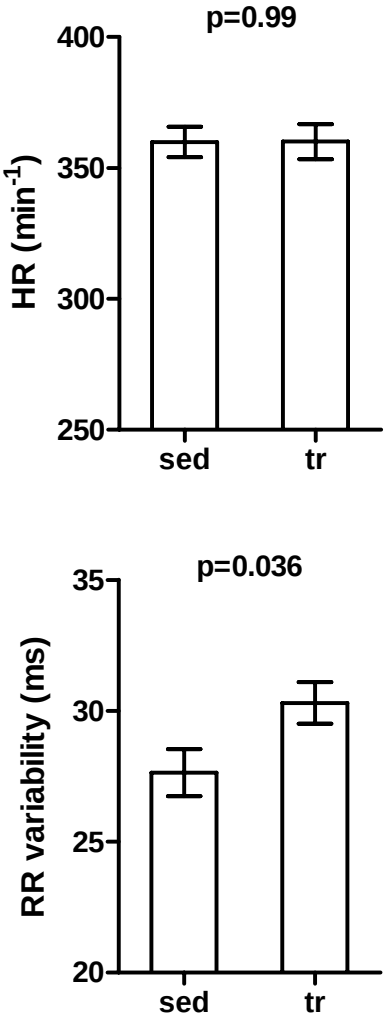
Fig. 2



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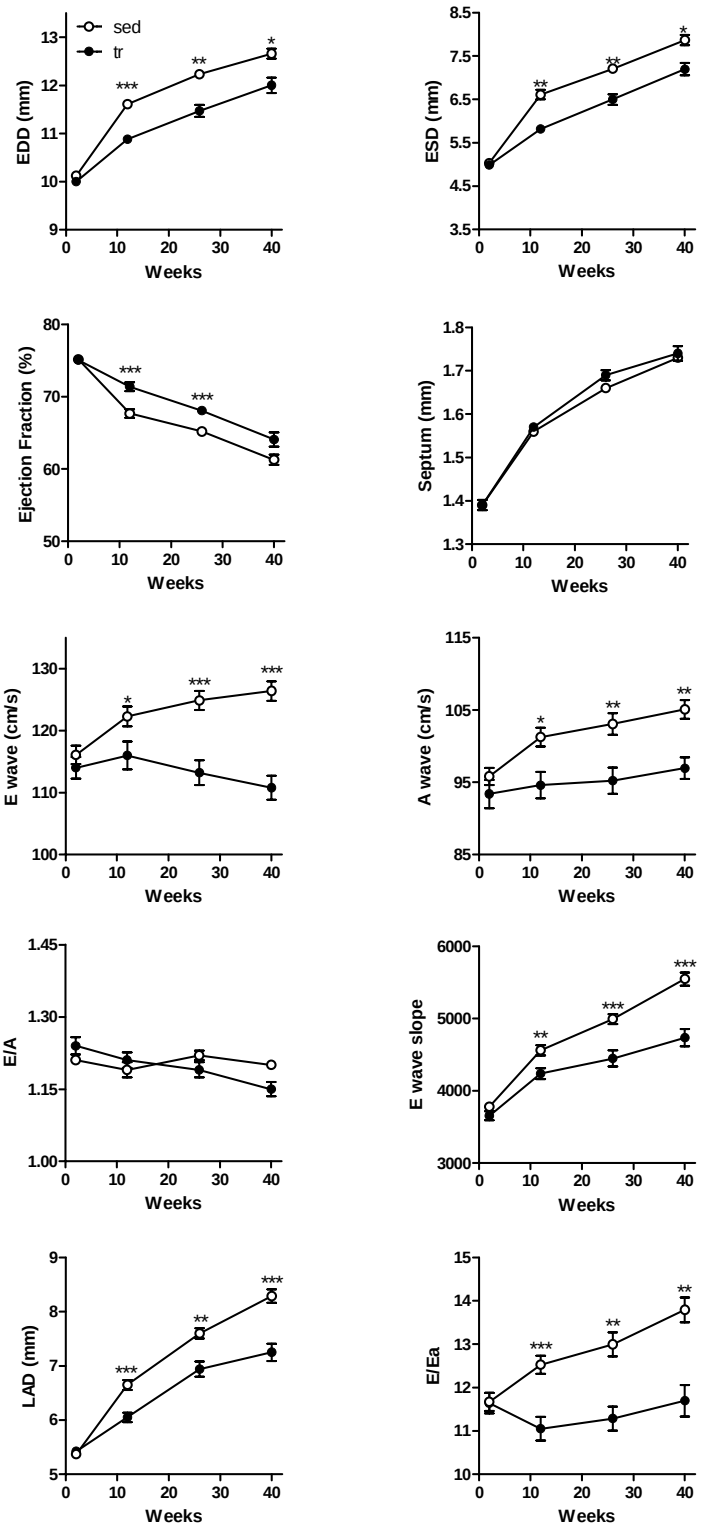

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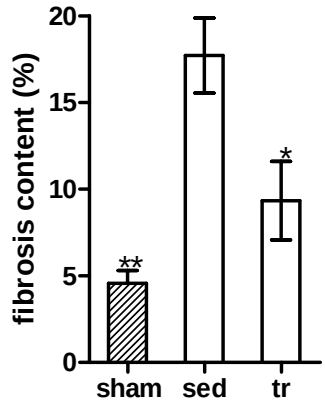

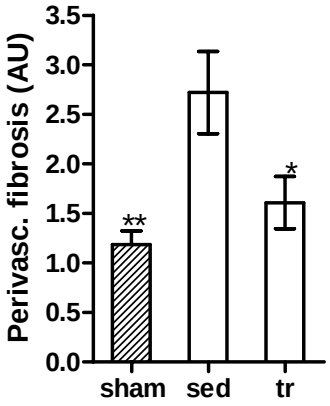

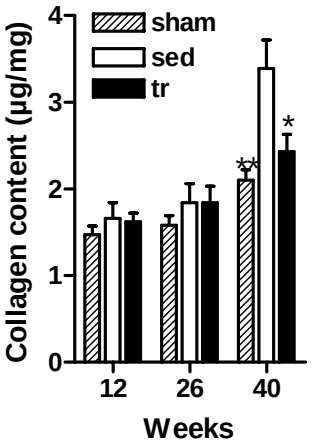

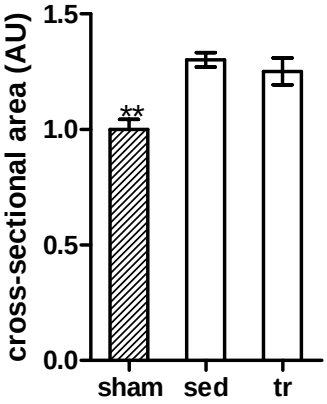

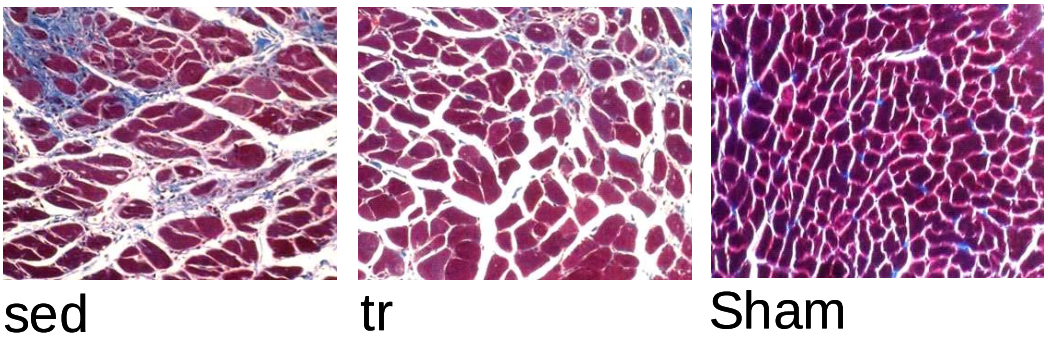

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Fig. 5

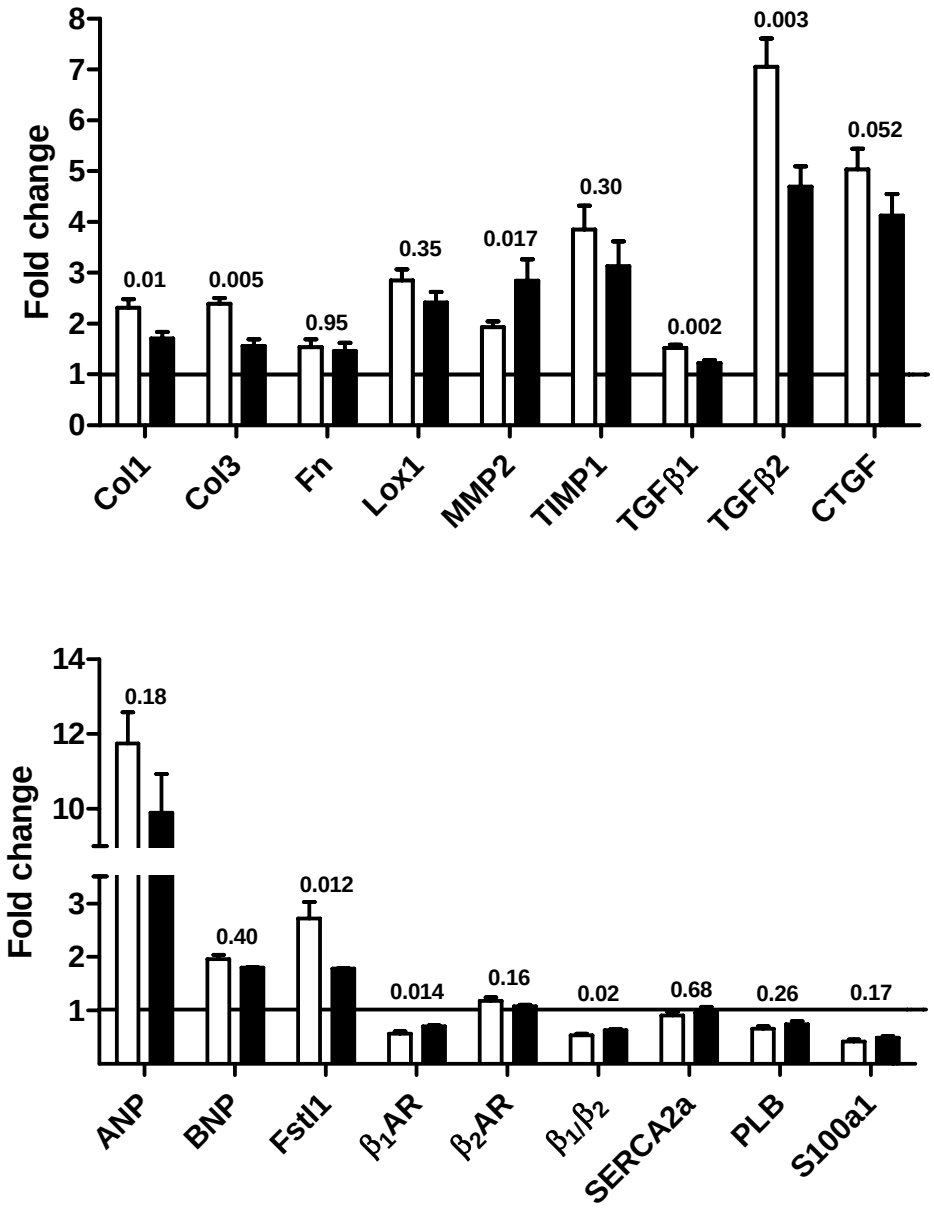

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Fig. 6