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The Effect of Different Programs of Exercise on The Expression of Genes Associated with Endurance and Energy Metabolism*

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Abstract

Physical exercise promotes the energy metabolism of the body. While physiological changes occuring in human body after regular exercise is well defined, it is not known how the body's response changes during the time course of the exercise. Here, we investigated how the acute and chronic exercise alters expressions of genes related to energy metabolism and endurance. Our study investigated the effects of acute and chronic exercise on the expression of genes related to energy metabolism (*AMPD1, PPARA*) and endurance (*ADRB2*). Study group; was formed with 24 people: 12 healthy females and 12 healthy males. Maximal oxygen use capacities of the participants were determined by the Bruce test protocol at the beginning and end of the 8-week training program. After calculating their maximal oxygen use capacity, each participant was given an acute running exercise on the tread mill at the speed and incline that the participant would reach to his/her maxVO_2 until he/she exhausted. The same people were built to continuous runs (%50-70) once every 8 weeks, and two days of medium-term interval training program (%90-95). Peripheral blood samples were taken before and after acute exercise and immediately after chronic exercises. RNA isolation was performed using TRIzol Reagent from peripheral blood mononuclear cells. Gene expression was determined by Biomark Real-Time PCR (RT-PCR). Gene expression data was quantified by using both t-test and Mann-Whitney U tests. The statistical level of $p < 0.05$ was taken. Our results show that *ADRB2* and *AMPD1* gene expression values increase in women after acute exercise. There were changes in the mean values of *ADRB2, AMPD1, PPARA* gene expressions in both men and women after the 8-week training program compared to pre-acute exercise. Expression of *PPARA* gene significantly decreased after exercise compared to pre-exercise only for the female group. This study is important in developing ideas about gene expressions of genes related to energy training and endurance with different selections and different exercise programs. Our results; this suggests that different training programs on different genders are important in terms of giving an idea about the gene expressions of genes related to energy metabolism and endurance.
Keywords: Exercise, Gene Expression, Energy Metabolism, Endurance

Farklı Egzersiz Programlarının Dayanıklılık ve Enerji Metabolizması ile İlişkili Genlerin İfadesine Etkisi

Öz

Fiziksel egzersiz vücudun enerji metabolizmasını destekler. Düzenli egzersiz sonrası insan vücudunda meydana gelen fizyolojik değişiklikler iyi tanımlanmış olmasına rağmen, egzersiz sırasında vücudun tepkisinin nasıl değiştiği bilinmemektedir. Çalışmamızda enerji metabolizması (*AMPD1, PPARA*) ve dayanıklılık (*ADRB2*) ile ilgili genlerin ekspresyonu üzerine akut ve kronik egzersizin etkileri araştırıldı. Çalışma grubu, 12 kadın, 12 erkek toplam 24 kişiden oluşturuldu. Çalışmaya katılan kişilerin maksimal oksijen kullanma kapasiteleri, 8 haftalık antrenman programlarının başında ve sonunda Bruce test protokolü ile oransal olarak belirlendi. Maksimal oksijen kullanma kapasiteleri hesaplandıktan sonra, her katılımcıya koşu bandında kendi maksVO_2'sine ulaştığı hız ve eğimde, tükenene kadar akut koşu egzersizi yaptırıldı. Aynı kişilere 8 hafta boyunca haftada bir gün sürekli koşular (%50–70), iki gün orta süreli interval antrenman programı (%90-95) uygulandı. Akut egzersiz öncesi ve sonrası ile kronik egzersizlerden hemen sonra periferik kan örnekleri alındı. TRIzol yardımıyla RNA izolasyonu yapıldı. Kantitatif Real Time PCR cihazı ile mültipleks olarak genlerin ekspresyonu belirlendi. Karşılaştırmalar hesaplanmış gen ekspresyon değerleri ile nicel veriler için bağımsız iki örneklem t testi, Mann-Whitney U kullanılarak yapıldı. İstatistiksel anlamlılık düzeyi $p<0,05$ olarak alındı. Sonuçlarımız, akut egzersiz sonrası kadınlarda *ADRB2* ve *AMPD1* gen ekspresyon değerlerinin arttığını göstermektedir. Akut egzersiz öncesine göre 8 haftalık antrenman programından sonra hem erkek hem de kadınlarda *ADRB2, AMPD1, PPARA* gen ekspresyonlarının ortalama değerlerinde değişiklikler oldu. *PPARA* geninin ekspresyonu, sadece kadın grubu için egzersiz öncesi ile karşılaştırıldığında egzersizden sonra önemli ölçüde azalmıştır. Sonuçlarımız; farklı cinsiyetler üzerinde yapılan farklı antrenman programlarının enerji metabolizması ve dayanıklılık ile ilgili genelerin, gen ekspresyonları hakkında fikir vermesi konusunda önemli olduğunu düşündürmektedir.
Anahtar kelimeler: Egzersiz, Gen İfadesi, Enerji Metabolizması, Dayanıklılık

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INTRODUCTION

Genetics is a rapidly developing scientific discipline which influenced our world since its foundation with a vast spectrum. Researchers has recently started using genetic tools in the determination of sports skills. Physical performance is influenced by environmental factors such as nutrition, lifestyle, and climate and genetics. Therefore, athletic success is determined by the combination of these factors that involve genetics, epigenetics, training, nutrition, motivation, and other environmental conditions. Genetic traits are influenced by many factors, such as power, endurance, flexibility, coordination, muscle fiber size, temperament, and physical performance. Recently, the development of technology for genotyping and rapid DNA sequencing has led to the recognition of individual genetic variations that contribute to athletic performance (Ahmetov & Fedotovskaya, 2012). The beta-2 adrenegeric receptor (*ADRB2*), adenosine monophosphate deaminase 1 (*AMPD1*), peroxisome proliferator activating receptor (*PPAR*) -delta (broad *PPARD*) and PPAR-gamma coactivator (*ADRB2*) genes, which are associated with physical performance through rapid development of molecular investigations in sport 1 alpha (broad *PPARGC1A*) genes have been discovered (Ginevičienė, Jakaitiene, Tubelis & Kucinskas, 2014). It was reported that genes associated with muscle mass, muscular strength, exercise response to exuberant carbohydrate and fat metabolism, exercise intolerance, cardiovascular and respiratory fitness (Bray et al., 2009). The energy support of muscle activity is one of the factors that determines the work performance of a person. Adenosine monophosphate deaminase (*AMPD*) is an important regulator of energy metabolism in muscles during exercise (Lippi, Longa & Maffulli, 2010). Muscle energy metabolism plays an important role by stimulating the specific skeletal adenosine deaminase isoform (AMP), which is encoded by the adenosine monophosphate deaminase 1 (*AMPD1*) gene, after a short period of high-intensity exercise. Exercise has been shown to regulate the expression of genes encoding various enzymes in muscles and other tissues. Genetic research in sport helps to clarify various aspects of human biology and physiology such as regulation of protein levels under various conditions and RNA (Duniec, 2013). Despite the well-defined physiological reactions in the human organism after regular exercise, genetic backgrounds are still not well known. There are many ongoing research efforts to elucidate the effects of genetic regulations on exercise (Ginevičienė et al., 2014). Nowadays, with the development amd implementation of genetic technologies in many fields, it becomes increasingly important to carry out studies in sport and sports. In addition to inheritance, both structural and functional aspects of the body such as sports stimuli of individuals, sports training, sport traumatology, genetic doping provide surprising developments in the field of sports (Maciejewska, Sawczuk & Cieszczyk, 2011). Increased genetic studies may provide an advantage in physiology, morphology, athlete injuries, and perhaps sports psychology. They can help to clarify various aspects of human biology and physiology by explaining how the expression of genes that encode various enzymes and tissues in muscle are regulated during exercise training. It is reported that physical exercise can lead to changes in the expression of many human eukaryotic genes. However, it is still not known which mechanisms regulate the expression of genes involved in metabolic stress or metabolic adaptation (Pareja-Galeano, Sanchis-Gomar & García-Giménez, 2014).

The aim of this project is to investigate the effect of objective exercise on possible changes in the expression of selected genes related to energy metabolism (*AMPD1, PPARA*) and endurance (*ADRB2*). Thus, we investigated whether the increase or decrease in expression levels of these genes are related to exercise.

METHODS

Participants

The research was conducted with total 24 healthy people (12 females and 12 males. Our study protocol was approved by Erciyes University Ethical Committee (201268) and the study was conducted in accordance with Helsinki Declaration and local law.

Maximal Aerobic Capacity (VO_2 Max): The maximal oxygen uptake capacities were determined proportionally by applying the Bruce Test Protocol (Body weight, Lean body mass/ ml/kg/min) (Bruce et al., 1949) . The first blood sample was taken to measure the resting values of the volunteers after calculating the amount of oxygen consumed (VO_2 max) and 2 days later. After 10 minutes of warm-up and stretching exercise, maximal exercise running test was applied. Maximal exercise running test: According to the Bruce Test Protocol, each participant did the running exercise until it was exhausted, at the speed and slope it reached to VO_2 max. Exercise participants It was completed by taking its own declarations and looking at the target heart rate (220-age). Blood samples were taken after exercise to determine the effect of acute exercise.

Training Program: The participants took part in the training program 3 days a week for 8 weeks.

Continuous-Running Training Method: The participants were given a running exercise 1 day per week for 8 weeks between 25-60 min with 50-70% of the target heart rate.

Medium Term Interval Training Program: The participants were given medium-interval training program 2 days a week, for 8 weeks. The intensity of the training was determined depending on the heart rates of the volunteers (90-95%). The heights, weights, systolic and diastolic blood pressures and heart rates of the participants were recorded. Before and after the maximal exercise protocol, 10 ml peripheral blood samples in tubes having EDTA were taken.

RNA Isolation and Gene Expression Studies: Genetic studies were carried out at Erciyes University Genome and Stem Cell Center. RNA was isolated from 2ml blood samples taken from each group (TRIzol, Roche, Germany) (Catoire et al., 2012; Jemiolo & Trappe, 2014). The quality and quantity of RNA was measured by BioSpec-Nano Spectrometer. Complementary DNA (cDNA) was obtained from RNA by RT2 HT First Strand (Qiagen, Hilden, Germany) kit. cDNA was synthesized at 42°C 15 min and 95°C 5 min incubations. Gene expressions were determined by Biomark Real-Time PCR (RTPCR). Biomark Real-Time PCR (Qiagen, Hilden, Germany) was used for expression study. While expression study was being held, it was incubated at 95°C 10 min and throughout 40 cycles 95°C 15 sec, 60°C 60 sec. Delta delta Ct method ($2^{-\Delta\Delta CT}$) was used for the relative quantification of the samples (Catoire et al., 2012; Jemiolo & Trappe, 2014).

Statistical Analysis

Data were collected with Biomark Real Time PCR analysis software using linear baseline correction method and auto global Cq threshold method. Briefly, ΔCT was calculated by extracting the expression of each gene of interest from control gene expression. The maximum ΔCT value of that gene was then subtracted from each sample ΔCT value for each gene. ΔΔCT values were obtained at this point. Gene expression values are calculated by the following formula: $log_2(2^{-\Delta\Delta CT} + 1)$. GeNORM was used to evaluate the expression stability of the genes. Data normalization was performed by using the $2^{-\Delta\Delta CT}$ method. For all binary comparisons were performed using the Mann-Whitney U test with calculated gene expression values. Statistical significance level was taken as p <0, 05.

FINDINGS

Some physical and physiological characteristics of 24 healthy subjects, 12 male and 12 female, participated in the study and their blood samples were prepared and evaluated according to the methods mentioned in the related section. The average age and height of the participants in the study was 21.88 ± 2.44 years for females and 23.8 ± 4.1 years for males and 162.13 ± 5.83 cm for females and 174.7 ± 6.9 cm for males, respectively. More physical and physiological characteristics of the participants are given in Table 1.

Table 1. Some physical and physiological characteristics of the participants

Variables	Groups	Before Exercise Avg±SD	After Exercise Avg±SD	t	p
Weight (kg)	Female (n=12)	58.60 ± 2.04	56.24±1.65	4.2	**0.001****
	Male (n=12)	72.1 ± 9.7	71.3± 9.5	1.6	0.116
BMI (kg/m^2)	Female (n=12)	22.29 ± 2.43	21.37±1.98	4.5	**<0.001****
	Male (n=12)	23.5 ± 2.5	23.3±2.3	1.6	0.117
HeartRate (rate/min)	Female (n=12)	86.70 ± 6.7	98.90±7.98	5.3	**0.001****
	Male (n=12)	81.60 ± 8.4	98.20±6.32	5.1	**0.001****
VO2 max	Female (n=12)	35.74±2.5	46.16±3.25	5.1	**0.001****
	Male (n=12)	51.8 ± 4.7	56.5 ± 3.3	4.8	**0.001****

* Paired Samples T Test / SD.: Standard deviation / *p<0.05 **p<0.001
** B.E.=Before Exercise A.E.=After Exercise BMI= Body Mass Index

According to the statistical analysis, it was found that heart rates significantly increased both in females and males after exercise (p<0.001, Table 1). Female participants showed significant decrease in body weights (p<0.001) and body mass index (p<0.001) following exercise, whereas no significant difference was found in male participants (p>0.05).

Table 2. Change in gene expression before and after acute maximal exercise protocol in females and males

Gene	Groups	BE- Median - IQR	AE- Median - IQR	p-value*
ADRB2	Female	11.315 ± 2.768	12.489 ± 0.811	**<0.001****
	Male	12.696 ± 0.665	12.708 ± 0.759	0.630
AMPD1	Female	0 ± 13.634	14.791 ± 16.948	**0.033***
	Male	16.906 ± 2.95	16.689 ± 1.719	1.000

* Mann Whitney U test / IQR: Interquartile range *p<0.05 **p<0.001
** B.E.= Before Exercise, A.E.= After Exercise

The mean values of the gene expression before and after acute exercise are shown in Table 2. *ADRB2* and *AMPD1* gene expression values in females increased after acute exercise ($p<0.001$, $p<0.05$, Table 2).

Table 3. Change of gene expression before and after 8 weeks training program in both women and men

Gene	Groups	BE- Median - IQR	AE- Median - IQR	p-value*
ADRB2	Female	11.315 ± 2.768	12.455 ± 0.339	**<0.001****
	Male	12.696 ± 0.665	12.248 ± 3.712	**0.030***
AMPD1	Female	0 ± 13.634	17.951± 0.571	**<0.001****
	Male	16.906 ± 2.95	13.616 ± 15.212	**0.038***
PPARA	Female	7.794 ± 3.249	1.1± 4.392	**0.008***
	Male	7.833 ± 1.959	6.073 ± 5.356	0.078

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

** B.E.=Before Exercise, A.E.= After Exercise

There were changes in the mean values of *ADRB2, AMPD1, PPARA* relative gene expressions in both male and female 8-week after endurance training according to pre-acute exercise (**p<0.001, *p<0.05, Table 3). Expression of PPARA gene significantly decreased after exercise compared to pre-exercise only for the female group (*p<0.05, Table 3).

CONCLUSION

In this study, among the genes we identified, *AMPD1, PPARA* are particularly important for energy, carbohydrate and lipid metabolism (Liang & Ward, 2006). It becomes increasingly clear that carbohydrates, proteins and lipids play an important role in the regulation of energy metabolism (Nakamura, Yudell & Loor, 2014). Previous studies have suggested that there are significant gender differences in hormonal responses and energy metabolism during the exercise and no sex-dependent difference in energy metabolism during pre-exercise, post-exercise, or non-exercise were reported between women and men in their resting state. Adenosine monophosphate deaminase (*AMPD*) is an important regulator of energy metabolism in muscles during exercise (Fedotovskaya, Danilova & Ahmetov, 2013). It is stated that the special skeletal muscle adenosine deaminase isoform (*AMP*) encoded by the adenosine monophosphate deaminase 1 (*AMPD1*) gene is stimulated after short-term high-intensity exercises and plays an important role in muscle energy metabolism (Cıeszczyk, Ostanek and Leon, 2012). In our study, there was no difference in the *PPARA* gene that we determined regarding energy metabolism after the acute maximal exercise protocol in women, but only in *AMPD1*. The expression of B2-adrenergic receptor (*ADRB2*) was found to be significantly different after acute maximal exercise in females. For males, there were no significant changes in the expression of genes studied. Studies conducted by Tracy J. Horton and colleagues have shown that women achieve energy with less carbohydrate oxidation than men during long-term exercise (Horton et al., 1998). It has been reported that B2-adrenergic receptor (*ADRB2)* and bradykinin β2 receptor (*BDKRB2*), a major lipolytic receptor in human fat cells, regulate body weight and fat metabolism (Cho et al.,2015). It was also shown that human fat tissue is very important for the level of endurance exercises due to the contribution of regulation of lipid mobilization and energy expenditure. *ADRB2* gene was shown to have a protective effect against the obesity related adverse events (Bea et al., 2010). Oxidation of free fatty acids (FFA) is very important for performance in endurance exercises (Macho-Azcarate et al., 2002). When the carbohydrate reserves fall due to low plasma glucose and muscle glycogen levels, the

endocrine system accelerates the oxidation of lipids which is called lipolysis. The great majority of lipid oxidation occurs during the steady-state exercise. When the intensity and duration of the exercise increase, the lipid oxidation gradually increases if there is enough amount of glucose consumed (Macho-Azcarate et al., 2003; Kang et al., 2007). This showed that women achieve more lipid oxidation (40% VO2 max) during low-intensityexercise compared to men. However, there is no significant difference in lipid oxidation between men and women with high intensity exercise. The effects of exercise on fat metabolism continue during the post-exercise recovery period. While women were shown to be more dependent on fat metabolism during the exercise than the recovery period, it is the opposite for men (Henderson et al., 2007). Venables et al., (2005) reported that during the exercise, only 12% of the differences regarding maximal fat oxidation can be explained by physical activity, VO2 max and gender but not body fat in healthy men and women (Venables, Achten & Jeukendrup, 2005). Differences in the expression of the adrenergic receptor gene are very important in terms of exercise adaptation (Janikowska et al., 2014). Dependence of adaptation time to the individual characteristics suggests that changes observed in the expression of *ADRB2* gene may be due to hormonal differences, body composition and type of exercise.

We found significant differences in *ADRB2* and *AMPD1* gene expressions in both males and females after the chronic 8-week exercise program ($p < 0.05$, $p < 0.001$). Although, there was a significant ($p < 0.05$) difference in the expression of *PPARA* genes in women after the second maximal exercise protocol, no significance was found in males. Some studies have shown that physical activity reduces skeletal muscle *AMPD* activity (Thomaes et al., 2011). In addition, a decrease in *AMPD* activity in a sprint training was reported with an increase in the rate of fast-twitch fibers, indicating that the level of expression of the *AMPD* gene in the skeleton was dependent on the muscle fiber composition. It has been reported that the expression of the *AMPD* gene is influenced by the intensity of physical activity (Thomaes et al., 2011).

In another study, *AMPD1* was shown to be an important regulator of muscle energy metabolism during exercise, and it was reported that *AMPD1*, ATP consumption rate was activated during short-term, high-intensity exercise exceeding the potential of the cell (Cieszczyk et al., 2011). Ginevičienė et al., (2014) the distribution of 204 allele frequency of Lithuanian athletes were compared with 260 healthy untreated individuals with regards to the genotype of the *AMPD1* C34T polymorphism. It has been emphasized that the *AMPD1 Cl* allele may be a marker associated with physical performance, speed and power. Although previous studies were founde a decrease in the expression of *AMPD1* gene after exercise, we found an increase in the *AMPD1* gene expression. This increase suggests that the increased energy requirement with exercise is due to the different muscle fiber properties of the volunteers and the exercise programs applied. *PPARGC1A, PPARA (G / C)* and *PPARAD* genes related to energy metabolism are very important for every athlete regardless of the type of exercise. The *PPARGC1A* and *PPARA* genes, which regulate expression of various genes, are reported to modulate carbohydrate and especially insulin sensitivity by modulating lipid metabolism and altering glucose uptake in skeletal muscles (Ginevičienė et al., 2014). These results are associated with many different factors and may have different outcomes depending on single exercise session, individual characteristics, physical conditions, basal lipid values, modality of exercise, duration, intensity and time period after exercise (Eynon, Meckel and Alves, 2009; Hargreaves, Hawley & Jeukendrup, 2004; Holst et al., 2003). *PPARGC1A* mRNA levels increased in endurance training according to the studies conducted, suggesting that regulation

of gene expression may increase skeletal oxidative capacity by *PPARA* Cieszczyk et al., 2011; Tunstall et al. (2002) found that fatty acid oxidation was increased after 60-minutes ergometer bike exercise for 9 days (63% VO2 max), although they did not find any significant change in the expression of *PPAR, PGC-1* right after or 3 hours after the 60-minutes ergometer bike exercise. There was a significant increase in the rate of oxygen consumption after the exercise compare to the rate before the exercise in both males and females. We believe that oxygen uptake capacity (VO2 max) can be improved depending on the severity and intensity of the training, while the change in gene expression may also be effective.

Sometimes, long high-intens exercises, favorable hours, appropriate genotype may not be enough to train a champion. Therefore, human biology, physiology, morphology, environmental factors, nutrition, rest, and genetic research must be carried out alltogether. Based on our results, there are gender dependent differences in the expression of genes regarding to energy metabolism (*AMPD1, PPARA*) and endurance (*ADRB2*) upon completeion of 8 weeks of intense exercise. We can show that these differences can positively affect performance in women and men, especially considering the increasedoxygen utilization capacity. However, it is not known whether these increases and decreases are due to different exercises, physiological characteristics of volunteer groups participating in the study, nutritional and rest status, or daily activity levels. Additional work is necessary to make the distinction. Our findings showed that acute and chronic maximal exercise has a significant positive effect on gene expressions, especially in some genes (*AMPD1, ADRB2, PPARA*) before and after the maximal exercise protocol that we performed at the end of intense exercise routines.

This study is important in terms of providing insights into the gene expressions information involved in energy metabolism and durability with different sexes and different exercise protocols. Our results show that chronic exercise in particular alters the expression of genes in both men and women.

Suggestions

1. Controlling the nutritional and resting characteristics of the people included in the study
2. It is recommended to increase the number of people included in the study.

Conflicts of Interest: None of the authors has anything to declare.

Note: All co-authors approved the final version of the manuscript

Authors' Contribution: The authors took part equally in the whole study.

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