

Cardiac Arrhythmogenic Remodeling in a Rat Model of Long-Term Intensive Exercise Training Clinical Perspective

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Cardiac Arrhythmogenic Remodeling in a Rat Model of Long-Term Intensive Exercise Training

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Background—Recent clinical studies suggest that endurance sports may promote cardiac arrhythmias. The aim of this study was to use an animal model to evaluate whether sustained intensive exercise training induces potentially adverse myocardial remodeling and thus creates a potential substrate for arrhythmias.

Methods and Results—Male Wistar rats were conditioned to run vigorously for 4, 8, and 16 weeks; time-matched sedentary rats served as controls. Serial echocardiograms and in vivo electrophysiological studies at 16 weeks were obtained in both groups. After euthanasia, ventricular collagen deposition was quantified by histological and biochemical studies, and messenger RNA and protein expression of transforming growth factor- β 1, fibronectin-1, matrix metalloproteinase-2, tissue inhibitor of metalloproteinase-1, procollagen-I, and procollagen-III was evaluated in all 4 cardiac chambers. At 16 weeks, exercise rats developed eccentric hypertrophy and diastolic dysfunction, together with atrial dilation. In addition, collagen deposition in the right ventricle and messenger RNA and protein expression of fibrosis markers in both atria and right ventricle were significantly greater in exercise than in sedentary rats at 16 weeks. Ventricular tachycardia could be induced in 5 of 12 exercise rats (42%) and only 1 of 16 sedentary rats (6%; $P=0.05$). The fibrotic changes caused by 16 weeks of intensive exercise were reversed after an 8-week exercise cessation.

Conclusions—In this animal model, we documented cardiac fibrosis after long-term intensive exercise training, together with changes in ventricular function and increased arrhythmia inducibility. If our findings are confirmed in humans, the results would support the notion that long-term vigorous endurance exercise training may in some cases promote adverse remodeling and produce a substrate for cardiac arrhythmias. (*Circulation*. 2011;123:13-22.)

Key Words: arrhythmia ■ exercise ■ fibrosis

Regular physical activity confers benefits that are widely recognized such as improved cardiovascular risk profiles and prevention of coronary heart disease and diabetes mellitus.^{1,2} Regular exercise also directly and positively affects cardiac physiology (eg, increased myocardial oxygen supply and enhanced myocardial contractility), both in the general population³ and in patients with cardiovascular disease.⁴

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Long-term exercise induces hemodynamic changes and alters the loading conditions of the heart, with specific effects depending on the type of sport and intensity, that are most evident among athletes.⁵ Cardiac adaptations in highly trained subjects include increased left ventricular (LV) and right

ventricular (RV) diameters, enlarged left atrial (LA) dimensions, and increased cardiac mass and LV wall thickness.^{5,6} These changes, together with a preserved ejection fraction, have classically characterized the physiology of the “athlete’s heart.”⁵

Despite the evident benefits of an active lifestyle,¹⁻⁴ numerous observational studies have raised concerns that high-level exercise training may be associated with increased cardiac arrhythmia risk and even primary cardiac arrest.⁷ Initial observations from our group and others,⁸⁻¹³ later confirmed by a large epidemiological study,¹⁴ have shown that long-term endurance training may promote atrial fibrillation. Complex ventricular tachyarrhythmias can also occur in highly trained individuals¹⁵; according to recent studies^{16,17} they often originate from a mildly dysfunctional RV, even

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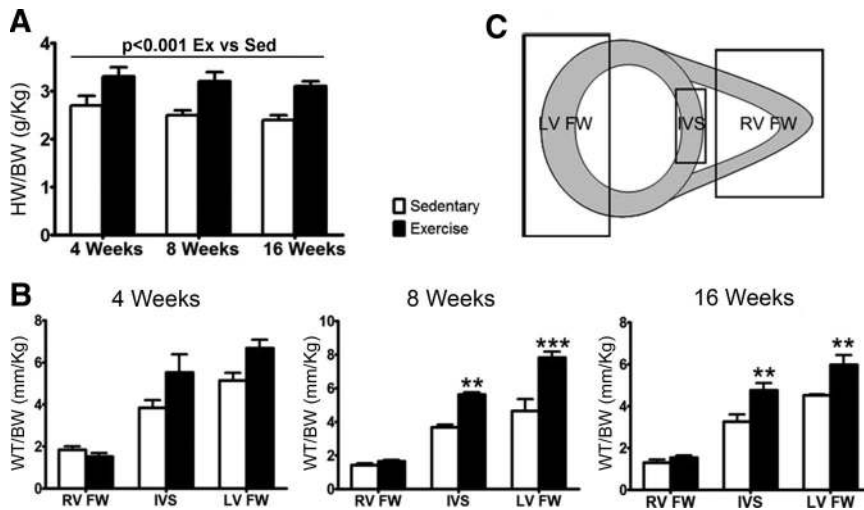


Figure 1. A, Mean \pm SEM cardiac mass changes indicated by heart weight/body weight (HW/BW) ratios (Sed: n=4, 4, and 6 for 4, 8, and 16 weeks, respectively; Ex: n=5, 5, and 8 for 4, 8, and 16 weeks; 2-way ANOVA). B, Schema indicating areas studied for ventricular hypertrophy assessment. C, Mean \pm SEM ventricular wall thickness (WT) indexed to body weight (n=4 rats per group; 2-way ANOVA, repeated measure=region). ** P <0.01, *** P <0.001, Bonferroni-adjusted t test (correction factor=3), Ex vs Sed.

after excluding RV pathologies like arrhythmogenic RV cardiomyopathy.

These findings raise the possibility that long-term endurance exercise may promote the development of certain cardiac arrhythmias. Some authors have speculated that the cardiac remodeling after sustained physical activity may create an arrhythmogenic substrate.^{8,17} Although information on athletes is insufficient, arrhythmia susceptibility has been extensively related to myocardial fibrosis in other clinical contexts.¹⁸ Tissue fibrosis appears as a reparative process for damaged myocardial parenchyma and results in accumulation of fibrillary collagen deposits, which may favor reentry and consequently arrhythmogenicity.¹⁸

The present study was designed to develop a rat model of long-term, intensive endurance exercise to test whether regular intense physical training can induce cardiac structural changes, particularly fibrosis, thereby generating a substrate for cardiac arrhythmias.

Methods

Experimental Design

This study conformed to European Community (Directive 86/609/EEC), Spanish, and Canadian guidelines for the use of experimental animals and was approved by the institutional animal research ethics committees. Pathogen-free, 4-week-old male Wistar rats weighing 100 to 125 g (Charles River Laboratories, France) were housed in a controlled environment (12/12-hour light/dark cycle) and fed rodent chow and tap water ad libitum.

Animals were randomly assigned to sedentary (Sed) or intensive exercise (Ex) groups. To assess time-course changes, animals in both groups were studied at 4, 8, and 16 weeks. The exercise program was based on a previously validated protocol.¹⁹ Ex rats underwent daily running training sessions on a treadmill. The treadmill had different lanes to serve as corridors for each animal and had a grid in the back that administered a small electric shock on contact to ensure that animals ran effectively. The electric shock was of constant intensity (0.3 to 2 mA), sufficient to encourage the animal to run without being harmful. The protocol included a 2-week progressive training program, starting with a 10-minute running session at 10 cm/s and increasing gradually to steady-state 60-minute running at 60 cm/s. Thereafter, animals were trained at this level 5 days a week for 4, 8, or 16 weeks. Investigators observed the treadmill sessions daily to ensure effective running. Only rats that mastered the running training and ran spontaneously with a maximum cumulative shock time of 15

seconds per 1-hour training session were included in the study. Sedentary rats were housed and fed in the same conditions.

An additional series of rats underwent 16 weeks of training followed by discontinuation of exercise (DEX) to assess the reversibility of exercise-induced changes. DEX rats were assessed after 2, 4, or 8 weeks of exercise cessation. Sedentary rats housed and fed in the same conditions over the same period served as DEX controls.

Animals were euthanized 3 days after the end of the training program to avoid immediate responses or after 2, 4, or 8 weeks from the last running session in the DEX groups. Hearts were quickly removed; weighed; dissected into LV, RV, LA, and right atrium (RA); and frozen in liquid nitrogen at -80°C or fixed for histological studies. For details on the echocardiography, electrophysiological study, tissue imaging, and biochemical studies, see the online-only Data Supplement.

Statistical Analysis

Data are expressed as mean \pm SEM. Statistical analysis was generally carried out with 2-way ANOVA with general linear model procedures using a univariate approach. Compound symmetry covariance structure was used for repeated measures analysis. The sphericity test, the Mauchly criterion, was used to test for departures from the assumption of compound symmetry and was consistent with the sphericity assumption in all instances. For heart weight and hydroxyproline experiments, exercise and time point were the main effects. Morphometric, real-time polymerase chain reaction and echocardiographic results were analyzed with 2-way, repeated measures ANOVA, with exercise as 1 main effect and either cardiac chamber (morphometric and real-time polymerase chain reaction experiments) or time point (echocardiography) as the repeated measures main effect. Picosirius red and hydroxyproline deconditioning studies were analyzed with 1-way ANOVA. In the case of a significant interaction by 2-way ANOVA or a significant difference on 1-way ANOVA, Bonferroni-corrected t tests were used to assess Sed versus Ex group differences. In the absence of interaction, P values are shown for significant differences in the main effect. Ex versus Sed immunoblots and electrophysiological testing results were compared by use of t tests for nonpaired samples. The Fisher exact test was used to compare frequency variables. SPSS version 17.0 was used for statistical analysis. Detailed specifications of statistical analysis in each figure are provided in the online-only Data Supplement. Two-tailed values of P <0.05 were considered significant.

Results

Cardiac Remodeling After Long-Term Intensive Exercise Training

Cardiac mass was significantly increased by exercise (Figure 1A). Values for individual cardiac chambers, available at 16

Table 1. Tissue Mass in Sed and Ex Rats After 16 Weeks of Training

	Sed Rats (n=9)	Ex Rats (n=10)
RA/BW, g/kg	0.091±0.006	0.144±0.020*
LA/BW, g/kg	0.083±0.010	0.153±0.020†
RV/BW, g/kg	0.357±0.019	0.433±0.029*
LV/BW, g/kg	1.837±0.057	2.212±0.098†
IVS, g/kg	0.444±0.026	0.644±0.100
LV FW, g/kg	1.394±0.052	1.568±0.160
LV/RV mass index	5.227±0.259	5.264±0.329

BW indicates body weight.

* $P < 0.05$, † $P < 0.01$, nonpaired t test, Ex versus Sed rats.

weeks, were significantly increased in Ex rats (Table 1). No significant changes were observed in the LV/RV mass ratio. Direct measurement of wall thickness (Figure 1B) confirmed significant increments in postmortem interventricular septum (IVS) and LV free wall (FW) thickness after 8 weeks of intensive exercise, which were maintained at 16 weeks (Figure 1C). No significant differences were observed in RV FW thickness.

To evaluate further cardiac morphological and functional adaptation to long-term intensive exercise, serial echocardiograms were performed in a subset of Ex and Sed rats. Because morphological LV hypertrophy was not observed before 8

weeks, echocardiograms were performed only at baseline and after 8 and 16 weeks of training. Ex rats developed concentric LV hypertrophy at 8 weeks, manifested by increased LV wall thicknesses and ratio of IVS to LV diameter at end diastole (for echocardiographic results, see Table 2), evolving to eccentric hypertrophy/ventricular dilatation at 16 weeks. Ex rats also showed evidence of LV diastolic dysfunction at 8 to 16 weeks (decreased S wave in pulmonary vein flow, increased LV isovolumic relaxation time corrected for R-R), along with LA enlargement. A slight but statistically significant decrease in LV systolic function was also observed in Ex rats at 16 weeks. Evidence of RV diastolic dysfunction also occurred at 8 to 16 weeks (decreased E-wave velocity, prolonged E-wave deceleration time).

Intensive Exercise Training Promotes Chamber-Specific Ultrastructural Remodeling

We next evaluated tissue fibrosis. Figure 2A shows representative photomicrographs of Picrosirius red-stained RV sections from Sed and Ex rats. Diffuse interstitial collagen deposition associated with disturbances in myocardial architecture was observed in RV FWs of Ex rats after 16 weeks of training. No differences were observed in the LV (Figure 1A in the online-only Data Supplement). Morphometric quantification confirmed a gradual increase in RV FW collagen with training (Figure 2B). No differences in collagen density

Table 2. Serial Echocardiographic Parameters in the Ex and Sed Groups

	Baseline		At 8 wk		At 16 wk	
	Sed (n=11)	Ex (n=12)	Sed (n=11)	Ex (n=12)	Sed (n=11)	Ex (n=12)
LV dimensions and function						
LVDd/BW, cm/kg	3.01±0.07	3.01±0.05	1.59±0.04	1.72±0.03	1.39±0.05	1.63±0.02‡
LVDs/BW, cm/kg	1.43±0.07	1.51±0.06	0.79±0.03	0.86±0.03	0.72±0.03	0.94±0.03†
IVS/BW, cm/kg	0.55±0.01	0.56±0.01	0.31±0.01	0.38±0.01‡	0.29±0.01	0.36±0.01‡
PW/BW, cm/kg	0.54±0.01	0.54±0.01	0.30±0.01	0.35±0.01†	0.27±0.01	0.32±0.01‡
LV mass/BW, g/kg	2.68±0.11	2.68±0.07	1.93±0.04	2.14±0.05	1.76±0.06	2.13±0.07†
IVS/LVDd*	0.19±0.01	0.19±0.00	0.20±0.01	0.22±0.00	0.21±0.01	0.22±0.01
EF, %	86.99±1.45	85.19±1.31	85.74±0.92	85.66±1.02	83.85±1.02	77.89±1.54†
S-wave PV, cm/s	32.44±1.53	32.71±1.53	33.39±1.48	31.93±1.45	37.06±1.46	30.45±1.20†
IVRTc, ms	1.21±0.06	1.12±0.07	1.21±0.06	1.43±0.07*	1.29±0.05	1.49±0.06
RV dimensions and function						
RVD/BW, cm/kg	1.29±0.03	1.27±0.05	0.68±0.02	0.72±0.03	0.60±0.02	0.69±0.02
RWWT/BW, cm/kg	0.10±0.02	0.11±0.03	0.06±0.01	0.06±0.01	0.06±0.01	0.07±0.01
TAPSE, cm	3.38±0.68	3.16±0.14	3.41±0.11	3.61±0.09	3.80±0.09	3.43±0.09
Sm, cm/s	8.13±0.68	8.57±0.40	9.42±0.45	9.21±0.47	10.37±0.59	8.99±0.36
E velocity, cm/s*	75.60±3.51	74.88±2.31	76.61±5.50	60.76±4.27	63.81±3.63	49.55±3.41
E-DT, ms	36.54±1.48	33.15±2.07	34.43±1.39	35.69±3.28	28.49±2.04	44.07±4.31‡
E/A ratio	1.08±0.06	1.20±0.07	1.03±0.05	0.88±0.07	1.03±0.06	0.87±0.05
Atrial dimensions						
LADs/BW, cm/kg	1.83±0.04	1.87±0.03	0.98±0.03	1.13±0.02†	0.89±0.03	1.14±0.02‡

LVDd indicates LV diameter at end diastole; BW, body weight; LVDs, LV diameter at end systole; PW, posterior wall thickness; EF, ejection fraction; PV, pulmonary vein; IVRTc, LV isovolumic relaxation time corrected for R-R; RVD, RV diameter at end diastole; RWWT, RV wall thickness; TAPSE, tricuspid annulus plane systolic excursion; Sm, RV lateral wall systolic motion velocity; E-DT, E-wave deceleration time; and LADs, LA diameter at end-systole.

* $P < 0.05$, † $P < 0.01$, ‡ $P < 0.001$, Ex rats versus Sed rats, 2-way repeated measures ANOVA.

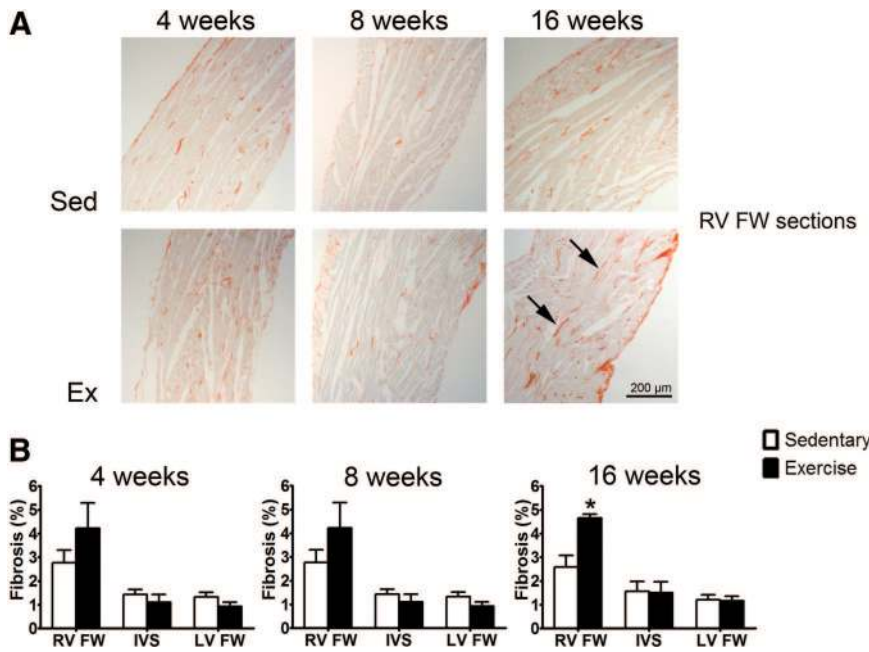


Figure 2. A, Picrosirius-stained photomicrographs of RV sections. In 16-week Ex rats, there is widespread interstitial collagen deposition with disarray of myocardial architecture (arrow). B, Mean \pm SEM collagen content in RV FW, IVS, and LV FW (n=4 per group/time point; 2-way ANOVA, repeated measure=region). * $P < 0.05$, Bonferroni-adjusted *t* test (correction factor=3), Ex vs Sed.

were observed in the IVS or LV FW. Increased collagen in the RV of Ex rats at 16 weeks was also noted on Masson trichrome-stained images (Figure 3A).

To independently quantify fibrous tissue content, the amount of hydroxyproline, a modified amino acid specifically found in collagen, was determined. After 16 weeks of

intensive exercise, animals showed significant increases in RV hydroxyproline content (Figure 3B), with no significant differences observed in the LV (Figure I in the online-only Data Supplement).

Messenger RNA (mRNA) expression of transforming growth factor- β 1 (TGF- β 1), fibronectin-1, matrix metalloproteinase-2 (MMP-2), tissue inhibitor of metalloproteinase-1 (TIMP1), procollagen-I, and procollagen-III was measured in all cardiac chambers of rats in the Sed and Ex groups. After 4 and 8 weeks of exercise, no significant changes were observed (Table I in the online-only Data Supplement). The results at 16 weeks are shown in Figure 4. TGF- β 1, fibronectin-1, and MMP-2 mRNA expression was significantly increased in the RA, LA, and RV of Ex rats compared with Sed rats (Figure 4A, 4B, and 4C, respectively). The only significant difference for TIMP1 mRNA expression was found in the RA (Figure 4D). Finally, mRNA expression of procollagen-I was significantly increased in the RA and RV of Ex rats (Figure 4E), whereas procollagen-III was significantly increased in both the RA and LA of Ex rats (Figure 4F).

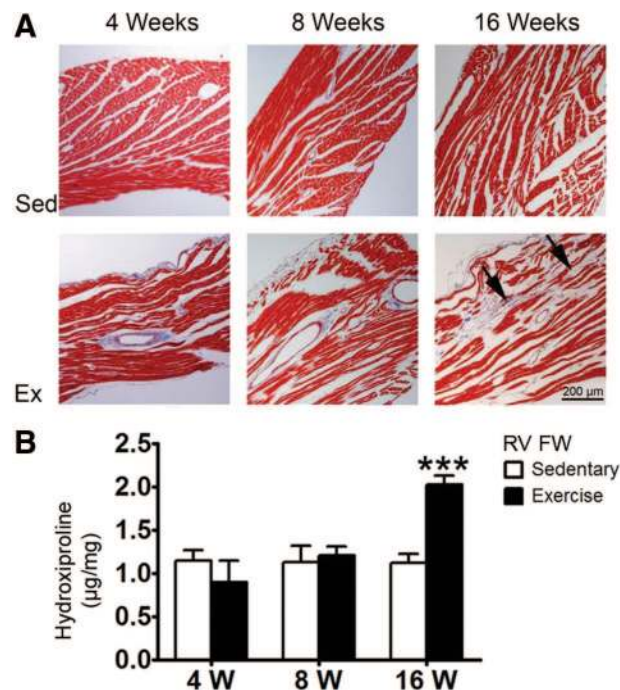


Figure 3. A, Masson trichrome-stained photomicrographs of right ventricular sections. Increased collagen deposition (blue staining, arrow) is present in the Ex group at 16 weeks. B, Mean \pm SEM hydroxyproline content in the RV FW. n=4 (Sed at 4 and 8 weeks), n=6 (Sed at 16 weeks), n=5 (Ex at 4 and 8 weeks), and n=8 (Ex at 16 weeks); 2-way ANOVA, no repeated measures. *** $P < 0.001$, Bonferroni-adjusted *t* test (correction factor=3), Ex vs Sed.

Alterations in protein expression corresponding to mRNA changes were assessed by Western blot analysis for TGF- β 1, fibronectin-1, MMP-2, TIMP1, collagen-I, and collagen-III. TGF- β 1 protein levels were significantly increased in both atria and RVs of Ex rats (Figure 5A). Fibronectin showed no significant changes (Figure 5B). MMP-2 protein expression was significantly increased in the RA and LA of Ex rats (Figure 5C), whereas TIMP1 was unchanged (Figure 5D). In parallel with results for procollagen-I mRNA expression, collagen-I protein levels were significantly greater in both the RA and RV of Ex rats (Figure 5E); however, collagen-III was unchanged (Figure 5F).

These results confirm the development of significant extracellular matrix (ECM) changes after 16 weeks of intensive endurance exercise, with fibrosis clear in the RV but not LV.

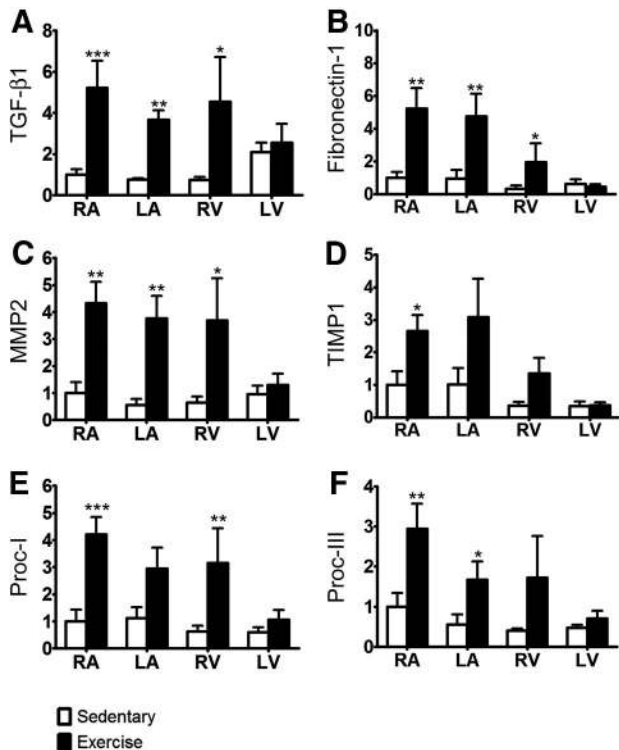


Figure 4. Mean \pm SEM mRNA-expression of fibrotic markers (A) TGF- β 1, (B) fibronectin-1, (C) MMP-2, (D) TIMP1, (E) procollagen-I (Proc-1), and (F) procollagen-III (Proc-III) at 16 weeks in the Sed and Ex groups, quantified by real-time polymerase chain reaction and normalized to β -actin. $n=6$ (Sed) and $n=8$ (Ex); 2-way ANOVA, repeated measure=region. * $P<0.05$, ** $P<0.01$, *** $P<0.001$, Bonferroni-adjusted t test (correction factor=4), Ex vs Sed.

Long-Term Intensive Exercise Increases Ventricular Arrhythmia Vulnerability

We then evaluated whether ventricular remodeling by 16 weeks of exercise led to changes in electrophysiological parameters and/or arrhythmia susceptibility. In vivo electrophysiological study was performed with a customized catheter inserted into the RV apex. Ex rats showed evidence of a slight delay in ventricular conduction, manifested by longer QRS duration (Table 3). No changes were noted in repolarization on the basis of ventricular effective refractory periods. During programmed stimulation with up to 3 extrastimuli, sustained ventricular tachyarrhythmias (>10 seconds) were induced in 5 of 12 Ex rats (42%) compared with 1 of 16 Sed rats (6%; $P=0.05$; Figure 6).

Remodeling Reverses With Detraining

To determine whether potentially adverse ventricular remodeling is reversible after exercise cessation, we compared rats allowed to recover after discontinuation of exercise (DEx groups) with age-matched Sed controls. Although showing some regression, cardiac mass remained significantly greater in all DEx groups compared with their Sed counterparts (Figure 7A).

Because all Sed groups (Sed at 16 weeks and Sed-DEx at 2, 4, and 8 weeks) presented equivalent data (not shown) for morphometric measurements, hydroxyproline levels, histology, and mRNA analyses, results for the 16-week Sed group

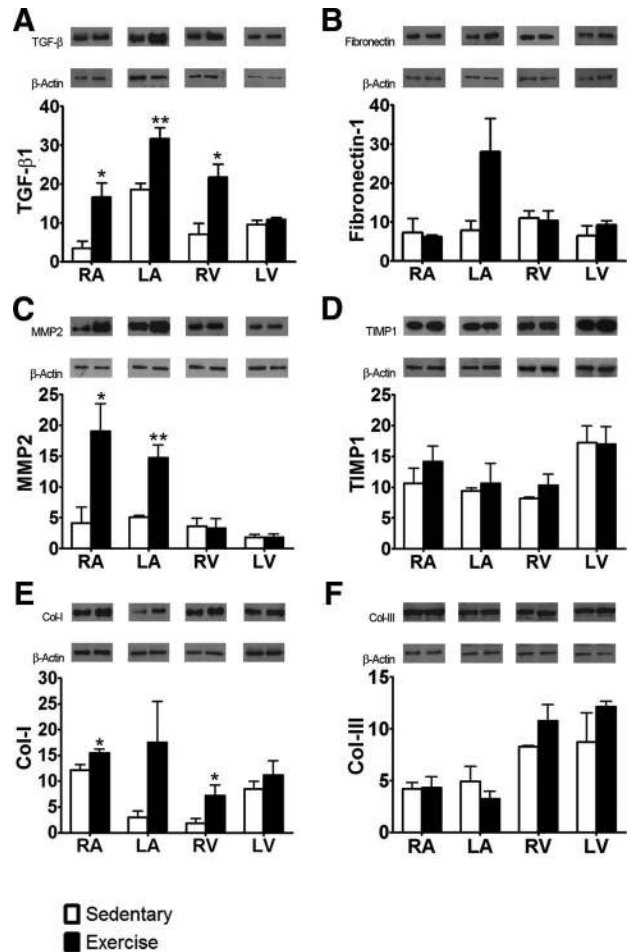


Figure 5. Mean \pm SEM protein levels of fibrotic markers (A) TGF- β 1, (B) fibronectin-1, (C) MMP-2, (D) TIMP1, (E) collagen-I (Col-I), and (F) collagen-III (Col-III) analyzed by immunoblot (examples shown above bar graphs) and normalized to β -actin in the Sed and Ex groups at 16 weeks. $n=6$ (Sed) and $n=8$ (Ex); non-paired t test, * $P<0.05$, ** $P<0.01$, Ex vs Sed.

were used for comparisons. Wall thickness increases induced by 16 weeks of intensive exercise resolved progressively in both the IVS and LV FW (Figure 7B). In contrast, no differences in RV wall thicknesses were found among the Ex, the Sed and all the DEx groups.

We then evaluated whether the fibrotic changes induced by 16 weeks of intensive exercise were also reversed by exercise discontinuation. Histopathology studies with Masson trichrome and Picosirius red confirmed a gradual decrease in collagen content during deconditioning (Figure 7C and 7D). Similarly, collagen quantification based on image analysis of

Table 3. Ventricular Electrophysiological Parameters at 16 Weeks

	Sed Rats ($n=10$), ms	Ex Rats ($n=11$), ms
QRS duration	23.5 \pm 0.4	25.2 \pm 0.6*
V-duration	17.1 \pm 0.5	18.2 \pm 0.6
VERP	39.8 \pm 1.1	43.2 \pm 1.3

V-duration indicates ventricular electrogram duration; VERP, ventricular effective refractory period.

* $P<0.05$, non-paired t test, Ex versus Sed group.

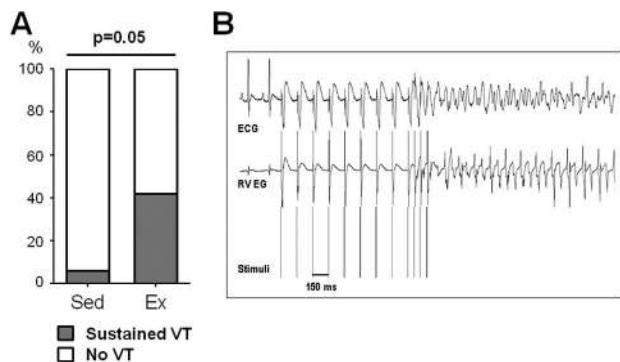


Figure 6. A, Inducibility of sustained (>10 seconds) ventricular arrhythmias by programmed electric stimulation; Fisher exact test, Ex vs Sed. B, Example of polymorphic ventricular tachyarrhythmias (VT) induction by ventricular stimulation in an Ex rat.

Picosirius red-stained tissues confirmed regression of fibrosis in the RV after deconditioning (Figure 7E). Correspondingly, hydroxyproline content in RV decreased progressively during deconditioning and became nonsignificantly different from Sed rats and significantly lower than in Ex rats at 16 weeks after exercise cessation (Figure 7F). In accordance

with these results, mRNA studies showed significant reversal in exercise-enhanced profibrotic markers within 2 weeks of deconditioning (Figure 8). Together, these results suggest substantial reversibility of vigorous endurance training-induced cardiac remodeling after the cessation of exercise training.

Discussion

The present study describes cardiac remodeling in a rat model of long-term, intensive exercise training, demonstrating changes in cardiac function, fibrous tissue content, fibrotic markers, and arrhythmia susceptibility following long-term endurance training, with substantial reversibility after exercise cessation. If results are similar in humans, then our findings suggest that long-term, intensive exercise can promote chamber-specific remodeling and provide a substrate for arrhythmogenesis.

Cardiac Remodeling After Intense Endurance Exercise-Training

As previously described in other models,^{19–21} we found significant LV hypertrophy at 8 weeks of training. At 16 weeks, LV dilatation was also observed, leading to eccentric

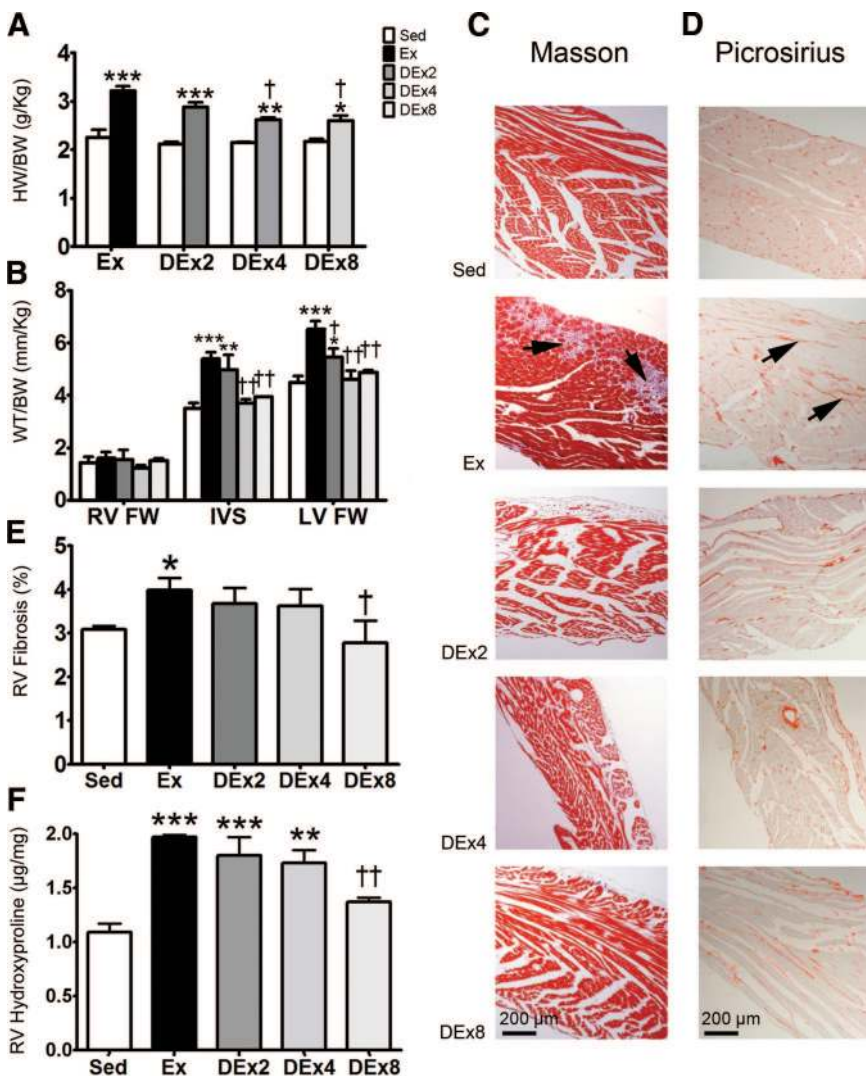


Figure 7. Reversibility of remodeling. Sed indicates time-matched sedentary control for 16-week Ex and 2-, 4-, 8-week exercise cessation groups in A and time-matched sedentary control for 16-week Ex in B through F. A, Mean±SEM heart weight/body weight (HW/BW) ratio (2-way ANOVA, no repeated measure; Bonferroni correction factor=7). B, Mean±SEM wall thickness/body weight ratio (2-way ANOVA, repeated measure=region; Bonferroni correction factor=21). C, Right ventricular Masson trichrome-stained photomicrographs. D, Picosirius red-stained photomicrographs. E, Mean±SEM collagen content (Picosirius red). F, Mean±SEM hydroxyproline content in RV. E and F analyses: 1-way ANOVA; Bonferroni correction factor=7.**P*<0.05, ***P*<0.01, ****P*<0.001, Ex vs Sed. †*P*<0.05, ††*P*<0.01, †††*P*<0.001, DEx vs Ex, Bonferroni-adjusted *t* test.

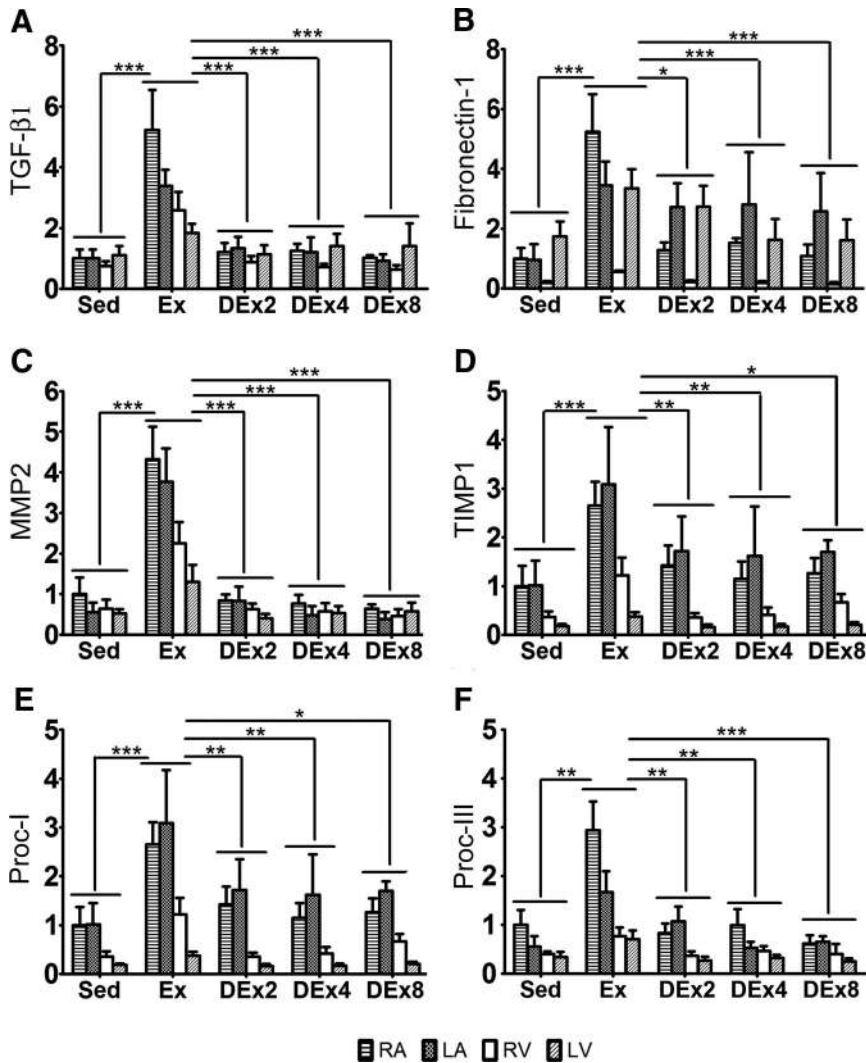


Figure 8. Mean \pm SEM mRNA expression of fibrotic markers (A) TGF- β 1, (B) fibronectin-1, (C) MMP-2, (D) TIMP1, (E) procollagen-I (Proc-I), and (F) procollagen-III (Proc-III) at 16 weeks in the Sed and Ex groups and in all DEx groups, quantified by real-time polymerase chain reaction and normalized to β -actin. n=4 (Sed) and n=6 (Ex and all DEx groups). Two-way ANOVA, repeated measure=region. *P<0.05, **P<0.01, ***P<0.001, Bonferroni-adjusted t test (correction factor=7) for main-effect group comparisons.

hypertrophy. LV hypertrophy may have accounted for the impaired diastolic function observed after 8 and 16 weeks of exercise, which in turn was associated with LA dilatation. All these findings are consistent with the features of the athlete's heart described in humans⁵ and support the potential relevance of our training program. Of note, we also observed RV diastolic dysfunction, with a trend to RV dilatation and systolic dysfunction after 16 weeks of exercise, findings that have recently been described in high-level endurance-sport practitioners.¹⁷

Chamber-Specific Myocardial Fibrosis After Intensive Exercise Training

Perhaps our most noteworthy finding is the demonstration of myocardial fibrosis after long-term, intensive exercise training. RV fibrosis was documented by collagen quantification in histological sections and analysis of hydroxyproline content. These observations were functionally paralleled by the development of RV diastolic dysfunction, with impaired relaxation potentially related to fibrotic infiltration. Moreover, we noted an increase in mRNA and protein expression of a series of fibrotic markers in the RV and in both atria. TGF- β 1 expression was increased in the RA, LA, and RV

after 16 weeks of intensive exercise. TGF- β 1 is a potent stimulator of collagen-producing cardiac myofibroblasts²² and leads to fibrosis development. Experimental studies have reported that both genetic ablation of TGF- β 1 in mice²³ and treatment with anti-TGF- β 1 antibodies²⁴ inhibit fibrosis development, indicating that TGF- β 1 plays a major role in collagen turnover. We also noted enhanced expression of other major components of the ECM control system, including fibronectin-1, collagens, MMP-2, and TIMP1. Collagen-I determines the stiffness of cardiac muscle, whereas collagen-III is more distensible. Thus, the ratio of collagen-I to collagen-III can be a marker of the ECM determinants of cardiac stiffness.²⁵ We observed a significant increase in collagen-I protein expression in right-sided cardiac chambers after 16 weeks of intensive exercise, whereas collagen-III expression remained unchanged, indicating that long-term, intensive exercise could increase cardiac stiffness in these chambers via altered ECM composition, a notion that was supported by echocardiographic evidence of diastolic dysfunction. These findings were accompanied by overexpression of mRNA and protein levels of MMP-2. MMP-2 is a proteolytic enzyme; activation of MMP-2 induces disruption of ECM proteins and promotes fibrogenesis. Together, these

results indicate the presence of a milieu favoring the development of myocardial interstitial fibrosis, characterized by alterations in fibroblast growth factors and ECM imbalance.

Although extensive data have been published on echocardiographic remodeling with exercise,^{6,26} less information is available on cardiac histological and biochemical remodeling in endurance athletes. A recent study demonstrated increased turnover of fibrosis markers in plasma from veteran athletes,²⁷ although cardiac origin was not assessed. Data in abstract form also suggest the development of ventricular fibrosis based on magnetic resonance imaging in endurance athletes.²⁸ Our finding of cardiac fibrosis represents the first direct evidence of potentially adverse cardiac remodeling after long-term, intensive exercise.

The mechanisms by which long-term, intensive exercise may promote cardiac fibrosis are unknown. It is possible that long-term cardiac overload plays a role by promoting physiological remodeling in early phases that may eventually become maladaptive in the long term. Experimental studies support the idea that physiological cardiac remodeling and pathological cardiac remodeling involve different signaling pathways,²⁹ but recent data have demonstrated that excessive stimulation of physiological systems can result in maladaptive responses.^{30,31} Whether this or other mechanisms are involved in the profibrotic cardiac remodeling observed in our model is a matter to be addressed in future studies.

Of note, tissue fibrosis in this model of long-term, intensive exercise was chamber specific (ie, the LV did not appear to be affected). There are 2 potential explanations for this finding. First, assuming that exercise increases loading conditions on all cardiac chambers,⁵ it is reasonable to suppose that greater profibrotic remodeling develops in chambers suffering greater degrees of overload. In this regard, clinical studies have described higher loading conditions on the RV than on the LV during endurance sports,³² leading to transient RV dysfunction immediately after exercise.³³ Second, it has been suggested that intrinsically thinner walls could make the atria, as well as the RV, more susceptible to remodeling.^{17,18} The absence of significant fibrosis in the LV agrees with previous models of long-term exercise^{20,21} and supports the normal functionality of the LV of the athlete's heart.⁵ Supporting this idea, an animal model of long-term volume overload with similarities to loading condition changes in endurance training showed regional overexpression of growth factors and increased collagen deposition in the RV but not the LV.³⁴

Intense Exercise Training and Arrhythmogenic Remodeling

Despite its benefits for overall health,¹⁻⁴ numerous clinical studies in recent years have suggested that long-term high-level exercise might be associated with an increased risk of cardiac arrhythmias, mainly atrial fibrillation and ventricular arrhythmias originating from the RV.⁸⁻¹⁶ One important aspect of this study is that the remodeling observed after long-term, intensive exercise training could represent a potential substrate for arrhythmias. Cardiac fibrosis and associated myocardial disarray provide electric heterogeneity and promote reentrant circuits and arrhythmogenesis.¹⁸ It has

been reported that atrial overexpression of TGF- β 1 in transgenic mice is sufficient to generate a fibrotic substrate that supports atrial fibrillation.³⁵ Similarly, increases in procollagen and MMPs have been related to increased risks of atrial and ventricular arrhythmias.³⁶⁻³⁸ We assessed this hypothesis by evaluating the inducibility of ventricular arrhythmias during in vivo programmed stimulation studies and noted inducible sustained ventricular tachyarrhythmias in 42% of rats subjected to intensive exercise for 16 weeks, compared with only 6% of sedentary rats. In the presence of increased QRS duration, indicating ventricular conduction slowing, and the absence of changes in electrophysiological parameters reflecting repolarization-like ventricular refractory period, these results suggest that the cardiac fibrosis observed in our model could play a role in producing the increased arrhythmia susceptibility we observed in exercise rats.

Reversibility

Clinical studies have reported regression of the morphological changes characteristic of the athlete's heart after long-term detraining.³⁹ The reversibility of arrhythmogenic remodeling is of potentially great clinical importance, because it would imply that deleterious rhythm consequences of long-term endurance training can be expected to disappear after cessation of intense physical training. We accordingly assessed whether a period of rest could allow reversion of the profibrotic changes induced by endurance training in our model. The results of our exercise discontinuation study demonstrate that, after 8 weeks of detraining, virtually all the abnormal cardiac remodeling parameters resulting from intense exercise training regressed to control levels.

More studies are needed to ascertain the mechanisms that participate in both the promotion and the reversal of the fibrotic remodeling associated with long-term exercise and detraining, respectively. In addition, follow-up clinical studies are indicated to establish whether similar remodeling changes can be demonstrated in humans and, if so, whether they are reversible.

Potential Limitations

We cannot exclude the possibility that our exercise training protocol involving conditioning shocks might have induced emotional stress in Ex rats. Maximum efforts were taken to minimize stress responses. Rats that did not adapt to treadmill exercise or received excessive shocks (>15 s/h) were excluded from the study.

It is difficult to estimate precisely how our exercise program translates into human activity. As a rough approximation, considering that the typical rat life expectancy is 2 to 2.5 years, our 18-week exercise protocol (2 weeks of progressive training plus 16 weeks of intensive exercise) would be equivalent to \approx 10 years of daily exercise training in humans. According to previous studies in rodents,^{40,41} the intensity of our program would correspond to \approx 85% of maximum oxygen uptake, equivalent to physical activity at \approx 90% of predicted maximum heart rate in humans.⁴² Therefore, our results cannot be directly extrapolated to milder or more moderate forms of exercise. In addition, we studied only remodeling reversal with complete exercise cessation,

which is unlikely in high-level athletes. Whether similar recovery is achieved by simply reducing the intensity of a training program is uncertain.

Only young male rats were tested in this study. Age- and gender-related factors could significantly influence exercise-related cardiac remodeling and were not analyzed in our study. Further work in other animal models, studies of age and gender effects on exercise-induced remodeling, and follow-up analyses in human populations would be of great interest.

The functional consequences of cardiac remodeling have been specifically assessed by passive pressure-strain curves in papillary muscles or LV pressure-volume curves.⁴³ Because of limited availability of hearts and the need to obtain tissue samples for histological and biochemical studies, we decided to study both functional and morphological consequences of long-term intensive exercise by performing serial echocardiograms, thus obtaining a maximum of information while being able to use each rat as its own control.

Conclusions

This study shows that long-term intense endurance training promotes heart chamber-specific remodeling and ventricular arrhythmia susceptibility in an animal model. Cessation of endurance training was able to arrest and even reverse this pathological process. These findings, if reproduced in humans, could have potentially important implications for arrhythmia risk and its management in individuals involved in high-level athletic training and practice.

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Disclosures

None.

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CLINICAL PERSPECTIVE

Despite the well-recognized benefits of exercise training in healthy individuals and in patients with cardiovascular disease, increasing evidence has suggested that long-term high-level exercise practice (as in athletic contexts) can increase the risk of developing cardiac arrhythmias. Both atrial tachyarrhythmias (particularly atrial fibrillation) and (much more rarely) potentially malignant ventricular arrhythmias have been associated with sustained high-level endurance training. There have been debates about whether these arrhythmias are due to undiagnosed underlying cardiac arrhythmogenic diseases, with long-term exercise being a triggering factor, or whether high-intensity long-term exercise can actually be a primary cause of arrhythmia susceptibility. To provide insights into the ability of sustained high-level exercise to cause arrhythmogenic cardiac remodeling, we applied an experimental model in which male rats were trained to run vigorously 1 hour daily for 16 weeks and compared them with a parallel group of sedentary control rats. We found that intense long-term exercise induced morphological and functional changes characteristic of the “athlete’s heart” as described in humans, along with extracellular matrix changes and fibrosis affecting all chambers except the left ventricle. Ventricular arrhythmia susceptibility to programmed electric stimulation was enhanced in exercise-trained rats. The fibrotic changes caused by 16 weeks of vigorous exercise training were reversible within several weeks of exercise cessation. These results, if confirmed in humans, suggest that long-term vigorous endurance exercise training may cause cardiac remodeling that serves as a substrate for arrhythmia vulnerability. Our findings may have important potential implications for arrhythmia risk assessment and management in individuals performing high-level exercise training.

SUPPLEMENTAL MATERIAL**Cardiac Arrhythmogenic Remodeling in a Rat Model of Chronic Intensive Exercise Training**

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Supplementary Methods, Figure legends, Figures and Tables

SUPPLEMENTAL METHODS

Echocardiography

Transthoracic echocardiographic studies were performed at baseline, 8 weeks, and 16 weeks in both exercise (Ex) and sedentary (Sed) groups, in all cases with the rats having been at rest during a minimum of 6 hours. The procedure was performed under general anesthesia with 2% isoflurane, using a phased-array probe 10S (4.5-11.5 Megahertz) in a Vivid 7 Dimension system (GE Healthcare Ultrasound, Horten, Norway). The M-mode spectrum was traced in parasternal long axis view at the level of the aortic valve, left atrial dimension at end-cardiac systole (LADs) and right ventricular (RV) dimension (RVD) and wall thickness (RVWT) at end-cardiac diastole were measured in this view. The M-mode spectrum was also obtained in parasternal short axis view at the level of papillary muscle, and left ventricular (LV) dimension at both end-cardiac diastole (LVDd) and systole (LVDs) were measured. The thickness of LV anterior wall and that of LV posterior wall at end-cardiac diastole were also measured in this view. Given that animals differed in size during the study, all dimensions were indexed for body weight. LV mass was calculated and corrected for small animals using the formula suggested by Reffelmann et al.¹ The Teicholz method was employed to calculate LV volumes ($LVV = (7/(2.4+D)) \cdot D^3$, where D is LV diastolic and/or systolic dimension). LV ejection fraction (EF) was calculated using the formula packed in the Vivid 7 system ($(LVVd-LVVs)/LVVd \cdot 100$). Pulsed-wave Doppler (PW) was used to record trans-mitral, trans-tricuspid, and pulmonary venous flow (TMF, TTF, PVF) in apical 4-chamber view. Peak velocity in early filling E and E wave deceleration time (E DT) were measured in TMF and TTF, and peak velocity in systolic S wave and diastolic D wave were measured in PVF. Mitral lateral, septal, and tricuspid lateral annulus moving velocity during early filling E_m were derived by tissue Doppler imaging (TDI) in apical 4-chamber view. Tricuspid annulus plane systolic excursion (TAPSE) was measured by M-mode echocardiography, and RV lateral wall systolic moving velocity S_m was obtained by TDI in apical 4-chamber view. Continuous-wave (CW) Doppler at the conjunction of LV inflow and outflow was recorded in apical 5-chamber view, LV isovolumic relaxation time (IVRT) was

measured, and corrected (IVRTc) by R-R intervals taken from simultaneously recorded ECGs.² The average of 3 consecutive cardiac cycles was used for each measurement, with the operator being blinded to treatment assignment. Special care was taken to get similar imaging at follow up study.

In vivo electrophysiological study

At 16 weeks, a subgroup of rats from Ex and Sed groups underwent in vivo electrophysiological study (EPS). The procedure was performed under general anesthesia with 2% isoflurane. After analgesia with buprenorphine (0.03-0.05 mg/Kg), a 1.9F octapolar electrocatheter (Scisense FTS-1913A-1018, London (ON), Canada) was introduced into the right ventricle through the right jugular vein. Surface ECG (lead I) and intracardiac electrograms were recorded on a computer through an analog-digital converter (IOX 1.585, EMKA Technologies, Paris, France) for monitoring and later analysis and measurement. Programmed right-ventricular stimulation was performed at a cycle length of 150 ms to determine the ventricular effective refractory period (VERP). QRS duration and the duration of the ventricular intracavitary electrogram were measured as indices of ventricular conduction. For assessment of inducibility of ventricular arrhythmias, double and triple extrastimulation techniques were administered to a minimal coupling interval of 30 ms, during spontaneous sinus rhythm and following a 9-beat train at a cycle length of 150 ms. Right-ventricular burst pacing at rates of 80 to 60 ms cycle length was also performed if no sustained arrhythmias were induced with 2-3 extrastimuli. Sustained ventricular tachycardia (VT) was defined as episode of ventricular arrhythmia lasting ≥ 10 s induced by ventricular stimulation.

Histology and morphometry

Total cardiac mass was assessed by heart-weight-to-body-weight ratio. Individual-chamber mass was assessed by chamber-weight-to-body-weight ratios. Relative LV to RV hypertrophy

was evaluated according to the formula proposed by Fulton (LV free-wall (FW) weight+interventricular-septum (IVS) weight/RV weight).³

For histological studies, the hearts were perfused with a fixative solution (10% neutral-buffered formalin) at a pressure of 80 cm H₂O, immersed in the fixative for 12 – 24 h, and embedded in paraffin. Ventricular hypertrophy was evaluated morphometrically by direct measurement of ventricular wall thickness in all the heart sections (analySIS Image Processing software, Soft Imaging System, Germany) at RV FW, IVS and LV FW levels. Differences in ventricular-size were controlled by indexing wall-thickness to body-weight.

Heart sections were stained with Masson's trichrome to identify connective tissue and collagen deposition. Additionally, sections from the RV and LV were stained with picosirius-red for quantification of collagen deposition using analySIS Image Processing software (Soft Imaging System GMBH, Germany), as previously described.⁴ Perivascular collagen was excluded from this measurement.

Hydroxyproline content

Ventricular hydroxyproline content was measured using the method described by Woessner.⁵ Samples of RV and LV were homogenized and then hydrolyzed in 6M HCl for 18 h at 110°C. The hydrolysate was then neutralized with 2.5M NaOH and analyzed for hydroxyproline content after addition of chloramine T, perchloric acid and dimethylaminobenzaldehyde. Samples were read for absorbance at 550 nm in a spectrophotometer. Results are expressed as µg of hydroxyproline per mg dried tissue sample.

mRNA analysis

Total RNA was extracted from 50 to 100 mg of a section of the right atrium (RA), left atrium (LA), RV and LV using Trizol® reagent (Invitrogen Corporation, CA, USA) according to the manufacturer's protocol. RNA integrity and loading amounts were assessed by examining

UV/VIS at a multiple wave lengths following the ND-3300 user manual V2.5, instructions (ND-3300, NanoDrop Technologies, USA). Analysis of transforming growth factor- β 1 (TGF- β 1), Fibronectin-1, metalloproteinase 2 (MMP2), tissue inhibitor of metalloproteinases 1 (TIMP1), procollagen-1 (Proc-I) and procollagen-3 (Proc-III) mRNA expression was obtained by Real-Time PCR. One μ g total mRNA was converted to cDNA with the iScript cDNA (Bio-Rad Laboratories, CA, USA) according to the manufacturer's protocol. 100 ng of cDNA was amplified by the iCycler IQTM version 3.1 (Bio-Rad Laboratories, CA, USA) using Applied Biosystems (Applied Biosystems, CA, USA) TaqMan gene expression assays (Rn00572010-m1 for TGF- β 1, Rn00569575-m1 for fibronectin, Rn02532334-s1 for MMP2, Rn00587558-m1 for TIMP1, Rn01526721-m1 for Proc-I, Rn01437675-m1 for Proc-III and Rn00667869-m1 for Actin, which was used as a housekeeping reference). Data were analyzed with the Δ Ct method as previously described.⁶

SDS-PAGE and Western blot

Protein samples were extracted using Nonidet P-40 buffer. SDS-PAGE was performed on 5%-13% acrylamide gels. Proteins were electrotransferred to nitrocellulose membrane and probed with primary antibodies. The antibodies used included mouse monoclonal anti-TGF- β 1 (ab27969, dilution 1/2000), mouse monoclonal anti-MMP2 (ab7032 dilution 1/1000), rabbit polyclonal to TIMP1 (ab61224 dilution 1/1000), mouse monoclonal to collagen-I (ab6308 dilution 1/1000) (all of them acquired from Abcam plc, Cambridge, UK); rabbit polyclonal anti-fibronectin (BP8025, dilution 1/1000) (Acris Antibodies GmbH, Herford, Germany) and rabbit polyclonal to collagen-III (dilution 1/500) (Santa Cruz Biotechnology, Ca, USA), and mouse monoclonal anti-actin (dilution (1/1000) (Chemicon-Millipore Co, MA, USA), which served as a housekeeping reference. The membranes were incubated with the corresponding peroxidase-conjugated secondary antibodies, washed, and then incubated with ECL reagents (GE Healthcare Europe GmbH; Freiburg; GE) before exposure to high performance

chemiluminescence films. Gels were calibrated using Bio-Rad standard proteins (Hercules, CA) with markers covering a 7-240 kDa range.

Films were scanned by using image-editing software NIH ImageJ software for densitometric analysis of immunoreactive bands.

Statistical analysis

Data are expressed as mean \pm SEM. Statistical analysis was generally carried out with two-way ANOVA using general linear models (GLM procedures), with the exceptions described in detail below. In the case of a significant interaction by 2-way ANOVA or a significant difference on 1-way ANOVA, Bonferroni-corrected t-tests were used to assess Sed versus Ex group-differences, except as otherwise indicated. All P-values shown were obtained by multiplying the P value for the non-paired t-test by the correction factors indicated below. In the absence of interaction, P-values are shown for significant main-effect differences. Ex versus Sed immunoblots and electrophysiological-testing results were compared with t-tests for non-paired samples. Fisher's exact test was used to compare frequency-variables. Statistical analysis was performed with SPSS v17.0.

Analyses of repeated measures were performed using the univariate general linear model approach (GLM procedures) and a compound symmetry covariance structure. The sphericity test, Mauchly's criterion, was used to test departures from the assumption of compound symmetry. The sphericity test was consistent with the sphericity assumption in all instances.

Detailed specifications of statistical analysis in each figure:

Figure 1A: An analysis of variance model, including exercise group (sedentary, exercise), time-point (4 weeks, 8 weeks and 16 weeks) and the interaction term (exercise x time-point) was performed. Each heart weight was obtained on different hearts at 4, 8 and 16 weeks, so time point was not considered a repeated measures factor. The interaction was not significant,

so the overall group effect is presented. Exercise group effect was significant and is depicted in the figure.

Figure 1C: A repeated measure analysis model, including exercise group (sedentary, exercise), repeating factor ventricular wall (as results were obtained for right ventricular free wall, interventricular septum and left ventricular free wall in each heart) and the interaction term (exercise x ventricular wall) was performed at each time point (4 weeks, 8 weeks and 16 weeks). At 4 weeks, neither the interaction term nor the exercise group effect were significant. At 8 and 16 weeks, interaction term was significant at the 0.05 level, so comparisons between exercise groups within each ventricular wall were done. The Bonferroni-corrected t-test (correction factor 3) was used to control for the family-wise error rate by reporting the adjusted p-value for every comparison.

Figure 2B: A repeated measure analysis model, including exercise group (sedentary, exercise), repeating factor ventricular wall (since results were obtained for right ventricular free wall, interventricular septum and left ventricular free wall in each heart) and the interaction term (exercise x ventricular wall) was performed at each time-point. At 4 and 8 weeks, neither the interaction term nor the exercise-group effect were significant. At 16 weeks, the interaction term was significant at the 0.01 level, so comparisons between exercise groups (sedentary vs exercise) within each ventricular wall were done. The Bonferroni-corrected t-test (correction factor 3) was used to control for the family-wise error rate by reporting the adjusted p-value for every comparison

Figure 3B A two-way analysis of variance model, including exercise group (sedentary, exercise), time-point (4 weeks, 8 weeks or 16 weeks) and the interaction term (exercise x ventricular wall) was performed. Each measurement was obtained from separate hearts at 4, 8 and 16 weeks, so time point was not considered a repeated measures factor. The interaction term was significant at the 0.01 level, so comparisons between exercise groups (sedentary vs exercise) within each time-point were done. The Bonferroni-adjusted t-test (correction factor 3)

was used to control for the family-wise error rate by reporting the adjusted p-value for every comparison

Figure 4: A repeated measure analysis of variance model, including exercise group (sedentary, exercise), repeating factor cardiac chamber (right atrium, left atrium, right ventricle and left ventricle) and the interaction term (exercise x cardiac chamber) was performed for each individual fibrosis marker. The interaction term was significant for all the fibrosis markers at the 0.05 level, so comparisons between exercise groups (sedentary, exercise) within each cardiac chamber were done (four comparisons per chamber). The Bonferroni-corrected t-test (correction factor 4) was used to control for the family-wise error rate by reporting the adjusted p-value for every comparison

Figure 5: In Figure 5, all Western blots for all Sed and Ex samples for one region were obtained on a single gel to ensure comparability. Because technical factors can greatly affect results between gels, the results for one region (which were done on one gel) cannot be compared in a valid way to results from another region (which because of limits on the number of samples that can be loaded on each gel had to be performed on a separate gel). Therefore, there were no repeated measures in Figure 5 and Sed vs Ex were compared by nonpaired t-test within each region.

Figure 6: The proportions of inducibility of ventricular arrhythmias were compared with the Fisher exact test.

Figure 7A: An analysis of variance model, including exercise group (sedentary, exercise), deconditioning-time (exercise, deconditioning 2 weeks, deconditioning 4 weeks and deconditioning 8 weeks) and the interaction term (exercise x deconditioning-time) was performed. Each heart weight was obtained on separate hearts at 4 weeks, 8 weeks, 16 weeks and deconditioning time points, so time point was not considered a repeated measures factor. The interaction was significant at the 0.05 level. Consequently, comparisons between exercise groups (exercise vs sedentary) within each deconditioning time-point (4 comparisons), and between the exercise group at different deconditioning-times (Ex vs DEx2,

Ex vs DEx4 and EX vs DEx8, 3 comparisons) were done. The Bonferroni-adjusted t-test (correction factor 7) was used to control for the family-wise error rate by reporting the adjusted p-value for every comparison.

Figure 7B: A repeated measures analysis of variance model, including exercise-deconditioning group (sedentary, exercise, deconditioning 2 weeks, deconditioning 4 weeks and deconditioning 8 weeks), repeating factor ventricular wall (since results were obtained for right ventricular free wall, interventricular septum and left ventricular free wall in each heart) and the interaction term (exercise-deconditioning x ventricular wall) was performed. Measurements were obtained on separate hearts at 4 weeks, 8 weeks, 16 weeks and deconditioning time points, so time point was not considered a repeated measures factor. The interaction was significant at the 0.01 level, so comparisons between exercise groups (Sed vs Ex, DEx2, DEx4 and DEx8, and Ex vs DEx2, DEx4 and DEx8, accounting for 7 comparisons) within each ventricular wall (3 ventricular walls) were done. The Bonferroni-corrected t-test (correction factor 21) was used to control for the family-wise error rate by reporting the adjusted p-value for every comparison.

Figure 7E and 7F: For these data sets, results for only one sedentary group (corresponding to 16-week exercise) were available. One-way ANOVA was therefore applied. Measurements were obtained on separate hearts at 4 weeks, 8 weeks, 16 weeks and deconditioning time points, so time point was not considered a repeated measures factor. The main-effect factor was group and was a statistically-significant determinant of the dependent variable. Pairwise comparisons (Bonferroni-corrected t-tests) were performed between Sed and each Ex or DEx group (4 comparisons) and between Ex and each DEx group (3 comparisons); thus, a Bonferroni correction factor of 7 was used.

Figure 8: A repeated measures analysis of variance model, including exercise-deconditioning group (sedentary, exercise, deconditioning 2 weeks, deconditioning 4 weeks, deconditioning 8 weeks), repeating factor cardiac chamber (right atrium, left atrium, right ventricle, left ventricle) and the interaction term (exercise/deconditioning x cardiac chamber) were performed for each

fibrosis marker (each panel). Measurements were obtained on separate hearts at 4 weeks, 8 weeks, 16 weeks and deconditioning time points, so time point was not considered a repeated measures factor. The interaction term was not significant for any of the fibrosis markers, so only the overall group effect is presented. Exercise/deconditioning group effect was significant for all fibrosis markers at the level of 0.001, so comparisons among exercise/deconditioning groups were obtained (Sed vs Ex, DEx2, DEx4 and DEx8 to look for differences in comparison to baseline, and Ex vs DEx2, DEx4 and DEx8 to establish the DEx groups with significant recovery, accounting for a total of 7 comparisons per fibrosis marker). The Bonferroni-corrected t-test (correction factor 7) was used to control for the family-wise error rate by reporting the adjusted p-value for every comparison.

Table 1: Comparisons by non-paired t-test between Ex and Sed.

Table 2: A repeated measures analysis of variance, including exercise group (sedentary, exercise), repeating factor time-point (each rat has echocardiographic data at baseline, 8 and 16 weeks) and the interaction term (exercise x time-point) was performed. Interaction was significant at the 0.05 level for LVDd/BW, LVDs/BW, IVS/BW, PW/BW, LV mass/BW, EF, S wave PV, IVRTc, E DT, and LADs/BW. For these, comparisons between groups (Ex, Sed) were performed at each time-point (baseline, 4 weeks, 8 weeks). The Bonferroni-corrected t-test was used to control the family-wise error rate (correction factor 3). Interaction was not significant but there was a significant group effect (Ex vs Sed) for IVS/LVDd and E veloc.

Table 3: Comparisons by non-paired t-test between Ex and Sed.

Online Table 1: A repeated measure analysis of variance model, including exercise group (sedentary, exercise), cardiac chamber (right atrium, left atrium, right ventricle and left ventricle) and the interaction term (exercise x cardiac chamber) was performed for each separate fibrosis markers. Neither interaction term nor exercise group effect was found to be significant in any of the fibrosis markers.

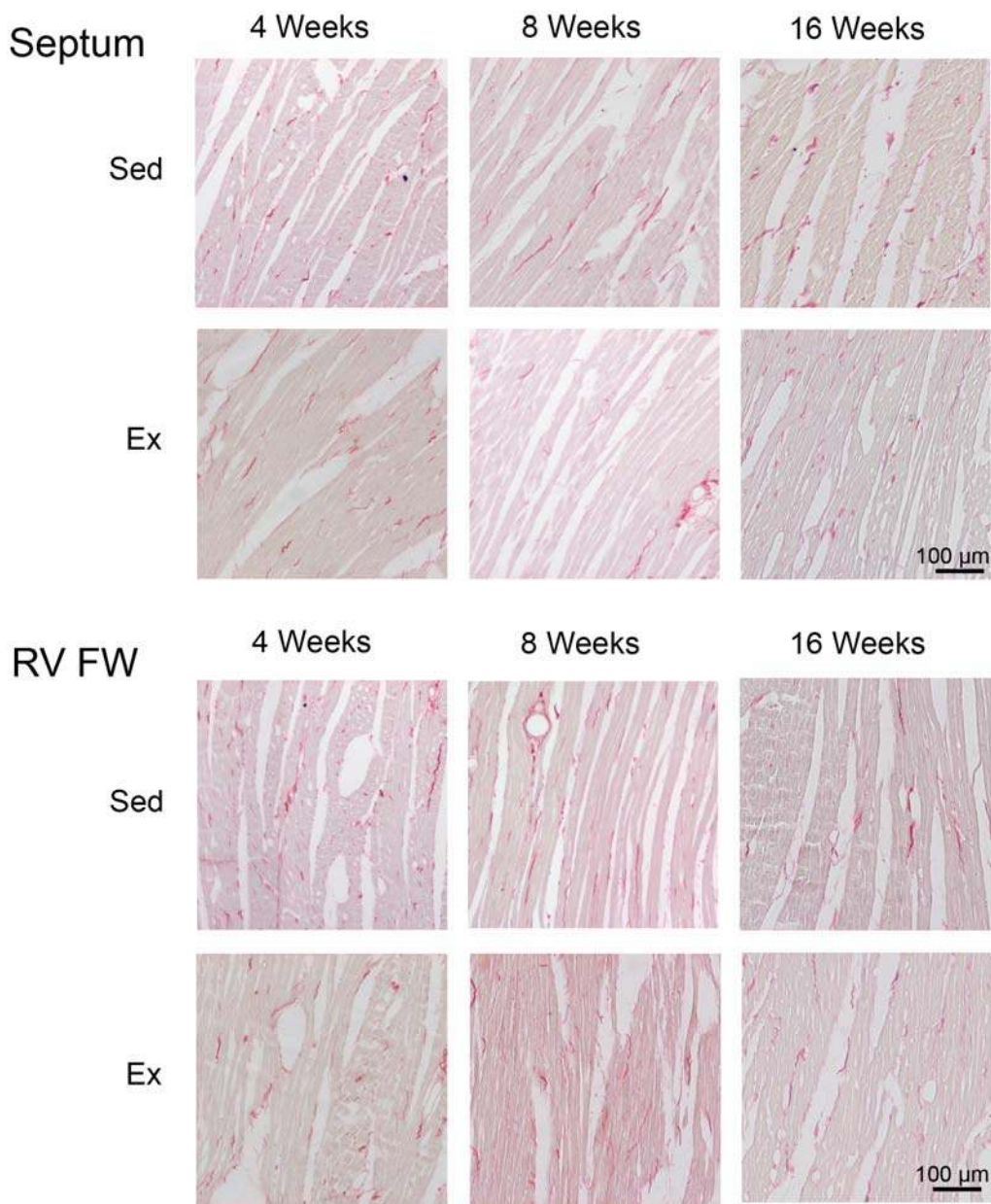
Online Figure 1: An analysis of variance model, including exercise group (sedentary, exercise), time-point and the interaction term (exercise x ventricular wall) was performed. Analyses were performed on separate hearts at 4, 8 and 16 weeks, so time point was not considered a repeated measures factor. Neither interaction term nor exercise group effects were found to be significant for any of the time-points.

SUPPLEMENTAL TABLES

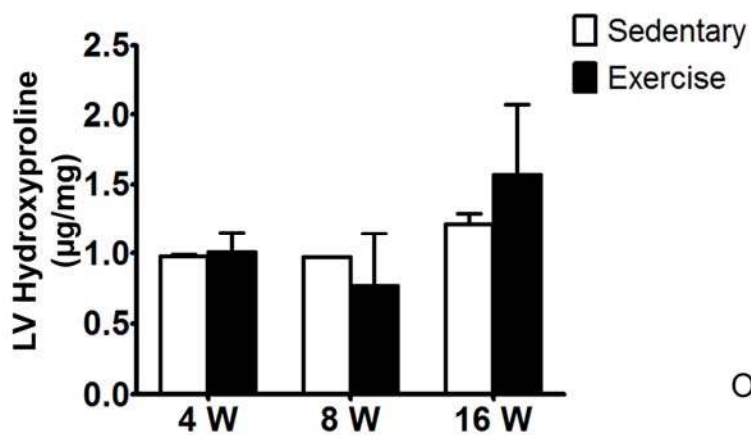
Table 1. mRNA expression of TGF- β 1, fibronectin-1, MMP2, TIMP1, procollagen-I, procollagen-III in the four cardiac chambers at 4 and 8 weeks in Sed and Ex groups. Results are normalized to actin mRNA expression. Values are mean \pm SEM of 4 animals (Sed at 4 and 8 weeks) and 5 animals (Ex at 4 and 8 weeks). There were no statistically-significant differences. Statistical analysis was by 2-way ANOVA.

	Right atrium		Left atrium		Right ventricle		Left ventricle		
	Sedentary (n=4)	Exercise (n=5)	Sedentary (n=4)	Exercise (n=5)	Sedentary (n=4)	Exercise (n=5)	Sedentary (n=4)	Exercise (n=5)	
4 weeks	TGFβ <i>Relative expression</i>	1.00 \pm 0.40	0.71 \pm 0.27	1.00 \pm 0.50	1.99 \pm 0.48	1.00 \pm 0.32	1.04 \pm 0.20	1.00 \pm 0.38	0.41 \pm 0.41
	Fibronectin <i>Relative expression</i>	1.00 \pm 0.35	0.97 \pm 0.37	1.00 \pm 0.31	1.42 \pm 0.34	1.00 \pm 0.62	0.96 \pm 0.45	1.00 \pm 0.32	0.62 \pm 0.21
	MMP2 <i>Relative expression</i>	1.00 \pm 0.42	1.06 \pm 0.50	1.00 \pm 0.30	2.01 \pm 0.77	1.00 \pm 0.71	2.28 \pm 0.77	1.00 \pm 0.47	0.38 \pm 0.07
	TIMP1 <i>Relative expression</i>	1.00 \pm 0.67	0.54 \pm 0.32	1.00 \pm 0.43	1.74 \pm 0.42	1.00 \pm 0.46	1.07 \pm 0.05	1.00 \pm 0.48	0.39 \pm 0.20
	Proc-I <i>Relative expression</i>	1.00 \pm 0.76	0.77 \pm 0.76	1.00 \pm 0.56	2.26 \pm 0.11	1.00 \pm 0.68	1.33 \pm 0.69	1.00 \pm 0.66	1.16 \pm 0.83
	Proc-III <i>Relative expression</i>	1.00 \pm 1.04	0.95 \pm 1.04	1.00 \pm 0.29	1.62 \pm 0.39	1.00 \pm 0.61	0.90 \pm 0.30	1.00 \pm 0.53	1.03 \pm 0.53
8 weeks	TGFβ <i>Relative expression</i>	1.00 \pm 0.34	1.44 \pm 0.62	1.00 \pm 0.56	3.76 \pm 1.09	1.00 \pm 0.56	1.91 \pm 0.73	1.00 \pm 0.47	2.19 \pm 0.43
	Fibronectin <i>Relative expression</i>	1.00 \pm 1.04	2.57 \pm 2.83	1.00 \pm 0.39	6.29 \pm 2.26	1.00 \pm 0.56	5.01 \pm 2.62	1.00 \pm 0.19	1.89 \pm 0.32
	MMP2 <i>Relative expression</i>	1.00 \pm 0.56	1.84 \pm 0.83	1.00 \pm 0.15	3.42 \pm 0.99	1.00 \pm 0.51	1.52 \pm 0.58	1.00 \pm 0.64	0.93 \pm 0.36
	TIMP1 <i>Relative expression</i>	1.00 \pm 0.12	1.63 \pm 0.77	1.00 \pm 0.10	3.97 \pm 1.34	1.00 \pm 0.43	4.51 \pm 1.52	1.00 \pm 0.34	3.09 \pm 1.32
	Proc-I <i>Relative expression</i>	1.00 \pm 0.38	3.48 \pm 1.33	1.00 \pm 0.34	0.98 \pm 0.62	1.00 \pm 0.43	2.53 \pm 0.37	1.00 \pm 0.34	1.25 \pm 0.24
	Proc-III <i>Relative expression</i>	1.00 \pm 0.43	2.55 \pm 0.62	1.00 \pm 0.38	2.14 \pm 1.10	1.00 \pm 0.34	1.95 \pm 0.29	1.00 \pm 0.64	1.34 \pm 0.63

A



B



Online Figure 1

SUPPLEMENTAL FIGURE LEGENDS

Supplementary Figure 1: (A) Representative picosirius-red stained photomicrographs of left ventricular sections obtained from the interventricular septum and the left ventricular free wall. (B) Mean \pm SEM hydroxyproline-content in left ventricle (whole tissue). n=4 (Sed, 4 and 8 weeks), n=6 (Sed, 16 weeks), n=5 (Ex, 4 and 8 weeks) and n=8 (Ex 16 weeks). Two-way ANOVA (exercise and timepoint as main factors).

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