



The effect of intense intermittent training with and without taking vitamin E on mRNA expression of p53/PTEN tumor suppressing genes in prostate glands of male rats

Mohammad Esmail Afzalpour^a, Nader Tanideh^b, Masood Sepehrimanesh^c
and Amin Allah Dashtivan^{a,*}

^aDepartment of Physical Education & Sport Sciences, University of Birjand, Birjand, Iran

^bStem Cell and Transgenic Technology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

^cGastroenterohepatology Research Center, Shiraz University of Medical Sciences, Shiraz, Iran

*Corresponding E-mail: amin.dashtivan@yahoo.com

ABSTRACT

Physical activity and diet are the most important modifiable determinants of cancer risk. The objective of this study was to examine the effect of intense intermittent training with and without taking vitamin E on expression of p53 and PTEN tumor suppressing genes in the prostate gland of male rats. For this purpose, 50 Sprague-Dawley male rats were randomly assigned into 5 groups: [1] control (CON, n = 10), [2] sham (S, n = 10), [3] intense intermittent training (IIT, n = 10), [4] intense intermittent training + vitamin E (IIT + VE, n = 10), [5] vitamin E (VE, n = 10). Protocol of this study was implemented for 6 days per week for 6 weeks, with observing the overload principle on the motorized treadmill. After implementing training protocol, expression rate of p53 and PTEN genes reduced significantly ($p < 0.000$, $p < 0.031$, respectively). Taking vitamin E with intermittent training caused significant reduction in p53 expression ($p < 0.013$), while it caused significant increase in expression of PTEN ($p < 0.035$). These results showed that intense intermittent training reduces expression of p53 and PTEN tumor suppressing genes and taking supplementation vitamin E along with this type of training could cause different effects in expression of these tumor suppressor genes.

Keywords: p53; PTEN; vitamin E; intense intermittent training; prostate glands

INTRODUCTION

Prostate cancer is the second most common cancer in men around the world [1]. Lifestyle and especially dietary and physical activity are among the factors that could reduce the risk of prostate cancer progression and death. Many prospective studies in healthy people show that intense physical activity is correlated with a reduced risk of prostate cancer [2].

p53 and PTEN tumor suppressor genes are most common inactivated and mutated genes in different types of cancer [3]. The p53 gene encodes p53 tumor suppressing protein and this protein is called as "guardian of the genome". When the p53 gene is damaged, tumor suppression is severely reduced [4, 5]. Many stresses lead to stabilization and activation of p53, including oxidative stress [6], damage to DNA, hypoxia, deprivation of food and DNA replication [7]. In response to cellular stresses, p53 tumor suppressor gene encodes a transcription factor [8] leading to inhibition of cell cycle progression, induced aging, differentiation or apoptosis [9], depending on the type of cell, the cell environment and oncogenic alterations. Phosphatase and tensin homolog gene [PTEN] encodes the PTEN tumor

suppressing protein [10]. This protein regulates cellular processes such as cell proliferation and death through the phosphoinositide 3-kinase/protein kinase B/mammalian target of rapamycin [11, 12]. PTEN gene acts as tumor suppressor through negative regulation of signaling pathway AKT/PKB, and it is considered as the most common tumor suppressor gene deleted in prostate cancer [13].

p53 and PTEN control cell proliferation and death and they are often expressed simultaneously in a variety of tumors [14, 15]. Due to the short half-life of p53, its suppressor function depends strongly on this stabilization [16]. In normal cells, p53 levels are kept at negligible levels by ubiquitin-mediated proteolysis. p53 and PTEN may form a positive feedback loop. By binding to PTEN promoter and thus activating PTEN receptor, p53 can regulate it. In detail, p53 can upregulate PTEN by binding to the PTEN promoter, thereby activating PTEN transcription [15]. In terms of mechanism, PTEN inhibits AKT-mediated phosphorylation of MDM2 to prevent MDM2 from translocation into the nucleus and p53 breakdown [17]. MDM2 is an important negative regulator of p53 [17, 18]. Thus, PTEN can stabilize p53. Therefore, it can be stated that AKT activation with PTEN deletion may lead to the quickly breakdown of p53, leading to more PTEN-dependent tumorigenesis [19]. However, there are controversial views on the importance of this regulation [20].

Germline mutation in p53 and PTEN leads to Li-Fraumeni syndrome and Cowden syndrome, respectively [21]. In recent years, physical activity has been considered as supportive treatment after diagnosis of cancer. There is clear evidence suggesting that training improves psychological and physiological consequences and reduces cardiovascular disease and mortality rate of non-cancerous people significantly. However, the possible impact of physical activity in preventing or reducing prostate cancer has not been revealed [22]. Therefore, the aim of our study was to investigate the effect of intermittent training with and without E on p53 and PTEN tumor suppressor genes in the prostate gland.

MATERIALS AND METHODS

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 School of Medicine Sciences, Shiraz University (SU), Iran. In this study, 50 three-month-old male Sprague-Dawley rats (180–220 g) were purchased from Center of Comparative & Experimental Medicine (Shiraz 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) intense intermittent training (IIT, $n = 10$), (3) intense intermittent training + vitamin E (IIT + VE, $n = 10$), (4) vitamin E (VE, $n = 10$), and (5) sham (S, $n = 10$) groups.

Exercise training protocol

Protocol of this study was implemented for 6 days in week for 6 weeks, with observing the overload principle on the motorized treadmill. The intermittent training program consisted of a 3-min warm-up at $16.2 \text{ m}\cdot\text{min}^{-1}$, followed by intervals of running at $54 \text{ m}\cdot\text{min}^{-1}$ for 30 s, alternated with running at $16.2 \text{ m}\cdot\text{min}^{-1}$ for 60 s, performed 3 days per week. Initially, 3 intervals were completed and increased to 20 intervals by the 4th week. On alternate days (3 days per week), rats completed a warm-up, followed by running for 3 min at $40.5 \text{ m}\cdot\text{min}^{-1}$ separated by 60 s at $16.2 \text{ m}\cdot\text{min}^{-1}$. Initially two repetitions were performed and increased to six repetitions by the 4th week. These intensities were subsequently maintained for 4 weeks (23).

Vitamin E supplementation

In order to supplement vitamin E in this study, 25-gram succinate package of Sigma Company ((+) - α -Tocopherol acid succinate, Sigma-Aldrich) was used. Six days per week and three hours before the implementation (24), 60 mg of vitamin E per kg body weight was given to rats of VE, IIT + VE groups by gavage (25). Sesame oil was used to prepare vitamin E (60 mg in 1 ml of sesame oil) (26). Additionally, 1 ml sesame oil was given for sham group rats per kg of body weight by gavage.

Tissue sampling

Forty and eight hours after the last training session, rats were anesthetized by Ether and killed. Then, their prostate was removed and rinsed by physiological serum, and it was kept at -80°C for subsequent analyzes.

Total RNA extraction and cDNA synthesis

The total RNA from prostate tissue were obtained 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. 500ng of total RNA was used for cDNA synthesis with a final volume of 20 µL, using Prime Script™ RT reagent Kit (EURx, E0801-03) following the manufacturer's instructions.

Real-time 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 forward and reverse primers listed in Table 1 were designed using the NCBI-primer BLAST database. The PCR was performed with 12.5 µl 2X SYBR /ROX qPCR Mix and 10 pmol forward and reverse primers specific for the respective genes, in a total volume of 25 µl. The following reaction conditions were applied: 10 min at 95°C, 45 cycles of 15 second at 95°C and 1minute at 60°C, and a melting curve protocol (plates read when increased 0.5°C every 5 second from 65°C to 95°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 amplifications were measured.

Table 1. Sequence of primers used for real-time RT-PCR analysis

Accession No.	Gene symbol	Forward	Reverse
NM_030989.3	P53	5'-ATTTACCCCTTAAGATCCGTGGG-3'	5'-AGACTGGCCCTTCTTGGTCT-3'
NM_031606.1	PTEN	5'-GGAAAGGACGGACTGGTGTA-3'	5'-AGTGCCACTGGTCTGTAATCC-3'
NM_012512.2	B2M	5'-TACGTGTCTCAGTCCACCC-3'	5'-TTGATTACATGTCTCGGTCCCA-3'

Measurement of prostate vitamin E

Prostate vitamin E levels were measured by HPLC, as described previously (27). 50 mg tissue were powdered with liquid nitrogen. 5 ml cold absolute ethanol were added. 10 ml cold hexane was added and then vortex. After that, Centrifuged for 2500 rpm, 15 min, 5 C°. The upper layer was removed and injected to HPLC instrument.

Statistical analysis

All data were expressed as means ± SE. 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 One-way analysis of Variance (ANOVA) test was used for comparisons among groups. When main effects were found, comparisons between means were made using a *LSD post hoc* test. The GraphPad software (Prism, 6.01) also was used to draw the graphs. A *P* value less than 0.05 was considered statistically significant.

RESULTS

Measuring vitamin E in the prostate of all groups showed that levels of vitamin E in the group VE showed significant increase compared to other groups ($p < 0.000$) (Figure 1). After performing intense intermittent training for 6 weeks, p53 gene expression in IIT and IIT + VE groups significantly decreased compared to the CON group (respectively, $p < 0.000$, $p < 0.013$). However, decrease in IIT + VE group, was lower than IIT group (Figure 2b). PTEN gene expression level after 6 weeks of intense intermittent training in IIT group decreased significantly compared to control group ($p < 0.031$). In IIT + VE group, the expression level of PTEN after 6 weeks training increased significantly compared to IIT and CON groups (respectively, $p < 0.001$, $p < 0.035$) (Figure 3b). p53 and PTEN expression decrease after 6 weeks of intense intermittent training in IIT group was statistically significant compared to sham group (S) (respectively, $p < 0.000$, $p < 0.016$). Figures 2a and 3a show results of electrophoresis gel of all PCR products of the samples.

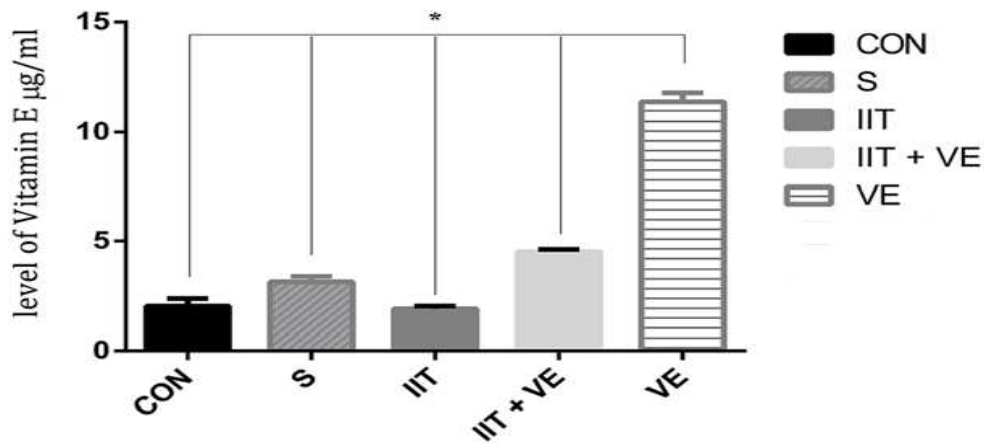


Figure 1 shows levels of vitamin E in prostate tissue of rats in the control (CON), sham (S), intense intermittent training (IIT), intense intermittent training + vitamin E (IIT + VE) and vitamin E (VE). As shown, E vitamin level in the VE group (*) increased significantly compared to all groups ($p < 0.000$)

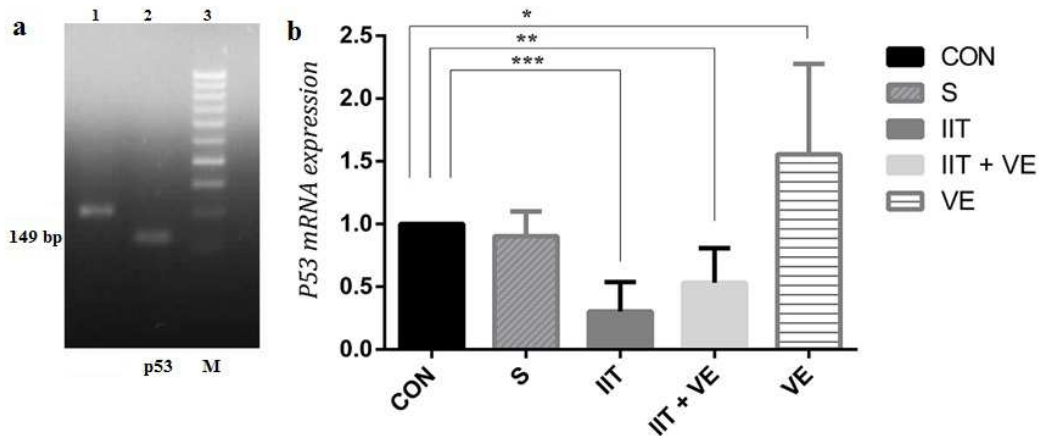


Figure 2. (a) Gel electrophoresis of PCR product for p53 gene (b) As shown, mRNA expression of p53 gene VE group (*), IIT+VE (**), and IIT group (***) has significant difference with that in control group (respectively, $*p < 0.002$, $**p < 0.013$, $***p < 0.000$)

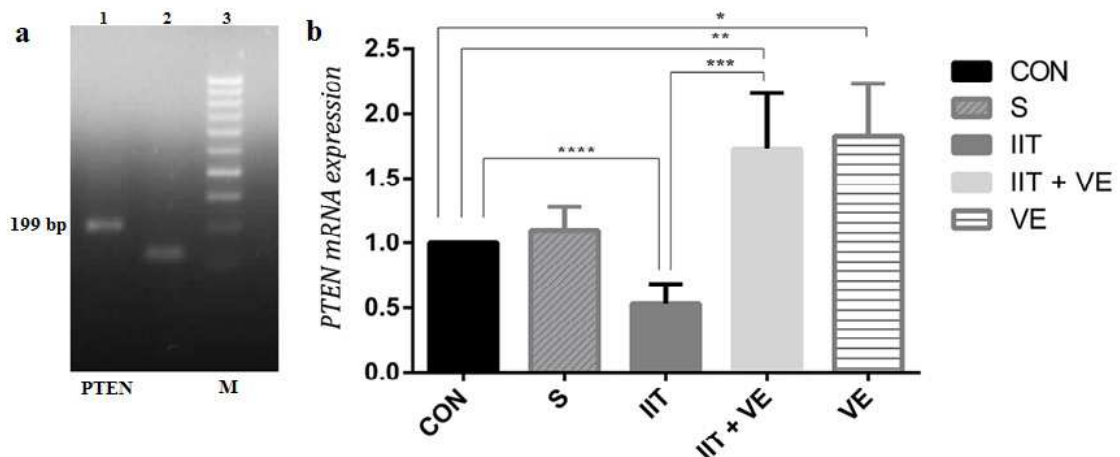


Figure 3. (a) Gel electrophoresis of PCR product for PTEN gene (b) As shown, PTEN expression level showed significant difference in VE group compared to CON group (*), IIT+VE group compared to CON group (**), IIT+VE group compared to IIT group (***), and IIT group compared to CON group (****) (respectively, $*p < 0.013$, $**p < 0.035$, $***p < 0.001$, and $****p < 0.031$)

DISCUSSION

The results of our research showed that after six weeks of intense intermittent training, the expression level of p53 and PTEN tumor suppressor genes was significantly reduced in the prostate tissue. As similar studies have not been conducted on the effect of intense intermittent training on p53 and PTEN gene expression level in prostate tissue, we were forced to refer findings of other tissues and other trainings such as continuous training to interpret and analyze the results.

p53 gene expression level after implementing 6 weeks of intermittent training in IIT group and IIT + VE group were significantly reduced compared to the CON group. The decrease in the expression of p53 was also seen in continuous training (28-30). Although some studies including those conducted by Jiang et al (2014) investigated the effect of chronic intermittent training in this regard, these studies were conducted on rats with myocardial infarction and the effect of these types of training on these rats was examined. Jiang et al (2014) examined the effect of 8 weeks of aerobic intermittent training on rats with myocardial infarction (MI). They investigated the protective effect of intermittent training on myocardial mitochondria in post-MI rats focusing on mitochondria. After MI, p53 expression level increased significantly. However, after implementing the training program, they observed that the ERK1/2-JNK-P53 signaling pathway was deactivated (31). Bartlett et al (2012) compared the effect of one session of high intensity intermittent training and one session of moderate continuous training matched with each other in terms of workload. They hypothesized that intermittent training stimulates signaling pathways related to mitochondrial biogenesis more than as continuous training does. Their samples included Vastus lateralis of active men. Their results showed that physical activity (both intense intermittent running and moderate continuous running) increases P53^{Ser15} phosphorylation in skeletal muscle of man 3 hours after training (in the period that it seemingly belongs to upstream AMPK signaling and / or p38 MAPK) (32). According to what was said, it is clear that one intense session of exercise as physical stressor increases p53 expression. However, the interesting thing is how chronic training reduces the p53 gene expression. It is proven that regular training reduces oxidative stress and enhances the antioxidant defense (30, 33). Free radicals and reactive oxygen species (ROS) are essential for our health playing various regulatory role in cells (34). They also stimulated the signaling of many genes encoding transcription factors, differentiation, and increased antioxidant enzymes (35, 36). Therefore, as one of the reasons for the increase of p53 is increased oxidative stress, we can expect that expression of p53 gene also to be reduced due to the reduction in oxidative stress.

On the other hand, expression of PTEN after 6 weeks of intermittent training was reduced in IIT group ($p < 0.031$), but it significantly increased in the group IIT + VE ($p < 0.035$). It has been shown that p53 can be connected to PTEN promoter areas and activate them in terms of transcription (15, 37). Thus, according to the direct relationship between p53 and PTEN, one of the reason to reduce PTEN expression might be reduced expression of p53. On the other hand, as mentioned earlier, regular training reduces the level of oxidative stress and this in turn can reduce the level of expression of PTEN in the prostate.

Many studies have shown that free radicals play important role in damage to genome DNA (38-40) and incidence of certain cancers (41). As physical activity is one of the factors in producing free radicals (42-44), one of the possible reasons in decreased expression of p53 and PTEN can be damage to the DNA of these genes.

It seems that vitamin E along with intermittent training regulates PTEN gene positively. Increased expression of PTEN in some studies used other supplements was also observed (45). Smolarek et al. (2012) examined the effect of taking various supplements of tocopherol (α , β , γ , and δ) in inhibition of breast cancer tumorigenesis in animal models having estrogen-receptor-positive. In this study, levels of PTEN mRNA in rat consuming β , γ , and δ increased, but it did not change in rats consuming α -tocopherol. The researchers concluded that in the tumors of mammals, γ and δ -tocopherol, but not α -tocopherol, increase levels of PTEN and p53 pathway. The relationship between PTEN and p53 may be an important mechanism for the inhibition of tumorigenesis by γ and δ -tocopherol *in vivo* (46). Gupta et al (2015) investigated the effects of γ -tocopherol-rich mixture in two different animal models. The results showed that γ -tocopherol consuming increases PTEN expression (45). It seems that vitamin E causes over-expression of this gene in IIT + VE group, because it did not happen in IIT group. Cellular effects of vitamin E is mainly based on the antioxidant activity of this vitamin, but it has been shown that vitamin E, in addition to modulating various signaling pathways, is effective in expression of genes involved in cell proliferation and inflammatory processes (47). The mechanism by which vitamin E causes over-expression in PTEN is not yet

known. It is due to fact that lack of sufficient background on diagnosis of mechanisms involved in this positive regulation is difficult.

CONCLUSION

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

Perspectives

The main finding in this study was that the regular intermittent training can reduce the expression of p53 and PTEN tumor suppressor genes. On the other hand, nutrition is a very important factor to alter the expression of these tumor suppressor genes. Therefore, in order to gain optimum results, it is recommended that the optimum level of training with the use of antioxidant supplements should be used.

Acknowledgement

This study was conducted under support of University of Birjand and Shiraz University of Medical Sciences. Thereby, we appreciate Dr. Jamhiri, Mr. Amirzadehfard, and Mr. Moradpour as well as Mrs. Zeraatiannejad and Mrs. Nejabat for their cooperation in all phases of research.

Conflict of interest

The authors declare that there are no conflicts of interest.

REFERENCES

- [1] Ferlay J, Soerjomataram I, Dikshit R, Eser S, Mathers C, Rebelo M, et al. Cancer incidence and mortality worldwide: sources, methods and major patterns in GLOBOCAN 2012. *International journal of cancer*. 2015; 136(5) : E359-E86.
- [2] Peisch SF, Van Blarigan EL, Chan JM, Stampfer MJ, Kenfield SA. Prostate cancer progression and mortality: a review of diet and lifestyle factors. *World Journal of Urology*. 2016; 1-8.
- [3] Dong JT. Prevalent mutations in prostate cancer. *Journal of cellular biochemistry*. 2006; 97(3): 433-47.
- [4] Read A, Strachan T. Chapter 18: Cancer Genetics. *Human molecular genetics*. 1999; 2.
- [5] Zhang X, Miao X, Guo Y, Tan W, Zhou Y, Sun T, et al. Genetic polymorphisms in cell cycle regulatory genes MDM2 and TP53 are associated with susceptibility to lung cancer. *Human mutation*. 2006; 27(1): 110-7.
- [6] Han E-S, Muller FL, Pérez VI, Qi W, Liang H, Xi L, et al. The in vivo gene expression signature of oxidative stress. *Physiological genomics*. 2008; 34(1): 112-26.
- [7] Pietsch EC, Sykes SM, McMahon SB, Murphy ME. The p53 family and programmed cell death. *Oncogene*. 2008; 27(50): 6507-21.
- [8] Levine AJ. p53, the cellular gatekeeper for growth and division. *cell*. 1997; 88(3): 323-31.
- [9] Vousden KH, Lu X. Live or let die: the cell's response to p53. *Nature Reviews Cancer*. 2002; 2(8): 594-604.
- [10] Steck P. MA Pers house, SA Jas ser, WK Yung, H. Lin, AH Li gon, et al., Identifi ca tion of a can di date tumour sup pres sor gene, MMAC1, at chro mo some 10q23. 3 that is mutated in multiple advanced can cers. *Nat Genet*. 1997; 15: 356-62.
- [11] Blumenthal GM, Dennis PA. PTEN hamartoma tumor syndromes. *European Journal of Human Genetics*. 2008; 16(11): 1289-300.
- [12] Feilotter HE, Nagai MA, Boag AH, Eng C, Mulligan LM. Analysis of PTEN and the 10q23 region in primary prostate carcinomas. *Oncogene*. 1998; 16(13): 1743-8.
- [13] Phin S, Moore M, Cotter PD. Genomic rearrangements of PTEN in prostate cancer. *Frontiers in oncology*. 2013; 3:240.
- [14] Bargonetti J, Manfredi JJ. Multiple roles of the tumor suppressor p53. *Current opinion in oncology*. 2002; 14(1): 86-91.
- [15] Di Cristofano A, Pandolfi PP. The multiple roles of PTEN in tumor suppression. *Cell*. 2000; 100(4): 387-90.
- [16] Cristofano AD, Pesce B, Cordon-Cardo C, Pandolfi PP. Pten is essential for embryonic development and tumour suppression. *Nature genetics*. 1998; 19(4): 348-55.

- [17] Zhou BP, Liao Y, Xia W, Zou Y, Spohn B, Hung M-C. HER-2/neu induces p53 ubiquitination via Akt-mediated MDM2 phosphorylation. *Nature cell biology*. 2001; 3(11): 973-82.
- [18] Ogawara Y, Kishishita S, Obata T, Isazawa Y, Suzuki T, Tanaka K, et al. Akt enhances Mdm2-mediated ubiquitination and degradation of p53. *Journal of Biological Chemistry*. 2002; 277(24): 21843-50.
- [19] Downward J. Mechanisms and consequences of activation of protein kinase B/Akt. *Current opinion in cell biology*. 1998; 10(2): 262-7.
- [20] Sheng X, Koul D, Liu J-L, Liu T-J, Yung WA. Promoter analysis of tumor suppressor gene PTEN: identification of minimum promoter region. *Biochemical and biophysical research communications*. 2002; 292(2): 422-6.
- [21] Nakanishi A, Kitagishi Y, Ogura Y, Matsuda S. The tumor suppressor PTEN interacts with p53 in hereditary cancer (Review). *International journal of oncology*. 2014; 44(6): 1813-9.
- [22] Warburton DE, Nicol CW, Bredin SS. Health benefits of physical activity: the evidence. *Canadian medical association journal*. 2006; 174(6): 801-9.
- [23] Chilibeck P, Bell G, Farrar R, Martin T. Higher mitochondrial fatty acid oxidation following intermittent versus continuous endurance exercise training. *Canadian journal of physiology and pharmacology*. 1998; 76(9): 891-4.
- [24] Metin G, Atukeren P, GüMüSTAS MK, Belce A, Kayserilioglu A. The effect of vitamin E treatment on oxidative stress generated in trained rats. *The Tohoku journal of experimental medicine*. 2002; 198(1): 47-53.
- [25] Valenca SS, Bezerra FS, Romana-Souza B, Paiva RO, Costa AMA, Porto LC. Supplementation with vitamins C and E improves mouse lung repair. *The Journal of nutritional biochemistry*. 2008; 19(9): 604-11.
- [26] Malafa MP, Fokum FD, Mowlavi A, Abusief M, King M. Vitamin E inhibits melanoma growth in mice. *Surgery*. 2002; 131(1): 85-91.
- [27] Kramer JK, Blais L, Fouchard RC, Melnyk RA, Kallury KM. A rapid method for the determination of vitamin E forms in tissues and diet by high-performance liquid chromatography using a normal-phase diol column. *Lipids*. 1997; 32(3): 323-30.
- [28] Ziaaldini MM, Koltai E, Csende Z, Goto S, Boldogh I, Taylor AW, et al. Exercise training increases anabolic and attenuates catabolic and apoptotic processes in aged skeletal muscle of male rats. *Experimental gerontology*. 2015; 67:9-14.
- [29] Al-Jarrah M, Ahmad MB, Maayah M, Al-Khatib A. Effect of Exercise Training on the Expression of p53 and iNOS in the Cardiac Muscle of Type I Diabetic Rats. *Journal of Endocrinology and Metabolism*. 2012; 2(4-5): 176-80.
- [30] Qi Z, He J, Zhang Y, Shao Y, Ding S. Exercise training attenuates oxidative stress and decreases p53 protein content in skeletal muscle of type 2 diabetic Goto-Kakizaki rats. *Free Radical Biology and Medicine*. 2011; 50(7): 794-800.
- [31] Jiang H-K, Wang Y-H, Sun L, He X, Zhao M, Feng Z-H, et al. Aerobic interval training attenuates mitochondrial dysfunction in rats post-myocardial infarction: roles of mitochondrial network dynamics. *International journal of molecular sciences*. 2014; 15(4): 5304-22.
- [32] Bartlett JD, Joo CH, Jeong T-S, Louhelainen J, Cochran AJ, Gibala MJ, et al. Matched work high-intensity interval and continuous running induce similar increases in PGC-1 α mRNA, AMPK, p38, and p53 phosphorylation in human skeletal muscle. *Journal of applied physiology*. 2012; 112(7): 1135-43.
- [33] Gomes EC, Silva AN, Oliveira MRd. Oxidants, antioxidants, and the beneficial roles of exercise-induced production of reactive species. *Oxidative medicine and cellular longevity*. 2012; 2012.
- [34] Freitas M, Gomes A, Porto G, Fernandes E. Nickel induces oxidative burst, NF- κ B activation and interleukin-8 production in human neutrophils. *JBIC Journal of Biological Inorganic Chemistry*. 2010; 15(8): 1275-83.
- [35] Sen CK. Antioxidant and redox regulation of cellular signaling: introduction. *Medicine and Science in Sports and Exercise*. 2001; 33(3): 368-70.
- [36] Powers SK, Jackson MJ. Exercise-induced oxidative stress: cellular mechanisms and impact on muscle force production. *Physiological reviews*. 2008; 88(4): 1243-76.
- [37] Stambolic V, MacPherson D, Sas D, Lin Y, Snow B, Jang Y, et al. Regulation of PTEN transcription by p53. *Molecular cell*. 2001; 8(2): 317-25.
- [38] Cadet J, Douki T, Ravanat J-L. Oxidatively generated base damage to cellular DNA. *Free Radical Biology and Medicine*. 2010; 49(1): 9-21.
- [39] D'Errico M, Parlanti E, Dogliotti E. Mechanism of oxidative DNA damage repair and relevance to human pathology. *Mutation Research/Reviews in Mutation Research*. 2008; 659(1): 4-14.
- [40] Evans MD, Dizdaroglu M, Cooke MS. Oxidative DNA damage and disease: induction, repair and significance. *Mutation Research/Reviews in Mutation Research*. 2004; 567(1): 1-61.

- [41] Totter JR. Spontaneous cancer and its possible relationship to oxygen metabolism. *Proceedings of the National Academy of Sciences*. 1980; 77(4): 1763-7.
- [42] Ashton T, Young IS, Peters JR, Jones E, Jackson SK, Davies B, et al. Electron spin resonance spectroscopy, exercise, and oxidative stress: an ascorbic acid intervention study. *Journal of Applied Physiology*. 1999; 87(6): 2032-6.
- [43] Bailey DM, Young IS, McEneny J, Lawrenson L, Kim J, Barden J, et al. Regulation of free radical outflow from an isolated muscle bed in exercising humans. *American Journal of Physiology-Heart and Circulatory Physiology*. 2004; 287(4): H1689-H99.
- [44] Bailey DM, Lawrenson L, Mceneny J, Young IS, James PE, Jackson SK, et al. Electron paramagnetic spectroscopic evidence of exercise-induced free radical accumulation in human skeletal muscle. *Free radical research*. 2007; 41(2): 182-90.
- [45] Gupta SD, Sae-Tan S, Wahler J, So JY, Bak MJ, Cheng LC, et al. Dietary γ -Tocopherol-Rich Mixture Inhibits Estrogen-Induced Mammary Tumorigenesis by Modulating Estrogen Metabolism, Antioxidant Response, and PPAR γ . *Cancer Prevention Research*. 2015; 8(9): 807-16.
- [46] Smolarek AK, So JY, Burgess B, Kong A-NT, Reuhl K, Lin Y, et al. Dietary Administration of δ -and γ -Tocopherol Inhibits Tumorigenesis in the Animal Model of Estrogen Receptor-Positive, but not HER-2 Breast Cancer. *Cancer Prevention Research*. 2012; 5(11): 1310-20.
- [47] Azzi A. Molecular mechanism of α -tocopherol action. *Free Radical Biology and Medicine*. 2007; 43(1): 16-21.