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Effects of High Intensity Interval Training and Intermittent Fasting on *VEGF* Gene Expression in the Cardiac Muscle Tissue of Obese Male Rats

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ABSTRACT

Introduction: The present study aim to investigate the combined effect of high intensity interval training (HIIT) and intermittent fasting (IF) on the expression vascular endothelial grow factor (VEGF) gene in the cardiac muscle tissue of obese male rats.

Methods: This study was conducted on 22 male Wistar rats that were kept on high fat -diet for 12 weeks. Afterwards the animals were randomly divided into four groups, including control, HIIT (three session for six week, 80 - 95% MRT), IF (three days for six week, 12h ours) and HIIT with IF (both protocols for six week). Real-time polymerase chain reaction (RT- PCR) was used to measure *VEGF* gene expression. Data analysis was performed using one –way analysis of variance ANOVA least significance test ($p<0.05$).

Results: no significant difference was observed between the groups *VEGF* expression ($p>0.52$).

Conclusion: According to the results HIIT with IF and HIIT or IF alone had no significant effects on not significant on *VEGF* expression in the cardiac muscle tissue of the obese male rats.

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Introduction

The American Obesity Association defines obesity as a chronic metabolic disease caused by fat accumulation in the body. The World Health Organization (WHO) announced obesity as a global epidemic in 1997 (1). Recently, the prevalence of obesity has increased in different countries and the number of obese patientsis is projected to reach 1.12 billion by 2030 globally (1). Evidence suggests that obesity is a risk factor for cardiovascular diseases, diabetes and other metabolic disorders.

Today, the benefits of exercise are well-established, and physical activity is known to enhance health and prevent disease. Physiological responses following by exercise reduce the incidence of chronic diseases, (including cardiovascular diseases) and may also improve athletic performance. Increased blood flow is a form of adaptation to exercise, which is associated with improved capillary density and maximum oxygen consumption (2). In this context, the vascular endothelial grow factor (VEGF) pathway plays a pivotal role in the process of restructuring blood vessels reconstruction and the blood flow (2).

VEGF is a key factor in the growth of endothelial cells and is significantly involved in cell proliferation and regeneration. Furthermore VEGF signals are expressed through Tyrosine Kinase VEGF1 and VEGF2 receptors in the arteries (3). Among endothelial receptors, VEGF-2 plays a key role in angiogenesis (3).

High -intensity interval training (HIIT), consists of high intensity training sessions with a period rest between the workouts, which are performed at the intensity of 80 – 100% of the maximum heart rate or maximum aerobic capacity, for 60 - 240 seconds depending on the maximum capacity of the individual (4). Recent studies suggest that HIIT is an effective intervention in increasing cardiovascular fitness. For instance, Ramos et al. (2015) reported that HIIT has far greater effects on the cardiovascular function compared to aerobic training (5). In addition, HIIT is reported to be involved in reducing oxidative stress, inflammation, and insulin sensitivity, which may cause cardiovascular diseases (6). On the other hand, different types of

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training such as traditional training with moderate intensity and high volume have proven effective in weight loss and fat oxidation, while research suggests that HIIT is more beneficial than other a weight-loss training protocols in terms of improving fat oxidation within a short period (6). Furthermore, Studies have shown that exercise plays a key role in activating the VEGF pathway (7). In this regard, Karbalaeifar et al. (2016) reported that six weeks of HIIT could increase angiogenesis and improve myocardial function in male Wister rats after myocardial infarction (8). In another study, Mohamed Tah et al. (2016) stated that HIIT increases VEGF concentration (9).

Recent findings indicate that fasting has beneficial effects on health and may be considered as a therapeutic intervention (10). Intermittent fasting (IF) includes short- term or long-term dietary periods with severe energy restriction (75% - 100% reduction in calorie intake on fasting days) which increases free access to water and food on non-fasting day (11). The Metabolic benefits from IF of could increase endothelial growth factors and stimulate the process of angiogenesis and vascular healing (10).

Previous studies have proposed conflicting results about the effect of HIIT and fasting on the VEGF pathway of the cardiac tissue. The heart tissue has the highest mitochondrial volume and the highest capillary density in the body. A comparison of different training methods indicates that compared to traditional training, HIIT yields similar responses to skeletal muscle metabolic adaptations, HIIT also saving time and periodic exercise has been observed to induce more efficient. Studies have also highlighted the importance of fasting interventions in improving vascular function and activating the anti-obesity angiogenesis process. In a review study, Peter et al. (12) reported that despite the obvious fact that cells need o_2 for aerobic metabolism blood vessels also play a pivotal role in providing o_2 during metabolism and angiogenesis (12). Evidence in this regard suggests that training interventions increase oxygen consumption and the body tissue's demand for more blood flow increases during training. Today research points to the key role of fasting in angiogenesis and improving the blood flow to various body tissues. The present study, aimed to investigate the combined effect of HIIT and IF on VEGF gene expression in the cardiac tissue of obese male rats.

Materials and Methods

This laboratory study was approved by the ethics committee of Baqiyatallah University of Medical Sciences Tehran Iran (ethics code BMSU.REC.1398.022). The study was conducted on 22 male Wistar rats aged four weeks and weighing120-130garams. The animals were obtained from the animal Home Center of Baqiyatallah University of Medical Sciences, Initially, the rats were kept on a high-fat diet for 12 weeks. Following that, they were randomly divided into four groups including control (n=4), HIIT (n=6), intermittent fasting (n=6), HIIT with intermittent fasting (n=6). The rats adapted to their new environment for one week and maintained in four polyethylene cages with metal mesh lids within a 12-hour light/dark cycle (7- 19 hours) at the mean temperature of 2.22°C and C and 50% relative humidity they had free access to food and water as well.

High-fat Diet

The rodents received a standard pelletized diet (Behparvar Company Tehran, Iran). The high-fat diet was maintained after adaptation for weight gain and was composed of 5% rodents base food and 30% sheep's oil (5+30%=35). For the first 12 weeks, all the rats had free access to the high-fat diet and sufficient water. After the rats reached the mean weight of 320±20 grams, we evaluated the rats in the HIIT, IF and HIIT with IF groups.

HIIT Protocol

The HIIT protocol was designed based on previous studies (13). At the first stage, the rats were trained for one week (3-5 sessions five minutes) at the rate of 10 meters and 0o slop. Following that, a moderately adjusted periodic HIIT protocol was implemented including five minutes of warm-up and cool-down 40% maximum running speed one minute of intense alternations with 80 - 95%maximum running speed an active rest for one minute and 55% maximum running speed for six weeks (three sessions per week).The subjects performed the maximum running speed test before the first week and at the end of the third week based on the new meanscores obtained to continue the protocol. Notably, the maximum running speed test was performed based on the Study by Machdo et al (14) - (Table 1).

IF Protocol

The IF protocol was designed based on previous studies (15). The Subjects underwent a fasting protocol for six weeks (three alternative days in fasting mode at 7pm - 7am). While fasting, the rats only had access to water and they had the rats had free access to water and food on the other days.

Table 1. Intense periodic exercise protocol

Exercise weeks	Intense rotation the number of sets	Speed	slow rotation the number of sets	Speed
first week	5	34	4	23
second week	6	36	5	23
third week	7	38	6	23
fourth week	8	43	7	27
fifth week	9	46	8	27
sixth week	10	46	9	27

Tissue Removal

About 48 hours after the last training session and the day of implementing the research protocols, the rats fasted for 10 - 12 hours. Afterwards, they were, anesthetized by the combined injection of ketamine (10 g) and xylazine (100 g).To prevent the slightest harassment of the animals, blood was initially taken from the cardiac muscle tissue using a 3 cc syringe. Following that, the cardiac muscle tissue was removed and kept frozen at the temperature of 80 °C for tissue analysis.

Table 2. Primer Sequence of VEGF-A and GAPDH genes in cardiac muscle tissue

Primer	Primer sequence
VEGF-A	F: 5´-TGAGACCCTGGTGGACATCTT-3 R: 5 - GTAGACGTCCATGAACTTCAC-3
GAPDH	F:CAAGTTCAACGGCACAGTCA R:CCCCATTTGATGTTAGCGGG

VEGF-A Gene Expression Analysis

Real-time polymerase chain reaction (RT – PCR) was used to analyze the expression of the VEGF-A gene. To design the primers, the sequence of VEGF-A encoding genes was initially extracted from the National Center for Biotechnology Information (NCBI). Afterwards, oligo version 7 was used to design the desired primer.After optimizing the RT- PCR device, relative were made in the VEGF-A and GAPDH genes (internal control gene) using the table primers listed in Table 2.

RNA Extraction, cDNA Synthesis, PCR Reaction

After extracting the entire RNA from the cardiac tissue, a SuperplusRNA extractionKit was used for analysis.CDNA was also provided in two stages using a Max First Strand c DNA Synthesis Kit.The PCR was performed using the device (Corbett, Easy Plex Analyzer device, Australia) and Master Mix Ampliqon.Each reaction was performed twice for each sample. The 2-DDCT method was also used for the Relative Quantification of VEGF-A GeneExpression.All the analyses were performed separately one each sample.

$Relative\ fold\ change\ in\ gene\ expression_{=2}{}^{-\Delta\Delta CT}$

$\Delta CT = CT\ target\ gene - CT\ reference\ gene$

$\Delta\Delta CT = \Delta CT\ test\ sample - \Delta CT\ Control\ sample$

Statistical Analysis

Data analysis was performed in SPSS version 25 using descriptive statistics to calculate the mean and standard deviation, Then, one-way analysis of variance (ANOVA) was also applied to compare the study groups at the significance level of $p < 0.05$.

Table 3. Shows the weight (g) of the study groups. HIIT high intensity interval training, IF, Intermittent Fasting, CON control.

Group	Mean	SD
HIIT	315	34.76
IF	333.40	16.21
HIIT& IF	325.25	39.06
Control	362	19.42

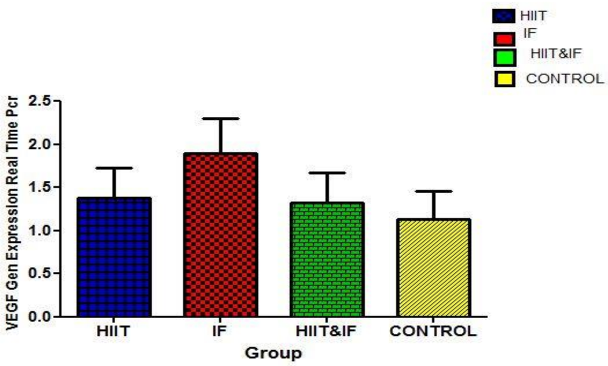

Figure 1. Shows the VEGF-A gene expression in the heart muscle tissue of obese male rats. HIIT high intensity interval training, IF, Intermittent Fasting, CON control

Results

Table 3 shows the mean weight of the rats in different of study groups. Figure 1 the results of one way ANOVA indicated no significant difference between the groups in this regard (p>0.52).

Discussion

The present study aimed to evaluate the combined effect of HIIT and IF on the expression of VEGF gene in cardiac muscle tissue. The obtained results indicated significant difference between the study groups in this regard (p>0.52). According to Bayati et al. (16) HIIT had a significant effect on the rate of change in VEGF protein in human skeletal muscles. In the mentioned study, the VEGF pathway was reported to be activated by PGC-1α to adapt angiogenesis to HIIT. Therefore, it could be inferred that PGC-1α induction is dependent on beta-adrenergic induction mediated by ERRa, other activation pathways for VEGF gene expression include the such as the HIF pathway which increases gene expression of VEGF due to the lack of oxygen and the activity of AMPK metabolic sensors, which are sensitive to metabolic insufficiency (17), In another study, Chinsombo et al.(18) showed that after training metabolic factors such as PGC-1α play a key role in activating VEGFas an angiogenesis factor (18). According to the findings of Arani et al. (19) the PGC-1α pathway effects the in the angiogenesis process by activating ERRα. Based on the aforementioned findings, it could be stated that the PGC-1α pathway and AMPK sensors significantly affect alternative angiogenesis signalin depending on the tissue type and training protocol. According to the studies by Bayti et al. (16) Chinsomio et al. (18), and Arani et al. (19), the most important variables to be considered in this regard are the tissue type, alternative angiogenesis signaling training protocol.

Previous studies have highlighted the importance of various,training parameters (e.g.. intensity, duration and volume) in training interventions in terms of activating the angiogenesis pathway and improving vascular function. Our findings in this regard indicated, there was no significant increase in VEGF gene expression in the cardiac muscle tissue following HIIT. Nevertheless, several studies confirm the effectiveness of HIIT in facilitating the angiogenesis process in the heart tissue. For instance Shabani et al. (20) reported that due to the nature of the cardiovascular system, HIIT maximizes the oxidative capacity of the cardiovascular system thereby leading to the accumulation of lactic acid, which inhibits the angiogenesis process (20). Therefore, HIIT with the intensity of 80> could be an influential factor in the inhibition of angiogenesis activation and decreased vascular function in the cardiac muscle tissue.

Studies regarding endurance training (e.g.. running, prolonged swimming) have demonstrated that such interventions could stimulate a significant increase in the oxygen and nutrient demand for the formation of new capillaries in the myocardium (21) In addition, changes in the blood flow, muscle concentration, and oxygen levels are associated with the mechanical occurrence of hemodynamics, which is a key signal in activating vascular wall regeneration and proliferation the formation of endothelial cells and angiogenesis. In other words, there is a reciprocal response between the skeletal and cardiac muscles in order to respond synergistically to the stimuli of training (21).

In a study conducted by Bellaforeet al. (21) endurance training was reported to an increase the VEGFR1 receptor in the cardiac muscle tissue thereby activating the angiogenesis signal pathways (21). In the present study, the HIIT training protocol and the associated training stimulation might have decreased the activation of angiogenesis. The volume and duration of training interventions are of great importance for create better adaptations in different body tissues after training in different tissues of the body. Morland et al. (22), observed a significant increase in VEGF in a study following the effect of seven weeks of HIIT (five sessions per week) (22). As a result, the angiogenesis pathway was activated and the vascular function was reported to improve. In the current research, HIIT, was performed for six weeks (three sessions per week), and the volume and duration of the HIIT training protocol may have decreased sufficient stimulus to upset the balance of angiogenesis process and improve vascular function from the rest time to the training time.

Previous studies have highlighted the correlation between fasting and signaling pathways such as PPARs, which consists of PPARsα, PPARsβ, PPARsγ isoforms with different roles (12). Two of these isoforms are involved in inhibiting angiogenesis, while one promotes cell growth (12). The findings of the current research indicated the effects of IF on signaling pathways (e.g. PPARs) and the inhibiting of VEGF after six week IF notably.

Factors such as, duration (12hours), volume and type fasting (every other day) should be considered when it comes to fasting protocols. It could be stated that our IF protocol (12 hours) could sufficiently stimulate angiogenesis and improve blood flow. In addition, the type (intermittent) and volume of the fasting protocol (six weeks) in the present study might have been effective in reducing angiogenesis and the blood flow.

Conclusion

According to the result HIIT with IF and HIIT and IF alone had no significant effect on the pathway signaling of the VEGF in the cardiac muscle tissue of the obsess rats, for the activation of this cellular pathway in the heart tissue of obese rats further research is required with other types of training (e.g.. aerobic exercise) and long-term fasting protocols.

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