


Original Article

Dynamic changes of hemodynamic parameters and cardiac transcription of sirtuins in adaptive and mal-adaptive phases of pressure overload-induced hypertrophy in rats

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Abstract

Introduction: The aim of the study was to investigate the structural and hemodynamic changes as well as cardiac transcriptional profile of the key regulatory proteins, sirtuins family (SIRT1-7), in adaptive and mal-adaptive phases of left ventricular hypertrophy (LVH).

Methods: LVH was induced in male Wistar rats (190±20g) by abdominal aortic banding. The third and sixteenth weeks post-surgery were considered as adaptive and mal-adaptive phases of hypertrophy (H3w and H16w groups, respectively). Blood pressure (BP) was recorded through the carotid artery catheter. Cell area and fibrosis were assessed using haematoxylin/eosin and Masson trichrome staining, respectively. The sirtuins mRNA levels were quantified using quantitative RT-PCR technique.

Results: H3w rats had a higher systolic and diastolic BP, cardiomyocytes area and heart to body weight ratio (HW/BW) compared with control (intact animals). Although cell size and HW/BW increased in the H16w group, systolic and diastolic BP did not change significantly in comparison with control. In H3w group, SIRT1/3/6/7 mRNAs levels increased significantly. In H16w group, SIRT1/3/5/6/7 mRNAs levels declined in comparison with H3w group. SIRT2 and SIRT4 mRNA levels did not change significantly among the experimental groups.

Conclusion: During progression of cardiac hypertrophy transcriptional profile of sirtuins changes in parallel to structural and hemodynamic parameters. Therefore, it can validate sirtuins as the pharmacological targets for the treatment of pathological LVH.

Keywords:

Hypertension;
LVH;
Adaptive hypertrophy;
Mal-adaptive hypertrophy;
Sirtuins 1-7

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Introduction

Left ventricular hypertrophy (LVH), characterized by increased cardiomyocytes volume and remodeling of

intercellular matrix, may reflect, at least initially, a compensatory response of the heart to hemodynamic overload following hypertension, myocardial infarction and valvular heart disease (Samak et al., 2016). Hypertension, which is estimated to cause about

12.8% of all death worldwide, has been known as the most common cause of LVH, as prevalence of LVH in hypertensive patient is about 40-60% (Martinez et al., 2003; Lazzeroni et al., 2016). Following uncontrolled hypertension, high peripheral resistance load placed on the left ventricle results in hypertrophy response in the heart.

During the early phase of LVH, cardiac performance increases to adapt the heart with the increased workload. However, sustained LVH can develop into decompensating or mal-adaptive stage with prolonged volume or pressure stress. The consequences of this persistent hypertrophy include activation of pro-apoptotic and pro-fibrotic pathways, abnormal calcium handling, progressive inflammation, imbalance between the myocardial oxygen supply and consumption and ion current disturbance. Therefore, prolonged LVH will ultimately result in deterioration of contractile performance of the heart and arrhythmia and eventually heart failure and sudden death (Kehat and Molkenkin, 2010).

Pressure overload-induced LVH progression is accompanied by alteration in heart geometry includes increase of heart weight, LV weight, LV thickness and interventricular septum thickness (concentric hypertrophy). In the late phase of LVH, the eccentric phenotype of hypertrophy is appeared in the heart which is characterized by dilatation of the left ventricular chamber and weakened heart. In compensated phase of hypertrophy, cardiac hypercontractility preserves the systolic function with higher ejection fraction. However, while LVH progresses to decompensated phase, ejection fraction progressively reduced (Lazzeroni et al., 2016).

During progression of LVH, excessive deposition of extracellular matrix proteins contributes in myocyte misalignment, distorted heart architecture and reduced tissue compliance which ultimately leads to cardiac failure. The negative correlation between ejection fraction and interstitial fibrosis has been shown in patient with pressure overload-induced LVH (Hein et al., 2003). Despite the significant progress in reducing LVH-induced heart failure, the prevalence of this disease and the consequent mortality rate is still high worldwide. Considering the disastrous complication of LVH progression, new therapeutic approaches for treatment of this disease are highly needed.

A hallmark of hypertrophy progression from adaptive (early) to mal-adaptive (late) phase is the genetic reprogramming of several cellular signaling in the cardiomyocytes. Identification of genes involved in cardiac hypertrophy progression improves our overall understanding of the molecular differences between adaptive and mal-adaptive phases of cardiac hypertrophy leading to discovery of new therapeutic approaches (Lai et al., 2013).

Emerging studies have revealed that histone/protein deacetylases (sirtuins) contribute to the regulation of physiological processes such as metabolism, cell growth, cell survival and stress response in the heart (Matsushima and Sadoshima 2015; Katsi et al., 2016). Seven sirtuins (SIRT1-7) have been identified in mammalian cells. The first one, SIRT1, which is a nucleocytoplasmic protein, regulates distinct metabolic and cell survival pathways in the heart. SIRT1 plays an important role in the regulation of physiological and pathological cardiac cell growth, but the underlying mechanism is poorly understood (Planavila et al., 2011; Lai et al., 2014).

The role of SIRT2 in cardiovascular system has been investigated in recent studies. SIRT2 deacetylases histones, tubulin and a broad range of transcription factors, thereby contributing to the control of genomic stability, metabolism and inflammation (Inoue et al., 2007). It has been shown that SIRT2 is involved in microtubule stabilization in diabetic cardiomyopathy (Yuan et al., 2015) and hypertension-induced vascular remodeling (Hashimoto-Komatsu et al., 2011). Recently, it has been shown that SIRT2 is a negative regulator of pathological hypertrophy through deacetylation and inactivation of the nuclear factor of activated T-cells (NFAT) transcription factor (Sarikhani et al., 2018).

Mitochondrial sirtuin, SIRT3, has been shown to protect the heart against oxidative stress by suppressing cellular levels of reactive oxygen species (ROS) and improvement of mitochondrial function (Chen et al., 2015a). The cardioprotective effects of another mitochondrial sirtuin, SIRT4, are currently under debate. For example, SIRT4 ameliorated hypoxia-induced apoptosis in cardiomyoblast by influencing caspase activity (Liu et al., 2013a); however, promoted pathological hypertrophy and fibrosis by inhibition of manganese superoxide dismutase activity (Luo et al., 2017).

The crucial role of SIRT5 in regulation of cardiac

function has been shown in recent studies (Sadhukhan et al., 2016). It has been demonstrated that SIRT5 was down-regulated in cardiomyocytes following oxidative stress and SIRT5 knocking down led to decreased cell viability and increased apoptosis (Liu et al., 2013b; Sadhukhan et al., 2016; Hershberger et al., 2017). SIRT5 knockout mice had reduced fatty acid and glucose oxidation as well as increased mortality when undergoing transverse aortic constriction to induce cardiac hypertrophy (Hershberger et al., 2017).

Studies have revealed that the nuclear protein SIRT6 exerts cardioprotective effects. Sundaresan et al. investigated the role of SIRT6 in cardiac hypertrophy progression in human hearts and animal models of LVH. They showed that SIRT6 deficient mice exhibited left ventricular hypertrophy and cardiac failure. Whereas, SIRT6 over-expression protected the hearts against hypertrophy, as characterized by decrease of HW/BW, cardiomyocyte size and fibrosis. Furthermore, cardiac expression of SIRT6 reduced in human failing hearts in comparison with normal hearts (Sundaresan et al., 2012). The association between genetic variant in the SIRT6 gene and atherosclerotic plaque has also been shown (Dong et al., 2011).

There is less information about the cardiovascular effects of the last sirtuin, SIRT7, which is localized in the nucleoli. Some novel cardioprotective effects of SIRT7 have been shown in recent studies. Following myocardial ischemia, SIRT7-deficient mice showed lower survival rate because of the cardiac rupture (Araki et al., 2015). Vakhrusheva et al. (2008) showed SIRT7 deficient mice developed cardiac hypertrophy which was accompanied by myocardial fibrosis, inflammation and increased apoptosis. Decrease of cardiac transcription of SIRT7 has also been reported in aged hearts (Wronska et al., 2016).

In general, disturbances in the sirtuins expression or activity is strictly linked to the pathological processes in the heart. However, each of sirtuins has a distinct regulatory pathway and proposing the same expressional profile for all of the sirtuins in different heart diseases is difficult. Consequently, understanding the transcriptional and expressional alteration of these proteins in cardiovascular diseases such as hypertension and LVH could allow for targeting these proteins as the novel paradigm in anti-hypertrophic drug discovery.

Despite the importance of cardiovascular effects of sirtuins, there is no report on the comparison of sirtuins transcriptional profiles between early and progressive phases of LVH. Therefore, the aim of the present study is to investigate the cardiomyocytes size, fibrosis and hemodynamic parameters (systolic and diastolic blood pressure, heart rate) as well as cardiac transcriptional levels of sirtuin family in adaptive and mal-adaptive phases of pressure overload-induced hypertrophy in rats.

Materials and methods

Animal care and experimental protocol

All experimental procedures in this study were performed in accordance with the ethical guidelines for animal research approved by the Ethics Committee for Animal Experiments of Shahid Sadoughi University of Medical Sciences, Yazd, Iran. Thirty two, 8-week-old male Wistar rats (190 ± 20 g) were purchased from the animal house of Shahid Sadoughi University of Medical Sciences, Yazd, Iran. Animals were housed under standard conditions of temperature and humidity with a 12 h light/dark cycle and free access to food and water.

Animals were randomly divided into four experimental groups ($n=8$ in each group): (I) control group, intact animals without any intervention; (II) H3w group, the animals underwent partial banding of suprarenal abdominal aorta. Three weeks post-surgery, the rats were in the early or adaptive phase of cardiac hypertrophy; (III) H16w group, aorta banding was performed similar to the group II. Sixteen weeks post-surgery, the rats were considered to be in the late or mal-adaptive phase of hypertrophy and (IV) sham-operated group, the rats were subjected to surgery procedure without aortic banding.

Pressure overload-induced hypertrophy model

Abdominal aorta banding was performed to create pressure overload-induced hypertrophy model in H3w and H16w groups. Briefly, the animals were anesthetized by an intraperitoneal injection of ketamine (70mg/kg) and xylazine (10mg/kg). The rats were kept on a heating pad during the surgery. After shaving, the left flank was incised from the last rib to upper border of hip. Abdominal supra renal aorta was exposed and surgically isolated from surrounding tissue. For partial banding of artery, a 21-gauge

Table 1: Primer sequences used for quantitative RT-PCR.

Gene	Sequences(5'->3')
β-actin	Forward : TTA-TTC-TGC-TCC-TGC-TTT-TC Reverse: GTG-GAT-TGT-TCT-GGA-GAC-TC
SIRT1	Forward : AGC-TGG-GGT-TTC-TGT-TTC-CTG-TGG Reverse : TCG-AAC-ATG-GCT-TGA-GGA-TCT-GGGA
SIRT2	Forward : AGG-GAC-AAG-GAG-CAG-GGT-TC Reverse: GAA-GAG-AGA-CAG-CGG-CAG-GAC
SIRT3	Forward : GAG-GTT-CTT-GCT-GCA-TGT-GGT-TG Reverse : AGT-TTC-CCG-CTG-CAC-AAG-GTC
SIRT4	Forward : TTG-TGC-CAG-CAA-GTC-CTC-CTC Reverse: GTC-TCT-TGG-AAA-GGG-TGA-TGA-AGC
SIRT4	Forward : TCC-AGC-GTC-CAC-ACG-AAA-CC Reverse: AAC-ACC-AGC-TCC-TGA-GAT-GAT-GAC
SIRT6	Forward : GCT-GGA-GCC-CAA-GGA-GGA-ATC Reverse : AGT-AAC-AAA-GTG-AGA-CCA-CGA-GAG
SIRT7	Forward : GAG-CCA-ACC-CTC-ACC-CAC-ATG Reverse: ACG-CAG-GAG-GTA-CAG-ACT-TCA-ATG

Table 2: Hemodynamic parameters in experimental groups.

Groups	SBP (mmHg)	DBP (mmHg)	MAP (mmHg)	HR (Beats/min)
Ctl	107.1±7.5	71.2±6.5	83.16±6.3	321±18
Sham	111.6±8.3	84.0±4.9	93.2±6.1	317±23
H3W	151±8.7***	119.6±7.6**	130**±8.2	289±16
H16W	129.2±6.9	91.3±5.4	103.9±7.4	308±21

Systolic (SBP), diastolic (DBP) and mean arterial (MAP) blood pressure and heart rate (HR) three and sixteen weeks (w) after abdominal aortic banding to induce hypertrophy (H) (H3W and H16w groups respectively). In sham group the rats were subjected to surgery procedure without aortic banding. Intact animals served as control (Ctl) (n=8 in each group). ** $P < 0.01$ and *** $P < 0.001$ vs. Ctl

needle was placed beside the artery and the suture was tied around it, then the needle was removed.

In H3w group, three weeks and in H16w group, sixteen weeks after artery banding, the animals were anesthetized again and blood pressure was recorded directly by cannulation of carotid artery through a transducer connected to the power lab system (AD Instrument, USA). In hypertrophied groups, animals without induction of hypertension were excluded from the study. In order to monitor the heart rate, electrocardiogram (lead II) was recorded using needle electrodes placed subcutaneously.

After measuring the blood pressure, hearts were immediately removed, cleaned in ice-cold saline and

weighed to calculate the heart weight- to -body weight ratio (HW/BW). The left ventricle was separated and transferred to liquid nitrogen and then frozen at -80°C. Three samples of each group were fixed in 4% paraformaldehyde for histological study and five samples were used for molecular studies (Seymour et al., 2015; Dorri et al., 2017).

Histopathological examination of the heart tissue

Paraformaldehyde fixed left ventricle tissues were processed and embedded in paraffin. The blocks were sectioned transversely (cross section at the papillary muscle level, 6µm thickness) and stained with hematoxylin/eosin and Masson's trichrome for

the assessment of cardiomyocyte size and fibrosis, respectively. Images were captured by a Nikon microscope equipped with a Sony, Syber-shot, DSCWX200 camera. Areas of 150 cells per group (50 cells per heart) were measured using Image J software (Seymour et al., 2015; Dorri et al., 2017).

Quantitative RT-PCR technique

Tissue samples were homogenized and total RNA was isolated using Trizol reagent (CinnaGen, Iran). First-strand cDNA was synthesized by RevertAid™ M-MuLV reverse transcriptase enzyme, in accordance with manufacturer's instructions (Vivantis, Korea). Quantitative RT-PCR reaction was performed using the specific primers in the Rotor Gene system (Qiagen, Germany). The relative expression of target genes was analyzed according to the $2^{-\Delta\Delta Ct}$ method. B-actin was used as the reference for normalization of genes expression. The primer sequences are listed in Table 1.

Statistical analysis

Data distribution was tested by the D'Agostino-Pearson normality test and analyzed by one-way analysis of variance (ANOVA). Multiple comparison analysis was performed using the Tukey test as Post-Hoc test. All statistical analysis was done using Graph Pad Prism (5.04) software. Data were presented as mean±SEM and statistical significance was considered to be at $P<0.05$ (95% confidence level).

Results

Hemodynamic parameters, HW/BW ratio and cardiomyocyte size in adaptive and mal-adaptive phases of cardiac hypertrophy

To realize the effect of adaptive and mal-adaptive hypertrophy on blood pressure, left carotid artery was cannulated and blood pressure was recorded directly. As shown in table 2, systolic, diastolic and mean blood pressure in H3w group was 151 ± 8.7 , 119.6 ± 7.6 and 130 ± 8.2 mmHg, respectively, which was significantly higher than that in the control group ($P<0.001$, $P<0.01$ and $P<0.01$, respectively). The systolic and diastolic blood pressure in H16w group was not statistically different with the control group. Heart rate did not change significantly among the experimental groups.

Regarding the HW/BW, there was a significant difference between H3w and control groups ($P<0.001$). HW/BW ratio in H16w group was also indicated a significant difference with the control group ($P<0.05$). Left ventricular tissue of hypertrophied hearts, both in early and late phases of hypertrophy, exhibited interstitial fibrosis (Fig. 1). Furthermore, cardiomyocytes size increased in H3w and H16w groups by $49.8\pm 8\%$ and $60.1\pm 7.2\%$, respectively, which showed a significant difference in comparison with control hearts ($P<0.001$, Fig. 2). There was no significant difference between control and sham groups regarding all the hypertrophy associated markers (blood pressure, HW/BW and cardiomyocyte area).

Sirtuins transcription levels in adaptive and mal-adaptive phases of cardiac hypertrophy

In H3w group, the SIRT1, SIRT3, SIRT6 and SIRT7 mRNAs levels increased by $75.7\pm 13.9\%$, $58.13\pm 14\%$, $41.38\pm 11\%$ and $72.29\pm 11.1\%$, respectively, which showed significant differences in comparison with the control group (SIRT1, 3 and 6 at $P<0.05$; SIRT7 at $P<0.01$). However, in H16 group SIRT1, SIRT3, SIRT5, SIRT6 and SIRT7 mRNAs levels down-regulated significantly in comparison with H3w group (SIRT5 at $P<0.01$ and other SIRTs at $P<0.001$). SIRT2 and SIRT4 transcription levels did not change significantly in both H3w and H16w groups (Fig. 3).

Discussion

The results of first part of our study revealed that in the early phase of LVH, systolic and diastolic blood pressure, mean arterial pressure, HW/BW and cardiomyocyte size increased. In the late phase of cardiac hypertrophy, despite increased cell area and heart weight, hypertension which was observed in early phase was disappeared probably due to cardiac dysfunction and decrease of ejection fraction during progression of hypertrophy to heart failure (Hein et al., 2003; Lazzeroni et al., 2016). Collagen deposition in left ventricular tissue was observable in both phases of hypertrophy. Our results regarding the hemodynamic and structural changes in the heart during progression of hypertrophy are consistent with previous studies. Ku et al. (2014) showed that cardiomyocytes size and HW/BW increased six and ten weeks after aortic banding but it weakened and

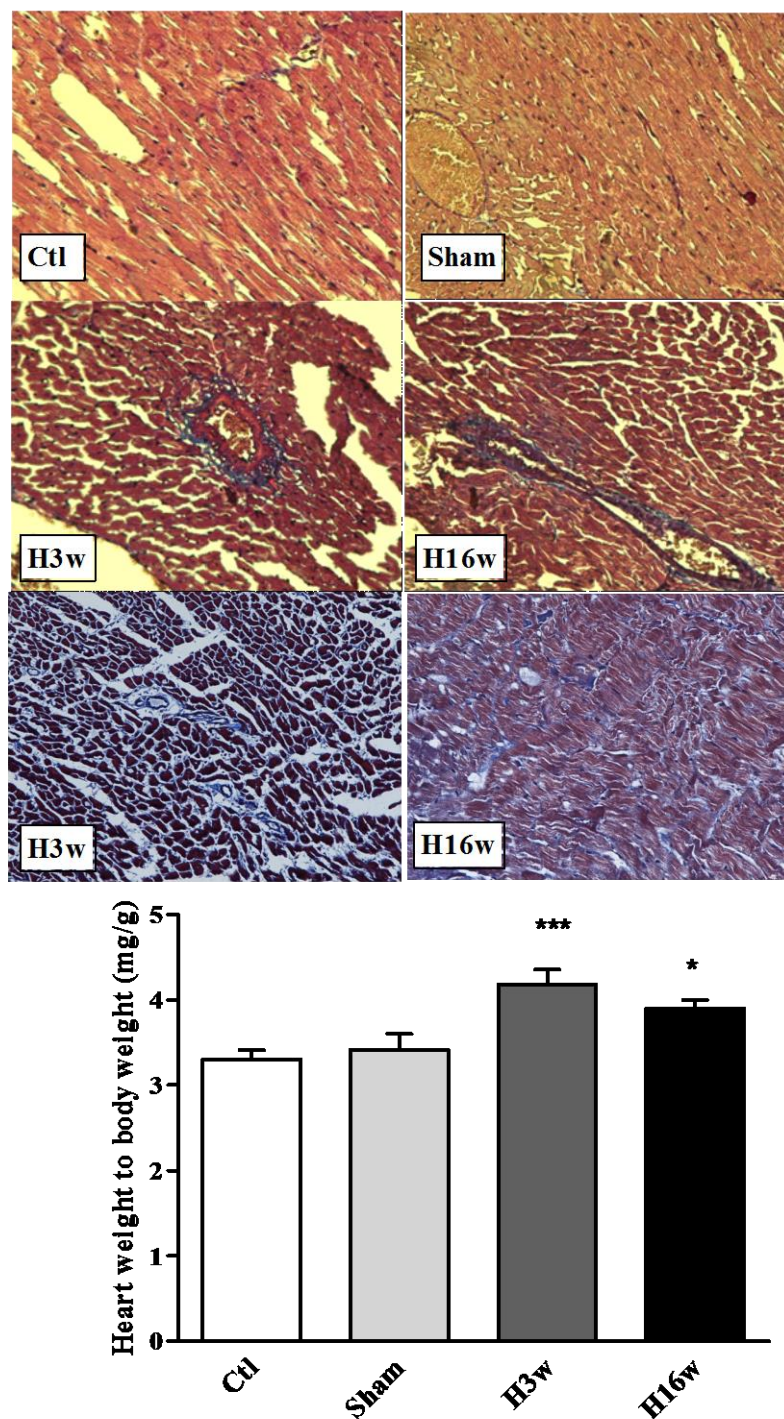


Fig.1. Comparison of the MDA (A) and total thiol (B) contents and SOD (C) and catalase (D) activities in renal tissue of five groups. Data are presented as mean \pm SEM (n= 8 in each group). ** P <0.01 and *** P <0.001 compared to control group. # P <0.05, ## P <0.01 and ### P <0.001 compared to DOX group. Co:control, DOX: doxorubicin, Vit E: vitamin E and PM: Plantago major

progressed to failure ten weeks after aortic banding in rats. In other studies, twelve and sixteen weeks after abdominal aortic constriction, heart failure was induced in rats but HR and mean arterial blood pressure did not change among the experimental groups (Wang et al., 2005; Liao et al., 2007). Our previous studies have also shown that three weeks after aortic banding mean arterial pressure, HW/BW

and cardiomyocyte area increased and transcription levels of atrial and brain natriuretic peptides was up-regulated (Sadeghzadeh et al., 2018; Dorri et al., 2017).

The next finding of our study was that the transcriptional profile of sirtuins is different in adaptive and mal-adaptive phases of cardiac hypertrophy. In the adaptive phase, SIRT1 mRNA was up-regulated,

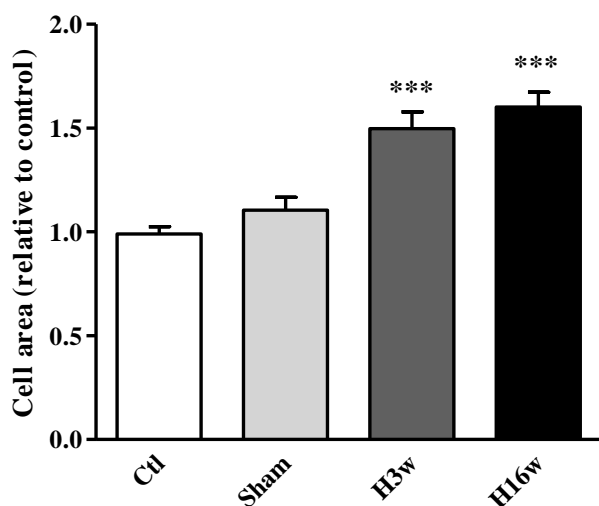


Fig.2. Cardiomyocytes area of experimental groups. Cardiomyocytes size increased three and sixteen weeks (w) after abdominal aortic banding-induced hypertrophy (H) (H3W and H16w groups, respectively). Intact animals served as control (Ctl). The sham group underwent the surgical procedure without aortic banding. *** $P < 0.001$ vs. Ctl.

but after progression to mal-adaptive phase, transcription of SIRT1 was substantially down-regulated. SIRT1 is a NAD(+)-dependent deacetylase with a diverse number of substrates which regulates a variety of physiological and pathological processes in cardiovascular system. Cardioprotective effects of SIRT1 have been shown in different aspects of cardiac hypertrophy and failure. SIRT1 protects the heart against oxidative stress and improves cardiac function in failing heart, by increase of manganese superoxide dismutase (Mn-SOD) (Tanno et al., 2010; Bagul et al., 2015). SIRT1 also deacetylates and deactivates nuclear factor- κ B (NF- κ B) to decrease transcription of NADPH oxidase as the main source of ROS production in diabetic cardiomyopathy (Tanno et al., 2010; Bagul et al., 2015). Resveratrol, SIRT1 activator, increased the activity of antioxidant factors, SOD, catalase and glutathione peroxidase and suppresses over-activity of NADPH oxidase in cardiomyocytes in a diabetic and ischemia models (Guo et al., 2015; Safari et al., 2015). Our recent study has shown that SIRT1 is involved in the cardioprotective effect of ischemia preconditioning in rats (Safari et al., 2017).

Alteration of cardiac expression of SIRT1 was reported in some previous studies. Li et al. (2009) showed that cardiac levels of SIRT1 mRNA and protein increased in spontaneously hypertensive rats.

In a study on patients with heart failure, SIRT1 mRNA down-regulated in peripheral leukocytes. Decrease of SIRT1 correlated with oxidative stress in failing heart (Akkafa et al., 2015). In agreement with previous studies, our results showed that SIRT1 mRNA was up-regulated in cardiac tissue in the early phase of hypertrophy, suggesting an adaptive response of the heart to pressure overload stress in order to increase its protection against pathophysiological events. However, down-regulation of SIRT1 transcription in mal-adaptive phase of hypertrophy may exacerbate cardiac dysfunction during progression to heart failure.

Another mechanism involved in pathophysiology of hypertrophy is inflammation. Suprarenal-aortic constriction-induced hypertrophy triggers prevascular inflammation (Baumgarten et al., 2002). Pro-inflammatory cytokines such as interleukin 1(IL-1), IL-6 and tumor necrosis factor- α are implicated in the progression of cardiac failure (Anker and von Haehling, 2004). Activation of SIRT1 by the natural polyphenol, resveratrol, reduced the serum and cardiac levels of proinflammatory cytokine, high mobility group box-1 in diabetic mice (Wu et al., 2016). SIRT1 also prevented up-regulation of pro-inflammatory cytokines and repressed NF- κ B activity in hypertrophied cardiomyocytes through peroxisome proliferator-activated receptor- α (PPAR α) (Planavila et al., 2011).

SIRT1 influences other aspects of cardiac hypertrophy such as angiogenesis. During cardiac hypertrophy, tissue growth should be coordinated with angiogenesis to meet the metabolic needs of the heart. Impaired angiogenesis in hypertrophied heart leads to the progression from adaptive to mal-adaptive cardiac hypertrophy (Shiojima et al., 2005). Experimental studies confirmed the regulatory role of SIRT1 in angiogenesis. SIRT1 (-/-) mice exhibited decreased cardiac capillary density and impaired angiogenic response to vascular endothelial growth factor (VEGF) (Maizel et al., 2014). Potente et al. (2007) show that disruption of SIRT1 inhibits sprouting angiogenesis and results in defective vascular growth. They suggest that SIRT1 regulates the angiogenic activity of endothelial cells through deacetylation of the forkhead transcription factor Foxo1. Furthermore, SIRT1 improves metabolic function of the heart. Treatment of rats with resveratrol after hypertension-induced heart failure,

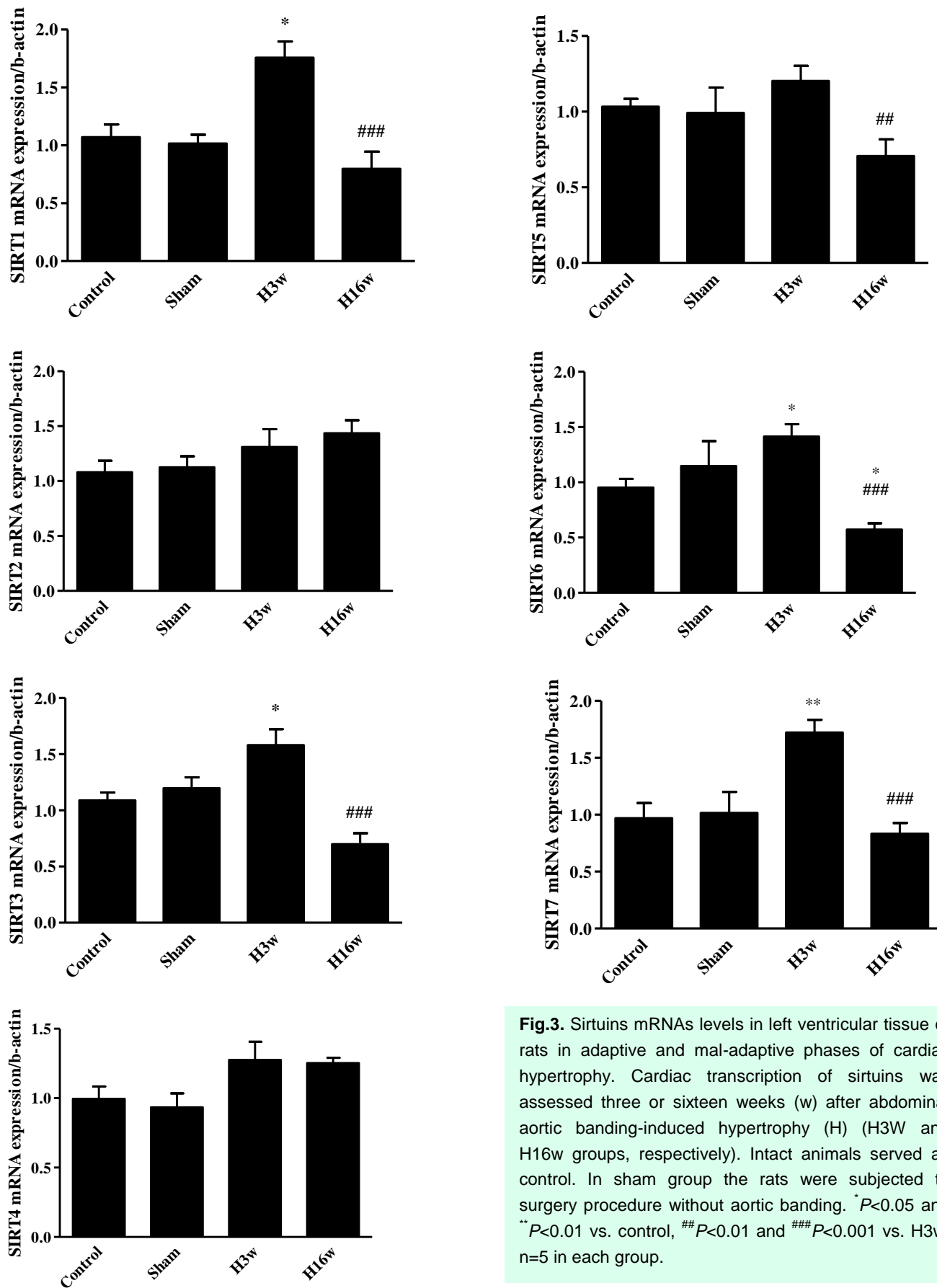


Fig.3. Sirtuins mRNAs levels in left ventricular tissue of rats in adaptive and mal-adaptive phases of cardiac hypertrophy. Cardiac transcription of sirtuins was assessed three or sixteen weeks (w) after abdominal aortic banding-induced hypertrophy (H) (H3W and H16w groups, respectively). Intact animals served as control. In sham group the rats were subjected to surgery procedure without aortic banding. * $P < 0.05$ and ** $P < 0.01$ vs. control, ## $P < 0.01$ and ### $P < 0.001$ vs. H3w. $n = 5$ in each group.

preserved metabolic function of the failing heart by modulating fatty acid oxidation and PPAR α expression as well as improvement of mitochondrial respiration and biogenesis (Rimbaud et al., 2011). Our previous study has shown that SIRT1 activator, resveratrol, decreases BP, HW/BW and cardiomyocyte area in hypertrophied heart. This anti-hypertrophic effect was accompanied by down-regulation of angiotensin type 1 receptor (Dorri et al., 2017). SIRT1 activation also exhibited anti-hypertrophic effect in age-induced cardiac hypertrophy (Dehghani et al., 2018).

In our study SIRT2 mRNA level did not change during progression of hypertrophy. Our data is inconsistent with the results of a recent study in which Tang et al. demonstrated that SIRT2 was down-regulated in hypertrophied hearts of rats. This conflicting result may be due in part to difference in the type of hypertrophy as in their study hypertrophy was induced by angiotensin II and in response to aging (Tang et al., 2017). In a recent study by Sarikhani et al. (2018), it has been revealed that SIRT2 protein content is reduced during cardiac hypertrophy. Also, sirt2-deficient mice exhibited age associated ventricular hypertrophy which was characterized by fibrosis and cardiac dysfunction. whereas, SIRT2 over-expression ameliorated cardiac hypertrophy response by inhibition of transcription factor NFAT.

The transcriptional profile of mitochondrial sirtuins (SIRT3, SIRT4 and SIRT5) was also evaluated in this study. Our results showed that in adaptive phase of hypertrophy, SIRT3 mRNA level was up-regulated. In another study, we showed that SIRT3 protein, but not mRNA level, decreased in the left ventricular tissue following acute ischemia. Ischemia reperfusion is a typical situation of oxidative stress which affects the heart in a different way (Klishadi et al., 2015). In the current study, progression of hypertrophy was associated with decrease of SIRT3 mRNA level. Our result is in agreement with that of a study by Chen et al. (2015b) in which they showed that, six weeks after transverse aortic constriction in mouse, cardiac level of mitochondrial form of SIRT3 decreased.

Emerging evidence suggests that SIRT3 is the main protein deacetylase in the mitochondria that plays a pivotal role in regulation of mitochondrial function in cardiovascular system. Also, SIRT3 deacetylates and thereby regulates diverse proteins in nucleus and cytoplasm. In a study by Sundaresan et al. it was

shown that SIRT3 blocked the hypertrophy by deacetylation and activation of transcription factor forkhead box O3a (FOXO3a) which resulted in up-regulation of Mn-SOD as well as catalase and decrease of cellular ROS level (Sundaresan et al., 2009). Also, it has been shown that SIRT3 activity decreased in diabetic hearts, leading to an increase in the acetylation of Mn-SOD. Restoring the cardiac level of SIRT3 caused increase SOD activity and reduce ROS level in diabetic heart (Sultana et al., 2016).

Angiogenic effects of SIRT3 have also been shown in some studies. For example, in the study by Hou et al. (2015) the peptide, apelin, enhanced angiogenesis in diabetes mellitus via SIRT3, so that in SIRT3 knockout mice it failed to increase capillary density as well as angiopoitin protein and VEGF levels. Recently, Wei et al. (2017) have shown that cardiac over-expression of SIRT3 led to improvement of vessel sprouting and micro vessels formation in mice infused with the pro-hypertrophic factor angiotensin II. Under stress condition, nuclear and mitochondrial pools of SIRT3 increase to protect the cardiomyocytes against stress-induced injury. SIRT3 blocks pro-apoptotic factor Bax through deacetylation of Ku70 (Sundaresan et al., 2008).

Regulation of autophagy in the heart is another novel action of SIRT3. Autophagy acts as a regulated renewal/recycling process and plays a pivotal role in cardiomyocytes survival by selective clearance of long-lived proteins and damaged organelles. Decrease of autophagy has been identified in hypertrophy progression (Nishida et al., 2016). Li et al. (2016) have demonstrated that SIRT3 knockout mice, when infused with the pro-hypertrophic factor angiotensin II, showed cardiac dysfunction and impaired autophagy. Activation of SIRT3 improved autophagy through FOXO1 signaling pathway and lead to cardiac improvement in these animals.

In our study, SIRT4 mRNA level did not change significantly in either early or late phases of cardiac hypertrophy. Although most studies confirm that sirtuins protect the cardiovascular system against oxidative stress, the data about the role of SIRT4 is different. Luo et al. demonstrated that SIRT4 knockout mice were more resistant to hypertrophy and fibrosis. They have shown that SIRT4 inhibits the binding of Mn-SOD to SIRT3, thus increasing ROS production in cardiomyocytes (Luo et al., 2017). In a

model of transverse aortic concentration-induced hypertrophy, SIRT4 increased oxidative stress and accelerated the progression to heart failure (Koentges et al., 2017).

To date, few studies reported the role of SIRT5 in cardiovascular physiology and pathology. SIRT5 over-expression protects the cardiomyocytes against oxidative stress. When cardiomyocytes undergo oxidative stress, they express lower level of SIRT5 which is accompanied by a decrease in cell viability. SIRT5 silencing promotes apoptosis in this oxidative stress situation. Whereas, SIRT5 over-expression protects the cardiomyocytes against oxidative stress-induced damage (Liu et al., 2013b). SIRT5 knockout mice were vulnerable to aortic banding-induced hypertrophy (Hershberger et al., 2017). In our study, SIRT5 mRNA levels did not change in the early phase of hypertrophy, but decreased dramatically in the mal-adaptive phase. Based on the cardioprotective effects of SIRT5, it is suggested that down-regulation of this mitochondrial sirtuin in failing heart may be involved in pathophysiology of cardiac dysfunction.

Regarding the transcriptional levels of the two last members of sirtuins, SIRT6 and SIRT7, our data also revealed that cardiac SIRT6 and SIRT7 mRNAs were up-regulated in the adaptive phase of pressure overload-induced hypertrophy. However progression of hypertrophy to mal-adaptive phase was accompanied by down-regulation of these sirtuins. SIRT6 and SIRT7 are deacetylase and mono-ADP ribosyltransferase enzymes with novel cardioprotective effects. SIRT6 activates transcription factor FOXO3a to upregulate antioxidant factors and reduce the ROS level in the heart following ischemia reperfusion (Wang et al., 2016). In a recent study, Lu et al. (2016) found that SIRT6 protected the heart against isoproterenol-induced hypertrophy through activation of autophagy. There is less information on the role of SIRT7 in antioxidant signaling. It has been shown that cardiomyocytes derived from SIRT7^{-/-} mice are more susceptible to hydrogen peroxide-induced oxidative stress. SIRT7^{-/-} mice have increased number of T lymphocyte, monocyte and granulocyte in their blood, inflammatory infiltration in myocardium and increased cardiac level of IL-4, IL-12 and IL-13, suggesting the anti-inflammatory effect of SIRT7 (Vakhrusheva et al., 2008).

Li et al. found that 28 days after aortic constriction,

SIRT6 mRNA and protein levels decreased in myocardial tissue of mouse. The difference in results may be due to the different animal species, as the authors confirmed the failure of heart in their study, but in rats, four weeks after aortic banding is considered as early phase and it takes longer time to develop into heart failure (Li et al., 2017). It seems that SIRT7 is up-regulated in response to oxidative stress, as cardiac level of SIRT7 transcription increased on day three after left ascending coronary artery ligation and ischemia induction in the mice (Araki et al., 2015). In our study, up-regulation of SIRT7 was also observed in the early phase of hypertrophy in which the heart is exposed to hemodynamic stresses. However, in the late phase of hypertrophy, SIRT7 mRNA was down-regulated, suggesting that decreased SIRT7 level may make the heart prone to oxidative damage. Down-regulation of SIRT7 mRNA has also been shown in hearts of aged (24-month-old) rats (Wronska et al., 2016).

With regard to the potential cardioprotective role of sirtuins, we can suggest that up-regulation of SIRT1, SIRT3, SIRT6 and SIRT7 in the early phase of hypertrophy presents the compensatory response of the heart to pressure overload. In other word, in this phase of hypertrophy, several survival pathways work together in an effort to maintain the normal cardiac performance in response to excess workload. On the other hand, suppression of these sirtuins in the decompensation phase of hypertrophy can make the heart more vulnerable to hypertrophy induced damages such as oxidative stress, inflammation and apoptosis which lead to heart failure. Further investigation will be needed to understand the exact molecular and cellular signaling that contribute to the effects of sirtuins in cardiac hypertrophy and to the cooperation or reciprocal function of sirtuin family in this disease. In future experiments, measurement of protein level as well as enzymatic activity of these potent deacetylases can provide accurate information in this regard.

The present study has two potential limitations. First, the echocardiogram parameters including left ventricular end diastolic pressure, left ventricle end-systolic pressure and ejection fraction have not been measured. Second, the expressional changes of sirtuins at protein level as well as enzymatic activity of sirtuins have not been investigated. The directions and limitations stem from the current project should

be considered in future studies.

Conclusion

Overall, our results revealed that progression of pressure overload-induced LVH is accompanied with structural and hemodynamic changes. Furthermore, the transcriptional profile of SIRT1, 3, 5, 6 and 7 is different in early and late phase of LVH. It could be concluded that sirtuins may be the therapeutic targets for pressure over load-induced cardiac hypertrophy.

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Conflict of interest

The authors declare that they have no conflict of interest.

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