Published online 2016 Winter. **Research Article**

Impact of exercise endurance training on *purβ* gene expression and cardiac function

Maryam Anjomshoa[1], Naser Rostamzadeh[2], Omid Rostamzadeh[3], Ayoob Rostamzadeh[1,*]

[1]Department of Anatomical Sciences, Shahrekord University of Medical Sciences, Shahrekord, Iran.
[2]Department of Physical Education and Sport Sciences, Shahid Rajaee Teacher Training University, Tehran, Iran.
[3]Department of Occupational Therapy, Faculty of Rehabilitation Sciences, Iran University of Medical Sciences, Tehran, Iran.

Corresponding author*: Ayoob Rostamzadeh,** Department of Anatomical Sciences, Faculty of Medicine, Shahrekord University of Medical Sciences, Rahmatiye Town, Shahrekord, Iran. Tel: +989187225635. Email: ayoobrostamzade@gmail.com, Fax: +983813334911.

Abstract

Introduction: Endurance training has significant effects on the renewal of heart tissue, including myosin heavy chain (MHC) proteins. On the other side, Purine-rich element-binding protein β (*purβ)* decreases the αMHC gene expression. The aim of this study was to determine the impact of exercise endurance training on *purβ* gene expression in the heart of Wistar rats.
Methods: Fourteen rats have been kept under controlled conditions and after familiarizing with training protocol, they were divided into control groups and experimental groups. The experimental group performed a 10-week treadmill running program for 30 min/day, 5 days/week. 48 hours after the last training session, the rats were anesthetized and the heart and their left ventricle were taken out and *purβ* expression was measured using real time PCR method. All data were analyzed using *t* test.
Results: In this study, the results of M-mode echocardiography showed that endurance training led to cardiac hypertrophy. After endurance training, the heart weight, especially the left ventricular weight significantly increased. The *purβ* gene expression significantly decreased in the left ventricular tissue of endurance-trained rats.
Conclusion: The results of this study revealed that endurance training has considerable effects on heart size and *purβ* gene expression. The *purβ* gene also repressed αMHC gene expression; it seems that the changes in heart structure related to αMHC gene expression.

Keywords: Gene expression, *purβ* gene, Cardiac plasticity, Endurance training

Introduction

In response to different environmental demands, cardiac structures and functions considerably changed. Physical activities have a drastic effect on heart remolding and gene expression in heart tissue (1). Heart adaptation in response to endurance training is related to the variety of stimuli imposed to the heart, so morphological changes in the heart are different in response to each activity (2). Adaptation of athlete's heart to endurance training includes changes such as increased wall thickness and left ventricular mass (3, 4). Also relative cavity diameter of heart in endurance runners is higher compared to sprint runners (3). Structural changes in left ventricle of heart demonstrated adaptation to hemodynamic overload induced by endurance training. Work capacity during exercise is positively influenced by preload increase after endurance training, while increased afterload due to isometric training in strength-trained athletes, determines higher systemic resistance during physical effort (4). There are different signaling pathways in heart that mediate the heart growth response. These signaling circuits directly control hypertrophic growth by altering gene expression in cardiac myocytes (5). There are wide varieties of physiological and pathological stimuli that affect the relative proportions of the two forms of the motor protein myosin heavy chain (MHC). The expression of faster MHC motor protein, α, (αMHC) in heart leads to produce more power than slower MHC motor protein, β, (βMHC) and so the heart power and contractility would increase (6). In left ventricle of normal hearts, the αMHC mRNA was expressed at considerable levels and in the end stage of heart failure, the αMHC mRNA expression, decreases up to 15-fold and also these changes would occur in the level of MHC protein. In nonfailing hearts, the αMHC protein represented about 7% of the total MHC protein, while in failing hearts, there was effectively no detectable αMHC protein in the left ventricles (6). No myocardial collagen produces in hypertrophy caused by exercise (7). Physiological and pathological cardiac hypertrophy has opposite changes in βMHC gene expressions (8).

The Purine-rich element-binding (*purβ*) protein is single-strand DNA binding proteins that can bind to DNA as the homodimer or heterodimer. This protein participates in cell differentiation, proliferation and apoptosis and cell-specific gene regulation (9). The *purβ* mediate repression of βMHC gene expression. The levels of *purβ* in heart failure would increase and is important in regulating the transcription of αMHC gene (10). The aim of this study was to investigative the effects of 10 weeks of endurance training on *Purβ* gene expression in the heart tissue of Wistar rats.

Material and methods

Animals care

Fourteen male Wistar rats of 5 weeks old (200±20gr) were obtained from the Pasteur institute (Tehran, Iran). All animals were kept at room temperature (22±3°C) with a 12-h light/dark cycle until they reach puberty. Within this time, rats were maintained in 4 equal cages. At the end of this step, the average and standard deviation of rat's weights was 231±24 gr. All rats were familiarized with treadmill running for 5 sessions/10 days. At the end of this session, rats were divided randomly into two groups, experimental group and control group (7 rats in each group).

Training protocol

According to previous studies, we designed an endurance training protocol for rats as previously described (11, 12), so that endurance training could cause cardiac hypertrophy. The rats trained 6 days/week for 4 weeks with speed, grade and duration progressively increased. To prevent the rats from stopping, an electric grid at the rear of the belt was used as the running stimulus. The rats began training at 12 m/min and 0% grade for 5 min in first day. By the 2nd weeks duration and speed were increased until the rats ran at 30 m/min for 60 min/day. During 5–8 weeks the speed and duration were maintained and grade was gradually increased to 5%. Finally, 48 hours after the end of the last exercise session, rats were anesthetized with a combination of Ketamine (50 mg/kg) and Xylazine (5 mg/kg) and under sterile surgical methods; their heart and left ventricle were isolated and placed into 1.5 ml microtubes. Then heart tissue explants were moved to a liquid nitrogen tank for cryopreservation. The frozen heart explants were homogenized in liquid nitrogen using a mortar. In this study, m-mode was used to demonstrate the effects of endurance training on the heart structure. Furthermore, many studies used heart weight/body weight ratio, and heart weight/body surface area (BSA) ratio to evaluate cardiac hypertrophy (13, 14). In this study to evaluate the cardiac hypertrophy, two factors were used for normalization the left ventricular weight. Weight and length of animal (from mouth to root of tail) were measured in the anesthetized state for BSA calculations (15). The heart and left ventricle were weighed separately using a digital analytical balance (Sartorius, model-BL210S; Japan). BSA of rats was evaluated using the following formula:

$BSA = 6.67 \times W^{0.7} \times [0.34 / (\sqrt[3]{W/L})]$. W= body weight (gr); L= body length (cm)

Animals and ethics

Animal experimentations were approved by the Ethical Committee of Shahid Rajaee Teacher training University and carried out in an ethically proper way by following the provided guidelines.

RNA extraction

Total RNA was extracted from heart tissue as previously described (16). Briefly, 1 ml TRIZOL Reagent (Invitrogen, Carlsbad, CA, USA) was added to 100 mg heart tissue. Then the RNA samples were treated with RNase free DNase to remove any residual DNA.

First-strand cDNA synthesis and Real-Time Quantification of Gene Expression

Total extracted RNA was transcribed to the first strand complementary DNA (cDNA) with the cDNA Synthesis Kit (Thermo Scientific, Schwerte, Germany) according to manufacturer's instructions. Real time RT-PCR was performed using the Rotor Gene System (Applied Biosystems, Darmstadt, Germany).

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First-strand cDNA synthesis and Real-Time Quantification of Gene Expression

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Table 1. The characteristic of used primers in this study.

Name		Sequence 5-3	NCBI Reference Sequence	Product size
Gapdh	F	AACCCATCACCATCTTCCAG	NM_017008.4	74
	R	CACGACATACTCAGCACCAG		
Purβ	F	GTGAGGAAGTGGATGAGGATTG	NM_001017503.1	100
	R	GGACGAGTAGGAAAGGGAAC		

Statistical analysis

The transformed data of RT-PCR expression of *purβ* gene were analyzed by Shapiro-Wilk test of SPSS 20. Then the differences between groups were compared by *t* test. Differences were considered as significant at *P<0.05*.

Results

The results of this study showed that after 10 weeks of endurance training, a hypertrophy occurred in left ventricle, which was supported by evaluating the left ventricular weight/body weight ratio and left ventricular weight/body surface ratio (Figures 1 and 2). Figure 1 show the left ventricular weight/BSA ratio in the experimental group (0.168±0.008) is significantly higher in comparison with control group (0.153±0.006) (*p=0.006*). On the other hand, figure 2 revealed that the heart and left ventricular weight of experimental group is higher than the control group, so that the left ventricular weight/ body weight ratio in experimental group was significantly higher (2/3±0.18) compared to control group (2.049±0.12) (*p=0.04*).

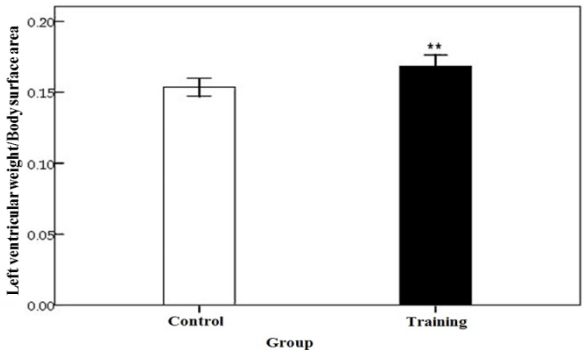

Figure 1. Analysis of hypertrophy indexes show that the left ventricular weight/ BSA ratio in the experimental group, is higher compared to control group (*P=0.006*). Error bars shows 95% CI.

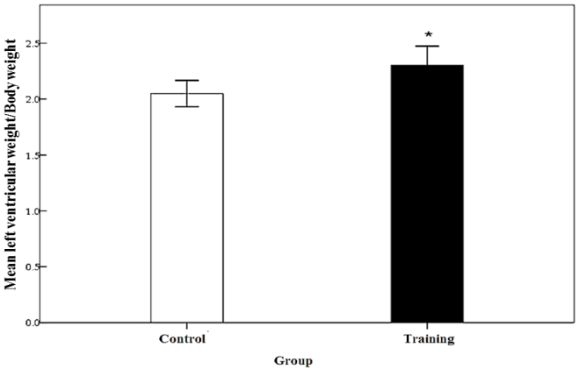

Figure 2. Analysis of hypertrophy indexes show that the left ventricular weight/ body weight ratio in experimental group, is higher compared to control group (*P=0.04*). Error bars shows 95% CI.

Also, the results of the t-test (t=-4.35) demonstrated that the average *Purβ* gene expression in heart of experimental group significantly decreased to 54% in comparison with control group after 10 weeks of endurance training (*p=0.008*) (Figure 3).

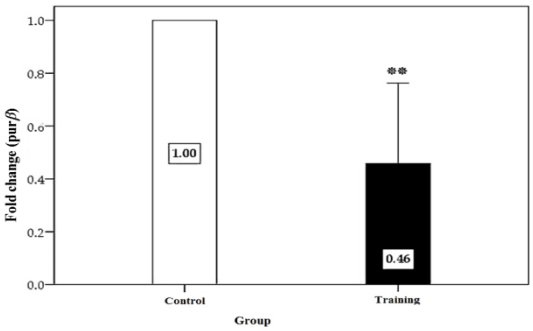

Figure 3. Results demonstrated that the average *Purβ* gene expression in heart of experimental group decreased in contrast to control group after 10 weeks of endurance training (*P=0.008*). Error bars shows 95% CI.

Discussion

Currently, the various aspects of exercise-induced cardiac remodeling, including ventricular chamber enlargement, myocardial hypertrophy have been recognized (21), but the cellular pathways responsible for cardiac remodeling remain poorly understood. The results of the present study demonstrated that endurance training led to reduction of *Purβ* gene expression in left ventricle to 46%. To date, except this study any previous information has been reported about a role endurance training on *purβ* gene expression, so the results of the present study was discussed based on the studies that identified other effects of this gene. In the rat, αMHC is the predominant isoform in adult hearts, has high ATPase activity, and is associated with increased shortening velocity of the cardiac myocytes. Therefore, changes in the αMHC in the cardiac ventricle alter the contractile properties of the heart (22). Miyata et al. (2000) showed that αMHC mRNA was expressed at considerable levels in the nonfailing heart and was considerably decreased in heart failure and pathological cardiac hypertrophy (6). van Rooij et al. (2007) showed that down-regulation of αMHC gene is a process that occurs in the pathological cardiac hypertrophy (23). The *purβ* protein is a single-strand DNA binding protein that can bind to DNA as the homodimer or heterodimer. The *purβ* protein is also highly expressed in cardiac myocytes, and their level changes in hear failure. Gupta et al. (2003) described Overexpression of *purβ* down regulates *αMHC* gene expression in heart tissue (9). The *purβ* protein cooperated with other cardiac factors binding to the αMHC gene promoter in a negative manner. The *purβ* protein has been shown to bind to other cardiac myogenic factors which also play prominent roles in *αMHC* gene expression. The studies of Gupta el al. (2003) also showed that *purβ* protein is capable of binding to αMHC mRNA and attenuate its translational efficiency and expression of *purβ* protein in failing hearts suppressed the αMHC mRNA (9). In cell renewal, the activity of *Purβ* gene and the level of protein increase, however, it decreases in response to mechanical overload. In heart failure the *Purβ* level increased, which is contributed to the transcription of αMHC (10). The results of McCarthy's study confirm that increase of *Purβ* expression led to muscles atrophy (24). Stevenson et al. (2003) showed that suppression of MHC genes associated with increased expression of *Purβ* gene (25). Comparing the results of McCarthy with the present report demonstrated that *Purβ* has major roles in the cardiac myocytes in response to cardiac hypertrophy. In the present study, we did not evaluate the *Purβ* protein level that is the final factor for the effect of endurance training on the heart tissue, so for future studies, evaluating the *Purβ* protein level in heart tissue after endurance training would be recommended. In conclusion, the results of the present study demonstrated that endurance training decreased the *Purβ* gene expression. Cardiac remodeling (including changes in dimensions, mass and shape of the hearts) in response to endurance training is in association with neurohormonal activation and changes in of heart size, specially left ventricle, which in turn. Considering different effects of endurance training on the cardiac hypertrophy, *Purβ* gene expression and also the effect of *Purβ* in the αMHC expression, it seems that heart remodeling in response to endurance training is related to αMHC expression.

Acknowledgements

The authors would like to thank the staff of the deputy research in the Shahid Rajaee Teacher training University for their kind cooperation.

Conflict of Interest

The authors declare that they have no conflict of interests.

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