http://jrhc.miau.ac.ir

Report of Health Care **Original Article**
Volume 3, Issue 3, 2017, p. 37- 45

The Effect of Exercise with Different Intensity on Platelet- Derived Growth Factor Gene Expression in Visceral and Subcutaneous Adipose Tissue of Rats

Mostafa Rahimi [*1], Sanaz Ghaforyan [2], Mohammad Reza Asad [2], Abouzar Abbasi [3]
1. Physical Education and Sport Sciences Department, Shahrekord University, Shahrekord, Iran
2. Exercise Physiology Department, University of Payam Noor, Iran
3. Exercise Physiology Department, Destruction 1 of Shiraz Education Department, Shiraz, Iran

Received: 25 April 2017
Accepted: 5 August 2017
Published online: 1September 2017

***Corresponding author:**
Mostafa Rahimi. Physical Education and Sport Sciences Department, Shahrekord University, Shahrekord, Iran

Phone: +989132811620

Fax: +983832324401

Email:
mostafa.rahimi20@gmail.com

Competing interests: The authors declare that no competing interests exist.

Citation: Rahimi M, Ghaforyan S, Asad MR, Abbasi A. The effect of exercise with different intensity on platelet- derived growth factor gene expression in visceral and subcutaneous adipose tissue of rats. Rep Health Care. 2017; 3 (3): 37-45.

Abstract

Introduction: Angiogenesis in adipose tissue plays an important role in the development or reduction of this tissue. Since exercise is essential in preventing obesity, the purpose of the present study was to investigate the effects of high intensity interval training (HIIT) and moderate intensity continues training (MICT) on platelet derived growth factor (PDGF) gene expression in visceral and subcutaneous adipose tissues in male Wistar rats.

Methods: In this experimental study, 24 male Wistar rats with 250-350 gram body masses were selected. As the study sample, the rats were divided into four groups: (1) basal control, (2) 8 weeks control, (3) MICT and (4) HIIT. Training program consisted of 8 weeks and 5 days per week running of MICT (15 to 30 m/min for 15 to 60 minutes) or HIIT (4 to 8 single-minute intervals of intense activity at a speed of 28-55 m/min and one-minute intervals of mild activity at a speed of 12-30 m/min). Adipose tissue samples were removed 48 hours after the last training session and PDGF gene expression was measured by Real-Time PCR methods. Kruskal-Wallis, one way ANOVA and Tukey post hoc tests were used to analyze the results at the significance level of 0.05.

Results: Results indicated that rats' weight of control group significantly increased (P= 0.001), but in HIIT (P = 0.34) and MICT (P = 0.22) groups, physical training prevented weight gain. In addition, there were significant effects neither subcutaneous (P = 0.38) nor visceral (P = 0.38) adipose tissues by HIIT and MICT on PDGF gene expression. However, both types of exercise activities, especially the HIIT exercise, increased the PDGF gene expression about 2.5 to 3 times in both adipose tissues.

Conclusion: It seems that the HIIT and MICT for 8 weeks do not have significant effects on PDGF gene expression in subcutaneous and visceral adipose tissues, although further studies are needed to clarify the issue.

Keywords: Training, Platelet-Derived Growth Factor, Adipose Tissue

Introduction

Adipose tissue in mammals is considered as a storage site of excess calories in the form of triglyceride, and is also an important endocrine organ that controls homeostasis of the whole body metabolism (1). A unique feature of adipose tissue is the ability to increase or decrease significantly the volume and size of cells. The development of adipose tissue is the result of an increase in the size of adipose cells, as well as the differentiation of progenitor cells to become new fat cells (1). Similar to all other tissues, changes in the adipose tissue mass should be associated with parallel changes in vascular circulation (1- 3). A sufficient supply of blood vessels to supply food and oxygen to all cells in the tissue and remove production waste is required, since it allows the tissue to create a functional interaction with other organs through the production of hormones and growth factors. In the case of adipose tissue, inadequate supply of blood vessels can cause triglyceride storage impairment by preventing access to circulating

blood lipoproteins under nutritional conditions, and also prevent the delivery of sufficient fuel to other organs in the fasting period (1). Angiogenesis is the process of creating new blood vessels from the vascular system available. Changes in angiogenesis can potentially disrupt the development of adipose tissue, thus providing a novel therapeutic approach to the treatment and prevention of obesity (2). So far, few studies have looked at angiogenesis in adipose tissue. In some studies, it has been argued that the adipose tissue remodeling (4) and other tissues (5, 6) depend on angiogenesis. In a study, Rupnick *et al.* (2002), suggesting the growth of tissues being dependent on their neovascularization, gave obese mice an anti-angiogenic drug and observed that this treatment led to a dose-dependent reversal of weight loss and reduced fat mass (4). In this study, the apparent vascular remodeling in adipose tissue was observed with decreasing endothelial proliferation and increased programmed death (apoptosis) in treated mice compared to control group. Therefore, the researchers found that the fat tissue mass is sensitive to angiogenesis inhibitors and can be controlled by the vessels (4). Under normal physiological conditions of the body, angiogenesis is closely monitored by two types of angiogenic and angiostatic agents (7, 8). Up to the present, more than 30 factors have been identified to stimulate and stop angiogenesis. Among the stimulating agents, vascular endothelial growth factor (VEGF), VEGF receptor (VEGFR) and platelet derived growth factor (PDGF) are more important and studied (9). Platelet-derived growth factors (PDGFs) are associated with the formation and strengthening of vessels. PDGFs include a family of mitogenic transplantation cells, such as smooth muscle cells, fibroblasts, and glial cells, which increase collagen production and control angiogenesis (7, 9). In the early stages of neovascularization and angiogenesis, factors such as fibroblast growth factor (FGF2) and VEGF play a start-up role and, by connecting with their own receptors, can promote the process of angiogenesis. As the process of the angiogenesis progresses in its final stages, when the blood vessels and circulatory system develop and mature, the role of PDGF becomes more pronounced. Recent studies have shown that PDGF is involved in stimulating angiogenesis when increasing the size of adipose tissue (10). Therefore, it seems that the evaluation of PDGF response is helpful in determining the fate of angiogenesis and in general, the circulation system compatibility (7, 9). Review of literature suggests that few studies have investigated the effect of exercise on the angiogenesis of adipose tissue (11, 12). In several studies, most often the role of VEGF in both visceral and subcutaneous adipose tissue in rats has been investigated (12-14), and only in one study, the effect of 6-week endurance training on PDGF in subcutaneous adipose tissue was investigated (14). Besides, visceral fat seems to be more active in comparison with subcutaneous fat in the metabolic rate (12). On the contrary, exercise activity has been shown to have a greater impact on adipokines and angiogenesis agents produced from subcutaneous adipose tissue than visceral fat (15). Recent studies have suggested that various models of sports exercises as a physiological stress can affect the regulation of angiogenesis of adipose tissue (12, 14), as Ramose *et al.* (2015) in an analytical overview to compare the effect of HIIT and MICT on vascular function concluded that HIIT exercises had a greater effect on vascular function (16). Considering the beneficial effects of high intensity interval training on adipose tissue, determining angiogenic and angiostatic responses of adipose tissue to these types of exercises and comparing them with continued exercises can help to understand the mechanisms of these training patterns in reducing obesity and related diseases. If exercise and specific physical activity patterns can be effective in stimulating or inhibiting angiogenesis, they can act as a non-

pharmacological new regulatory approach to fatty tissue growth. In this regard, the purpose of this study is to investigate the effect of exercise with different intensity on the expression of PDGF in visceral and subcutaneous adipose tissue of rats.

Methods

In this experimental study, 24 Wistar healthy male rats weighing 250-300 grams was selected. After two weeks of familiarity with the laboratory environment, they were divided into four groups including: basal control group (n = 6), control of 8 weeks (n = 6), HIIT (n = 6) and MICT (n = 6). Additionally, in this study, the ethical principles were followed in accordance with the principles of working with laboratory animals at Baqiyatallah University of Medical Sciences, and with the Code of Ethics Committee ES-124-94. The rats were kept under control in laboratory conditions (mean temperature of 22 ± 3 ° C, darkness cycle 12:12 hours) and had free access to water and food. Basal control group was sacrificed at the beginning of the study and their subcutaneous and visceral fat tissue was taken. 8 weeks control group alongside the experimental groups was kept without participation in any training program for eight weeks. In order to familiarize the rats in the training groups with treadmill and to reduce the stress caused by this, the rats participated in a training program for 6 sessions in two weeks at a low speed (10-20 m / min) and ten minutes duration. The training program was designed and implemented in experimental groups with running on treadmill for eight weeks and five sessions per week in accordance with the principles of practice and derived from the previous studies (17, 18). The training protocol in the MICT group included running at speeds of 15 to 30 m/min (equivalent to 50 to 75% of maximal oxygen consumption) and for 15 to 60 minutes. The HIIT training protocol consisted of 4 to 8 single-minute intervals of intense activity at a speed of 28-55 m/min (approximately 70 to 100% of maximum oxygen consumption), and one-minute intervals of mild activity at a speed of 12-30 m /min (approximately 50-70% of the maximum oxygen consumption). The program was warmed up and cooled for 5 and 3 minutes, respectively, at the beginning and end of the training program. 48 hours after the last training session and after 12 hours of fasting, the rats were anesthetized by intraperitoneal injection using xylazine (10 mg/kg) and ketamine (100 mg/kg) anesthetics and subcutaneous and visceral (intra-abdominal) adipose tissues were extracted. Then, after being washed with normal cold saline, tissues were immediately frozen in liquid nitrogen and stored for molecular cellular experiments in a -80 ° C freezer. The level of PDGF gene expression was measured by Real-time RT-PCR. In this method, after extracting the whole RNA using trisol, the purity of the RNA was measured by spectrophotometer (WPA Biowave II, UK). The cDNA was made in two steps using a kit (Fermentase, GmBH, Germany). Polymerase chain reaction (PCR) was performed using LifeTechnologies, USA and the SYBER® Green I PCR Master Mix kit. Primer sequences were prepared from the National Center for Bioinformatics Information (NCBI) database, and then PDGF gene primers were designed using preprimer, primer 3 and oligo online programs. In this study, the GAPDH gene was also measured as a reference. The characteristics of the primers used are given in Table 1.

The $2^{-\Delta\Delta CT}$ method was used to evaluate the quantitative relative expression of PDGF gene.

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

ΔCT = CT target gene – CT reference gene

$\Delta\Delta CT$ = ΔCT test sample – ΔCT Control sample

Using SPSS 20 software, data were analyzed at the significance level of 0.05. The normal distribution of data was checked by the shapiro wilks test. Non-parametric Kruskal-Wallis statistical test was used to analyze the results due to the lack of homogeneity of variances.

Table1. Characteristics of the primers used to evaluation of PDGF gene.

Primers sequence	primers
F: 5/ - TCTGGGTAGGAGAATCCAAACTT-3/	PDGF
R: 5/ - ACTTCTGAGTGTTGGGACTGT -3/	
F: 5/ - ACACCCGCTCATCAATCTTT -3/	GAPDH
R: 5/ - AGGTCCACGACTCTGTTGCT -3/	

Bp: base pair, F: Forward, R: Reverse

The body weight of rats was measured at the first week and before sacrificed. The weight of rats was measured in pre-test and post-test and analyzed by one way ANOVA and Tukey tests.

Results

The mean and standard deviation of the body weight of the rats are presented in Table 2 in the basic and before sacrifice levels. Statistical analysis with ANOVA and Tukey post-hoc tests showed that the body weight of the control group increased significantly after 8 weeks (P = 0.001), but the MICT (P = 0.34) and HIIT (P = 0.22) has prevented this weight gain.

The results of Kruskal-Wallis test showed that 8 weeks of MICT and HIIT did not have a significant effect on PDGF gene expression in subcutaneous fat tissue (P = 0.38) (Fig. 1). However, HIIT had a more effect on the expression of this gene. Also, the Kruskal-Wallis test showed that in visceral adipose tissue, HIIT and MICT did not have a significant effect on PDGF gene expression (P = 0.38) (Fig. 2). Interestingly, in this tissue, the effect of the HIIT training is greater than the MICT training, and has led to a greater increase in the PDGF gene expression.

Discussion

The results of this study showed that MICT and HIIT activity prevented the weight gain of the rats compared to the control group. In the present study, although changes in PDGF gene expression in adipose tissue after 8 weeks of MICT and HIIT were not statistically significant, increasing PDGF gene expression in subcutaneous and visceral adipose tissue after HIIT was significant. This means that the HIIT increased about 2.8 and 2.3 times the expression of the gene in the subcutaneous and visceral adipose tissue, respectively. So far, in several studies, the role of various sports activities has been studied on contributing proteins in the angiogenesis of skeletal and serum muscle tissue in human and animal subjects. In contrast, the effect of exercise on angiogenesis of adipose tissue has been studied only in a few limited studies (12-14). In the only study on the PDGF protein by Czarkowska *et al.* (2011), similar to the findings of the present study, increased expression of PDGF-A gene in subcutaneous adipose tissue in rats was seen three hours

Table 2. Body weight of rats in groups before and after 8 weeks

Groups		CO	CO8W	MICT	HIIT
Body	Basic	237.5 ± 6.87	233.4 ± 7.15	234.7 ± 7.64	236.9 ± 8.85
weight (gr)	8 weeks	-------	295.8 ± 8.8 *	241.5 ± 10.3	258.4 ± 11.5

Data is presented as mean and standard deviation. *: Significant difference compared to baseline data. CO: base line control group; CO8W: 8 weeks control group; MICT: moderate intensity continuous training; HIIT: high intensity interval training

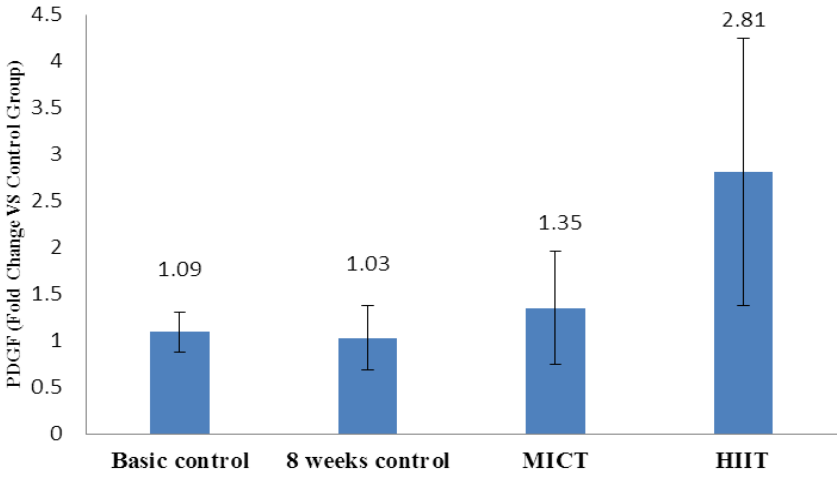

Figure 1. PDGF gene expression of subcutaneous adipose tissue
MICT: moderate intensity continuous training; HIIT: high intensity interval training

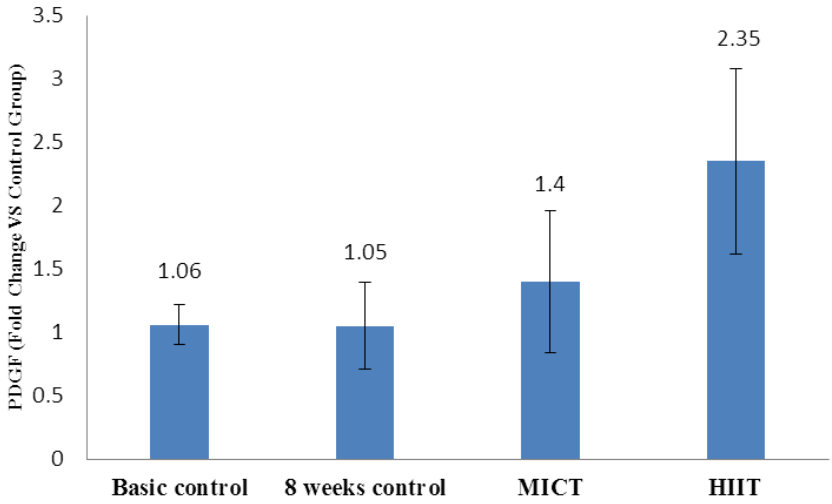

Figure 2. PDGF gene expression in visceral adipose tissue
MICT: moderate intensity continuous training; HIIT: high intensity interval training

after the completion of six weeks of endurance training, however a change in the protein level of PDGF-A in subcutaneous adipose tissue was not observed at this time (14). PDGF, as a strong mitogen in the body, plays an important role in regulating vital cell activities such as cell division, angiogenesis, cell proliferation and cell migration. Therefore, modest changes in levels of PDGF in the human body have clinical and physiological significance. In addition, studies have shown that PDGF is involved in stimulating angiogenesis when increasing the size of adipose tissue (10). Regarding the role of the VEGF gene, Hatano *et al.* (2011) reported an increase in the VEGF gene and its receptor in visceral fat tissue of male rats after nine weeks of endurance training (13). In addition, Disanzo and You (2014) reported that after eight weeks of endurance training, the level of VEGF-A gene in the visceral fat tissue of rats was increased, but no changes in the amount of this gene were

found in subcutaneous fat tissue (12). Concerning the metabolic and angiogenic activity of adipose tissue in different regions of the body, there are a few yet inconsistent results. It has been reported in a study that sports activity is likely to affect levels of VEGF and lactate metabolism in visceral adipose tissue, which supports the idea that visceral fat may be metabolically more potent than subcutaneous fat (12). On the contrary, endurance training has been shown to have a greater effect on adipokines produced from subcutaneous adipose tissue than visceral fat (15). For example, performing optional exercise for four weeks on rotating wheels in rats significantly increased the expression of several cytokines (interleukin 6 (IL-6), Tumor necrosis factor-α (TNF-α) and interleukin-1 receptor antagonist (IL-1 Ra)) in subcutaneous fat, while no changes in visceral fat tissue were established. This phenomenon suggests that subcutaneous fat may play a special role in the response to the physical exercise (19). This has also been confirmed in other studies on adipokines such as IL-18 and visfatin (14). In addition, diet and body mass index also affect the angiogenesis of adipose tissue. Animal studies have shown that diet-induced obesity increases levels of VEGF-A gene in the visceral fat tissue (15). In human studies, it has been shown that obese subjects have lower levels of VEGF-A gene in abdominal adipose tissue (20), and in obese subjects with higher body mass index, VEGF-A release rates have been lower in adipose tissue (21). Another issue is that it seems that various mechanisms and agents stimulate the process of angiogenesis in different tissues. Recently, Walton *et al.* (2015) observed the effect of 12 weeks of cycling in 45 minutes on 26 patients with insulin resistance. Although this type of exercise led to an increase in some angiogenic indexes (Cyclophilin A, Angiopoietin 1 (Angpt1), Angiopoietin 1 (Angpt2), cluster of differentiation 31 (CD31), Hypoxia-inducible factor 1-alpha (HIF-1α), Tyrosine kinase with immunoglobulin-like and EGF-like domains 1 (TIE1), TIE2, VEGFA) in skeletal muscle tissue, this training pattern did not change the angiogenic index of subcutaneous adipose tissue in the abdominal region (22). The process of angiogenesis can potentially accelerate or disrupt the development of adipose tissue, so this can be considered as a novel therapeutic method for the prevention and treatment of obesity (2). The findings from recent studies report that exercise can manipulate regulation of angiogenesis in adipose tissue (12, 14). The optimal effectiveness of exercise in stimulating or inhibiting angiogenesis can be considered as a non-pharmacological strategy for regulating the growth of adipose tissue. In the adipose tissue, growth factors including VEGF and PDGF play an important role. In addition, it has been reported that there is a significant correlation between body mass index and Transforming Growth Factor beta 1 (TGF-β1) presence and release in subcutaneous fat tissue. This factor affects the metabolism of fat cells and potentially inhibits differentiability of lipid progenitor cells to adipose cells, as well as adiposeness (14). Among all the contributing factors in lipid angiogenesis, VEGF seems to play a more important role. When the new adipose tissue, adiposeness, is formed, VEGF increases the angiogenesis in the tissue. Also, the blockage of the VEGF2 receptor (VEGFR2) limits the formation of adipose tissue in obesity caused by nutrition (14). Although several mechanisms increase the expression of genes involved in angiogenesis, and many of them are unknown, it seems that the induction of HIF-1 plays an important role in activating these genes, especially VEGF (14). In addition, it has been shown that factors such as hypoxia and non-hypoxia may be responsible for increasing HIF-1 in the tissues. Other factors such as free fatty acids (FFA) and IL-6 may reach the adipose tissue from other tissues in the endocrine form and cause angiogenesis (14). Increasing the size and number of fat cells without adequate blood flow causes hypoxia in

the tissue. In the situation of obesity, fat cells move toward anaerobic metabolism, which leads to increased lactate production. Increasing lactate and some other factors indicate hypoxia in adipose tissue (12, 15). In rodents, it has been reported that, with obesity or caloric restriction, lactate levels of visceral fat are increased and decreased, respectively (15). Therefore, the lack of oxygen in fat cells appears to cause chronic inflammation in adipose tissue during obesity (12, 15). Interestingly, human studies have shown that obese people have lower levels of oxygen consumption in adipose tissue compared with lean subjects (12). The amount of lactate produced is reduced by improving the blood flow to the adipose tissue or increasing the oxygen consumption by improving the biogenesis of the fat cells. However, sports activity in rats has been reported to increase the blood flow of adipose tissue and, by increasing the expression of the PGC-1α gene, adjusts mitochondria biogenesis (12). On the other hand, a variety of sports activities can have different effects. Meanwhile, intensity of exercise is one of the important factors influencing the response and adaptability of tissues to exercise. Several studies have suggested that extreme sports have been able to produce favorable changes in visceral adipose tissue (23, 24). In the present study, MICT and HIIT had a nearly different effect on angiogenesis, despite the doubling of the time of MICT compared to HIIT training (15 to 60 minutes versus 7 to 15 minutes). The significance of this is due to the instructions from the American College of Exercise Medicine (ACSM) and the World Health Organization (WHO) which recommend 150 minutes of moderate intensity or 75 minutes of intense activity to gain health benefits from exercise and prevent chronic diseases (25, 26). In a review analysis article, Ohkawara *et al.* (2007), revisiting the findings on the effect of exercise on visceral fat, indicated that the intensity of aerobic exercise based on the four MET was significantly related to the percentage of visceral fat reduction. It was also reported that there is a significant relationship between weight losses and reduced visceral fat, although visceral fat loss can occur without weight loss. Accordingly, the researchers suggested that aerobic exercise should be about ten MET per week, which may include fast walking, jogging, and the use of an ergometer to reduce visceral fat, because there is a dose-dependent releation between aerobic exercise and the reduction of visceral fat in obese people with no metabolic disorders (24). According to the results of this research and studies close to the present study, it can be suggested that the effect of exercise on the angiogenesis of adipose tissue is more dependent on the intensity of exercise than it is related to the duration of exercise. Research in the field of angiogenesis in adipose tissue, such as a toddler, is still at the beginning steps, and the clinical use of regulating and controlling the angiogenesis of adipose tissue opens the horizon for future studies in this area. Performing exercise as one of the main factors regulating the growth of adipose tissue and determining the role of different patterns of exercise with the severity, duration and frequency of different sessions can play a pivotal role in understanding the mechanisms of angiogenesis control and possible clinical angiogenesis of adipose tissue. As a result, identification of a suitable sports pattern that can induce angiogenic or angiostatic changes in this tissue requires further studies and achieving this may be able to provide a new non-pharmacological approach in the treatment and prevention of obesity.

Conclusion

It seems that the HIIT and MICT for 8 weeks do not have significant effects on PDGF gene expression in subcutaneous and visceral adipose tissues. But there was an increase in this gene expression especially after HIIT protocol, although further studies are needed to clarify the issue.

Ethical issues

Not applicable.

Authors' contributions

All authors equally contributed to the writing and revision of this paper.

Acknowledgments

We are very grateful from Baqiyatallah University of Medical Sciences and Tehran University of Medical Sciences for laboratory assistance and genes measurements and all those who helped us in this research.

References

1. Rojas-Rodriguez RO, Gealekman ME, Kruse B, Rosenthal K, Rao S, Min A, and et al. Adipose tissue angiogenesis assay. Methods Enzymol. 2014; 537: 75.
2. Christiaens V and Lijnen HR. Angiogenesis and development of adipose tissue. Mol Cell Endocrinol. 2010; 318 (1): 2-9.
3. Lijnen C.V.H.R and K.L. Center for Molecular and Vascular Biology, Leuven, Belgium, Angiogenesis and development of adipose tissue. Mol Cell Endocrinol. 2010; 2 (318): 1-20.
4. Rupnick MA, D Panigrahy C-Y, Zhang SM, Dallabrida BB, Lowell R, Langer, and et al. Adipose tissue mass can be regulated through the vasculature. Proc Natl Acad Sci. 2002; 99 (16): 10730-10735.
5. Folkman J., Editorial :Is tissue mass regulated by vascular endothelial cells? Prostate as the first evidence. Endocrinology. 1998; 139 (2): 441-442.
6. Franck-Lissbrant IS, Häggström J-E, Damber, and Bergh A. Testosterone Stimulates Angiogenesis and Vascular Regrowth in the Ventral Prostate in Castrated Adult Rats 1. Endocrinology. 1998; 139 (2): 451-456.
7. Das SK, Vasudevan DM. Essential factors associated with hepatic angiogenesis. Life Sci. 2007; 81: 1555-1564.
8. Tahergurabi Z and Khazaei M. Obesity and Angiogenesis. J Res Med Sci. 2012; 29: 173.
9. Korivi M, Hou CW, Chen CY, Lee Jo-Ping, Kesireddy SR, Kuo CH. Angiogenesis: Role of exercise training and aging. Adapt. Med, 2010; 2: 29-41.
10. Pang CZ, Gao J, Yin J, Zhang W, Jia and J Ye. Macrophage infiltration into adipose tissue may promote angiogenesis for adipose tissue remodeling in obesity. Am J Physiol Endocrinol Metab. 2008; 295 (2): E313-E322.
11. Cullberg KB, Christiansen T, Paulsen S, Bruun JM, Pedersen SB and Richelsen B. Effect of weight loss and exercise on angiogenic factors in the circulation and in adipose tissue in obese subjects. Obesity. 2010; 21 (3): 454-460.
12. Disanzo BL and You T. Effects of exercise training on indicators of adipose tissue angiogenesis and hypoxia in obese rats. Metabolism. 2014; 63 (4): 452-455.
13. Hatano D, Ogasawara J, Endoh S, Sakurai T, Nomura S, Kizaki T, and et al. Effect of exercise training on the density of endothelial cells in the white adipose tissue of rats. Scand J Med Sci Sports. 2011; 21 (6): e115-e121.
14. Czarkowska-Paczek B, Zendzian-Piotrowska M, Bartlomiejczyk I, PrzybylskiJ, and Gorski J. The influence of physical exercise on the generation of TGF-β, PDGF-AA, and VEGF-A in adipose tissue. Eur J Appl Physiol. 2011; 111 (5): 875-881.
15. Ye J, Gao Z, Yin J, and He Q. Hypoxia is a potential risk factor for chronic inflammation and adiponectin reduction in adipose tissue of ob/ob and dietary obese mice. Am J Physiol Endocrinol Metab. 2007; 293 (4): E1118-E1128.
16. Ramos JS, Dalleck LC, Tjonna AE, Beetham KS, and Coombes JS. The impact of high-intensity interval training

versus moderate-intensity continuous training on vascular function: a systematic review and meta-analysis. Sports Med. 2015; 45 (5): 679-692.
17. Lee Y, Min K, Talbert EE, Kavazis AN, Smuder AJ, Willis WT, and et al. Exercise protects cardiac mitochondria against ischemia-reperfusion injury. Med Sci Sports Exerc. 2012; 44 (3): 397-405.
18. Rahimi M, Shekarforoush S, Asgari AR, Khoshbaten A, Rajabi H, Bazgir B, and et al. The effect of high intensity interval training on cardioprotection against ischemia-reperfusion injury in wistar rats. EXCLI journal. 2015; 14: 237.
19. Gollisch KS, Brandauer J, Jessen N, Toyoda T, Nayer A, Hirshman MF, and et al. Effects of exercise training on subcutaneous and visceral adipose tissue in normal-and high-fat diet-fed rats. Am J Physiol Endocrinol Metab. 2009; 297 (2): E495.
20. Pasarica M, Sereda OR, Redman LM, Albarado DC, Hymel DT, Roan LE, and et al. Reduced adipose tissue oxygenation in human obesity. Diabetes. 2009; 58 (3): 718-725.
21. Fain JN, Madan AK, Hiler ML, Cheema P, and Bahouth SW. Comparison of the release of adipokines by adipose tissue, adipose tissue matrix, and adipocytes from visceral and subcutaneous abdominal adipose tissues of obese humans. Endocrinology. 2004; 145 (5): 2273-2282.
22. Walton RG, Finlin BS, Mula J, Long DE, Zhu B, Fry CS, and et al. Insuline-resistant subjects have normal angiogenic response to aerobic exercise training in skeletal muscle, but not in adipose tissue. Physiological rep. 2015; 3 (6): e12415.
23. Coker RH, Williams RH, Kortebein PM, Sullivan DH, and Evans WJ. Influence of exercise intensity on abdominal fat and adiponectin in elderly adults. Metab Syndr Relat Disord. 2009; 7 (4): 363-368.
24. Ohkawara K, Tanaka S, Miyachi M, Ishikawa-Takata K, and Tabata I. A dose–response relation between aerobic exercise and visceral fat reduction: systematic review of clinical trials. Int J Obes. 2007; 31 (12): 1786-1797.
25. ACSM F.e.a. Exercise for Persons with Cardiovascular Disease. 2016; Available from: https://www.acsm.org/docs/current-comments/exercise-for-persons-with-cardiovascular-disease.pdf?sfvrsn=6.
26. WHO and 2010. Global Recommendations on Physical Activity For Health. Cataloguing-in-Publication Data. 2010.