

Research paper

The effect of endurance training with and without vitamin E on expression of p53 and PTEN tumor suppressing genes in prostate glands of male rats

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Received 25 November 2016; accepted 24 March 2017

Available online 1 April 2017

Abstract

The aim of this study was to investigate the effect of endurance training with and without vitamin E on the expression of p53 and Phosphatase and tension homolog (PTEN) tumor suppressor genes of prostate glands in male rats. For this purpose, 50 Sprague–Dawley male rats were randomly assigned into 5 groups: (1) control group (CON, n = 10), (2) sham (S, n = 10), (3) endurance training (ET, n = 10), (4) endurance training + vitamin E (ET + VE, n = 10), (5) vitamin E (VE, n = 10). Endurance training protocol was implemented for 6 weeks, 6 days per week, in accordance with the overload principle. To measure expression changes of p53 and PTEN genes in rats' prostate, real-time PCR method was used and HPLC method was used to measure vitamin E in this tissue. After 6 weeks of taking vitamin E, its level in all groups, except for group VE ($p < 0.000$) did not significantly increase. After implementing training protocol, p53 expression reduced significantly in ET group ($p < 0.026$). Vitamin E supplementation along with endurance training did not cause any significant change either p53 or PTEN (respectively; $p < 0.2$, $p < 0.11$). Instead, vitamin E supplementation without endurance training caused significant increase in PTEN, but did not cause any significant changes in p53 (respectively; $p < 0.016$, $p < 0.15$). These results indicate that endurance training reduces p53 and PTEN tumor suppressing genes expression, and taking vitamin E supplement could increase expression of these genes in some extent.

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Keywords: p53; PTEN; Endurance training; Vitamin E; Prostate gland

1. Introduction

Studies show that poor diet, low levels of physical activity and obesity play an important role in different types of cancer, including prostate cancer progression [1–4]. Higher levels of physical activity are associated with reduced rate of death caused by prostate cancer [5]. Physical activity might have preventive effects before and after the diagnosis of cancer [6], which means it may affect both the initiation and progression

of tumor [7]. Tumor suppressor genes or anti-oncogenes protect cells against cancer [8]. Among these genes, suppressor genes of p53 and PTEN are the most common inactivated and mutated genes in different cancers [9].

p53 protein is a transcription factor stopping the G1 stage of cell cycle through activating several downstream genes. Inactivation of p53 gene is a common event in the development of many types of cancer [10]. p53 is activated in response to numerous stressors such as oxidative stress [11]. In response to stress, p53 activates physiological routes regulating cell cycle stopping, repairing the DNA, apoptosis, autophagy, and the metabolism of body [12,13]. In a normal cell, p53 is deactivated by its negative regulator MDM2. In the case of damage to DNA by other stresses, numerous routes

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lead to isolation of p53 and MDM2 complex. Due to activation, p53 induces cell cycle stopping for cell repair and survival or removal of damaged cells [14]. Recently, p53 has been proposed as a key molecular factor that regulates metabolism of substrates and mitochondrial biogenesis caused by training in skeletal muscle. Disorder in content and performance of mitochondria is associated with many damages, such as metabolic disorders, aging, type 2 diabetes, obesity and cancer as well as reduced training performance [15].

Phosphatase and tension homolog (PTEN) are one of the most common mutated tumor suppressors in human cancer [12,13]. Disorder in regulation of this tumor suppressor will lead to metabolic diseases and cancer [16]. Specifically, this gene is removed in more than 50% of cases in prostate cancer [17,18]. The protein regulates intracellular surfaces of phosphatidylinositol-3, 4 and 5-trisphosphate (PIP3) negatively in cells and it acts as tumor suppressor [13].

Recent studies show that there is a strong relationship between PTEN and p53. Both PTEN and p53 regulate cell proliferation and death [19,20]. Mechanistically, evidence suggests that activation of protein kinase B by PTEN plays a major role in modulating MDM2-dependent (Mouse double minute 2 homolog) p53 breakdown [21,22], but p53 is a short-lived protein that its stabilization is vital for its tumor suppressive function. Protein kinase B is physically associated with MDM2 and it phosphorylates MDM2 in serine residues. In other words, it is considered as a key step in the nuclear translocation of MDM2 and p53 breakdown through MDM2 [23,24]. Therefore, activation of protein kinase B in PTEN deficient cells causes faster breakdown of p53 that it would help to tumorigenesis due to the loss of PTEN. In addition, p53 can be connected to PTEN promoter region and activate it in terms of transcription [25]. However, there are controversial views considering the importance of such regulation [26]. Germ lines mutation in p53 and PTEN cause Li–Fraumeni syndrome and Cowden syndrome, respectively. There are some phenotypic overlaps in this syndrome [27].

Over the past few decades, many studies have been conducted on the relationship between training and prostate cancer risk. More than half of the studies conducted in this area show an inverse relationship between physical activity and prostate cancer [28–31], while other studies do not approve such relationship [32–35]. In the four studies, it has been found that training increases the prostate cancer risk [36–39]. In this regard, the objective of the present study was to investigate the effect of endurance training with and without taking vitamin E on the level of p53 and PTEN tumor suppressor genes of prostate gland.

2. Materials and methods

2.1. Animals

All experiments involving the animals were conducted according to the policy of Iranian Convention for the Protection of Vertebrate Animals Used for Experimental and other Scientific Purposes, and the protocol was approved by the Ethics

Committee of the Birjand University (BU), Iran. In this study, 50 three-month-old male Sprague–Dawley rats (180–220 g) were purchased from *Center of Comparative & Experimental Medicine* (Birjand University of Medical Sciences, Iran). All animals were housed on a 12-h light–dark cycle, with controlled humidity and room temperature (20–23 °C), and access to food and water *ad libitum*. Afterward, the rats were randomly divided into five groups including: (1) control (CON, $n = 10$), (2) Sham (S, $n = 10$), (3) endurance training (ET, $n = 10$), (4) endurance training + vitamin E (ET + VE, $n = 10$) and (5) vitamin E (VE, $n = 10$).

2.2. Exercise protocol

Treadmill training began with familiarization of rats with the apparatus for 10 days by placing them on the motorized-treadmill. Rats were run at a rate of 27 m min⁻¹. Initially, 20 min of running was performed with 2 min added per day until 60 min of daily continuous running was achieved. This intensity was subsequently maintained for 6 weeks [40]. If you have a look to SHEPHERD and GOLLNICK 1976 [41], you could adjust the oxygen uptake to your running intensity.

2.3. Supplementation vitamin E

In this study, succinate 25-g package of succinate vitamin E made by Sigma Company ((+) – α -Tocopherol acid, Sigma–Aldrich) was used. Sixty mg of vitamin E per kg body weight [42,43] was given 6 days per week and 3 h before training by gavage to rats group of VE, ICT + VE [44]. Sesame oil was used for the preparation of vitamin E (60 mg in 1 ml sesame oil). Additionally, 1 ml sesame oil per kilogram of body weight was given by gavage for Sham group rats [45].

2.4. Tissue sampling

In order to prevent acute effects last training session, 48 h after the last training session sampling was performed. In addition, in order to control rats nutritional states, they were fasting overnight before killing. The rats were anesthetized with Ether. The prostate tissue was removed and washed with saline and immediately frozen for later analysis kept in –80 freezer.

2.5. Total RNA extraction and cDNA synthesis

The total RNA from prostate tissue were extracted with TRIzol reagent (Invitrogen Life Technologies, Carlsbad, CA, USA) following the manufacturer's instructions. The total RNA concentration and purity were measured using a NanoDrop™ 2000c Spectrophotometer (Thermo Scientific, USA). The 1.5% agarose gel electrophoresis was performed to check the RNA integrity. 500 ng of total RNA was used for cDNA synthesis with a final volume of 20 μ L, using PrimeScript™ RT reagent Kit (EUR_X, E0801-03) following the manufacturer's instructions.

Table 1

The body weight of rats pre- and post-interventions in each group (n = 10). Values are expressed as mean \pm SD. CON = control group; S = sham group; ET = endurance training group; ET + VE = endurance training + vitamin E group; VE = vitamin E group.

	CON	S	ET	ET + VE	VE
Pre-intervention	217.6 \pm 16.17 (g)	217.4 \pm 18.85 (g)	213.8 \pm 19.04 (g)	224.5 \pm 13.18 (g)	227.6 \pm 13.38 (g)
Post-intervention	307.1 \pm 16.51 (g)	291.6 \pm 15.67 (g)	242.9 \pm 16.50 (g)	290.0 \pm 31.13 (g)	298.8 \pm 34.10 (g)

2.6. Real-time quantitative PCR

To measure the relative mRNA expression, real-time PCR was performed with an StepOne real-time PCR system (ABI, Applied Biosystems, USA) with SYBR Green High ROX (RealQ-PCR 2x Master Mix, Ampliqon, Denmark). The housekeeping gene *B2M* was used as a reference gene for normalization. The primers sequences were as follows: *p53* (NM_030989.3): 5'-ATTCACCCTTAAGATCCGTGGG-3' and 5'-AGACTGGCCCTTCTTGGTCT-3'; *PTEN* (NM_031606.1): 5'-GGAAAGGACGGACTGGTGTA-3' and 5'-AGT GCCACTGGTCTGTAATCC-3'; *B2M* (NM_012512.2): 5'-TACGTGTCTCAGTTCCACCC-3' and 5'-TTGATTACATGT CTCGGTCCCA-3'. The qPCR was performed with 12.5 μ l 2X SYBR/ROX qPCR Mix and 10 pmol forward and reverse primers specific for the respective genes, in a total volume of 25 μ l. The following reaction conditions were applied: 10 min at 95 $^{\circ}$ C, 45 cycles of 15 s at 95 $^{\circ}$ C and 1 min at 60 $^{\circ}$ C, and a melting curve protocol (plates read when increased 0.5 $^{\circ}$ C every 5 s from 65 $^{\circ}$ C to 95 $^{\circ}$ C) for amplicon specificity verification. All amplifications were run in triplicate, and any doubtful curves were excluded. The amplification efficiency for *p53*, *PTEN* and *B2M* was estimated by real-time PCR with different diluted cDNA template. The threshold cycle (Ct) values from all samples were measured.

2.7. Measurement of prostate vitamin E

Prostate vitamin E levels were measured by HPLC, as described previously [46]. 50 mg tissue were powdered with liquid nitrogen. 5 ml cold absolute ethanol was added. 10 ml cold hexane was added and then vortex. After that, centrifuged for 2500 rpm, 15 min, 5 $^{\circ}$ C. The upper layer was removed and injected to HPLC instrument.

2.8. Statistical analysis

All data were expressed as means \pm SD. The comparative $2^{-\Delta\Delta CT}$ method for relative quantitative analysis was used, and the results are expressed as a fold change of expression levels. The mean value of triplicates was applied for all calculations. All statistical calculations were performed with the SPSS 22.0 software package (SPSS Inc.). The influence of independent variables, including endurance training and vitamin E, was analyzed using a two-way analysis of variance (ANOVA) followed by Tukey tests. A one-way analysis of variance (ANOVA) and Tukey post hoc test were used for analysis of body weight and vitamin E levels among groups. The GraphPad software (Prism, 6.01) also was used to draw the

graphs. A *P* value less than 0.05 was considered statistically significant.

3. Result

Table 1 shows the body weight of rats pre- and post-interventions in each group. After 6 weeks of endurance training, body weight of rats decreased significantly in ET group compared to other groups ($p < 0.000$) (Fig. 1). Measurement of prostate vitamin E for using HPLC method showed that level of vitamin E in the group of vitamin E (VE) is significantly higher than other groups ($p < 0.000$) (Fig. 2). After performing endurance training for 6 weeks, *p53* gene expression in ET group reduced significantly compared to the CON group ($p < 0.026$) (Fig. 3b). In addition, *p53* gene expression level after 6 weeks of supplementation vitamin E in VE group increased significantly compared to ET and ET + VE groups (respectively; $p < 0.0001$, $p < 0.0008$). *PTEN* gene expression level after 6 weeks of endurance training reduced in ET group compared to control group, but the reduction was not significant ($p < 0.53$). In ET + VE group, the expression of *PTEN* after 6 weeks increased significantly compared to ET group ($P < 0.0029$), but these results were not statistically significant compared to control group ($p < 0.11$) (Fig. 4b). After six weeks supplementation with vitamin E, VE group had significantly higher *PTEN* gene expression than CON and ET groups (respectively; $p < 0.0161$, $p < 0.0001$).

4. Discussion

To our knowledge, this is the first controlled study investigating the effect of endurance training on *p53* and *PTEN*

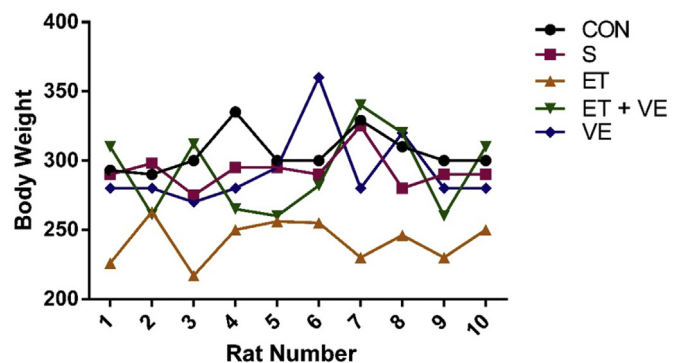


Fig. 1. Analysis of body weight of rats in each group after 6 weeks (n = 10). After 6 weeks, body weight of rats decreased significantly in ET group compared to other groups ($p < 0.000$).

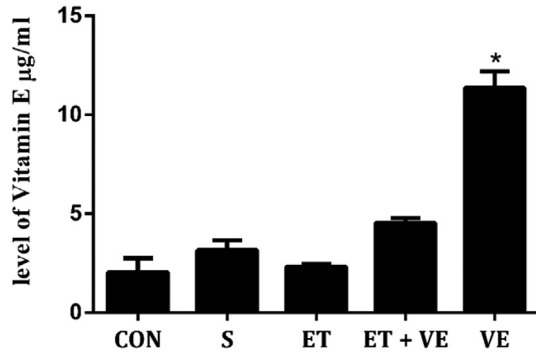


Fig. 2. The level of vitamin E in prostate tissue of rats. Values are expressed as mean \pm SD (n = 10). As can be seen, the level of vitamin E in VE group (*) is significantly higher than that in other groups (* $p < 0.000$). CON = control group; S = sham group; ET = endurance training group; ET + VE = endurance training + vitamin E group; VE = vitamin E group.

tumor suppressor genes of prostate gland in male rats. The results of the current study showed that the expression level of p53 gene was significantly decreased after six weeks of endurance training.

As we found no similar study to investigate the effect of endurance training on p53 gene expression of prostate tissue, we refer to results obtained on other tissues for analyzing the results unnecessarily. After performing endurance training for 6 weeks, p53 gene expression in ET and ET + VE groups decreased compared to CON group, but reduction in ET + VE group was not significant. The decreased levels of p53 after endurance training were also observed in other studies [47–49]. Ziaaldini et al. [47] evaluated the effect of 6 weeks of endurance training on a treadmill in the skeletal muscles of young rats (three months) and old rats (eight months). After 6

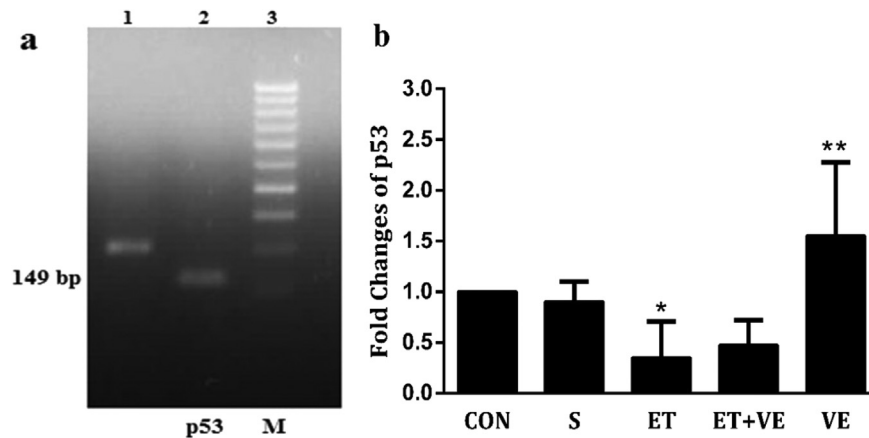


Fig. 3. (a) Gel electrophoresis of PCR product for p53 gene (b) Fold change in gene expression of p53. Values are expressed as mean \pm SD (n = 10). B2M gene was used for housekeeping gene (Ct = 28.63 \pm 2.92). The Ct mean of all groups are as follows; CON = 33.30 \pm 3.91; S = 35.01 \pm 4.26; ET = 35.75 \pm 3.33; ET + VE = 35.35 \pm 6.45; VE = 33.68 \pm 5.17. As can be seen, the expression of p53 in the ET group (*) decreased significantly compared to the CON group ($p < 0.026$). In VE group (**), the p53 expression was significantly increased compared to the ET and ET + VE groups (respectively; $p < 0.0001$ and $p < 0.0008$). CON = control group; S = sham group; ET = endurance training group; ET + VE = endurance training + vitamin E group; VE = vitamin E group.

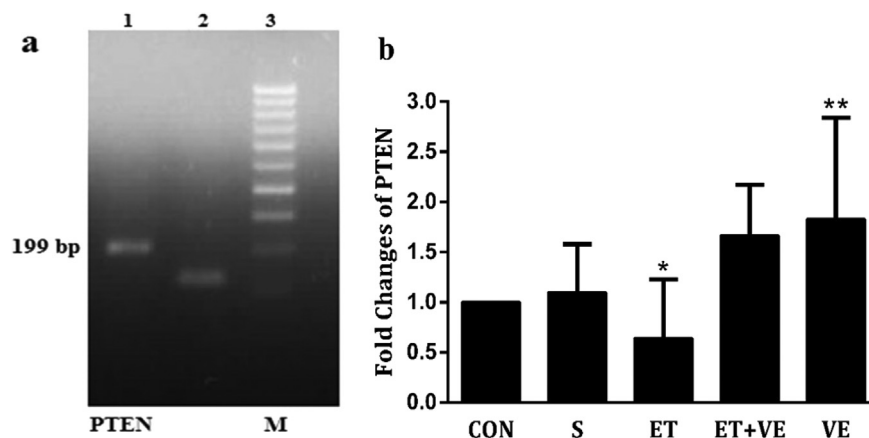


Fig. 4. (a) Gel electrophoresis of PCR product for PTEN gene (b) Fold change in gene expression of PTEN. Values are expressed as mean \pm SD (n = 10). B2M gene was used for housekeeping gene (Ct = 28.63 \pm 2.92). The Ct mean of all groups are as follows; CON = 25.73 \pm 1.94; S = 25.45 \pm 2.91; ET = 25.95 \pm 2.25; ET + VE = 25.12 \pm 1.71; VE = 26.00 \pm 3.12. As can be seen, the expression of PTEN in the ET group (*) decreased significantly compared to the ET + VE and VE groups (respectively; $p < 0.0029$ and $p < 0.0001$). The expression of p53 in the VE group (**) increased significantly compared to the CON group ($p < 0.0161$). CON = control group; S = sham group; ET = endurance training group; ET + VE = endurance training + vitamin E group; VE = vitamin E group.

weeks of endurance training, p53 levels reduced in both old and young rats, but this decrease was not significant. Al-Jarrah et al. [48] investigated the impact of endurance training on treadmill in heart muscles in diabetic and non-diabetic rats. The results of this research show that the level of p53 expression of heart muscle in diabetic control group increased significantly compared to inactive control group ($p < 0.02$), but after the implementation of the training protocol, the level of p53 expression in the diabetic group significantly reduced compared to their peers in control group ($p < 0.005$). Generally, significant difference was not observed between the expression of p53 in the healthy active control group active and healthy inactive control group.

Studies have shown that one session of physical training, including aerobic [50,51] or anaerobic [52,53] training, increases p53 activity. Saleem et al. [54] also observed that even an acute muscle contraction (in the form of exhausted electrical stimulation protocol) causes double change in p53 phosphorylation on serine 15 (a change that would be associated with increased stability and activity normally) immediately after training and exercise. This change also takes place in the state of phosphorylation, in collaboration with classical phosphorylation caused by AMPK and p38MAPK training. Thus, these kinases may play as upstream kinases in changing p53 activity. According to what was said, it is clear that an acute session of training, as a physical stressor, increases p53 expression. However, the interesting point is that how exercise or training reduces the expression of this gene in the long-term. It has been shown that regular training reduces oxidative stress and enhances antioxidant defense of the body [49]. Therefore, as one of the reasons for the increased expression of p53 is increased stress of oxidative, it can be expected that p53 gene expression level to be reduced due to reduction in stress of oxidative. On the other hand, the body weight in ET rats was significantly decreased after 6 weeks of endurance training. Therefore, one of the possible reasons for decrease in p53 expression could be this issue. But we didn't find any direct study about the relationship between losing weight and reduction in p53 gene expression. Instead, there are many studies showing that nutritional deprivation (thinness) increases expression of the p53 gene [55–57]. Therefore, the decrease in p53 expression cannot be unequivocally attributed to weight loss. Further studies are needed in this regard.

p53 expression level in VE group increased significantly compared to the ET and ET + VE groups. Cellular effects of vitamin E is essentially based on its antioxidant activity and it has been shown that vitamin E regulates the main genes expression related to cell proliferation and inflammatory processes in addition to regulating several signaling routes [58]. Moreover, obesity (i.e., high lipid levels) increase oxidative stress levels and lead to p53 induction [57]. Thus, one of the possible reasons for increased p53 expression level in VE group could be this issue.

In our study, the level of PTEN gene expression after 6 weeks of endurance training in ET decreased, but these

changes were not significant compared to the control group ($P < 0.53$). These results are consistent with results of other studies [59–61]. Ding et al. [59] examined the impact of 8 weeks of endurance training on PTEN in twin and soleus muscles of rats and they stated that after 8 weeks, mRNA expression of PTEN and PTEN protein decreased in twin muscle, but PTEN mRNA expression related to PTEN in soleus muscle after 8 weeks was unchanged, while it protein reduced. In this regard, Ma et al. [60] examined the effect of 8 weeks of swimming training on male Wistar rats and they observed that after 8 weeks, gene expression and PTEN protein decreased in the muscle of the left ventricle of rats significantly. It has been proven that PTEN has interaction with p53 in complicated way [27]. Although they have different functions, it has been shown that they have mutual co-operation so that it is thought that PTEN regulates stability of p53 and p53 increases the transcription of PTEN. However, as soon as PTEN is lost, p53 pathway is highly activated [62,63].

In addition, lack of PTEN loss is associated with lack of p53 and it will lead to cancer development [64]. PTEN gene can be regulated positively by early growth regulated transcription factor 1, peroxisome proliferator activated receptor γ (PPAR γ), p53, and activating transcription factor 2 (ATF2) [65,66]. However, transforming growth factor- β (TGF- β) and nuclear factor κ B (NF- κ B) and Jun can negatively regulate it [67,68]. As a result, p53 and MDM2 form a regulatory feedback loop in which p53 regulates MDM2 expression positively and regulates MDM2 protein level negatively. Therefore, PTEN could protect breakdown of p53 through MDM2 and p53 might increase transcription of PTEN [69]. Thus, inactivation of each of these genes leads to reduced protein levels of another gene. Thus, considering the direct relationship between p53 and PTEN, reduction in p53 expression could be one of the possible reasons for reduced levels of PTEN expression. On the other hand, as mentioned earlier, regular training reduces the stress level of oxidative, which this in turn can reduce the level of PTEN expression in the prostate.

In VE and ET + VE groups, PTEN expression level increased after 6 weeks of supplementation with vitamin E. It seems that taking vitamin E increases the expression of this gene, because it did not happen in ET and CON groups. The mechanism through which vitamin E increases the expression level of PTEN is still unknown. As one of the goals of p53 transcription is PTEN, one of the ways by which p53 indirectly inhibit the production of PIP3 is stimulating the expression of PTEN [69]. PTEN regulates p53 level as well as its activity positively through negative regulation of MDM2 transcription and connection activity of p53 [70]. However, in the absence of p53, PTEN might play the inhibitory role of carcinogenesis by contribution of MDM2 through regulating transcription of MDM2 and selection of isoforms [71]. With regard to what was said, as expression of PTEN is essential for stabilization of p53, it could be concluded that taking vitamin E along with training provides the best conditions for maintaining this feedback loop.

5. Conclusion

Our results showed that regular endurance training reduces the expression of p53 and PTEN suppressor genes. Reduced level of these tumor suppressing genes can be examined from three aspects (1); regular endurance training reduces free radicals level in organism and reduces the need for activation of these genes (2) regular endurance training and followed by production of free radicals causes damage to DNA of these genes, thereby reduced expression of them (3) Regular endurance training can lead to weight loss and thereby reduces the expression of these genes. On the other hand, vitamin E along with training, can cause over-expression of PTEN and prevent p53 from under-expression in some extent.

Conflict of interest

The authors declare the absence of conflict of interest in relation to the present work.

Acknowledgement

This study was conducted by contribution of University of Birjand and Shiraz University of Medical Sciences. Thereby, we appreciate men, including Dr. Jamhiri, Amirzadehfard, and Moradpour, and women, including Zeraatiannejad and Nejabat who cooperated at all stages of the study.

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