



ORIGINAL ARTICLE

The Effect of Eight Weeks High Intensity Intermittent Training and Blood Flow Restricted on Angiogenic Markers of Muscle in Male Runners

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ABSTRACT

Background. The aim of this study was to investigate the effect of eight weeks of HIIT and blood flow restricted (BFR) on protein expressions (VEGF and eNOS) in vastus lateralis of male runners. For this purpose, 15 runners (age: 23±3) voluntarily participating in this study were divided into three groups: 1) Control 2) HIIT and 3) HIIT+BFR. **Methods.** The experimental groups were practicing (three sessions a week and six attempts each session for eight weeks). Before and at the end of eight weeks, the biopsy samples were collected from vastus lateralis muscle and the protein expression levels of the VEGF and eNOS were studied by immunohistochemical method. **Results.** The findings of this study showed that the levels of the VEGF and eNOS were significantly increased in the experimental groups compared to the control group ($p<0.001$). There was also a significant difference between experimental groups in the VEGF protein expressions ($p<0.05$). **Conclusion.** In conclusion the HIIT and BFR training can effectively increase the protein expression levels of the VEGF and eNOS in vastus lateralis muscle of runners.

KEYWORDS: HIIT, BFR, VEGF, eNOS.

INTRODUCTION

In recent years, several studies have focused on specific forms of exercise, such as the high intensity intermittent training (HIIT) and blood flow restricted (BFR) that have examined the impact of these exercise models mainly on functional and behavioral Physiological variables (1-3). HIIT are used in the form of vigorous repetition exercises (generally with intensity exceeding 80% of maximum heart rate), along with alternate recovery periods, as a substitute for traditional endurance training (4). BFR exercises also as a training types via reducing local blood flow, lead to the accumulation of metabolic elements (5). Both the practice models can lead to adaptive responses in long term. Skeletal muscle angiogenesis is one of the adaptation responses

that reduce exercise induced stress (6). Angiogenesis is called the formation of new capillaries from existing vessels (7) and is a multifactorial process that can be identified only by understanding the molecular bases (6).

Multiple-fold increase in blood flow during exercise result in increased shear stress and lead to upregulation of eNOSmRNA (nitric oxide synthase mRNA) and protein expression (6). VEGF is also an important angiogenic factor that has been reported following an acute bout of a range of exercise modalities including: submaximal cycling exercise (8), HIIT (9), and sprint interval training (SIT) (10) increased expression at the mRNA levels. The increased expression of VEGF mRNA has been

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demonstrated between zero and six hours after the end of exercise, with levels returning to baseline within 20 -24 hours (11, 12) and is decreased following a period of training (12, 13). In comparison to mRNA expression, the muscle VEGF protein response to an acute bout of exercise and period of training is not well clear. Some reports show following an acute bout of submaximal continuous cycling a decrease (14) or no change in protein levels immediately (15) and up to two hours post exercise (8), whereas, other studies have demonstrated an increase in protein levels 2-4 hours post exercise (15, 16). However, in humans no increase in basal VEGF protein content have been reported following 4-8 weeks of either continuous or HIIT (9, 13, 17). Nevertheless, at one study did identify an increase in VEGF protein following short term (10 days) one legged knee extensor exercise (18). However, there were uncertainties about the impact of a course of HIIT on angiogenic factors yet.

On the other hand, the application of BFR can involve multiple primary contractile stimuli including: shear stress (19), skeletal muscle hypoxia (20), metabolic stress (21) and oxidative stress (22), all of which are important stimuli of angiogenesis. In a recent investigation, the BFR stimulus is applied as an additional stimulus in the recovery intervals during an interval training program (23). In this study 8 trained cyclists undertook one session of SIT either alone or with 2 min of post-exercise BFR during the recovery intervals on ergometer and did not observe any significant differences between two practice methods in VEGF or eNOS mRNA expression post-exercise. In another research, 21 trained male cyclists ($V_{O2max} = 62.8 \text{ ml.min}^{-1}.\text{kg}^{-1}$) undertook 4 weeks of SIT either alone ($n = 10$) or with post-exercise BFR ($n = 11$) (24). The results of this study despite the tendency to increase VEGF levels in the SIT+BFR group, did not reveal any changes in vastus lateralis muscle capillarity. The results of the study by Conceicao et al. (2016) also show that muscle VEGF mRNA expression occurs at a higher level after performing a single session of intense endurance training compared to moderate endurance training with BFR on an ergometer.

Due to the lack of sufficient information about the interactive effects of HIIT and BFR exercises on angiogenic variables of skeletal muscle, it seems that integration these training models can influence skeletal muscle angiogenesis in the long term over the individual application of each of these training methods. Since there is a growing scope for identifying and adopting new compact exercise methods that show greater adaptation responses, therefore the aim of present study was to assess combining HIIT and BFR effect on the immunohistochemical expression of angiogenic markers (VEGF and eNOS) of vastus lateralis in male mid-endurance runners.

MATERIALS AND METHOD

Study Design. The present applied research, semi-experimental study with inter-group design was conducted on med-endurance runners who were active under the supervision of the athletic board of Amol city-Iran in order to assess the effects of HIIT and HIIT+BFR on angiogenic factors (VEGF and eNOS) in vastus lateralis muscle.

Subjects. All experimental procedures were approved by the Mazandaran University Ethics Approvals (Human Participants) Sub-Committee (IR. UMZ. REC. 1397.048) and conformed in all respects with the Declaration of Helsinki. Participants were fully informed of the risks and discomforts associated with all experimental trials before providing written, informed consent. 15 amateur semi-endurance male runners (age 23 ± 3 yr) that worked in 800 and 1500 materials, volunteered to take part in the study. Inclusion criteria for this study were: having at least two years of training experience, not taking any ergogenic ingredients and supplements for at least six months, be full health and having a BMI below 25. In a pre-training briefing session about the research performing process, advices and recommendations were made and anthropometric measurements of runners including height, weight and limb length were recorded in the Physiology Laboratory of Mazandaran University (Table 1). Then runners were categorized into homogeneous groups through Bruce exhausting test and after recording the records, the runners were divided into three groups ($n = 5$) of control (Con), HIIT and HIIT + BFR.

Table 1. Anthropometric Characteristics of Runners

Foot Length	Thigh Environment	Waist Circumference	Subcutaneous Fat Thickness	BMI	Height	Weight
6.5 ± 102	7.2 ± 67	$4.5 \text{ cm} \pm 92$	3.1 ± 11.2	1.7 ± 23	$5 \text{ cm} \pm 172$	$4 \text{ kg} \pm 73$

Exercise Training Program. Unlike the control group did not participate in any training programs, experimental groups (HIIT and HIIT + BFR) had to practice the 10-20-30 exercise pattern for eight weeks and three sessions per week. The 10-20-30 training consisted of a 15-min warm-up at a low intensity followed by 6×5 min running periods interspersed by 2 min of rest. Each 5-min running period consisted of five consecutive 1-min intervals divided into 30, 20, and 10 s at an intensity corresponding to $\sim 30\%$, $\sim 60\%$, and $\sim 90\text{--}100\%$ of maximal running speed. The difference between the experimental groups was that the HIIT+BFR group had to install 12 cm wide bands at the upper thighs of both legs when performing the exercise and during the two-minute breaks would put them away.

Experimental Protocol. All of the runners in the experimental group were present in the medical laboratory 72 hours before the start of training and 72 hours after the last exercise session and needle biopsy were taken from them. The control group was sampled only at the first time. At first to perform the needle biopsy, each subject would lie open vault mode. Then the desired position was the external anterior lower third part vastus lateralis muscle, was injected and anesthetized with a syringe containing lidocaine. In the next step, using a biopsy needle, 1.6 mm slices were removed from the muscle and was rapidly discharged into the formalin-containing falcon tubes. Tissue samples were stored in these tubes for one month and then transferred to histological laboratory for immunohistochemistry studies. After transferring samples to histological laboratory, immunohistochemical expression of VEGF and eNOS proteins by Envision method and using VEGF (code SC-7269 manufactured by

Santa Cruz company of America, the accuracy of measurement 200 $\mu\text{g/ml}$) and eNOS (code Ab-76198 manufactured by Abcam company of America, the accuracy of measurement 300 $\mu\text{g/ml}$) specific antibodies were measured.

Statistical Analysis of Data. The Kolmogorov-Smirnov and Levene tests were used to ensure normal distribution of data and equality of error variances, respectively. The covariance analysis test was also used to examine intra-group changes. Statistical analysis was performed using software SPSS. 20 and $p < 0.05$ was considered as the level of significance.

RESULTS

VEGF. ANCOVA revealed that the effect of groups was significant ($f_{2,11} = 70.83$, $p < 0.001$, $\eta^2 = 0.93$); so that, VEGF increased significantly ($p < 0.001$) in both HIIT (47.07 ± 1.84) and HIIT+BFR (53.96 ± 1.95) groups in comparison with control group (22.92 ± 1.87). Besides, the difference of HIIT and HIIT+BFR was also significant ($p = 0.03$) (Figure 1).

Immunohistochemical expression of VEGF protein of the vastus lateralis muscle cells have been shown for experimental and control groups (Figure 2).

eNOS. ANCOVA revealed that the effect of groups was significant ($f_{2,11} = 181.5$, $p < 0.001$, $\eta^2 = 0.97$); so that, eNOS increased significantly ($p < 0.001$) in both HIIT (50.10 ± 1.11) and HIIT+BFR (51.42 ± 1.17) groups in comparison with control group (23.34 ± 1.15). But the difference of HIIT and HIIT+BFR was not significant ($p = 0.43$) (Figure 3).

In Figure 4 has been shown immunohistochemically expression of eNOS protein of the vastus lateralis muscle cells for experimental and control groups.

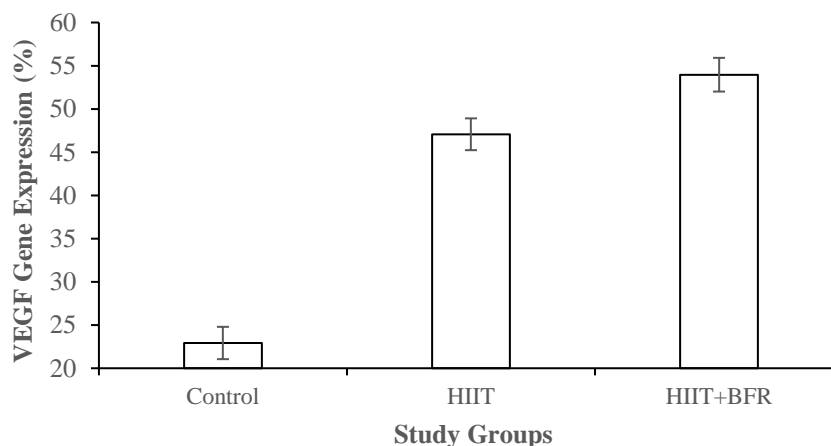


Figure 1. The Effect of 8 Weeks High Intensity Intermittent Training and Blood Flow Restriction on Muscular VEGF Protein Expression of Male Runners. Covariates Appearing in the Model Are Evaluated at the Following Values: eNOS. $\text{preTest} = 24.8267$. HIIT: high intensity intermittent training; BFR: blood flow restriction. *Significant different from Control group at $p < 0.001$. #Significant different from HIIT at $p = 0.03$.

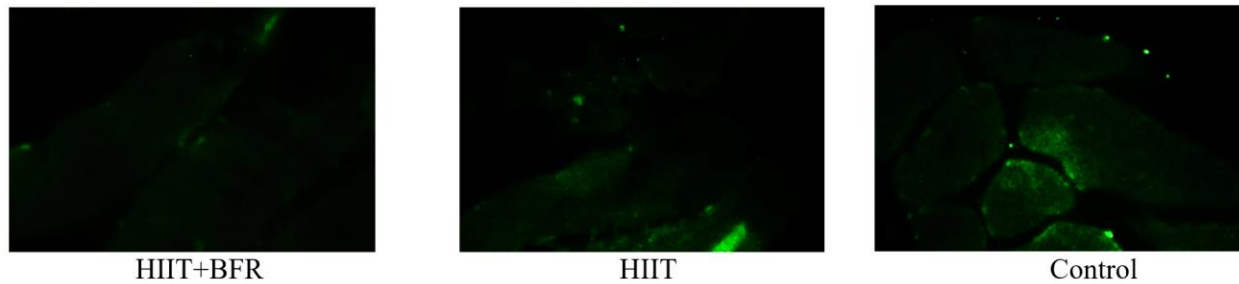


Figure 2. The Effect of 8 Weeks High Intensity Intermittent Training and Blood Flow Restriction on Muscular VEGF Protein Expression of Male Runners. Immunohistochemical Expression of VEGF in Vastus Lateralis Muscle Cells of Experimental and Control Groups. Green Spots Indicate VEGF Protein Expression.

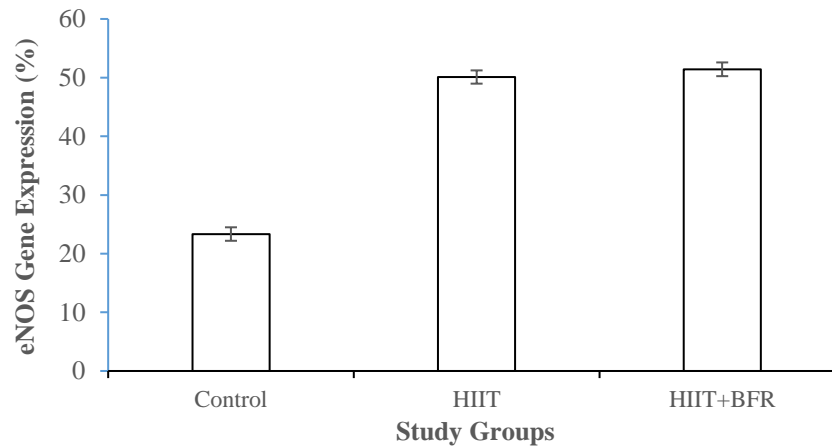


Figure 3. The Effect of 8 Weeks High Intensity Intermittent Training and Blood Flow Restriction on Muscular eNOS Protein Expression of Male Runners. Covariates Appearing in the Model Are Evaluated at the Following Values: eNOS. PreTest=24.8267. HIIT: high intensity intermittent training; BFR: blood flow restriction. *Significant different from Control group at $p < 0.001$.

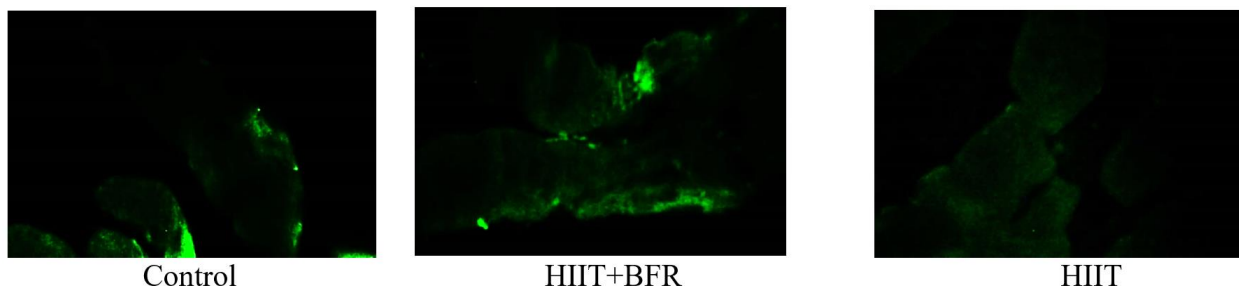


Figure 4. The Effect of 8 Weeks High Intensity Intermittent Training and Blood Flow Restriction on Muscular eNOS Protein Expression of Male Runners. Immunohistochemical Expression of eNOS in Vastus Lateralis Muscle Cells of Experimental and Control Groups. Green Spots Indicate VEGF Protein Expression.

DISCUSSION

The purpose of this study was to investigate the effect of eight weeks of HIIT and BFR training on VEGF and eNOS protein expressions in the vastus lateralis muscle of male med-endurance runners. The results of the present study show that the expression of VEGF and eNOS proteins increased significantly after eight weeks of HIIT and HIIT+BFR training compared to the control group. Contrary to the results, Michel et al (2018) did not report any change in the capillarization markers of well-trained cyclist

men's vastus lateralis muscles ($Vo_{2max} = 62.8$) that performed SIT alone or in combination with BFR for four weeks. The inconsistency of the results is probably related to factors such as the level of physical fitness of the subjects, the volume of exercises and the type of exercise protocol. In the study of Michel et al (2018), the training protocol was conducted twice a week for four weeks using cycle ergometer in professional cyclists, whereas in the present study, 10-20-30 training sessions were performed three times a week over eight weeks by amateur med-endurance runners. In humans it is generally considered that

angiogenesis manifests later than after 4-5 weeks of training (25) and based on the available evidences, exercise training volume or the total amount of time devoted to exercise affects capillary adaptations (6). In the current study, use of BFR exercises was also performed by installing stretch bands during the run training which differs to the mentioned study when applied it between the recoveries of SIT intervals. Taylor et al. (2016b) demonstrated enhanced HIF-1 α mRNA expression following a typical SIT session only when combined with post-exercise BFR, however, they did not report any enhancements in either VEGF or eNOS mRNA expression three hours post-exercise with the addition of BFR. The mentioned research suggests that post-exercise BFR is not a sufficient stimulus to enhance transcription of these genes. These findings are in contrast to BFR applied during low load resistance-type or low intensity endurance-type exercise, which has been shown to enhance the acute expression of both PGC-1 α and VEGF mRNA (2, 22). The exercise protocols in the aforementioned studies undertaken alone did not elicit increases in VEGF mRNA and only elicited modest increases in PGC-1 α mRNA expression of ~2-fold, compared to the 5-fold increase following SIT in the study of Taylor et al. (2016b). Therefore, it seems likely that when combined with standard intensity training protocols, which are already potent stimuli of these genes, there is reduced capacity of BFR protocols to further increase the transcriptional response. Exercise induced angiogenesis occurs by a combination of growth factors, hypoxia, shear stress and mechanical stress (6). Studies suggest that angiogenic signals start from within the contracting muscle and PGC-1 α as a master regulator of angiogenesis is involved in this process by interacting to the last nuclear receptor (ERR α). Mechanistically, the functional role of PGC-1 α in VEGF expression in response to exercise training depends on its upstream cascade or P38MAPK (26). Since ROS production is increased during HIIT execution, so it results in more activation of P38MAPK (27) which may eventually lead to more VEGF expression. On the other hand, increased blood flow to the blood vessel bed during exercise increases shear stress, which is a potential stimuli for increased NO and eNOS expressions (28, 29). In consistent to the results of this study, there are studies have assessed the effects of SIT on angiogenic factors

(VEGF and eNOS) (8, 30). Hoier et al. (2013) studied effects of four weeks of SIT on a cycle ergometer in healthy subjects that established to increase VEGFmRNA and eNOS expressions. In the other study, have assessed the structural and endothelial enzymatic changes in skeletal muscle micro-vessels of sixteen young sedentary males in response to ET and SIT for six weeks by Cocks et al. (2013). The expression levels of eNOS increased in both groups, with a significantly greater increase observed following SIT. However, in the present study unlike the VEGF protein values, the difference between the experimental groups (in eNOS protein expression) did not reach a significant level. It seems this discrepancy is related to BFR training which did not induce the adequate amount of shear stress. In other words, the use of flexible bands during HIIT training could not lead to the reactive hyperemia in artery beds when bond pressure was removed during in recoveries. BFR applied during exercise and at rest has been shown to elicit multiple adaptive signals, i.e. increased shear stress, reduced muscle oxygenation and increased markers of oxidative stress, all of which are considered key stimuli of exercise induced angiogenesis and mitochondrial biogenesis (20, 22, 31). Therefore, although the BFR used in the present study compared with the HIIT alone did not significantly affect the expression of eNOS protein, however, due to the significant increase in VEGF protein expression in the HIIT+ BFR group compared to HIIT, it seems other angiogenic pathways were incrementally affected and evoked.

Studies have demonstrated a greater proliferative effect arising from continuous moderate intensity exercise than intense interval training (9) and a reduction in skeletal muscle VEGF protein content when trained runners increased bouts of intense exercise at the expense of total training volume (17). These investigations suggest that training volume as opposed to training intensity is a greater stimulus for angiogenesis in already trained individuals, at least in the short term. Gliemann (2016) proposed that one reason for a reduction in the angiogenic stimulus with a reduced training volume was the reduction in the shear stress stimulus. Shear stress is a product of both magnitude and duration, but although high levels of shear stress are elicited during intense intervals, it seems likely that this is outweighed by the prolonged stimulus continuous moderate intensity exercise elicits (32).

In general, angiogenesis is a complex process involving a series of inhibitory-excitatory signaling cascades involved in this process. Therefore, the regulation of angiogenesis is not dependent on one or two factors and there are still need to more exact investigations. In conclusion the results of the present study indicate that an eight-week course of HIIT and HIIT+BFR can increase the expression of

the VEGF and eNOS proteins in the vastus lateralis muscle of male med-endurance runners.

APPLICABLE REMARKS

- The training methods used in this article by increase in muscle protein (VEGF and eNOS) expressions, lead to optimal muscle adaptations that can affect athletes' adaptability to HIIT and HIIT+BFR.

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