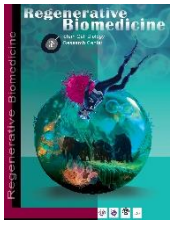

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Original Article

Age and Sex Differences in Cardiac Expression of Sirtuin-3 in Rats

Sadegh Naderi[1*], Mohammad Ebrahim Rezvani[1], Zeinab Hafizibarjin[1], Ali Moradi[2], Razie Najari[1] Fatemeh Safari[1,3]

1. Department of Physiology, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
2. Department of Biochemistry, Faculty of Medicine, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.
3. Stem Cell Biology Research Center, Yazd Reproductive Sciences Institute, Shahid Sadoughi University of Medical Sciences, Yazd, Iran.

***Corresponding Author:**
Naderi, Sadegh
Email:
naderisa95@gmail.com

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Abstract

Sirtuin-3 (SIRT3), a class III histone deacetylase plays a key role in regulating metabolism, oxidative stress and survival. This study investigated the cardiac level of SIRT3 in male and female rats during aging.

Wistar rats were divided into four experimental groups including old males, young males, old females and young females. Blood pressure was recorded through right carotid artery cannulation. Gene expression was measured using Real-time (RT-PCR technique and protein levels were evaluated using the ELISA test.

Our findings indicate that, both sexes experienced a similar increase in systolic pressure during aging without any significant difference. The heart weight-to-body weight ratio (HW/BW) of old male rats was significantly lower than that of young males ($P<0.01$) and old female rats ($P<0.05$). The cardiac level of SIRT3 did not show any significant changes during aging in either male or female rats. However, the hearts of young and old female rats exhibited higher expression of SIRT3 at both mRNA and protein levels.

The sex difference in age-related cardiovascular diseases is, at least in part, attributed to differences in the expression of regulatory proteins such as SIRT3.

Keywords: Aging, Blood pressure, HW/BW, Sex, SIRT3

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Introduction

Mitochondrial dysfunction, metabolic alterations, and oxidative stress are critical molecular mechanisms involved in the aging heart, contributing significantly to cardiac senescence and dysfunction. As cardiomyocytes age, they experience a decline in mitochondrial function characterized by impaired electron transport chain activity and reduced ATP production, which leads to inefficient energy metabolism that favors fatty acid oxidation over glucose utilization (1-3). This metabolic shift can result in the accumulation of free fatty acids and toxic lipid species, exacerbating oxidative stress through increased production of reactive oxygen species (ROS) within mitochondria. Elevated ROS not only damages mitochondrial proteins and DNA but also triggers cellular apoptosis and inflammation, further compromising cardiac function. Additionally, the accumulation of mitochondrial DNA mutations over time is linked to enhanced oxidative stress responses, activating pathways that promote cellular senescence and exacerbate age-related cardiac diseases (2,4). Sirtuin 3 (SIRT3) is a mitochondrial NAD(+)-dependent deacetylase that plays a pivotal role in regulating oxidative stress and mitochondrial health. Studies now highlight that SIRT3 contributes to protecting the heart from mitochondrial dysfunction, a key driver of age-related cardiovascular diseases. SIRT3 is also involved in modulating reactive oxygen species (ROS) production, crucial for mitigating oxidative stress that contributes to aging-related cellular damage (5, 6). Gender differences in the aging heart are significant and manifest in various structural and functional changes that influence cardiovascular health outcomes. Women generally exhibit a better preservation of myocardial structure with age compared to men, as evidenced by a lower rate of myocyte loss and hypertrophy in the female heart. Studies show that aging in women is associated with a stable number of ventricular myocytes, while men experience a loss of myocardial mass at an approximate rate of 1 gram per year, leading to detrimental effects on cardiac function (7). Furthermore, older women tend to develop heart failure with preserved ejection fraction (HFPEF) more frequently than men, which is linked to greater age-related concentric remodeling and diastolic dysfunction (8,9). Hormonal factors, particularly the decline in estrogen levels post-menopause, also contribute to these differences by affecting cardiac remodeling and vascular health (10, 11). Overall, these gender-specific patterns highlight the need for tailored approaches in the prevention and treatment of cardiovascular diseases in aging populations. This study aims to expand on these findings by investigating the age- and gender-related expression of SIRT3 at both the mRNA and protein levels in cardiac tissue, focusing on how these differences contribute to heart health in males and females across the lifespan. Understanding these mechanisms is crucial for developing targeted strategies to mitigate age-related cardiac decline and improve cardiovascular health in both sexes.

Materials and Methods

Experimental groups

Wistar rats were categorized into four experimental groups (n=9 each): (I) 24-

month-old male rats (old male group); (II) 3-month-old male rats (young male group); (III) 24-month-old female rats (old female group); and (IV) 3-month-old female rats (young female group). The animals were housed under controlled conditions, maintaining a 12-hour light/dark cycle at a temperature of 22°C. All procedures adhered to the ethical guidelines established by Shahid Sadoughi University of Medical Sciences.

Blood pressure recording

Animals were weighed and anesthetized with an intraperitoneal injection of ketamine (70-90 mg/kg) and xylazine (10 mg/kg)(12). Arterial pressure was measured by carotid cannulation through a catheter connected to the PowerLab system (ADInstruments PowerLab 4/30)(13). The hearts were then excised, rinsed with cold saline, weighed, and stored at -80°C for subsequent assays.

Histological analysis of hearts

Left ventricular tissues were fixed in 10% formaldehyde, dehydrated, embedded in paraffin, and sectioned to a thickness of 5 µm. To evaluate cell size, tissue samples were stained with hematoxylin and eosin (14). Photomicrographs were taken using a Nikon Eclipse ci-l microscope (Japan), and the area of cardiomyocytes was measured with ImageJ software.

Quantitative real time RT-PCR

To determine SIRT3 mRNA levels, real-time RT-PCR was employed. RNA was extracted from the left ventricle using RNX plus (Sinagen-Iran) and evaluated for both quantity and quality with a NanoDrop device (Biotech Instrument Model: Box998, USA) at a wavelength of 260-280 nm. The reverse transcription reaction utilized RevertAid™ M-MuLV reverse transcriptase enzyme (Fermentas, USA). In the presence of specific primers, cDNA was amplified using a Rotor-Gene Q real-time RT-PCR machine (Germany), with β-actin serving as the reference gene. The primer sequences for SIRT3 and β-actin are detailed in Table 1. Gene expression analysis was conducted using the 2-ΔΔ CT method.

Measuring SIRT3 protein level by ELISA Test

To quantify cardiac SIRT3 concentration, tissue samples were homogenized in a PBS solution at a ratio of 10 mg/100 µl and centrifuged at 4°C and 5000 rpm for 15 minutes. The resulting supernatant was analyzed for SIRT3 protein levels following the protocol outlined in the SIRT3 ELISA assay kit (MyBiosource, MBS047755, USA).

Statistical analysis

The impact of age and sex on blood pressure, heart weight to body weight ratio (HW/BW), gene expression, and protein levels was evaluated using two-way ANOVA followed by Tukey's post hoc test (GraphPad Prism-5 software). A p-value of less than 0.05 was deemed statistically significant. Data are presented as Mean ± SEM.

Results

Changes in Blood Pressure Among Experimental Groups

Statistical analysis indicates that, irrespective of sex, advancing age has a significant impact on systolic blood pressure. As presented in Table 2, systolic blood pressure significantly

Table 1. Primer sequences used for real time RT-PCR reaction

Gene	Forward Primer(5′-3′)	Reverse primer(5′-3′)
SIRT3	GAGGTTCTTGCTGCATGGTTG	AGTTTCCCGCTGCACAAGGTC
β-actin	GAACCCTAAGGCCAACCGTGAAAGAT	ACCGCTCGTTGCCAATAGTGATG

increased with age in both male (P<0.01) and female (P<0.05) rats. In contrast, changes in diastolic pressure due to age were not statistically significant in either sex, nor were there significant differences when comparing the sexes.

Changes in Cell Size and Heart Weight to Body Weight Ratio Among Experimental Groups

Regarding the heart weight to body weight ratio (HW/BW), results indicate that the HW/BW ratio in the old male group was significantly lower than that of the young male and old female groups (P < 0.001 and P < 0.01, respectively) (Table 2). The size of cardiomyocytes was assessed using ImageJ software, revealing an increase in cell size among older rats, particularly in old males compared to their younger counterparts (Figure 1).

Cardiac levels of SIRT3 in Experimental Groups

As illustrated in Figure 2-A, there were no significant differences in SIRT3 mRNA levels between rats of both sexes within the old and young groups. However, young females exhibited a higher cardiac SIRT3 mRNA level compared to the young male group (P<0.001). Additionally, the SIRT3 mRNA level in the old female group showed a significant increase compared to the old male group (P<0.05). Moreover, the level of SIRT3 protein in the left ventricular tissue of the young female group was significantly higher than that of the young male group (P<0.01). Furthermore, SIRT3 concentration in the old female group was significantly elevated compared to the old male group (P<0.05) (Figure 2-B).

Discussion

The first part of our study demonstrated that, despite an increase in heart weight in the old male group, the heart weight to body weight ratio (HW/BW) in this group was lower compared to both the young male and old female groups. This suggests that ventricular hypertrophy in the old male group may not progress in parallel with body weight gain, resulting in the heart having to work harder to supply blood to the organs. Additionally, staining results indicated that cell size increases with age in both sexes, with a more pronounced change observed in males. Further evidence of cardiac tissue changes is provided by Ji et al., who studied young and old rats and found that while myocardial cell size increases, the number of cells decreaseswith aging (15). Similarly, Melissari et al. conducted a study on the myocardium of 67 individuals aged 17 to 90 who died from cardiovascular diseases. They observed a decrease in cell numbers in both the left and right ventricles, along with an increase in cell size among older individuals (16). These

Table 2. Blood pressure and heart weight-to-body weight ratio in experimental groups. Systolic and diastolic blood pressure, heart weight-to-body weight ratio in young (3 months) male and female and old (24 months) male and female rats. *P<0.05, **P<0.001 *vs*. young male group. $^{\#}$P<0.05 *vs*. young female group. $^{\cdot}$P<0.05, *vs*. old female. Data are reported as Mean ± SEM.

Groups	Young male	Old male	Young female	Old female
Systolic Pressure (mm Hg)	116.1±1.9	140±5.6**	120±3	136.4±2.8$^{\#}$
Diastolic Pressure (mm Hg)	77.7±3.8	87.1±3.1	71.3 ±3.5	83.4±3.9
Heart weight/Body weight (mg/g)	3.6±.07	3.03±.05$^{*\cdot}$	3.51±.1	3.41±.08

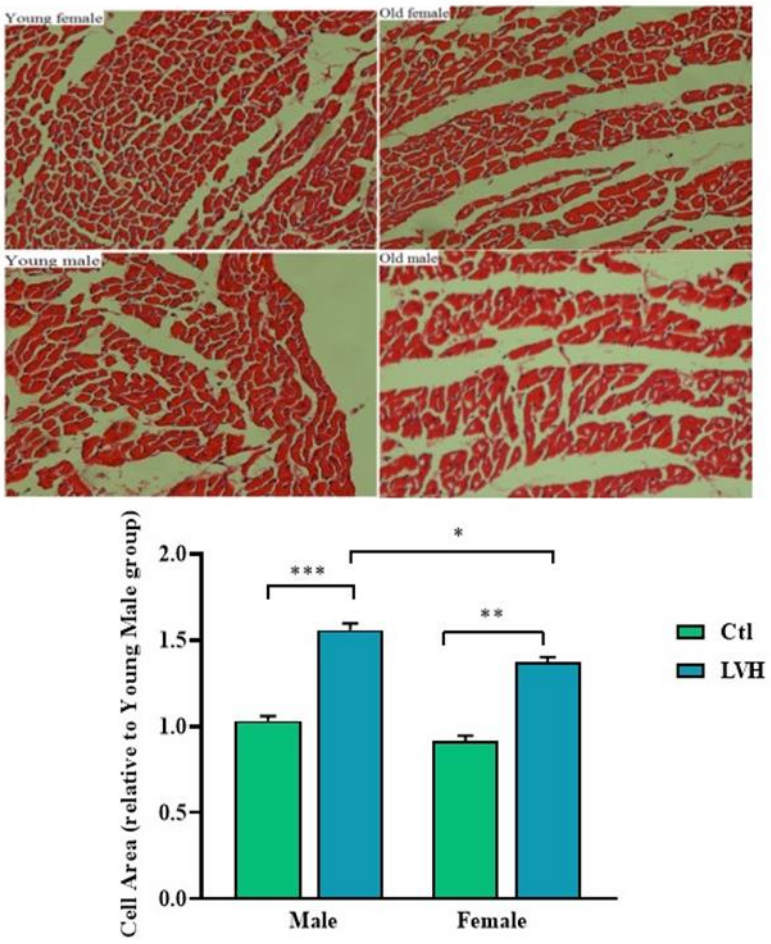

Figure 1. Left ventricular tissue cross sections of young (3 months) and old (24 months) rats stained with H&E (upper photomicrographs) to evaluate cardiomyocyte size. Data are expressed as Mean ± SEM.

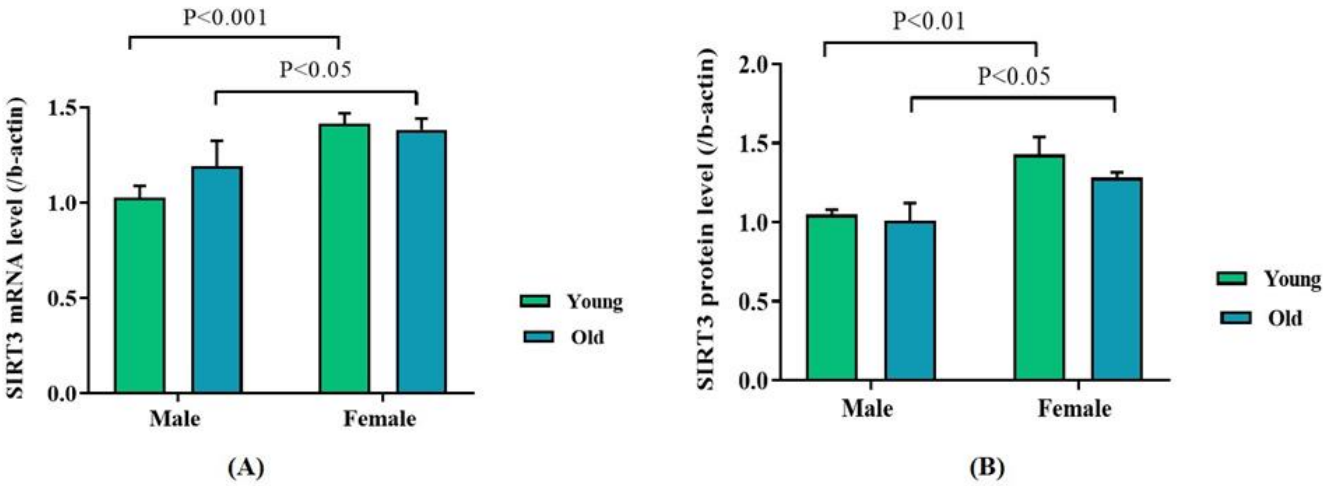

Figure 2. SIRT3 mRNA (A) and protein (B) levels in left ventricular tissue of young (3 months) and old (24 months) rats. Data are expressed as Mean ± SEM.

findings raise concerns about how these changes may contribute to the development and progression of ischemic diseases and heart failure. Bueno et al. showed that collagen content in both left and right ventricles progressively increases with age, which may be associated with stress on the ventricular wall (17).

In this study, we found that SIRT3 mRNA and protein expression were significantly higher in young female rats compared to their male counterparts. This difference persisted with aging between the two sexes. Research indicates that SIRT3 plays a crucial role under stress conditions and during cellular aging. The consumption of oxygen by cells leads to the production of reactive oxygen species (ROS), which are known contributors to aging due to their potential for protein and lipid degradation. Studies have shown that increased intracellular oxidants and the role of SIRT3 protein in cardiac function have shown that increased energy consumption and enhanced cardiac function occur in older rats with SIRT3 compared to those lacking it. Rats deficient in SIRT3 exhibited cardiac issues such as reduced contractility, decreased ejection fraction (EF), and a significant increase in end-diastolic volume (20). Another study on cardiac myocytes from SIRT3-deficient rats revealed an age-dependent increase in mitochondrial inflammation due to heightened mPTP opening. Thirteen-month-old rats lacking SIRT3 displayed accelerated symptoms of cardiac aging—such as hypertrophy and fibrosis—demonstrating for the first time that SIRT3 activity is essential for preventing mitochondrial dysfunction and cardiac hypertrophy during aging (21). By influencing K166 cyclophilin D, SIRT3 prevents mPTP opening during the aging process and reduces tissue stress (22). Studies indicate that Ku70 acetylation increases during oxidative stress and cellular genotoxicity, remaining acetylated over time. This condition facilitates Bax entry into mitochondria, triggering apoptosis. SIRT3 can block Ku70

acetylation under oxidative conditions; indeed, sirtuins—especially SIRT1 and SIRT3—can bind Ku70/Bax through Ku70 deacetylation, preventing Bax from entering mitochondria and initiating inflammatory and apoptotic processes (23).

Additionally, SIRT3 reduces ROS through various mechanisms, including counteracting ROS-stimulated inflammatory factors such as TNF and activating the antioxidant enzyme SOD2. Moreover, SIRT3 has detoxifying effects; for instance, superoxide dismutase—a mitochondrial antioxidant enzyme—is one of its deacetylation targets (5,6). The observation that SIRT3 protein levels do not decline with aging may indicate that increased stress and oxidative factors activate compensatory protective mechanisms. Gene expression and synthesis can be influenced by multiple factors; for example, Kwon et al. evaluated SIRT1 and SIRT3 expression across different organs using 6-month-old rats as young subjects and 24-month-old rats as older subjects. They found that while SIRT3 expression decreased in adipose tissue and kidneys with age, it increased in lungs and spleen; conversely, SIRT1 expression decreased in kidneys and skin but increased in lungs (24). In addition to age, organ type may also play a significant role in expression levels. It is possible that expression is organ-dependent; however, since expression levels were higher in 3- and 24-month-old females compared to old and young males, sex differences should also be considered. Given that sex is primarily represented through sex hormones in the body, one can postulate about the protective role of estrogen along with its influence on SIRT3 expression.

Continuing research on the relationship between estrogen and SIRT3 expression may yield a deeper understanding of the differential expression of this protein in male and female hearts.

Conclusion

These findings suggest that sex differences in age-related cardiovascular diseases can be attributed, at least in part, to variations in the expression of regulatory proteins such as SIRT3.

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Conflict of interest

The authors have no conflict of interest to declare.

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