ORGINAL ARTICLE

Association of Different Genotypes of VEGF Gene with Changes in Aerobic Capacity Following Endurance Training in Obese Women

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Abstract

Objective: Obesity is a chronic disease characterized by an excessive mass of adipose tissue in the body. The present study aimed to investigate the relationship between different genotypes of VEGF gene and changes in aerobic capacity following aerobic exercise in obese women.

Materials and Methods: In this study, 23 inactive women aged 34 to 43 years with BMI 30 and 35 were purposefully selected and participated in eight weeks of aerobic exercise including 4 sessions per week and 30 minutes per session with an intensity of 55 to 75% of maximum heart rate. Before and after the training period, aerobic power (VO2max) was measured by the modified Bruce test. Saliva Sample was collected at 12 hours of fasting to measure VEGF genotypes. To compare aerobic capacity between different genotypes, since we had three genotypes GG, CG, and CC, one-way analysis of variance was used.

Results: Although the mean amount of aerobic power changes of GG genotype was somewhat higher after eight weeks of aerobic training than the other two genotypes, this difference was not significant. (*P*-value= 0.663, *P*-value= 0.873 and *P*-value= 0.173, respectively).

Conclusion: Eight weeks of aerobic training leads to increased aerobic capacity in obese women and increased VEGF plays a role, but there is not seemingly a difference between different VEGF genotypes for these changes. In any case, since this study was conducted for the first time, we need more studies to draw a more accurate conclusion.

Keywords: Aerobic training, Angiogenesis, VEGF, Genotype, Aerobic power

Introduction

Obesity is a chronic disease characterized by an excessive mass of adipose tissue in the body (1). The change in lifestyle and eating habits of people towards the consumption of high-fat and energetic foods and reduced physical activity has led to increasing obesity and overweight in developed and developing countries (2). According to statistics published by the World Health Organization, obesity is one of the ten leading causes of death (3), The

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metabolic effects of obesity have made this prevalent complication as one of the most important risk factors for diseases such as diabetes, hypertension, coronary heart disease and osteoarthritis (4). On the other hand, exercise increases metabolism and decreases obesity (5).

Angiogenesis involves the growth of new capillaries in the muscle and is associated with the proliferation and migration of endothelial cells and occurs in two ways: germination or halving of existing capillaries (6,7). Vascular endothelial growth factor (VEGF), as the strongest and most important factor affecting angiogenesis, increases the migration and proliferation of endothelial cells, formation of the vascular network, differentiate endothelial cells, and germinate new capillaries from previous vessels (8). Hypoxia-inducing factor (HIF-1α) can activate the transcription of several genes, including the growth factor VEGF. Thus, VEGF is a target gene for HIF-1α that has been shown to regulate positively in response to hypoxia in the laboratory and in vivo (9). If the amount of intercellular oxygen is reduced, the enzymatic activities of prolyl hydroxylases are inhibited, and this condition leads to nuclear transcription and the formation of HIF-1α after the formation of the active heterodimer HIF-1β, which is essentially transcription.

Genetic factors compatible with hypoxia including angiogenesis, glycolysis, hematopoiesis, and catecholamines are involved (10). Therefore, HIF-1α and VEGF are essential signals for maintaining vascular density and oxygen supply in tissue hypoxia (11). The role of regular physical activity in health has been well established, which is a strong stimulant for cardiovascular and muscular adaptations and increases maximal oxygen consumption (VO2max), metabolism, increased athletic performance, reduced carbohydrate intake, and fat dependence. Improves insulin function, lowers blood pressure in patients with heart disease and hypertension, and improves cardiovascular fitness. Regarding the effect of endurance activity on the process of angiogenesis, extensive studies have been done before, which often refer to the positive effect of endurance activity on angiogenesis (12,13).

Hypoxia has been reported during exercise, and this hypoxia may increase myoglobin levels (14,15). Also, a positive and significant relationship has been seen between exercise and traction (16). Exercise increases nitric oxide in the heart muscle of heart patients, which is a strong vasodilator (17). Exercise can also be a physiological stimulus for the release of endothelial progenitor cells from the bone marrow. The most important mechanism concerning improving endothelial function due to exercise is increased vascular endothelial growth factor VEGF (18). Exercise increases the expression of the VEGF gene by causing hypoxia in skeletal muscle and the important role of this index in the recall and implantation of endothelial precursor cells in blood vessels has been proven (19). Also, Sliuka et al. (2014) measured changes in VEGF-mRNA in laboratory animals under aerobic and anaerobic exercise and concluded that both activities increased VEGF-mRNA levels (20). However, the association of VEGF changes following exercise with less aerobic power has been investigated.As angiogenesis leads to more blood supply and consequently more oxygen to the tissues, aerobic capacity should also be increased. This is especially important in people who are overweight and obese.

On the other hand, different VEGF genotypes may lead to different responses to changes in aerobic capacity following exercise. Physical activity is a complex phenotype that is affected by millions of environmental and genetic factors and it has long been known that diversity in physical function and athletic ability has very strong genetic components (21). The development of DNA sequencing and genotyping methods has made it possible to identify some individual genetic variations that indicate athletic performance (22,23-25).

The present study aimed to investigate the relationship between different genotypes of the

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VEGF gene and changes in aerobic capacity following aerobic exercise in obese women.

Materials and Methods

The present study was a semi experimental study. Among the Tehran Movement and Health Center clients, 23 inactive women aged 34 to 43 years with a body mass index (BMI) between 30 and 35 were purposefully available. One week before the start of the training protocol, a health medical questionnaire was used to ensure the health status of the subjects and no history of cardiovascular disease, diabetes, infectious diseases, allergies to smoking, or any other medications and supplements. Also, written consent was obtained from all subjects to participate in the study. Subjects performed exercise for eight weeks, 4 sessions per week and 30 minutes per session with an intensity of 55 to 75% of maximum heart rate. Thus, the subjects in the first two weeks with 55 to 60% of maximum heart rate and the second two weeks with 60 to 65% of maximum heart rate and 4 weeks left until the end of the training period with 65 to 75% of maximum heart rate. Ten minutes of warm-up and ten minutes of cooling were provided for each session, and participants' heart rates were monitored by a model polar heart rate monitor (POX 1000) made in Japan. Precise nutrition control was not possible and subjects were asked to follow a normal diet. 48 hours before and 48 hours after the training period, the aerobic capacity of all subjects was measured. Bruce test was used to measure aerobic power or VO2max according to the executive instructions. VO2max from Met was obtained by performing the Bruce test on a treadmill divided by 3.5. Also, 48 hours before the training period in the resting position (before the Bruce test) and in the 12-hour fasting state, saliva samples were collected from all subjects and sent to the Genetics Laboratory of the Growth Center affiliated to Tarbiat Modares University of Tehran for evaluation. The diagnosis was made and the samples were stored at -20 ° C. DNA was extracted from the saliva sample by salting out method. Tetra ARMS PCR technique is a simple and inexpensive method for genotype determination that was used in this study. Tetra-ARMS PCR is so sensitive to concentrations of reactants, including MgCl2, that if its concentration decreases, nonspecific bands appear. In this technique, four primers were used in a PCR tube and then the genotypes were determined by gel electrophoresis. This technique uses an awkward base-pair strategy to show the PCR reaction to amplify a specific allele. To design the primers, first, the genomic sequences of the VEGF gene along with the sequence around the polymorphic region were taken from the NCBI website. Then, with the help of primer design software and information service introduced by Collics and Ke in 2012 and available at http:11primer1.soton. ac.uk/primer1.html, Tetra ARMS PCR internal and external primers were designed. To perform electrophoresis on the samples, 2% agarose gel was prepared in TBE buffer. Each PCR sample was then mixed with 2 µl of loading buffer and poured into wells. In the first well, a suitable 100 bp DNA Ladder was used and the tank was connected to the power supply so that the wells were facing the negative pole. Thus, the samples were electrophoresed for 98 minutes at 98 volts. It should be noted that after DNA extraction from peripheral blood lymphocytes was extracted to confirm the quality of DNA and also after performing Tetra-ARMA PCR technique to determine the genotype, a gel imaging device was used. For statistical analysis, first, the data were described using mean and standard deviation and then to compare aerobic capacity between different genotypes, since we had three genotypes GG, CG, and CC, one-way analysis of variance statistical method independent was used at the level of *P*-value≤ 0.05. SPSS statistical software version 19 was also used to perform statistical calculations.

- VEGF F: 5′ ATCATGCGGATCAAA CCTCACC 3′ ;

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- VEGF R: 5′ GGTCTGCATTCACAT CTGCTATGC 3

Ethical considerations

This study has been approved by the Ethics Committee of Boroujerd Islamic Azad University (Ethics code: IR.IAU.B.REC. 1399.008.)

Results

As can be seen, there was no significant difference between the aerobic capacity of different genotypes before and after training and the number of changes from before to after training (*P*-value= 0.663, *P*-value= 0.873 and *P*-value=, respectively. 0.173). Although the mean amount of aerobic power changes of the GG genotype was somewhat higher after 8 weeks of aerobic training than the other two genotypes, this difference was not significant (Table 1).

Discussion

Based on the findings of the present study, the aerobic capacity of all genotypes increased before and after the training period, but there was no significant difference between the changes in aerobic capacity of the three groups of GG, CG, and CC genotypes. In confirmation of the present findings, Bouchard et al. (2011) also showed that 20 weeks of aerobic exercise leads to increased aerobic capacity of sedentary subjects (26). Bouchard et al. (2012) reported other similar findings (27).

In their study, Arkat et al (2015) investigated the effect of 5 weeks of treadmill training on VEGF expression in the heart muscle of diabetic rats. The results showed that VEGF decreased significantly in the inactive diabetic group compared to the inactive healthy control group, but treadmill exercise in both the diabetic and healthy control groups showed a significant increase in VEGF levels (28). In contrast, Dugan et al. (2014) reported results that contradicted their findings (29). In the study of Holloway et al. (2015), VEGF and eNOS were studied and the results showed that there was no significant effect of exercise on the process of angiogenesis (30). The reason for the difference in different results can be the difference in the intensity and duration of training as well as the samples.Exercise seems to induce VEGF genes by inducing hypoxia, thereby stimulating angiogenesis.

The hypoxia-inducing agent is not hydroxylated in hypoxia conditions and remains stable and migrates to the nucleus and induces factors affecting angiogenesis (14). Exercise-induced hypoxia also releases cytokines, which enter the endothelial cells and enter the vascular chain through an endothelial-derived relaxant (EDRF) called nitric oxide. Fibroblast growth factor 2 (FGF-2) overexpression is stimulated. On the other hand, the immediate increase in traction force due to exercise, mainly through the activation of ion channels, especially potassium channels, has caused the secretion of vasodilators, especially nitric oxide, which causes an upregulation of VEGF and VEGFR. -2 becomes (31).

As a result of exercise, there is an increase in muscle adaptation, especially a decrease in the degradation of creatine phosphate and an increase in gluconeogenesis. Also, anabolic

Table 1. Aerobic capacity changes of different genotypes

Genotype	Factor	Number	Mean (±SD)	F-value	*P*-value
Pre-test	GG	9	28.76 (± 5.03)	0.42	0.66
	CG	9	30.75 (± 7.27)		
	CC	5	21.28 (± 2.18)		
Post-test	GG	9	36.82 (± 5.29)	0.137	0.873
	CG	9	35.67 (± 8.75)		
	CC	5	34.92 (± 5.01)		
Changes	GG	9	8.06 (± 3.4)	1.91	0.173
	CG	9	4.92 (± 3.4)		
	CC	5	6.71 (± 3.3)		

*One-way analysis of variance

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hormones and adenosine are increased, which stimulates the expression of the VEGF gene (32). On the other hand, due to exercise and dephosphorylation of AMP by the Octo-5 nucleotidase, adenosine is produced from hypoxic tissues in the extracellular space adjacent to the parenchymal cell, which plays an important role in the process of angiogenesis (33).

Finally, all agents, by increasing VEGF and binding to its specific receptors on the endothelial cell, activate messages that cause endothelial cells to proliferate and migrate and increase vascular permeability (34). Finally, VEGF synthesizes DNA through the upregulation of anti-apoptotic elements, destroying the basement membrane and phosphorylating intercellular endothelial components and tightly binding, resulting in endothelial cell survival, proliferation, migration, and permeability, respectively will be (35). All of these processes lead to an increased capillary density of tissues, more blood supply to tissues, and naturally more oxygen and nutrients to tissues, the result of which is an increase in the aerobic capacity of obese people (36).

However, genetic data on the effect of changes in aerobic capacity is not large. In particular, we could not find a study on the association of VEGF genotypes with changes in aerobic capacity. What was observed in the present study was no difference in aerobic power changes between the three groups of individuals with GG, CG, and CC genotypes, but in this regard, we need more studies, and care should be taken in interpreting the findings. The results showed that, the average aerobic capacity of GG, CG, and CC genotypes were 28.76, 30.75, and 28.21 ml.kg.min, respectively, with the highest CG and lowest amount of CC was obtained but there was no significant difference between them.

In the post-test, the average aerobic capacity of these genotypes was 36.82, 35.67, and 34.92 ml.kg.min, respectively. Therefore, it is observed that in the post-test (after 8 weeks of aerobic training), the aerobic capacity of individuals in all three genotypes has increased, which was statistically significant. However, the rate of change in aerobic capacity from before to after training for GG, CG, and CC genotypes was 8.06, 4.92, and 6.71 ml.kg.min, respectively. As can be seen, the highest increase in aerobic capacity was related to the GG genotype and the lowest increase in aerobic capacity was related to the CG genotype. In any case, there was no significant difference between the increase in aerobic capacity of individuals with the three genotypes. Perhaps if a larger sample size is used in future studies, a significant difference can be observed between changes in aerobic capacity of different genotypes. In their meta-analysis of 35 articles, Camila et al. (2017) stated that 97 genes could predict the VO2max response to exercise (37). However, as mentioned, no study has probably been conducted to date to investigate the association of different VEGF genotypes with changes in aerobic capacity following exercise. Considering the importance of capillaries in increasing blood oxygen uptake and thus increasing VO2max, it is very important to study the role of different VEGF genotypes in changes in aerobic capacity following exercise, and since not many studies have been done in this field, it is better. Future studies will be conducted with a larger number of subjects. Also, one of the limitations of the present study was the lack of precise control of the subjects' diet, which due to the effect of nutrition on these adaptations resulting from exercise, is suggested to be considered in future studies.

Conclusions

Eight weeks of aerobic exercise may increase aerobic capacity in obese women, with increased VEGF followed by angiogenesis. However, there does not appear to be a difference between the different VEGF genotypes for these changes. In any case, since this study was conducted for the first time, we need more studies to draw a more accurate

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conclusion. We indicated that high endurance training increases Vo2max and cardiac output and improves ventilation according to age and gender, and is important for promoting better health and life in other hands to maintain homeostasis in this range, it is necessary for adolescents and young people who are looking to determine the sport according to their innate abilities, because it can be used as a scientific screening.

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Conflict of Interest
There are no conflicts of interest.

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