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The Effect of Interval Training Program on Nuclear Factor Erythroid-Derived 2-like 2 (NFE2L2/Nrf2) Gene Expression in Women

Aralıklı Antrenman Programının Kadınlarda NFE2L2/Nrf2 Gen İfadesine Etkisi

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ABSTRACT

Purpose in this work, to investigate whether interval training program has an effect on Nuclear factor erythroid-derived 2-like 2 (NFE2L2/Nrf2) gene expression in women.The research was made on 12 women. Participants were given a medium-term interval training program for 8 weeks, 3 days a week. The blood samples of the participants were collected before and after the 8 weeks of training. RNA isolation was performed using TRIzol Reagent from peripheral blood mononuclear cells. NFE2L2 gene expression was determined by Biomark Real-Time PCR (RT-PCR). The participants was a significant increase in heart rate and maximal oxygen use capacity (VO2 max) after the exercise ($p < 0.001$). There was a significant decrease in the body weight and body mass index of women after the exercise ($p < 0.001$). There was a decrease in NFE2L2 gene expressions after 8 weeks of the training program ($p < 0.05$,). It shows that interval exercise reduces NFE2L2 gene expression in women.

Key Words

Exercise, gene expression, NFE2L2 gene, interval training.

ÖZ

Aralıklı antrenman programının bayanlarda Nuclear factor erythroid-derived 2-like 2 (NFE2L2/Nrf2) gen ekspresyonuna etkisinin olup olmadığını araştırmaktır. Araştırma 12 kadın üzerinde yapıldı. Katılımcılara, haftada 3 gün olmak üzere 8 hafta süreyle orta süreli interval antrenman programı uygulandı. 8 haftalık antrenmanlar öncesi ve sonrası kan örnekleri alındı. Periferik kan mononükleer hücrelerinden TRIzol Reaktifi kullanılarak RNA izolasyonu yapıldı. NFE2L2 gen ekspresyonu Biomark Real-Time PCR (RT-PCR) ile belirlenmiştir. Çalışmaya katılan katılımcıların maksimal oksijen kullanma kapasitesinde (VO2 Max) egzersiz sonrası önemli ölçüde artış bulundu ($p<0.001$). Kadınların vücut ağırlıkları ve vücut kitle indeksinde azalma olmuştur ($p<0.001$). NFE2L2 gen ifadelerinde 8 haftalık antrenman programı öncesine göre sonrasında azalma olmuştur ($p<0.05$,). Aralıklı yapılan egzersizin bayanlarda NFE2L2 gen ekspresyonunu azalttığını işaret etmektedir.

Anahtar Kelimeler

Egzersiz, gen ekspresyonu, NFE2L2, aralıklı antrenmanlar.

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INTRODUCTION

According to many studies, it is reported that the increase in the physical activities of the people expands life-span by reducing the risk of death. Regular physical activity contributes to the treatment of various chronic diseases, especially lung, heart diseases, hypertension, metabolic disorders, especially type 2 diabetes and obesity, muscle, bone, joint injuries, cancer and neuropsychiatric disorders [1,2]. However, the type, intensity of exercise, competitions, traumas, and stress lead to both physiological and metabolic changes in the human body [3]. During the prolonged/intense exercise, the amount of consumed oxygen varies depending on the type and severity of the exercise varies, but generally by increasing in accordance with relaxation leads to the formation of free radicals (reactive oxygen species, ROS) that cause oxidative stress. ROSs are consistently built up in the body, but physical exercise leads to further increases in ROSs and cannot be removed sufficiently by the antioxidant defense system. They cause many damages by reacting with various molecules such as nucleic acids, proteins, membranes, cells and tissues [4,5]. Mitochondria, which participate dynamically in various muscle cell activities along with death, autophagy, differentiation, are generally accepted as the main source of reactive oxygen species (ROS) in skeletal muscles [6]. Some studies have emphasized that the ROSs, that emerged as a result of the reduction of oxygen in the normal metabolic stages, are not only the toxic. The significance of exercise in the process of adaptation, which is the most important impact on the human body, is specifically emphasized. In these studies, it is indicated that the increase in the levels of ROSs minimizes the damage by increasing the activity of the antioxidant enzymes during regular exercise, and emphasized that ROSs give more harm to the unprepared tissues [7,8]. Besides, ROSs play an important role in regulating the cell signaling and gene expression [9,10]. The transcription factor NFE2L2 is the main organizer of antioxidant defenses, regulating more than 200 cytoprotective genes as a response to oxidative stress. It is also reported that nuclear factor erythroid-derived 2-like 2 (NFE2L2), acute oxidative and nitrosative stress regulate antioxidant response and mitochondrial biogenesis. In addition, they also noted that NO and ROSs, that is produced through exercise, activated skeletal muscle NFE2L2 and required the NFE2L2 expression for normal mitochondrial biogenesis, acute exercise and antioxidant transcriptional response during regular training. The studies reported that NFE2L2 activators protect mice against metabolic diseases and prolong the lifespan of C. Elegans and M. Drosophila [11]. The purpose of this study is to investigate the effect of interval training program on Nuclear factor erythroid-derived 2-like 2 (NFE2L2/Nrf2) gene expression in women.

MATERIALS and METHODS

Participants:The research was made on 12 women who have similar ages and physical characteristics that did not exercise regularly, active, non-smoker, did not take any of food supplement and did not have any health problem, average of age was 21.88±2.44 years, average of height was 162.13±5.83 cm, and the average of weight was 58.60± 2.04 kg. Our study protocol was approved by Erciyes University Ethics Committee. Our work was conducted under the direction of Declaration of Helsinki and local laws.

Experimental Design

Maximal Aerobic Capacity (VO2 max): Maximal oxygen use capacities were determined proportionately (body weight / lean body mass / ml / kg / min) by applying the Bruce Test Protocol, which was performed before the training and at the end of the 8-week training.

Training Protocol: At the beginning of the study, participants were asked not to take any medication or nutritional supporters for 8 weeks, to avoid heavy physical activity 48 hours before the training and to be hungry at least 3 hours before the test time. Two days after the maximal oxygen use capacities were determined, 8-week training programs were started. Training Program: Participants were given a medium-duration interval training program in 3 days a week, for 8 weeks. The severity of the study was determined according to the volunteers' target heart rate (%90-95). Participants were received height, weight, systolic-diastolic blood pressure, heart rate and 10 ml peripheral blood samples with EDTA tubes before and after 8 weeks of exercise.

RNA Isolation and gene expression studies

2 ml venous blood samples were taken from the participants for the gene expression study. RNA samples were stored at the -80°C until analysis. Firstly, peripheral blood mononuclear cells were isolated using standard methods [12,13]. RNA isolation was performed using TRIzol Reactive (TRIzol, Roche, Almanya) from peripheral blo-

Table 1. Some of the physical and physiological characteristics of the participants.

Variables		BE.Avr±SD	AE.Avr±SD	t	P
Mass (kg)	(n=12)	58.60±2.04	56.24±1.65	4.2	0.001**
BMI (kg/m^2)	(n=12)	22.29±2.43	21.37±1.98	4.5	<0.001**
Heart Rate(pulse/min)	(n=12)	86.70±6.7	98.90±7.98	5.3	0.001**
Systolic H.P (mmHg)	(n=12)	114.90±19.9	107.70±12.17	1.2	0.269
Diastolic H.P (mmHg)	(n=12)	78.60±9.78	73.20±5.29	0.8	0.431
VO2 Max	(n=12)	35.74±2.5	46.16±3.25	5.1	0.001**

Paired Samples T Test / SD.: Standard Deviation / *p<0.05 **p<0.001

B.E.= Before Exercise A.E.= After Exercise B.M.I.= Body Mass Index

od mononuclear cells. The amount and quality of RNA samples were measured with a NanoDrop 2000 Spectrometer (Thermo Scientific, Waltham, MA). A reverse transcription polymerase chain reaction (RT-PCR) was used to detect the gene expressions through syntheses of the complementary DNA (cDNA) transcripts from the total RNA extracts. Complementary DNA (cDNA) was obtained from the RNA using RT2 HT First-Strand (Qiagen) kit. When complementary DNA was synthesized, it was left to incubate for 5 min at 95°C and for 15 min at 42°C. Nuclear factor erythroid-derived 2-like 2 (NFE2L2) gene expression was determined by Biomark Real-Time PCR (RT-PCR). Biomark Real-Time PCR (Qiagen) instrument was used for the expression study. The expression study was incubated at 95°C for 15 sec and 60°C for 60 sec through 95°C for 10 min and 40 cycles. Glyceraldehyde-3-phosphate dehydrogenase (GAPDH) was selected as the house keeping gene [14,15] .The data were collected with the Fluidigm Real-Time PCR analysis software and quantification cycle (Cq) values of 999. Cq values larger than 23 were removed since these readings were unreliable. The delta delta Ct (2-ΔΔCT) method was applied for the relative quantification of the samples that normalized with Glyceraldehyde-3-phosphate dehydrogenase [12]. Genetic studies were carried out at Erciyes University Genomic and Stem Cell Center.

Statistical Analysis

The Comparisons were performed using two independent sample T-test, the Mann-Whitney U test, for quantitative data and calculated gene expression values. The statistical significance level was taken as p <0,05.

Results

Some of the physical and physiological characteristics and the blood samples of the 12 participants, who participated in the study, were prepared and evaluated according to the methods mentioned in the related section. The average age of the participants was 21.88±2.44 years, and the average height was 162.13±5.83 cm (Table.1.).

There was no difference in systolic and diastolic blood pressures (mmHg) at the beginning and end of the 8-week training programs of participants attended the study (p>0.05 Table.1).

According to the statistical analysis results, there was a significant increase in heart rate and maximal oxygen use capacity (VO2 max) after exercise (p<0.001 Table.4.1). There was a significant reduction in body weight (p <0.001) and body mass index of women after the exercise (p<0.000).

The change in the Nuclear factor erythroid-derived 2-like 2 (NFE2L2) gene expression, performed in the analyses by the blood samples taken before and after the 8-week training program, is shown in the table below.

RESULTS and DISCUSSION

It is known that acute exercise systematically causes to oxidative, metabolic, mechanical and thermal stresses. It is reported that even a single exercise increases oxidative stress bioindicators in almost all tissues. ROSs contain one or more unpaired molecules with high energies. Normal cell metabolism converts about 1-2% oxygen molecules to ROSs.

This ROS is potentially toxic. Although there are significant contributions to the regulation of normal physical activities, such as ROS muscular contraction, the signifi-

Table 2. Female NFE2L2 gene expression change before and after the 8-week training program.

Gene	BE-W Median - IQR	8w.AE-W Median - IQR	P-value*
NFE2L2	15.807±2.986	12.11±1.087	<0.004

Mann Whitney U test / IQR: Interquartile range *p<0.05 **p<0.001

B.E.= Before Exercise A.E.= After Exercise W= Woman

There was a decrease in female NFE2L2 gene expressions after the 8-week training program than before (p<0.05, **p<0.001, Table-2.).

cant increases in the concentrations of ROS may disrupt normal cell function and cause to oxidative damage of various biomolecules (protein, lipid) and cellular DNA [16-19]. Due to its role in the organ, tissue damage and the aetiopathogenesis of various diseases, ROS has been a growing area of interest in medicine recently. NFE2L2 or Nrf2 regulates basal antioxidant and antioxidant reactions against stress in various organisms from ferment to Caenorhabditis elegans and mammals. The cell culture studies using C2C12 skeletal muscle cells report that Nrf2 is activated by ROS. In a study of wild-type mice, it was emphasized that a single acute exercise increased Nrf2 proteins, Nrf2-bound phase II enzymes and Nrf2 gene expression in the skeletal muscle [20,21]. Horie et al. used an electrical impulse stimulation (EPS) to imitate acute exercise and emphasized that Nrf2 expression is related to both intensity and duration of the stimulus [22]. Similar to the cell culture studies, studies on some animals showed that increases in Nrf2 signalization depend on the duration of exercise. Less than one hour of running treadmill exercise was reported to have no effect on Nrf2 mRNA or protein expression [23,24,]. The data on the NFE2L2 signal related to exercise is still very limited in humans, but a recent study indicated that acute exercise increases Nrf2 proteins at all cellular levels measured in peripheral blood mononuclear cells (PBMCs) in young and old men. Again in the same study, nuclear accumulation of Nrf2 was observed only in the young group and emphasized that aging may be related to Nrf2 disruption. Similarly, in middle-aged women, who do regular exercise, Nrf2 mRNA increased significantly 2 hours after 30 minutes of moderate treadmill exercise, and sedentary women did not show any change in Nrf2 gene expression as a response to exercise [25]. This may suggest that sport may play a role in preserving the response of acute Nrf2 and aging. The impact of regular exercise training on the NFE2L2 response is investigated in the studies more extensively than the acute exercise. It is showed that medium-intensity or high-intensity interval training for 4-24 weeks activates Nrf2 signalization in several tissues, including skeletal muscle, kidney, brain, liver, testicle, prostate and myocardium in rodents. It is stated that regular exercise in rats prevents the normal level of Nrf2 from falling due to the oxidative stress. At the same time, it is also emphasized that antioxidant supplement weakened the normal activation of NFE2L2 during the exercise [26] .

Gomes et al. [27] had Sprague-Dawley rats instructed to exercise using a jumping protocol with a weighted vest as a resistance training. While no change in Nrf2 expression was observed in young animals, a decrease in Nrf2 was indicated in elderly animals subjected to the same training, and this difference was initially explained by highness in the Nrf2 expression in elderly animals. There are contradictory results about NFE2L2 gene expression in the studies. In some studies; increasing with exercise, a decrease, and no difference is detected. It is emphasized that these different results in the studies affected by the age of the subjects and periods of sampling after the exercise [28]. In our study, it was found that NFE2L2 gene expression in females decreased after the regular exercises compared to basic level. This make us consider that the reduction resulted from the time interval in blood sampling, the initially high level of NFE2L2 and the duration, intensity and severity of the exercise.

It is indicated that regular exercise reduces the Nuclear factor erythroid-derived 2-like 2 (NFE2L2) gene expression in women. Both animal studies and human studies are almost limited to male subjects only. We believe that different exercise protocols and taking samples at different time intervals may be important to a better explanation of this variation, activation of NFE2L2 and to catch different reaction times and general variation of NFE2L2 in females

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References

1. J.N. Stathopoulos , J. G.Tzanninis , A.A. Philippou, M. Koutsilieris, Epigenetic regulation on gene expression induced by physical exercise. J. Musculoskelet. Neuronal. Interact.,13 (2013) 133-146.
2. P. Meier , M. Renga , H. Hoppeler, O. Baum, The impact of antioxidant supplements and endurance exercise on genes of the carbohydrate and lipid metabolism in skeletal muscle of mice, Cell Biochem. Funct., 31 (2013) 5-159.
3. G. Bangi, M. Delfabbro, C. Maurş, M. Corsi, G.Melegati. Haematological parameters in elite rugby players during a competitive season. Clin. Lab. Haem., 28 (2000) 183-188
4. Z. Rodak, J. Pucsuk, S. Boros, L. Josfai, A.W. Taylor, Changes in ürine 8-hytdroxydeoxyguanozine levels of super-marathon runners during a four-day race period, Life Sci., 66 (2000) 1763-1767.
5. Z. Rodak, T. Kaneko, S. Tahara ,H. Nakamoto, H. Ohno, M. Sasvari, C. Nyakas, S. Goto, The effect of exercise training on oxidative damage of lipids, proteins, and DNA in rat skeletal muscle: evidence for beneficial outcomes, Free Radic. Biol Med., 27 (1999) 69-74.
6. L. Packer, E. Cadenas, K.J.A. Davies, Free radicals and exercise: an introduction, Free Radic. Biol. Med., 44 (2008) 123–125.
7. G. Gandhi, G. Gunjan, Exercise-Induced Genetic Damage: A Review, Int. J. Hum. Genet., 9 (2009) 69-96
8. Z. Radak , Y.H. Chung , E. Koltai , A.W. Taylor, S. Goto, Exercise, oxidative stress and hormesis, Ageing Res. Rev., 7 (2007) 9-170.
9. M.C. Gomez-Cabrera, E. Domenech. J. Vina, Moderate exercise is an antioxidant: upregulation of antioxidant genes by training, Free Radic. Biol. Med., 44 (2008) 126-31.
10. C.L. Saw, Q. Wu, A.N. Kong, Anti-cancer and potential chemopreventive actions of ginseng by activating Nrf2 (NFE2L2) anti-oxidative stress/anti-inflammatory pathways, Chinese Medicine, 27(2010) 5-37
11. C. Nilsson, S. Aboud, K. Karlen, B. Hejdeman, W. Urassa, Optimal blood mononuclear cell isolation procedures for gamma interferon enzyme-linked immunospot testing of healthy Swedish and Tanzanian subjects, Clin. Vaccine Immunol., 15 (2008) 585-589.
12. F. Bayram, H. Diri, E. F Sener, M. Dundar, Y. Simsek, Genetic expressions of thrombophilic factors in patients with Sheehan's syndrome, Gynecol. Endocrinol., 32 (2016) 908-911
13. B. Jemiolo, S. Trappe, Single muscle fiber gene expression in human skeletal muscle: validation of internal contro lwith exercise, Biochem. Biophys. Res. Commun., 320 (2004) 1043-1050.
14. M. Catoire, M. Mensink, W.V. Boekschoten, M.Müller, P. Schrauwen, S.Kersten, Pronounced effects of acute endurance exercise on gene expression in resting and exercising human skeletal muscle, PloS One 7 (2012) e51066.
15. Z. Radak, S. Kumagai, A.W. Taylor, H. Hisashi, S. Goto, Effects of exercise on brain function: role of free radicals, Appl. Physiol. Nutrition., Metabol., 32 (2007) 942-946.
16. T.L Merry, M. Ristow, Nuclear factor erythroid-derived 2-like 2 (NFE2L2, Nrf2) mediates exercise-induced mitochondrial biogenesis and the anti-oxidant response in mice, J. Physiol., 594 (2016) 5195-5207
17. K. Nakatani, M. Komatsu, T. Kato, T. Yamanaka, H. Takekura, Habitual exercise induced resistance to oxidative stress, Free Radical. Res., 39 (2005) 905-911,
18. H. Orhan, B. Van Holland, B. Krab, J. Moeken, N.P.Vermeulen, J.H. Meerman, Evaluation of a multiparameter biomarker set for oxidative damage in man: Increased urinary excretion of lipid, protein and DNA oxidation products after one hour of exercise, Free Radical. Res., 38 (2004) 1269-1279.
19. R.J. Bloomer, A.H. Goldfarb, J.M. Mckenzie, Oxidative stress response to aerobic exercise: Comparison of antioxidant supplements, Med. Sci. Sports Exerc., 38 (2006) 1099-1105
20. M. Horie, E. Warabi, S. Komine, S. Oh, J. Shoda, Cytoprotective role of Nrf2 in electrical pulse stimulated C2C12 myotube, PLoS One., 10 (2015) e0144835.
21. P.C.G. Wang, Z. Li, D. Qi, S. Ding, Acute exercise stress promotes Ref/Nrf signaling and increases mitochondrial antioxidant activity in skeletal muscle, Exp. Physiol., 101 (2015) 410-20.
22. A.J. Done, M.J. Gage, N.C. Nieto, T. Traustadottir, Exercise-induced Nrf2-signaling is impaired in aging, Free Radic. Medic.,96 (2016) 130-138.
23. M. Narasimhan, J. Hong, N. Atieno, V.R. Muthusamy, C.J. Davidson, N. Abu-Rmaileh, R. Richardson, A.V. Gomes, J.R. Hoidal, N.S. Rajasekaran, Nrf2 deficiency promotes apoptosis and impairs PAX7/MyoD expression in aging skeletal muscle cells, Free Radic. Biol. Med.,71 (2014) 402–414.
24. I. Bos, P. De Boever, L. Int Panis, S.Sarren, R. Meeusen, Negative effects of ultrafine particle exposure during forced exercise on the expression of Brain-Derived Neurotrophic Factor in the hippocampus of rats, Neuroscience, 223 (2012) 131–139.
25. T. Li, S. He, S.Liu, Z. Kong, J. Wang, Y. Zhang, Effects of different exercise durations on Keap1-Nrf2-ARE pathway activation in mouse skeletal muscle, Free Radic. Res., 49 (2015) 1269-1274.
26. H.A. Scott, J.R. Latham, R. Callister, J.J. Pretto, K. Baines, N. Saltos, J.W. Uphan, L.G. Wood, Acute exercise is associated with reduced exhaled nitric oxide in physically inactive adults with asthma, Ann. Allergy Asthma Immunol., 114 (2015) 470-479.
27. F.C. Gomes, L.G. Chuffa, W.R. Scarano, P.F.F. Pinheiro, W.J.Favaro, R.F. Domeniconi, Nandrolone decanoate and resistance exercise training favor the occurrence of lesions and activate the inflammatory response in the ventral prostate, Andrology, 4 (2016) 473-480.
28. J. Done Aaron, T. Tinna, Nrf2 mediates redox adaptations to exercise, Redox. Biol., 10 (2016) 191-199.