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Obese and diabetic *KKAy* mice show increased mortality but improved cardiac function following myocardial infarction

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Abstract

Background—Introduction of the yellow obese gene (*A^y*) into mice (*KKAy*) results in obesity and diabetes by 5 weeks of age.

Methods—Using this model of type 2 diabetes, we evaluated male and female 6–8 month old wild type (WT, n=10) and *KKAy* (n=22) mice subjected to myocardial infarction (MI) and sacrificed at day (d) 7.

Results—Despite similar infarct sizes (50±4% for WT and 49±2% for *KKAy*, p=N.S.), the 7 d post-MI survival was 70% (n=7/10) in WT mice and 45% (n=10/22) in *KKAy* mice (p<0.05). Plasma glucose levels were 1.4 fold increased in *KKAy* mice at baseline, compared to WT (p<0.05). Glucose levels did not change in WT mice but decreased 38% in *KKAy* post-MI (p<0.05). End-diastolic and end-systolic dimensions post-MI were smaller and fractional shortening improved in the *KKAy* (5±1% in WT and 10±2% in *KKAy*, p<0.05 for all). The improved cardiac function in *KKAy* was accompanied by reduced macrophage numbers and collagen I and III levels (both p<0.05). *Griffonia (Bandeiraea) simplicifolia* lectin-I staining for vessel density demonstrated fewer vessels in *KKAy* infarcts (5.9±0.5%) compared to WT infarcts (7.3±0.1%, p<0.05).

Conclusion—In conclusion, our study in *KKAy* mice revealed a paradoxical reduced post-MI survival but improved cardiac function through reduced inflammation, extracellular matrix accumulation, and neovascularization in the infarct region. These results indicate a dual role effect of obesity in the post-MI response.

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All authors take responsibility for all aspects of the data and discussion presented.

Conflict of interest: None.

Keywords

obesity; diabetes; extracellular matrix; heart failure; inflammation; myocardial infarction

Introduction

The prevalence of obesity has increased substantially over the past 30 years, and the current obesity estimate indicates that over 35% of the population in developed countries is either obese or overweight [1]. Obesity, defined as a body mass index (BMI) of 30 or greater, induces a state of chronic low-grade inflammation, and adipose tissue derived inflammation has been shown to be a key component of insulin resistance and type 2 diabetes [2]. In humans, obesity is linked with hypertension and an increased risk of cardiovascular disease that decreases life expectancy [3, 4]. While obesity and diabetes are well-known risk factors for heart failure after myocardial infarction (MI), heart failure patients with BMIs >30 actually show improved survival compared to patients with BMI <25 [5, 6]. The mechanisms that explain this obesity paradox remain to be elucidated.

The *KKAy* mouse strain was developed by introducing the *A^y/a* mutation onto the inbred KK strain of native Japanese mice [7]. By five weeks of age, *KKAy* mice are hyperglycemic and hyperinsulinemic, with increased degranulation of β cells and hypertrophy of pancreatic islet cells that reflect increased production of insulin [8]. By 16 weeks of age, both sexes of *KKAy* mice show blood glucose levels approaching 400 mg/dl, indicative of marked hyperglycemia [8]. This model, therefore, closely mimics the obesity and type 2 diabetes phenotype seen in humans. The objective of the present study was to use this unique mouse model to determine the impact of obesity and hyperglycemia on MI. We focused on functional, morphological, and histological features of the post-MI left ventricle (LV).

Methods

All animal procedures were conducted according to the “Guide for the Care and Use of Laboratory Animals” (Eight Edition, 2011) and were approved by the Institutional Animal Care and Use Committee at the University of Texas Health Science Center at San Antonio.

Mice

The mice used in this study were 6 to 8 month old male and female mice (n= 17 wild type (WT), 9 male and 8 female, and n=32 *KKAy*, 20 male and 12 female. Of these, 7 WT and 10 *KKAy* mice were used for day (d) 0 controls. A total of 10 WT and 22 *KKAy* mice were enrolled into the 7d MI study. MI was induced by surgical ligation of the left anterior descending coronary artery, as described previously [9]. Buprenorphine (0.1 mg/kg, I.P.) was given immediately after the chest was closed. Survival was monitored daily, and all surviving mice underwent echocardiographic assessments during the terminal procedure. Non-surviving mice underwent autopsy evaluation to determine to cause of death.

Blood glucose measurements

Postprandial blood samples were collected during sacrifice to determine glucose levels. Glucose levels were measured using a glucose monitor according to manufacturer recommendations (Ascencia Elite™; Bayer, Mishawaka, IN, USA).

Transthoracic echocardiography

For the echocardiography analysis, 0.5–2% isoflurane in a 100% oxygen mix was used to anesthetize the mice. The electrocardiogram and heart rate were monitored continuously

during the imaging procedure. Images were acquired using the Vevo 770 high-resolution in vivo imaging system (Visual Sonics) and were taken at heart rates >400 beats/min to obtain measurements that were physiologically relevant. Measurements were taken from two-dimensional parasternal short axis (m-mode) recordings acquired mid-papillary. Echocardiographic studies were performed prior to sacrifice for both d0 controls and 7d post-MI mice. For each parameter, three images from consecutive cardiac cycles were measured and averaged.

Post-necropsy LV processing and infarct size analyses

At 7d post-MI, the mice were anesthetized under 1–2% isoflurane, and the mice were injected with heparin (4 units per g body weight; ip). After 5 minutes, the carotid artery was cut, and blood was collected and centrifuged for plasma collection. The coronary vasculature was flushed with cardioplegic solution to arrest the heart in diastole [10]. The heart and lung were removed, and the left and right ventricles were separated and weighed individually. The LV was further divided into base, mid-cavity, and apex sections, and all three LV sections and right ventricle were stained with 1% 2,3,5 triphenyltetrazolium chloride (TTC; Sigma Inc.) and photographed for infarct size determination. The % infarct size was calculated by the length of the infarct and non-infarct segments. In brief, infarct size was calculated from the TTC stained image by tracing the infarcted perimeter and the non-infarcted perimeter of the LV. The measurements were combined to calculate the total LV infarct size by using this formula.

$$\% \text{ LV infarct size} = (\text{total LV infarcted length} / \text{total LV diameter}) \times 100$$
The mid cavity section was fixed in 10% zinc-formalin and paraffin-embedded for histological examination. The lungs and tibias were removed, and the lung wet weights and tibia lengths were measured.

Plasma proteomic profiling

Plasma samples (100 µl) were analyzed by the RodentMAP® version 2.0 Antigens (Myriad Genetics Inc.), and concentrations of 58 analytes were measured by a Clinical Laboratory Improvement Amendments (CLIA) certified biomarker testing laboratory using reproducible, quantitative, multiplexed immunoassays [11]. Plasma analytes with values below the limit of detection were excluded from the analysis.

Histology and immunohistochemistry

Mid cavity transverse sections of the LV were embedded in paraffin, sectioned at 5 µm, and stained using hematoxylin and eosin. Immunohistochemistry was performed with the use of the Vectastain ABC kit (Vector Labs). HistoMark Black (KPL Inc.) was used to visualize positive staining, with eosin as a counterstain. Negative controls included no primary and IgG-matched isotype antibodies. The following primary antibodies were used: macrophages (Mac 3, Cedarlane CL8943AP, 1:100 dilution); collagen I (Sigma, clone Col-1, C 2456, 1:2000 dilution); and collagen III (Cosmo Bio Co. Ltd, LSL-LB-1393, 1:200 dilution). *Griffonia (Bandeiraea) Simplicifolia Lectin I* (GSL I, Vector Labs, B-1105, 1:50 dilution) binds specifically to galactosyl residues and was used to label the vascular endothelium [12]. Staining areas were quantified using Image-Pro software (Media Cybernetics) to calculate percentage of total area stained positive [13].

Statistical methods

Data are presented as mean±SEM. Multiple group comparisons were performed using the one-way ANOVA, followed by the Student Newman-Keuls post test. Two group analyses of WT and KKAy were compared using unpaired t-test. Statistical analyses were performed using Graph Pad Prism. A value of p<0.05 was considered statistically significant.

Results

***KKAy* mice showed reduced survival post-MI**

At 7d post-MI, the survival rates were 70% for WT (7/10) and 45% for *KKAy* (10/22; $p<0.05$). The increase in mortality in the *KKAy* was equally attributed to perioperative and postoperative mortality. Of the postoperative deaths in *KKAy* groups, 3 of the 5 (60%) were due to cardiac rupture. The 1 postoperative death in the WT group was due to cardiac rupture (Table 1; $p=n.s.$).

Body weights and glucose concentrations were higher in the *KKAy* mice at baseline and decreased to normal levels post-MI

The *KKAy* mice developed obesity (Figure 1a) and overt diabetes (Figure 1b). In response to MI, the body weights significantly decreased in the *KKAy* mice, while body weights did not change in the WT mice post-MI (Figure 1b). Hypoglycemia and hyperglycemia both increase the risk of heart failure after acute MI [14]. In the d0 control *KKAy* mice, blood glucose levels were 1.4 fold higher than WT d0 control levels, indicating pronounced overt diabetes (Figure 1b). In response to MI, blood glucose levels plummeted in the *KKAy* mice, while levels remained similar between the control and MI groups for the WT (Figure 1b).

Improved cardiac function in *KKAy* mice post-MI

Echocardiographic, necropsy, and infarct size analyses for WT and *KKAy* mice at 7d post-MI are shown in Table 2. The echocardiographic and morphometric analyses were analyzed separately by sex, and no differences seen for the WT male vs. female or *KKAy* male vs. female comparisons. For example, end systolic dimensions between male and female WT post-MI groups had a $p=0.55$, and the male and female *KKAy* post-MI groups had a $p=0.89$. For this reason, both sexes were combined for further analyses. At d0, the controls demonstrated no differences in LV mass, RV mass, and lung wet weights. Post-MI, both groups showed similar infarct sizes ($50\pm4\%$ for WT and $49\pm2\%$ for *KKAy*, $p=0.79$) and significant increases in LV mass compared with their respective d0 controls ($p<0.05$ for both).

In response to MI, WT and *KKAy* mice demonstrated significant increases in LV end-diastolic dimensions and end-systolic dimensions, and decreases in posterior wall thickness and fractional shortening compared with their respective d0 controls ($p<0.05$ for all). Despite the fact that the *KKAy* mice were obese and diabetic, the decreases in post-MI end-diastolic and end-systolic dimensions were attenuated compared to the WT mice at 7d post-MI. Due to the improved dimensions, fractional shortening was improved in the *KKAy* mice post-MI ($p<0.05$). The RV mass, LV mass, and lung wet weight all increased in the WT and *KKAy* post-MI groups, compared to the respective d0 controls ($p<0.05$ for all). The increases in RV and lung wet weights indicate pulmonary edema, consistent with post-MI cardiac remodeling.

Post-MI macrophage numbers were decreased in *KKAy* mice at d7 post-MI

To investigate potential mechanisms and to explain the improved cardiac function in the surviving *KKAy* mice post-MI, LV sections were stained for macrophages. Macrophages migrate to the site of injury post-MI to clear necrotic myocyte and apoptotic neutrophil debris [15, 16]. As such, macrophages coordinate the balance between pro- and anti-inflammatory actions to resolve inflammation [16]. At 7d post-MI, the *KKAy* mice exhibited fewer macrophages in the infarct region compared to WT post-MI mice ($p<0.05$, Figure 2). The reduced macrophage density suggests either debris was cleared earlier or that fewer macrophages infiltrated into the infarct area to resolve inflammation.

CD40, CD40 ligand, and macrophage-derived chemokine are differentially expressed post-MI in the *KKAy* mice

The plasma proteomic profiling data set is provided in the supplemental table 1. Of 58 plasma analytes evaluated, CD40, endothelin, lymphotactin, macrophage derived chemokine, myoglobin, serum glutamate oxaloacetic transaminase, RANTES (CCL5), tissue factor, and von Willebrand factor were different (all $p < 0.05$). All were reduced in the *KKAy* plasma, with the exception of macrophage derived chemokine and RANTES (CCL5) that increased. CD40 and CD40 ligand, eotaxin, epidermal growth factor, macrophage derived chemokine, myoglobin, oncostatin, serum glutamate oxaloacetic transaminase, and von Willebrand factor were different between WT and *KKAy* at day 7 post-MI (all $p < 0.05$). Of these, three analytes showed interesting patterns that varied by genotype: a) CD40 was lower in the *KKAy* mice at baseline and stayed low post-MI, while WT levels increased post-MI; b) CD40 ligand levels were similar at baseline, but post-MI WT levels increased while *KKAy* levels remained the same; and c) macrophage derived chemokine levels increased at baseline in the *KKAy* mice and increased post-MI in WT but not *KKAy*. CD40 is a pro-inflammatory stimulus that binds to the CD40 ligand expressed by macrophages, fibroblasts, endothelial cells, and smooth muscle cells [17]. Post-MI, the *KKAy* mice showed decreased CD40, CD40L, and MDC levels compared to WT, consistent with the reduced macrophages density measured by immunohistochemistry.

Reduced collagen I and III levels in *KKAy* mice post-MI

The interstitial spaces of the myocardium contain collagen type I and III fibers. Collagen I is about 85% and III is about 11% of the normal cardiac extracellular matrix [18]. Myocardial extracellular matrix deposition is an indispensable part of post-MI wound healing that also contributes to ventricular stiffness and dysfunction [18]. In the WT mice, there was increased expression of collagen I and III compared to *KKAy* mice at 7d post-MI (Figure 3a–d). The reduced deposition of collagen I and III would likely reduce stiffness in *KKAy* mice, which could explain the improved cardiac function post-MI.

Reduced neovascularization in the healing heart of *KKAy* mice

Neovascularization is an essential component of cardiac remodeling after MI, in order to establish new blood supply network for the infarcted region [19, 20]. Therefore, we measured vessel density in the healing myocardium of WT and *KKAy* mice at 7d after MI. Immunohistochemical studies using GSL-1 revealed fewer vessels in infarct area of *KKAy* mice compared to WT (Figure 4a and b), indicating increased granulation process following MI. Plasma vascular endothelial growth factor (VEGF)-A protein levels remain unchanged in the *KKAy* mice compared to WT at 7d post-MI. This evidence of reduced neovascularization in *KKAy* mice indicates that additional studies on other angiogenic factors and infarct area granulating factors in diabetes and obesity setting are warranted.

Discussion

Obesity and diabetes mellitus are common comorbidities in acute MI and heart failure patients [21, 22]. We used the *KKAy* mice to evaluate post-MI remodeling in a model of obesity and type 2 diabetes. *KKAy* mice develop spontaneous diabetes at an early age and polyuria that increases urine excretion volume from 1.5 ± 0.3 ml at 5 week to 9.5 ± 1.2 ml by 12 weeks of age [7, 8, 23]. The major findings of this study were that the *KKAy* mice post-MI showed: 1) increased mortality due to increase rupture rates, 2) attenuated inflammatory response, as evidenced by altered CD40, CD40 ligand, and macrophage derived chemokine levels and reduced macrophage infiltration into the infarcted area, 3) reduced collagen I and III levels, and 4) reduced neovascularization in the infarcted area of *KKAy* mice. These results indicate that diabetes worsen post-MI survival but attenuate LV remodeling in

surviving mice by modulating inflammation, collagen synthesis, and neovascularization. This study sheds light on mechanisms that help to explain the obesity paradox.

The decreased post-MI survival and increased rupture events in male *KKAy* mice are similar to rates observed in other strains. Gao *et al* reported that male mice are prone to post-MI mortality and rupture events [24]. Having increased testosterone levels in male mice contributes to acute early remodeling and rupture that is not seen in female mice or estrogen-treated castrated males [25]. Decreased post-MI survival and increased rupture events in the *KKAy* male mice may associate with chronic inflammation in the diabetic setting [26].

Frangogiannis and colleagues demonstrated that in post-MI inflammation, macrophages are capable of exerting stimulatory actions by secreting mediators that augment inflammation and inhibitory pro-resolving effects through the removal of inflammatory neutrophils [16, 27]. *KKAy* mice had higher levels of plasma macrophage derived chemokine at baseline that explains the non-significant increase in inflammatory response and attenuated LV dysfunction in *KKAy* mice. Post-MI WT and *KKAy* showed increased levels of several plasma analytes, including macrophage inflammatory protein 1 beta, macrophage inflammatory protein 1 gamma, macrophage inflammatory protein 2, myeloperoxidase and tissue inhibitor of matrix metalloproteinase compared to their respective controls. Surprisingly, *KKAy* mice showed fewer macrophages in the infarcted area. Pre-existing chronic inflammation in *KKAy* mice might sensitize signaling for inflammatory cell recruitment that is required for neutrophil clearance and removal of necrotic myocytes in post-MI remodeling event.

CD40 ligand is a transmembrane protein that belongs to the tumor necrosis factor superfamily and interacts with the CD40 receptor. Longitudinal and cross-sectional studies in humans shows that acute and chronic heart failure are characterized by evidently raised levels of CD40 and CD40 ligand [28]. Lower levels of CD40 and CD40 ligand in *KKAy* mice associates with lower ventricular dysfunction measured on 7d compared WT mice. This is the first report that reduced CD40–CD40L interactions in *KKAy* mice may decrease macrophage activation and attenuate ventricular dysfunction.

Post-MI myocardial repair is a dynamic process involving an inflammatory response that clears the necrotic tissue, and replaces with collagen, ultimately leading to granulated and healed scar. Macrophages comprise an important component of the inflammatory infiltrate. Infiltrated macrophages are not only responsible for eliminating the neutrophils to limit the expansion of the infarct, but also secrete growth factors, such as TGF- β 1, which regulate the activity of fibroblasts. Phenotypically modified fibroblasts, myofibroblasts, play a central role in progression of fibrosis and their ability to produce procollagens [29]. Previous study reports enhanced collagen synthesis in diabetic rodents and post-MI remodeling, [30] To our surprise, having enhanced baseline inflammation due to obesity and diabetes in *KKAy* mice enhanced the reparative phase leading to a more granulated scar and improved cardiac function. Among many collagens type I and III are major collagens. Type I collagen links mainly with thick fibers that confer tensile strength and resistance to stretch and deformation. Collagen type III allies with thin fibers and provides resilience depending on the cross-linking or alignment [18, 31]. Reduced collagen I and III deposition in *KKAy* mice may contribute to reduced stiffness and improved LV function in post-MI setting. Given reduced collagen I and III indicates early post-MI scar granulation in *KKAy* mice due to enhanced non-collagen extracellular matrix deposition. Furthermore, post-MI *KKAy* mice showed reduced vascularization and signs of earlier granulation tissue formation. Post-MI neovascularization relies heavily on increased VEGF as an angiogenic stimulus [32]. In the present investigation, post-MI changes in macrophages density and early scar granulation compensated for the reduced neovascularization. In the present investigation, post-MI

changes in macrophages density and early scar granulation compensate for reduced neovascularization. Our overall results introduce new questions as to the contribution of extracellular matrix proteins in the post-MI remodeling response in the setting of obesity.

Conclusion

We are the first to report dichotomous results in *KK* obese and diabetic mice in the post-MI setting, which mirrors clinical observations of the obesity paradox. *KK* mice showed increased post-MI mortality, while survivors had improved cardiac function accompanied by reduced macrophages, collagen I and III, and neovascularization. Our results provides a strong foundation for future studies to focus on proteins that stabilize the post-MI scar in the early inflammatory setting, which could lead to improved outcomes in heart failure patients.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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Summary

We observed dichotomous results in *KKAy* obese and diabetic mice in the post-MI setting, which mirrors clinical observations of the obesity paradox. *KKAy* mice showed increased post-MI mortality, while survivors had improved cardiac function accompanied by reduced macrophages, collagen I and III, and neovascularization. Our results provides a strong foundation for future studies to focus on proteins that stabilize the post-MI scar in the early inflammatory setting, which could lead to improved outcomes in heart failure patients.

Figure 1.

Body weight and glucose levels were increased in *KKAY* mice. (a) *KKAY* mice showed higher body weights at baseline but, decreased post-MI compared d0 *KKAY* controls. (b) Plasma glucose levels were higher in the *KKAY* mice at baseline, and decreased post-MI compared to d0 *KKAY* controls. The post-MI percent change in body weight and glucose levels are presented in (c) and (d), respectively. $n=7-9/\text{group}$, $*p<0.05$ vs. WT d0 control, $\nless p<0.05$ vs. *KKAY* d0 control, values are mean $\pm$ SEM.

Figure 2. Histological changes in LV of WT and *KKAy* mice post-MI. WT and *KKAy* mice showed inflammation with extensive necrotic changes compared to d0 controls. Hematoxylin and eosin stained LV images in WT (a, b) and *KKAy* (c, d) mice at d0 and post-MI d7. n=7–9/group (magnification is 40x, scale bar is 100 μm).

Figure 3.

Macrophages density was reduced in *KKAy* mice at d7 post-MI compared to WT. LV mid cavity sections were immunostained for macrophages at d0 and post-MI d7 (a, b WT and c, d *KKAy*). Post-MI *KKAy* mice showed reduced macrophages (circled brown stained cells) in the infarct region compared to the WT infarct region (magnification is 40x, scale bar is 100 μm). (e) Quantification of percent stained area, n= 4–5/group, *p<0.05 vs. WT d7 post-MI, Values are mean ± SEM.

Figure 4.

The *KKAY* mice showed reduced collagen I and III accumulation in the infarct region at d7 post-MI. Representative photomicrographs of collagen I and III immunohistochemistry shows reduced collagen deposition in *KKAY* infarct area compared to WT. (a) and (b) demonstrates collagen I and III staining in the WT infarct region, respectively, while (c) and (d) demonstrates collagen I and III staining in the *KKAY* infarct region, respectively, magnification is 40X, scale bar is 50 μ m. Each insert shows a 1.25X view of the post-MI LV infarcted wall.

Figure 5.

The *KKAY* mice showed reduced blood vessel density in the infarct region at d7 post-MI. LV mid cavity sections were stained with *Griffonia (Bandeiraea) simplicifolia* lectin-I (GSL-1) to depict blood vessels. Representative images of WT (a and b) and *KKAY* (c and d) mice stained with GSL-1 from remote and infarcted areas at d7 post-MI (magnification is 40x, scale bar is 100 μm). (e) The percent density of GSL-1 stained areas, n= 4/group, *p<0.05 vs. WT at 7d post-MI, Values are mean ± SEM. (f) Plasma VEGF-A (ng/ml) levels unchanged between WT and *KKAY* mice at d0 and at d7 post-MI.

Table 1

Post-MI survival is decreased in *KKAy* mice.

	Wild type (n=17, M/F=9/8)	%	<i>KKAy</i> (n=32, M/F=20/12)	%
Day 0 control	7	--	10	--
Post-MI 7 day survival	7	70	10	45*
Peri-operative mortality (died in the first 24 hours)	1	10	5	23
Post-operative mortality (died from days 1 to 7)	1	10	5	23
Non-MI survivor	1	10	2	9

MI- myocardial infarction; n- number of mice; M- male; F- female; MI- myocardial infarction.

Group comparisons were made by Fisher's exact test;

*
p<0.05 vs. WT.

Table 2

Echocardiography and necropsy analyses show an improved remodeling response in the *KKAy* mice post-MI. WT and *KKAy* showed no differences at baseline.

Parameters/Genotype	WT Day 0 control (n=7)	<i>KKAy</i> Day 0 control (n=10)	WT Day 7 Post-MI (n=7)	<i>KKAy</i> Day 7 Post-MI (n=10)
Age (months)	6 ± 1	6 ± 1	6 ± 1	7 ± 1
Heart Rate, beats/min	445 ± 16	410 ± 7	460 ± 40	496 ± 40
End-diastolic dimension, mm	3.71 ± 0.10	3.78 ± 0.14	6.16 ± 0.42 [*]	5.80 ± 0.34 ^{*¥}
End-systolic dimension, mm	2.38 ± 0.10	2.30 ± 0.20	5.88 ± 0.44 [*]	5.24 ± 0.37 ^{*¥}
LV free wall thickness s, mm	1.21 ± 0.03	1.34 ± 0.08	0.47 ± 0.07 [*]	0.52 ± 0.06 [*]
Fractional shortening, %	36 ± 2	40 ± 3	5 ± 1 [*]	10 ± 2 ^{*¥}
End diastolic volume, µl	49 ± 6	54 ± 4	139 ± 20 [*]	130 ± 19 [*]
End systolic volume, µl	19 ± 4	22 ± 3	122 ± 20 [*]	110 ± 11 [*]
Ejection fraction, %	62 ± 4	59 ± 4	14 ± 3 [*]	18 ± 3 [*]
LV mass, mg	81 ± 5	81 ± 4	99 ± 4 [*]	102 ± 7 [*]
RV mass, mg	21 ± 1	23 ± 2	28 ± 1 [*]	25 ± 2 [*]
Lung wet weight, mg	122 ± 6	130 ± 4	233 ± 25 [*]	217 ± 23 [*]
LV mass/tibia, mg/mm	4.8 ± 0.3	4.8 ± 0.2	5.8 ± 0.2 [*]	5.9 ± 0.4 [*]
LV remodeling index, µl/mg	--	--	1.41 ± 0.20	1.23 ± 0.12
Infarct size, %	--	--	50 ± 4	49 ± 2

Values are mean and ± SEM, n- number of mice. Controls are mice evaluated at Day 0.

Abbreviations are LV- left ventricle, MI- myocardial infarction, RV- right ventricle, and WT- wild type. LV remodeling index is end-diastolic volume/LV mass.

* p<0.05 vs. respective Day 0 control,

¥ p<0.05 vs. WT Day 7 Post-MI.