

## The effect of rehabilitation training on TRF1 and TRF2 in myocardial infarction patients

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### Abstract

**Introduction:** Telomeres are repetitive sequences of TTAGGG section that find at two ends of eukaryotic chromosomes and they shield chromosome ends. Telomere shortening in patients with myocardial infarction has been reported. Shelterin complex's role is essential in telomere length regulation. Telomeric repeat binding factors 1 and 2 (TRF1 and TRF2) are the most important shelterin complex proteins. The purpose of this study was to investigate the effect of eight weeks of rehabilitation training on TRF1 and TRF2 in myocardial infarction patients.

**Materials and methods:** In this Quasi-experimental pre-post intervention study, twenty male patients selected and randomly assigned to training (n=10) or control (n=10) groups. Rehabilitation training was eight weeks of concurrent training, 3 times per week. For TRF1 and TRF2 assessment, blood samples were taken half hour before first training session and 24 hours after the last training session. t-test was used for data analyses. Statistical significance was set at  $P < 0.05$ .

**Results:** The findings of the present study revealed that concurrent training increases TRF1 and TRF2 protein levels significantly ( $P = 0.005$  and  $P = 0.006$ , respectively).

**Conclusion:** It seems that rehabilitation training improves shelterin complex and enhances protection of the telomere and as a result, induces better repair of infarcted area. Therefore, rehabilitation training could be suggested to myocardial infarction patients as a non-pharmacological treatment.

**Keywords:** Rehabilitation training, TRF1, TRF2, Myocardial infarction, Telomere

### Introduction

Telomeres are repetitive sequences of TTAGGG and find at two ends of eukaryotic chromosomes. These DNA segments have critical functions such as shielding the ends of chromosomes and keep genome sustainability, preventing the chromosome's ends from end-to-end contact, preventing the chromosome's ends to be identified as the damaged part of double-stranded DNA by the DNA damage response tract, DNA restoration, and as results, precluding senescence, apoptosis, and finally cell death (1). One

of the factors which is involved in telomere length regulation is shelterin complex, which is composed of six proteins. The most important of them are Telomeric repeat binding factor 1 (TRF1) and Telomeric repeat binding factor 2 (TRF2). Other proteins are as follows: Tripeptidyl peptidase 1 (TPP1), Protector of telomeres 1 (POT1), human Repressor activator protein 1 (RAP1), and TRF1-Interacting nuclear factor 2 (TIN2). Shelterin complex plays a critical role in telomere homeostasis by fixing the

telomere's structure. shelterin prevents the cells to recognize their natural ends as broken DNA, suppress DNA repair pathways, and control the telomerase-based mechanism of telomere length retention (2).

The length of telomere is regulated by telomerase. Access to telomerase depends on shelterin complex. In mammals' cells, the levels of TRF1 and TRF2 and other components of shelterin would be increased as the number of TTAGGG sequences increases. Too much increase in TRF1 (by overexpression) will lead to ongoing erosion of telomeres, by telomerase inhibition. Therefore, the main role of TRF1, is the negative adjustment of telomere length (2, 3). Also, it has been reported that TRF1 could be interacted with telomere in the interphase and mitosis phases (4). TRF2 is vital to maintain the normal structure of chromosome ends. Moreover, TRF2 interacts with other factors and acts as a connection point for other proteins (2, 3).

Diseases such as cardiovascular diseases, are considered to be associated with inactivity and senescence. There are many studies which have associated the telomeres erosion in peripheral blood mononuclear cells (PBMCs) and other tissues with blood pressure and other dangerous factors in cardiovascular diseases. Thus, there is a strong physiological relation between telomere and its regulating factors and cardiovascular diseases, and we could use them as an index of effectiveness of treatments (5). Some researchers have proposed that physical exercises could change telomere length (6-8), but, the impact of exercise training on shelterin complex proteins have been examined only in few studies. Therefore, the purpose of the present study was to investigate the effect of 8 weeks of rehabilitation training on TRF1 and TRF2 in myocardial infarction patients.

## Materials and methods

This was a Quasi-experimental pre-post intervention study. From among the patients who came to Taleghani Hospital, 20 male patients were selected (Table 1). They were randomly assigned to training group (n=10) and control group (n=10). Having been familiarized with the procedure, they filled out personal information, medical history, and written consent forms. Then, their height and weight were measured. Exclusion criteria of this study were decompensated heart failure, unstable angina, ventricular arrhythmias, and orthopedic problems.

Inclusion criteria were as follows: 4-8 weeks after the heart disease, stability of the patient's conditions, no smoking and alcohol drinking during the study. The patients were notified about the possible dangers of the present study. The present study was conducted under the supervision of ethics committee of Taleghani Hospital.

**Rehabilitation training:** The experimental group executed the rehabilitation training protocol, three times per week, for eight weeks. First, the subjects walked slowly for 5 minutes as a warm-up. Then, in order to improve their muscular endurance, using free weights, and an apparatus with the intensity of  $13 \leq$  RPE (less than 30%, 1RM, 5 - 10 repeats, 1 to 3 sets), they engaged in resistance training. In this phase, using combined and multiple joints movements, hands, scapula, and legs muscles were exercised. The duration of resistant training was 10 - 15 minutes. We tried to gradually increase the intensity to RPE  $\leq 15$  (50 - 60 % of 1RM, 8 - 15 repeats, and 1 to 3 sets). It was followed by aerobic training for 25-35 minutes. Using modified Bruce protocol, participants  $VO_{2peak}$  were measured. Then, the participants trained on bicycle ergometer and treadmill with the intensity equal to 50 % of  $VO_{2peak}$  (12 -13 RPE, 60% of maximum heart rate). At the beginning of the exercise schedule, bicycle ergometer was used more, to prevent

orthopedic problems, and then treadmill was used more. But generally, the ratio of exercising with the bicycle or treadmill depended on the physical conditions of the participants. Aerobic training gradually increased to 80%  $VO_{2peak}$  (15-16 RPE, 90% of maximum heart rate). Then, the participants cooled down for 5 minutes (9-11). During training, participants were observed using heart monitoring. The participants of the control group lived their usual lives during this period. Blood samples of 10 CC were drawn from brachial vein (antecubital vein) 30 minutes before the first exercise session and 24 hours after the last exercise session. For TRF1 and TRF2 assessment, ELISA method and CUSABIO BIOTECH kits with a sensitivity of 14ml/pg (made in China) were used.

### Statistical analysis

In order to normalize the data, shapiro-wilk test was used. Next, In order to investigate the within group difference, a dependent t-test was used and in order to

pinpoint the between group difference, an independent t-test was used. In all tests, the significance level was considered as  $P < 0.05$ .

### Results

Characteristics of the subjects under study are shown in Table 1. Data of the mean and standard deviation of TRF1 and TRF2 levels are provided in Table 2.

After data analysis, it was found that there is a significant difference between pre-test and post-test TRF1 values in training group ( $P=0.005$ ), but there is no significant difference between pre-test and post-test TRF1 values in control group ( $P=0.304$ ). Independent t-test didn't show a significant difference between training and control groups ( $P=0.14$ ).

Moreover, a significant difference between pre-test and post-test TRF2 values in training group ( $P=0.001$ ), but no significant difference in control group ( $P=0.26$ ) were found. Independent t-test showed a significant difference between training and control groups ( $P=0.006$ ).

**Table 1.** Demographic data in AMI and control subjects.

Variable	Training (mean $\pm$ SD)	Control (mean $\pm$ SD)	P value
Age (year)	57.3 $\pm$ 5.56	58.4 $\pm$ 5.44	0.66
Height (cm)	172.2 $\pm$ 5.2	173.4 $\pm$ 5.57	0.62
Weight (kg)	76.9 $\pm$ 8.1	78.1 $\pm$ 7.2	0.74
Body mass index (Kg/m <sup>2</sup> )	25.9 $\pm$ 1.4	26.1 $\pm$ 1.6	0.93

**Table 2.** Mean and standard deviation of TRF1 and TRF2 levels.

Variable	Training (mean $\pm$ SD)		Control (mean $\pm$ SD)	
	Pre-test	Post-test	Pre-test	Post-test
TRF1(pg/ml)	3.28 $\pm$ 1.85	5.49 $\pm$ 1.59	4.22 $\pm$ 1.89	4.4 $\pm$ 1.56
TRF2(pg/ml)	4.45 $\pm$ 1.97	8.97 $\pm$ 2.63	4.54 $\pm$ 2.30	5.41 $\pm$ 2.41

### Discussion

The findings of the present study revealed that concurrent training increases TRF2 protein levels significantly ( $P=0.006$ ). TRF1 in experimental group also significantly increased but it was not statistically significant in comparison to control group ( $P>0.05$ ).

As mentioned, only few studies have been done on the effect of physical training on TRF1 and TRF2. Werner, et al. (2008) found that endurance training in the form of running in mice for 21 days increases telomerase activity and TRF2 levels (12). Laye, et al (2012) showed that after an ultra-marathon for seven days, TRF1

mRNA increases (13). Werner, et al. (2009), found that three optional exercises in mice will increase TRF1 and TRF2. Furthermore, they reported that TRF1 is higher in trained athletes compared with the control group. They concluded that exercise training will regulate the proteins that are connected with telomere length regulation (14). All the afore-mentioned studies are in line with the present study. But Ladlow et al. (2012) reported that one session of running on a treadmill for 30 minutes will decrease TRF1 gene expression in mice muscles (15). One of the factors which could explain the difference between the present study and Ladlow et al (2012) finding is the type of the participants, which in this study patients were used but in their study mice were used.

During the natural heart growth, in addition to myocytes hypertrophy, the production of new myocytes is higher than myocytes death which leads to the growth of the organ. Some studies showed that heart is an organ which its mitotic division stops after birth. If it is accepted that heart is an organ which is incapable to cell division, therefore, any significant change and conversion in a healthy heart myocytes will be refuted. But it has been reported that some of cardiac myocytes gradually die and have to be replaced. If the heart is unable to produce new myocytes, the cardiac mass will gradually disappear (16, 17). Both necrosis and apoptosis will affect the ischemic area (about 80% and 20%, respectively) after the occurrence of ischemia. But over time, these two kinds of cell deaths interference, compensation procedures start and the cardiac scar forms (16).

The partial loss of Myocardium, because of ischemia, will activate myocytes production and their hypertrophy. Regarding the production of new myocytes, some studies have reported that the new myocytes are formed due to cell proliferation of the remaining cells but others have shown that progenitor cells

differentiation will lead to the formation of new myocytes (16, 18). Telomeres are involved both in cell division and maintenance of cell survival. Therefore, telomeres have a fundamental role in any compensatory mechanism for the improvement of infarcted area (19, 20).

As it was previously mentioned, one of the most important factors which plays a role in telomere regulation is shelterin complex. The ability of cells to distinguish between their normal chromosome ends and DNA breaks is provided by shelterin, moreover, shelterin suppress DNA repair pathways, and it also adjust telomerase-based telomeres regulation (4). TRF1 is one of the shelterin complex proteins which is involved in the double stranded formation of telomere TTAGGG sequence. TRF1 plays an important role in collecting shelterin complex, together by connecting to TRF2. Due to changes in nucleotides/protein sequences, any deviation in TRF1 structure will directly influence its relation with other shelterin complex proteins. When TRF1 dimerization fragments, DNA connection changes and thus TTAGGG sequences are not protected and in general, lead to the incorrect accumulation of shelterin proteins (21).

TRF2 causes to turn the 3' single-stranded DNA end into double-stranded DNA, forming structures called t-loops. TRF2 also attaches to the repetitive sequences of TTAGGG. Loss of TRF2 function leads to increased activity of the DNA damage response pathway, end-to-end chromosome fusion and cellular senescence (22). T-loops protect telomere structures and regulate the telomerase activities. TRF2 causes the formation of t-loops. On the other hand, when TRF2 is inhibited, the quantity of TTAGGG repeated sequences of single-stranded DNA is declined by 30%–50% (23). Interestingly, cardiac apoptosis in human heart failure was associated specifically with defective expression of TRF2 and activation of the DNA damage checkpoint

kinase, Chk2. Also, it has been reported that exogenous TRF2 prevents oxidative stress. Moreover, TRF2 was suggested to mediate proapoptotic signaling in post-mitotic, non-cycling cardiomyocytes, and plays a role in controlling the migration of progenitor cells to the infarcted area (24). When the ends of telomeres are not protected by shelterin proteins, get exposed and vulnerable to DNA repair mechanisms and presumably to telomerase action, therefore, leading to telomere attrition, many of structural and molecular changes and cellular senescence (25).

### Conclusion

In general, because in the present study, TRF1 and TRF2 have been increased significantly, these conditions will improve shelterin complex and enhance

protection of the telomeres and as a result, better repair of infarcted area will be seen. Therefore, rehabilitation training could be suggested to myocardial infarction patients. One of the limitations of the present study is the small sample size. Another limitation, is the lack of measuring other shelterin proteins and more importantly the telomere length. Because shelterin complex proteins have a close interaction, future studies can measure other shelterin complex proteins and telomere length to help us understand the effect of exercise training on shelterin complex and telomere.

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### References

1. Ludlow TA, Ludlow WL, Roth MS. Do telomeres adapt to physiological stress? Exploring the effect of exercise on telomere length and telomere-related proteins, Review article. *Biomed Res Int.* 2013; 601368.
2. Shammas AM. Telomeres, lifestyle, cancer, and aging. *Curr Opin Clin Nutr Metab Care.* 2011; 14(1):28–34.
3. Wong LS, vanderHarst P, deBoer RA, Huzen J, vanGilst WH, vanVeldhuisen DJ. Aging, telomeres and heart failure. *Heart Fail Rev.* 2010; 15(5):479-86.
4. Palm W, de Lange T. How shelterin protects mammalian telomeres. *Annu Rev Genet.* 2008; 42:301-34.
5. Ludlow AT, Roth SM. Physical activity and telomere biology: exploring the link with aging-related disease prevention. *J Aging Res.* 2011; 2011:790378.
6. Cherkas LF, Hunkin JL, Kato BS, Richards JB, Gardner JP, Surdulescu GL, et al. The association between physical activity in leisure time and leukocyte telomere length. *Arch Intern Med.* 2008;168(2):154–8.
7. LaRocca TJ, Seals DR, Pierce GL. Leukocyte telomere length is preserved with aging in endurance exercise-trained adults and related to maximal aerobic capacity. *Mech Ageing Dev.* 2010; 131(2):165-7.
8. Kim JH, Ko JH, Lee DC, Lim I, Bang H. Habitual physical exercise has beneficial effects on telomere length in postmenopausal women. *Menopause.* 2012; 19(10):1109-15.
9. Fletcher GF, Ades PA, Kligfield P, Arena R, Balady GJ, Bittner VA, et al. Heart association exercise standards for testing and training: A scientific statement from the American. *Circulation.* 2013; 128(8):873-934.
10. Pescatello LS, Riebe D, Arena R, American College of Sports Medicine. *ACSM's Guidelines for Exercise Testing and Prescription.* 9th ed. Baltimore (MD): Lippincott Williams & Wilkins; 2014.
11. Mendes M. General principles of exercise testing in cardiac rehabilitation. en: Niebauer J, Editor. *Cardiac Rehabilitation Manual.*



- Springer-Verlag London Limited; 2011.
12. Werner C, Hanhoun M, Widmann T, Kazakov A, Semenov A, Pöss J, et al. Effects of physical exercise on myocardial telomere-regulating proteins, survival pathways, and apoptosis. *J Am Coll Cardiol*. 2008;52(6):470-82.
  13. Laye MJ, Solomon TPJ, Karstoft K, Pedersen KK, Nielsen SD, Pedersen BK. Increased shelterin mRNA expression in peripheral blood mononuclear cells and skeletal muscle following an ultra-long-distance running event. *J Appl Physiol*. 2012; 112(5):773-81.
  14. Werner C, Fürster T, Widmann T, Pöss J, Roggia C, Hanhoun M, et al. Physical exercise prevents cellular senescence in circulating leukocytes and in the vessel wall. *Circulation*. 2009; 120(24):2438-47.
  15. Ludlow AT, Lima LCJ, Wang J, Hanson ED, Guth LM, Spangenburg EE, et al. Exercise alters mRNA expression of telomere-repeat binding factor 1 in skeletal muscle via p38 MAPK. *J Appl Physiol*. 2012; 113(11):1737-46.
  16. Barile L, Gherghiceanu M, Popescu L, Moccetti T, Vassalli G. Human cardiospheres as a source of multipotent stem and progenitor cells. *Stem Cells Int*. 2013; 2013: 916837.
  17. Wollert KC, Meyer GP, Lotz J, Ringes-Lichtenberg S, Lippolt P, Breidenbach C, et al. Intracoronary autologous bone-marrow cell transfer after myocardial infarction: the BOOST randomised controlled clinical trial. *Lancet*. 2004; 364(9429):141-8.
  18. Leri A, Rota M, Pasqualini F, Goichberg P, Anversa P. Origin of cardiomyocytes in the adult heart. *Circ Res*. 2015; 116:150-66.
  19. D'Amario D, Fiorini C, Campbell P, Goichberg P, Sanada F, Zheng H, et al. Functionally-competent cardiac stem cells can be isolated from endomyocardial biopsies of patients with advanced cardiomyopathies. *Circ Res*. 2011;108(7):857-61.
  20. Richardson G, Breault D, Horrocks G, Cormack S, Hole N, Owens A. Telomerase expression in the mammalian heart. *FASEB J*. 2012; 26(12):4832-40.
  21. Jason AS, Mary FC, Feng W, Carolyn MP. Maintaining the end: Roles of telomere proteins in end protection, telomere replication and length regulation. *Mutat Res*. 2012; 730(1-2):12-9.
  22. Wood AM, Rendtlew Danielsen JM, Lucas CA, Rice EL, Scalzo D, Shimi T, et al. TRF2 and lamin A/C interact to facilitate the functional organization of chromosome ends. *Nat Commun*. 2014; 5:5467.
  23. de Lange T. Shelterin: the protein complex that shapes and safeguards human telomeres. *Genes Dev*. 2005; 19(18):2100-10.
  24. Oh H, Wang SC, Prahash A, Sano M, Moravec CS, Taffet GE, et al. Telomere attrition and Chk2 activation in human heart failure. *Proc Natl Acad Sci U S A*. 2003; 100(9):5378-83.
  25. Patel TN, Vasani R, Gupta D, Patel J, Trivedi M. Shelterin proteins and cancer. *Asian Pac J Cancer Prev*. 2015; 16(8):3085-90.