Original Article

Moderate Aerobic Training Inhibits Middle-Aged Induced Cardiac Calcineurin-NFAT Signaling by Improving TGF-ß, NPR-A, SERCA2, and TRPC6 in Wistar Rats

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Abstract

Objective: The purpose of this study was to investigate the effect of moderate-intensity training on the calcineurin/nuclear factor of activated t-cells (NFAT) pathway and factors affecting it in the middle-age Wistar rats.

Materials and Methods: In this experimental study, 40 young (n=10, 4-month-old) and middle-aged (n=30, 13-15 months old) Wistar rats were included in this experimental study. All young and 10 middle-aged rats did not training and served as a control comparision; while the remaining 20 middle-aged rats were trained at moderate intensity for 4-weeks (n=10) or 8-weeks (n=10) on a treadmill (speed: 16 m/minutes, slope: 0%, distance: 830 m, duration: 54 minutes).

Results: Calcineurin tissue expression was increased in the middle-aged control rats compared to the young control rats (P=0.001). Expression of sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERC2A), natriuretic peptide receptor-A (NPR-A), phospholamban (PLB), plasma membrane Ca^{2+} ATPase (PMCA4b), and p-AKT was significantly decreased in the heart tissue of middle-aged control compared to the young control rats (P=0.001). Furthermore, transforming growth factor beta (TGF-β), including transient receptor potential canonical 6 (TRPC6), were up-regulated in the heart tissue of middle-aged control compared to the young control rats (P=0.001). However, aerobic training inhibited this pathway and reversed all changes in the trained middle-aged rats.

Conclusion: Aerobic training effectively inhibited the calcineurin/NFATc pathway and modulated intracellular Ca^{2+} levels at least partially by restoring NPR-A, SERCA2, p-PLB, and p-AKT, and decreasing TRPC6 and TGF-β levels.

Keywords: NPR-A, SERCA, TGF-β, TRPC6

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Introduction

Cardiac aging is often accompanied by adverse structural and functional changes such as increased left ventricular wall thickness (hypertrophy), prolonged diastolic duration, valvular degeneration, cardiac fibrosis, and compromised ventricular contractility (1), termed pathological cardiac hypertrophy (2). One of the major signaling pathways regulating the cardiac hypertrophy process is the calcineurin/nuclear factor of the activated T-cells (NFATc) signaling pathway (3). Increased activity of NFATc has been reported in elderly rats (4). Inhibition of the activation of NFATc has been suggested as a promising therapeutic strategy in pathological myocardial hypertrophy (5, 6). Evidence shows that pathological cardiac hypertrophy induces intracellular Ca^{2+} release and activation of calcineurin (7). Consequently, activated calcineurin mediates NFATc dephosphorylation leading to its translocation to the nucleus and activation of NFATn (6, 8). The activation of NFATn is followed by the transcription of several essential genes contributing to cardiac hypertrophy as well as the channels for entering Ca^{2+} penetration into the cell, including transient receptor potential canonical 6 (TRPC6) (8).

The TRPC channel- family, is a Ca^{2+}-permeable cation channel presenting in the plasma membrane of many tissues, including the heart tissue. TRPC6 contains NFAT-responsive elements in their promoters, playing an essential role in enhancing and maintaining gene expression through the feed-forward circuit (9). Unlike TRPC6, plasma membrane Ca^{2+} ATPase (PMCA) is a transporter protein in the cellular plasma membrane that maintains an appropriate cytoplasmic Ca^{2+} level via removing Ca^{2+} from the cell (10, 11). PMCA4, the most expressed PMCA isoform in the cardiomyocytes, interacts with a catalytic subunit of the calcineurin,, disabling its downstream signals and antagonizing cardiac hypertrophy (12).

Moreover, intracellular pumps such as sarco/endoplasmic reticulum Ca^{2+}-ATPase (SERCA2) play an essential role in regulating calcineurin/NFATc signaling, possibly by decreasing cytosolic Ca^{2+} levels via transferring Ca^{2+} from the cytosol to sarcoplasmic lumens with ATP hydrolysis (13, 14). Several studies have reported that SERCA2 expression decreases with aging, pathologic cardiac hypertrophy, heart failure, and vascular

proliferative remodeling (13, 15). It is well established that a reduction in SERCA2 can impair Ca^{2+} cycling due to an increase in cytosolic Ca^{2+} resulting in cardiac hypertrophy. On the other hand, restoring SERCA2 expression improves various features of heart failure (16-18). Phospholamban (PLB) is one of the proteins involved in regulating SERCA2 pump activity in the heart. Indeed, its phosphorylation increases SERCA pump activity, while dephosphorylation of PLB shuts down the SERCA pump (19). Other factors influencing the calcineurin/NFATc pathway include natriuretic peptide receptor-A (NPR-A) through SERCA2 activity (20, 21); transforming growth factor-beta (TGF-β) through increasing reactive oxygen species (ROS) (21), and p-AKT through increasing the phosphorylated form of NFATc and reducing NFATc (22). Evidence shows that TGF-β expression is up-regulated in aging, myocardial infarction, and cardiac hypertrophy (23).

The literature is limited on training effects on pathological cardiac hypertrophy and subsequent cardiac function (24, 25). Our previous study showed that aerobic exercise increases anti-aging protein and improves MAPK signaling and cardiac hypertrophy (24). But the mechanism of the effect of aging and training on Ca^{2+} channels and pumps in cardiac tissue is unclear. Given the impact of aerobic exercise on improving heart function, the purpose of this study was to examine the effect of aging and moderate-intensity aerobic training on the calcineurin/NFATc pathway and its regulators in the heart tissue of middle-age Wistar rats.

Materials and Methods

Antibodies and reagents

In this expreimental study, polyclonal rabbit antibodies, including anti-Akt, anti-p-Akt, anti-TGFβ, anti-p-PLB, anti-NFATc, anti-p-NFATc, anti-H1, anti-SERCA2, anti-PMAC4b, anti-NPR-A, anti-TRPC6, and anti-β-Actin, as well as goat anti-rabbit IgG-HRP (sc-2030) secondary antibody, were purchased from Santa Cruz Biotechnology, Inc (Santa Cruz, CA). All other reagents and chemicals were obtained from Sigma-Aldrich, USA.

Animals

Forty male Wistar rats, including ten young rats (4 months old) and thirty middle-aged rats (13-15 months old) were obtained from Pasteur Institute (Tehran, Iran). All animals were kept in the animal house of Neurosciences Research Center (Tabriz, Iran) for ten days on a 12 hours light/dark cycle, 22.0 ± 2°C temperatures, humidity 60%, and water and food ad libitum, to adapt to the new condition.

Ethics statement

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Islamic Azad University Jolfa Branch (96.03.1189).

Study design

Ten untrained young rats and ten untrained middle-aged rats were allocated into young control and middle-aged control groups, respectively. The remaining, twenty middle-aged rats were subjected to an aerobic training program, in which half of the animals were randomly trained for four weeks (old-T4 group), and half of them were trained for eight weeks (old-T8 group).

Aerobic training protocol

The middle-aged rats were familiarized with the aerobic training program on a rodent's treadmill for five days (Technic Azma, Iran). At the start of training, exercises began with an initial speed of 11 m/min, a slope of 0%, and distance traveled of 180 m for 13 minutes that progressed to a speed of 14 m/minutes, a slope of 0%, and distance traveled of 460 meters for 34 minutes by the fourth week, and a speed of 16 m/minutes, a slope of 0%, and distance traveled of 830 meters for 54 min by the eighth week (26).

Sampling

Twenty-four hours following the last training session, the trained animals were anesthetized with ketamine (60 mg/kg) and xylazine (10 mg/kg) and-euthanized. Subsequently, left ventricular heart tissues were collected, weighed, and stored either at -80°C for immunoblotting or in formaldehyde 10% for histological examination. The young control and middle-aged control rats were-euthanized at the beginning of the study.

Immunoblotting assay

We homogenized the frozen cardiac tissue samples on ice with a rotor blender (Fisher) in pre-cold RIPA lysis buffer (50 mM Tris-HCl, pH=8.0, 0.1% sodium dodecyl sulfate, 150 mM sodium chloride, 0.5% sodium deoxycholate, and 1.0% NP-40) along with- a protease and phosphatase inhibitor cocktail (Sigma Aldrich). We centrifuged the crude homogenate at 12000 ×g for 20 minutes at 4°C to procure the supernatants. We identified the protein concentration in the supernatant with a Bradford assay kit (Sigma Aldrich). We mixed an equal amount of protein (50 µg) (1:1) with twice the sample loading buffer (Sigma Aldrich) and boiled the solution for 5 minutes. We separated the proteins by electrophoresis on a denaturing sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred them onto a polyvinylidenedifluoride (PVDF) membrane. We blocked the membranes for 1-2 hours in the blocking buffer containing 3% bovine serum albumin in phosphate-buffered saline solution and 0.1% Tween-20 (PBST). We probed the membranes with a primary antibody diluted (1:500) in blocking buffer solution overnight at 4°C. After washing, we incubated the membrane with horseradish peroxidase (HRP) conjugated goat anti-rabbit secondary antibody solution for 1 hour at room temperature. We detected the antibody-antigen complex with enhanced chemiluminescence (ECL, Amersham, UK) detection kit that we visualized by exposure to x-ray film (Fuji, Japan). We used the Image J 1.62 software (National Institutes of

Health, USA) to quantify the signal intensity of each band with β-Actin as the loading control.

Immunohistochemical staining

We used IHC to assess the heart's tissue expression of calcineurin. We embedded formalin-fixed heart tissue samples in paraffin and then cut them into 4 µm sections and mounted them on slides. We deparaffinized the sections in xylene and rehydrated them in PBS. In addition, we pre-incubated the sections with normal bovine serum for 30 minutes to prevent non-specific binding, followed by incubation with calcineurin primary polyclonal rabbit antibody (Santa Cruz, CA, USA) at 4ºC. After three 40 minutes washes in PBS, we incubated the sections with biotinylated goat anti-rabbit IgG secondary antibody for 60 minutes at room temperature. After another 10 minutes wash in PBS, we incubated the slides with 0.5 mg/ml diaminobenzidine tetrahydrochloride 2-hydrate (DAB, Boster bio-engineering, USA)+H_2O_2 for 5 minutes (27). The total number of positively stained cells from five sections per each animal were counted using a light microscope (x10) in a blind way.

Statistical analysis

Descriptive statistics were determined for all variables. The Shapiro-Wilk test determined the normal distribution of data. Since the data distribution was normal, data were analyzed using appropriate Linear Mixed Models (based on fixed effects) to define the relationship between markers and Repeated-Measure design with Bonferroni Post hoc test for the intragroup' comparisons. Also, t test analyses were used to compare differences between the young control and middle-age control groups. Statistical significance was set at the alpha of $\alpha \leq 0.05$ for all tests. All data was analyzed using the Statistical Package for the Social Sciences 22 software (SPSS, Chicago, IL, USA).

Results

Calcineurin tissue expression

As shown in Figure 1, calcineurin tissue expression increased in the heart tissue of the middle-aged control rats compared to the young control rats (P<0.001). Nevertheless, the aerobic training program led to a significant decrease in heart calcineurin expression at the 4th (P<0.001) and 8th week (P<0.001) of training in the middle-aged rats compared to middle-aged control rats.

p-NFATc NFATc and NPRA protein levels

The results of the immunoblotting assay showed that phosphorylated levels of NFATc (p-NFATc) were significantly decreased in the heart tissue of the middle-aged control rats compared to the young control rats (P= 0.001), while protein expression of NFATc was increased as compared to the young control rats (P=0.001, Fig.2). However, aerobic training for eight weeks significantly increased p-NFATc levels in the middle-aged rats compared to the middle-aged control rats (P=0.001). Moreover, aerobic training markedly decreased protein expression of NFATc in themiddle-aged-T4 and middle-aged-T8 groups (for both P=0.001, Fig.2B, C). Data are presented as mean ± SEM.

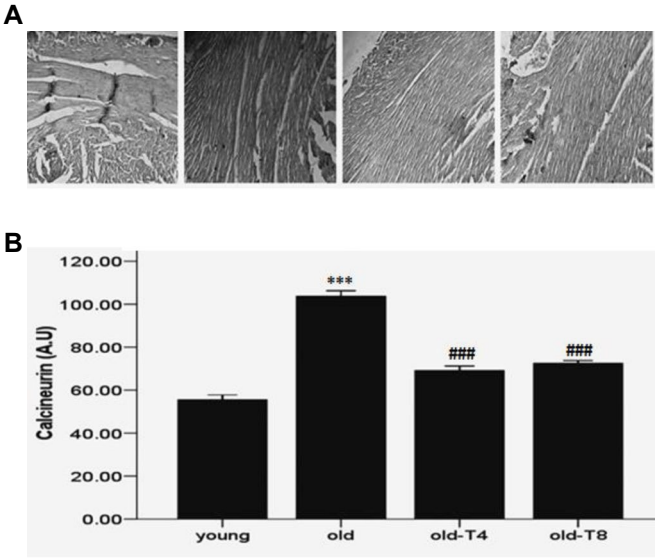

Fig.1: Effect of aerobic training on tissue expression of calcineurin. **A.** Immunohistochemistry staining of calcineurin in the heart tissue. **B.** The number of calcineurin-positive cells in the heart tissue (brown granules in the cytoplasm). Data are presented as mean ± SEM (n=3). ***; P<0.001 vs. young group and ###; P< 0.001 vs. old group.

NPR-A protein levels

As Figure 2 shows, aging significantly decreased NPR-A protein levels in the heart tissue among the middle-aged control rats compared to the young group (P=0.001). Nonetheless, aerobic training increased NPR-A protein expression in themiddle-aged-T4 (P=0.001) andmiddle-aged-T8 (P=0.001) groups compared to the middle-aged control group (Fig.2D).

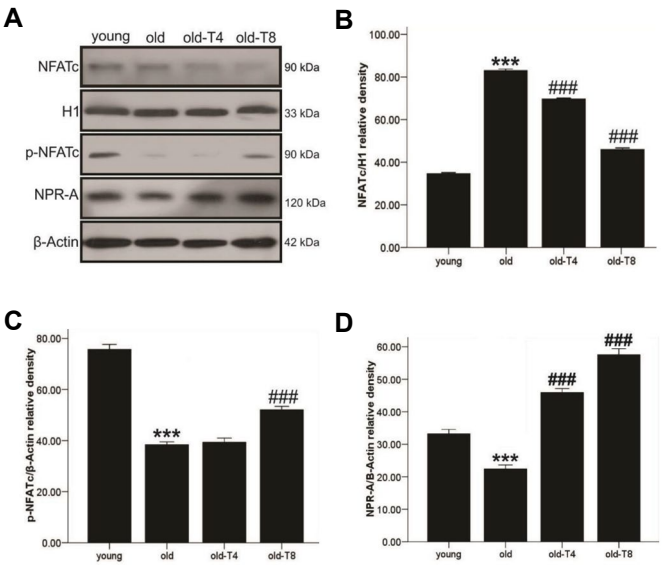

Fig.2: Effect of aerobic training on protein expression of phospho-NFATc (p-NFATc), NFATc, and NPR-A in the heart tissue. **A.** Immunoblotting images of p-NFATc, NFATc, NPRA, H1, and β-Actin in the heart tissue. **B.** NFATc/H1 ratio, **C.** p-NFATc/β-Actin ratio, and **D.** NPRA/ β-Actin ratio. β-Actin and H1 proteins were used as internal loading controls. Data are expressed as mean ± SEM. ***; P<0.001 vs. young group and ###; P<0.001 vs. old group.

SERCA2, PMCA4b, and TRPC6 protein expressions

Expression of SERCA2, TRPC6, and PMCA4b are shown in Figure 3A. The protein expression of SERCA2 (Fig.3B) were significantly decreased (P=0.001), TRPC6 protein levels (Fig.3C) were remarkably increased (P=0.001) and PMCA4b (Fig.3D) were significantly decreased (P=0.001) in the heart tissue of middle-aged control compared to the young control rats. On the other hand, aerobic training significantly increased SERCA2 (P=0.001) levels in themiddle-aged-T8 group and decreased TRPC6 (P=0.01) levels in the middle-aged -T4 and old-T8 groups (P=0.001) compared to the control middle-aged rats. However, aerobic training did not affect PMCA4b protein expression in themiddle-aged-T4, and T8 groups compared to middle-aged control groups.

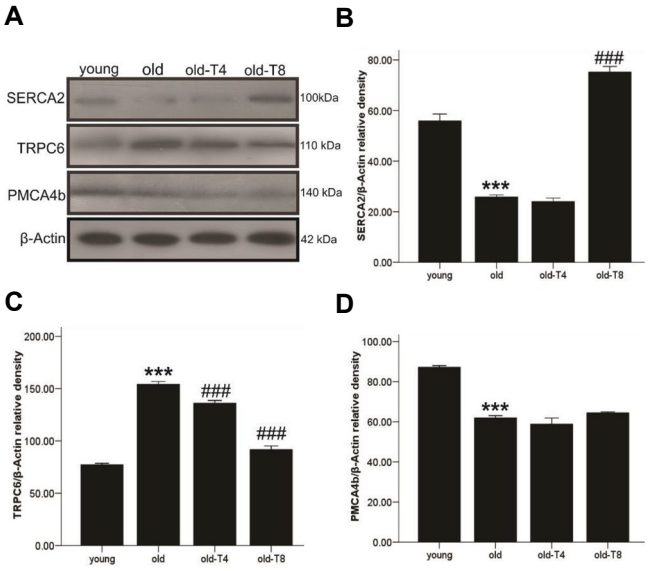

Fig.3: Effect of aerobic training on protein expression- of SERCA2, TRPC6, and PMCA4b in the heart tissue. **A.** Immunoblotting images of SERCA2, TRPC6, PMCA4b, and β-Actin in the heart tissue. Protein levels of **B.** SERCA2, **C.** TRPC6, and **D.** PMCA4b. β-Actin was used as an internal loading control. Data are expressed as mean ± SEM. ***; P< 0.001 vs. young group and ###; P<0.001 vs. old group.

p-AKT p-PLB TGF-β protein expression

Expression of p-AKT, p-PLB, and TGF-β are shown in Figure 4A. We found that p-AKT (P=0.001, Fig.4B) and p-PLB (P=0.001, Fig.4C) levels were significantly decreased while TGF-β protein expression was significantly (P=0.001, Fig.4D) increased in the heart tissue of the middle-aged control group compared to the young control group. However, aerobic training led to a significant increase in p-AKT (P=0.05) and p-PLB (P=0.001) levels and a considerable decrease in TGF-β (P=0.001) protein levels in the heart tissue of the old-T8 group as compared to the old control rats (Fig.4).

The relationship between components of the Calcineurin/NFATc pathway in middle-aged rats following aerobic training

As a result of training, there was a significant positive relationship between Calcineurin and NFATc expression (P=0.001) and an inverse relationship between p-NFTAc and NFTAc (P=0.025) expression in the middle-aged rats.

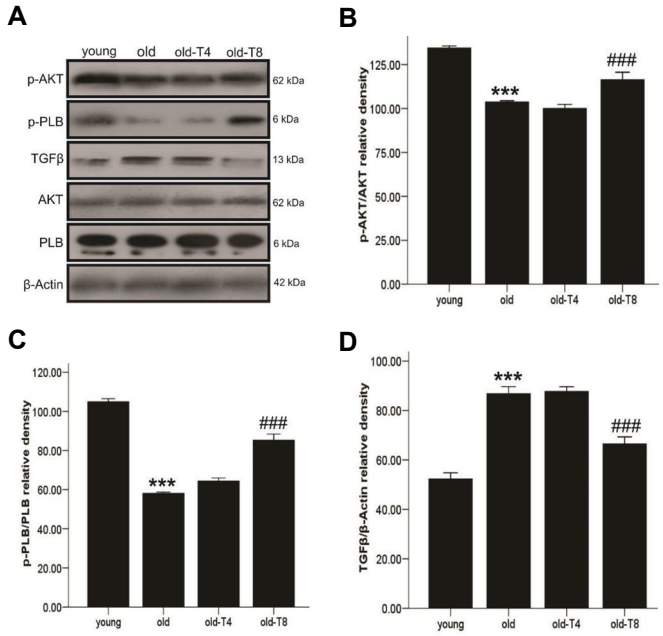

Fig.4: Effect of aerobic training on phospho-AKT (p-AKT) and phospho-phospholamban (p-PLB) levels as well as TGF-β protein expression in the heart tissue. **A.** Immunoblotting images of p-AKT, AKT, PLB, p-PLB, TGF-β, and β-Actin identified by Western blotting. **B.** p-AKT/AKT ratio, **C.** p-PLB/PLB ratio, and **D.** TGF-β protein expression in the heart tissue. β-Actin was used as an internal loading control. Data are expressed as mean ± SEM. ***; P< 0.001 vs. young group and ###; P<0.001 vs. old group.

Also, a significant positive relationship between TRPC6 and Calcineurin expression (r=0.65, P=0.03), an inverse relationship between TRPC6 and p-NFTAc (P=0.001), and a positive relationship between TRPC6 and NFTAc (P=0.001) in trained middle-aged rats were observed.

There was a significant positive relationship between TGF-β and Calcineurin expression (P=0.048), an inverse relationship between TGF-β and p-NFAT, and a positive relationship between TGF-β and NFAT (respectively, P=0.001 and P=0.003) in trained middle-aged rats.

Also, a significant inverse relationship between NPR-A and Calcineurin (P=0.001), a positive relationship between NPR-A and p-NFTAc, and a significant inverse relationship between NPR-A and NFTAc (respectively, P= 0.008 and P= 0.001) were observed in trained middle-aged rats.

We found a inverse significant relationship between SERCA2 and Calcineurin (P=0.031) and between SERCA2 and NFTAc and positive relationship between SERCA2 and p-NFTAc (respectively, P=0.006 and P=0.001) in trained middle-aged rats.

There was a significant inverse relationship between p-PLB and Calcineurin (P=0.049), a significant positive relationship between p-PLB and p-NFAT (P=0.001), and

a significant inverse relationship between p-PLB and NFAT (P=0.001). Also, there was a significant inverse relationship between p-AKT and NFATc (P=0.03, Table 1) in trained middle-aged rats.

Table 1: The relationship between research markers with Calcineurin-NFAT signaling in trained middle-aged Wistar rats

Marker-1	Marker-2		
	Calcineurin P value#	p-NFATc P value#	NFATc P value#
Calcineurin	-	0.003**	0.025**
TRPC6	0.03**	0.001**	0.001**
TGF-β	0.048	0.001**	0.003**
NPR-A	0.001**	0.008**	0.001**
PMCA4b	0.85	0.126	0.346
SERCa2	0.031**	0.001**	0.001**
p-PLB	-	0.001**	0.001**
p-AKT	0.65	0.061	0.03**

#; By liner mixed model (α≤0.05).

On the other hand, there was a significant relationship between NPR-A and p-PLB (r=0.826, P=0.002), SERCA2 (P=0.013) and TRPC6 (P=0.001) in trained middle-aged rats.

Also, a significant inverse relationship was observed between TGF-β and p-AKT (P=0.012), p-PLB (P= 0.003), and SERCA2 (P=0.001) in trained middle-aged rats.

A significant positive relationship was observed between p-PLB and SERCA2 (P=0.001) in trained middle-aged rats (Table 2).

Table 2: The relationship between research markers with each other in trained middle-aged rats

Marker-1	Marker-2	P value#
NPRA	p-PLB	0.002
	SERCa2	0.013
	TRPC6	0.001
TGF-β	p-AKT	0.012
	p-PLB	0.003
	SERCa2	0.001
p-PLB	SERCa2	0.001

#; By liner mixed model differences between.

Discussion

We found that aerobic training inhibited the calcineurin/NFATc signaling pathway and modulated intracellular Ca^{2+} levels by restoring NPR-A, SERCA2, p-PLB, and p-AKT and decreasing TRPC6 and TGF-β levels in cardiac tissue. Our previous study demonstrated that eight weeks of moderate-intensity aerobic training modulated pathological cardiac hypertrophy to physiological hypertrophy in middle-aged rats (24). Seo et al. (28) showed that training-induced cardiac remodeling is associated with gene regulatory mechanisms and cellular signaling pathways underlying cellular, molecular, and metabolic adaptations.

We found that aerobic training reduced calcineurin and NFATc expression, while increasing p-NFATc levels, suggesting calcineurin/NFATc signaling was inhibited. Oliveira et al. (25) reported that 8 weeks of moderate training deactivated the calcineurin/NFATc pathway, reduced heart weight, and improved cardiac function in a genetic mice model of heart failure. Sustained activation of the Ca^{2+}-sensitive signal transduction pathways such as the calcineurin pathway is induced by aging and is associated with pathological hypertrophy. Consistent with this observation, we found that p-NFATc (inactive type of NFATc) was decreased while calcineurin and NFATc were increased in the heart tissue of middle-aged control rats compared to the young control rats. Similarly, previous studies have reported that the activity of the calcineurin/NFATc signaling pathway is increased under the influence of aging, accelerating cardiac aging (1, 29).

The pumps and channels are also critical in regulating intracellular Ca^{2+} content (30). PMCA4b inhibits calcineurin leading to an attenuation of the local Ca^{2+} signals involved in cardiomyocyte hypertrophy (31). Our findings showed that PMCA4b was significantly decreased in middle-aged control rats' heart tissue compared to the young control group. However, aerobic training did not influence PMCA4b protein levels. Moreover, the protein expression of TRPC6 was markedly increased in the middle-aged control group compared to young control groups, which was attenuated by aerobic training. Also, the training-induced reduction in TRPC6 was accompanied by reductions in calcineurin and NFATc and elevation in p-NFATc levels. According to previous studies, increased activity of TRPC6 can lead to an increase in ROS levels (32). However, aerobic training reduced ROS in the heart of middle-aged Wistar rats (24). It seems that aerobic training-induced reductions in TRPC6 levels occur by reducing Ca^{2+} entry and ROS production.

Also, we found that middle-aged control rats had lower SERCA2 levels than young rats, which was increased by eight weeks of aerobic training. SERCA2 is down-regulated during cardiovascular disorders such as pathologic cardiac hypertrophy, heart failure, and vascular proliferative remodeling (15). Since SERCA can decrease cytosolic Ca^{2+} levels by pumping it from the cytosol to

sarcoplasmic lumens, a reduction in SERCA2 expression impairs Ca^{2+} cycling resulting in cardiac hypertrophy (13). In contrast, restoration of SERCA2A expression can improve various features of heart failure (17). ROS and PLB are effective in reducing SERCA2 (33). Therefore, the reduction of ROS and PLB by aerobic training can restore SERCA2 levels.

Phosphorylation of PLB increases SERCA pump activity, while dephosphorylation of PLB acts as a brake on the SERCA pump and decreases the affinity of SERCA2 for Ca^{2+} (19, 34). Our results also showed that p-PLB levels were reduced in the middle-aged control rats compared to the young control group, butwere increased by eight weeks of aerobic training. Also, there was a positive relationship between the increase in p-PLB and SERCA2 and an inverse relationship between p-PLB and Calcineurin-NFATc pathway. NPR-A and TGF-β also regulate p-PLB activity. NPR-A inhibits the calcineurin/NFATc pathway. In the current study, NPR-A decreased in the middle-aged control rats. Reducing the expression of NPR-A leads to an increase in the size of heart cells in a pathologic form and increases the cardiomyocyte cross-sectional area (20).

In contrast, we found that aerobic training increased NPR-A in the heart tissue of middle-aged rats. In this regard, we found a significant inverse relationship between NPR-A and TRPC6. The reduced activity of the Calcineurin-NFATc signaling pathway decreases the expression of TRPC6 (35). Therefore , NPR-A affects TRPC6 levels by decreasing the activity of the Calcineurin-NFATc pathway.

GSK-3 B inhibition by AKT allows NFAT dephosphorylation and its nuclear translocation (36). In the present study, p-AKT was also decreased in the middle-aged control rats compared to young control rats but was increased by aerobic training in the middle-aged rats. In line with our results, some studies have reported activation of AKT pathway by activity which mediates cardiac adaptation to exercise (25, 37). Increased p-AKT levels increased the phosphorylated form of NFATc and reduced NFATc. In the regulation of AKT activity, the role of TGF-β is essential. TGF-β is an activator of ROS and mitogen-activated protein kinase (MAPK) (22, 38), which are considered as enhancers of AKT activity (22). Moreover, TGF-β promotes apoptosis cell death in the cardiomyocyte and pathological cardiac hypertrophy (39). We found that aerobic training decreased TGF-β levels and increased p-AKTlevels in the middle-aged rats. On the other hand, we found a significant inverse relationship between TGF-β changes induced by aerobic training and the levels of p-PLB and SERCA2 in middle-aged rats. Since ROS induced by TGF-β can enhance the PLB activity (22, 40), it is likely that decreased TGF-β leads to an increase in p-PLB and SERCA2 expression.

Conclusion

Overall, we found that increases in the calcineurin/NFATc activity occurred in middle-aged control rats compared to young rats, resulting in impairment in intracellular calcium homeostasis. However, aerobic training modulated the middle-aged-related increase in intracellular Ca^{2+} by inhibiting the calcineurin/NFATc pathway and modulating intracellular Ca^{2+} levels by restoring NPR-A, SERCA2, p-PLB, and p-AKT, and reducing TRPC6 and TGF-β levels. Identification of molecular mechanisms underlying age-associated cardiac hypertrophy may allow for targeted therapies to improve cardiac function in patients with heart disease.

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Authors' Contributions

B.B.; Performed exercise training, analyzed the data and wrote the manuscript. R.B.; Contributed to all experimental work, data and statistical analysis. P.K.; Contributed to all experimental work and interpretation of data. L.S.P.; Contributed to interpretation of data, and drafted and edited the manuscript. All authors read and approved the final manuscript.

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