Journal of Sports Physiology and Athletic Conditioning (J.S.P.A.C) 2023; 3 (9): 17-28

Research Article

Investigating the effect of aerobic exercise and octopamine on HIF-1 gene and protein expression and the permeability of white cells into visceral adipose tissue in rats fed with heated oil

Fateme Shokri[1], Mohammad Ali Azarbayjani*[2], Maghsoud Peeri[3], Farshad Ghazalian[4]

1, Bachelor of Physical Education, Tehran University of Medical Sciences
2,3. Department of Sport Physiology, Central Tehran Branch, Islamic Azad University, Tehran, Iran
4. Department of Sports Physiology, Science and Research Branch, Islamic Azad University, Tehran, Iran

Received: 16 July 2023
Revised: 20 August 2023
Accepted: 14 September 2023

Keywords:
aerobic exercise, adipose tissue, heated oil, fed rats

Abstract

Background: the use of heated oils has become an integral part of today's nutrition. Studies show that with the development of obesity, capillarization in adipose tissue does not occur in line with changes in cell size. Therefore, adipose tissue in obese people is always associated with lack of oxygen and it causes systemic inflammation by releasing inflammatory mediators. The purpose of this study is to investigate the effect of aerobic exercise and octopamine on HIF-1 protein concentration in visceral fat and white cells in rats fed with deeply-heated oil.

Materials and Methods: In an experimental trial, 30 male Wistar rats were divided into five groups: healthy control, control-heated oil, aerobic exercise-heated oil, octopamine-heated oil and exercise, and octopamine-heated oil. Octopamine was given to rats by IP intraperitoneal injection daily for four weeks and five days a week. Aerobic exercise was also performed for four weeks and five days a week with moderate intensity on the treadmill. Forty-eight hours after the last intervention, the rats were anesthetized and visceral adipose tissue was removed from the body to measure HIF-1a gene expression.

Results: As a result of receiving deeply-heated oil, the expression of HIF-1 gene and protein in visceral fat increased significantly (P=0.001), but the number of white cells in visceral adipose tissue increased significantly (P=0.001). Aerobic exercise significantly decreased HIF-1 gene and protein expression (P=0.01). In addition, octopamine supplementation had no significant effect on HIF-1 gene expression of visceral fat of white cells in rats poisoned with deeply-heated oil. Receiving octopamine also decreased HIF-1 gene and protein expression (P=0.002). In addition, exercise significantly reduced the number of white cells (P=0.001). Octopamine could significantly reduce the expression of HIF-1 protein and the number of white cells. The interaction of exercise and octopamine was significant for the expression of HIF-1 protein and the number of white cells.

Conclusion: The results of this study showed that aerobic exercise and octopamine improve the angiogenesis process of the visceral adipose tissue that had been disrupted by heated oils, and reduce the damage caused by feeding with deeply-heated oils.

***Corresponding author:** Mohammad Ali Azarbayjani
Address: Department of Sports Physiology, Central Tehran Branch, Islamic Azad University, Tehran, Iran.
Email: ali.azarbayjani@gmail.com **Tell**: 00982122481622-3
M A: 0000-0002-3502-7487

1. Introduction

Overweight and obesity are the main risk factors for chronic diseases such as diabetes, cardiovascular diseases and cancer. At the beginning, this problem was seen only in high-income countries, but now it is increasing alarmingly in low- and middle-income countries, especially in urban societies. Overweight and obesity are defined as "abnormal and excessive accumulation of fat" which poses a health risk (1-4). This disorder is the foundation of diseases related to it such as diabetes and atherosclerosis, high blood pressure, cardiovascular diseases and cancer, which are considered as the main causes of death in societies with a western lifestyle. The growth of adipose tissue is accompanied by fundamental changes in the structure of adipose tissue. The main feature of these changes is the ability to greatly decrease and increase the size of this tissue. The growth of adipose tissue is related to its blood vessels. In addition, it has been shown that the growth of adipose tissue is related to angiogenesis. One of the specific characteristics of adipose tissue is its ability to grow excessively in response to a positive energy balance and decrease in size when stored calories move to other body organs to be consumed. The growth of adipose tissue occurs as a result of the increase in the size of adipose tissue cells or the differentiation of precursor cells into fat cells. Based on the findings of recent limited studies, it has been suggested that different patterns of exercise as a physiological stress can have different effects on the regulation of adipose tissue angiogenesis. If exercise and specific patterns of physical activity can be effective in stimulating and inhibiting angiogenesis, they can play a role as a new non-pharmacological approach in regulating the growth of adipose tissue.

Since both aerobic exercise and octopamine can increase lipolysis and oxidation of fatty acids through different mechanisms, it can be expected that the size of fat cells will decrease, and the balance between the size of the cell and the capillaries around it will be established. However, the studies show that in the conditions of feeding with high-fat food, the effect of these two interventions on the indicators of capillarization in visceral adipose tissue is not known. Based on this, the aim of this study is to explain the simultaneous effects of aerobic exercise and octopamine on HIF gene and protein expression and the number of white cells in visceral adipose tissue (5). Excessive consumption of energy, which is observed in a high-fat diet, causes metabolic adaptation in adipose tissue and skeletal muscle to increase the oxygen demand in the tissue. In this situation, the regeneration of the new capillary network should be stimulated. But in reality, impaired capillarization in both skeletal muscle tissue and white adipose tissue is disturbed in the conditions of feeding with high-fat food (6). In addition, the reduction of capillarization in white adipose tissue has been reported as one of the main factors in the pathogenesis of adipose tissue in conditions of obesity and feeding with high-fat food (7). It has been suggested that the reduction of capillarization in adipose tissue leads to metabolic complications caused by obesity. On the contrary, increasing capillary density in skeletal muscles or adipose tissue (8) improves not only peripheral metabolism but also the metabolic state of the whole body

These findings indicate that the reduction of angiogenesis may be the cause of the disruption of homeostasis caused by obesity signals in adipose tissue and skeletal muscle and plays a significant role in the development of obesity-related disorders. In their study, Rudnicki et al. (2018) investigated the effect of feeding with high-fat food on the capillarization process of visceral adipose tissue in male and female rats. The findings of this study showed that the visceral adipose tissue of female rats showed a higher amount of capillarization and vascular factors in response to a high-fat diet compared to male rats. This phenotype was associated with maintaining metabolic balance at both tissue and systemic levels. Based on these findings, it was found that the sex-specific difference in the regulation of adipose tissue capillarization is very significant, which may contribute to a person's susceptibility to causing fat dysfunction and obesity-related metabolic disorders (9).

In this regard, Bolinder et al. (2000) reported that the blood flow of adipose tissue and muscles in obese people is approximately 30-40% lower than in non-obese people, which can be due to the inadequacy of the capillary network in obese people compared to non-obese people (10). In line with the reduction of capillarization in adipose tissue in conditions of obesity, Goossens et al. (2011) showed that in obese people, in addition to the low blood flow of adipose tissue, the oxygen consumption of this tissue is also lower than that of non-obese people (11). Goossens and Blaak (2015) again showed that in the condition of obesity, the oxygen supply to adipose tissue in obese people is disturbed and as a result, fat cells do not receive enough oxygen (12).

Brahimi-Horn and Pouysségur (2007) showed that in hypertrophied white fat cells, the diameter of the cell exceeds the normal distance of oxygen diffusion from the width of the tissue. Based on this, it is clear that in this condition, larger white fat cells will have less ability to receive oxygen (13).

But Goossens et al. (2012) showed that in the adipose tissue of obese people, there is only a very small proportion of fat cells with a diameter of more than 100 micrometers (14).

These findings show that in humans, adipose tissue hypoxia is more due to a decrease in blood flow to the adipose tissue rather than a change in cell size. Therefore, the contribution of fat cell size increase to hypoxia response is controversial (15).

2. Materials and Methods

1.2 Animals

In an experimental trial, 30 male Wistar rats in the weight range of 300 to 350 grams were selected as subjects from the Histogene Research Center. These animals were kept in transparent polycarbonate cages with dimensions of 42x26.5x15 cm, temperature of 24 degrees Celsius, humidity of 50% and 12:12 light–dark cycle with proper ventilation. In addition, during the research, the animals were fed with daily pellet food and had free access to city tap water through special bottles. After familiarizing the subjects with the laboratory environment, they were randomly divided into five groups: healthy control group, control group receiving deeply-heated oil, aerobic exercise group receiving deeply-heated oil, octopamine group receiving deeply-heated oil, and aerobic exercise and octopamine group receiving deeply-heated oil. All the measures performed in this study were designed and implemented based on the instructions for the care and use of laboratory animals in scientific affairs approved by the Ministry of Health and Medical Education of the Islamic Republic of Iran.

2.2 Preparation of heated oil

Eight liters of margarine oil were exposed to a temperature of 190 to 200 degrees Celsius for 4 days, 8 hours a day. On the first day, the oil was exposed to heat in an unmixed form. But from the second day onwards, during eight hours of heat, some chicken nuggets and carbohydrates were fried inside the oil every day (8).

2.3 Octopamine

Octopamine was obtained from Sigma-Aldrich with Cat no 0.0250. Its solvent was 9% normal saline (NaCl), which was measured as 18 cc of normal saline in 0.2764 grams by a scale (C:0001) and was dissolved in a homogenizer (Sonicator). Rats received 81 µmol octopamine per each kg of their weight (dissolved in 9% normal saline) intraperitoneally for 4 weeks and 5 days per week.

Octopamine is a biogenic monoamine that is structurally and functionally very close to noradrenaline. Octopamine acts as a neurotransmitter in invertebrates and is a trace amine with uncertain properties in vertebrates and affects adrenergic and dopaminergic systems. Octopamine is present in various plants and fruits, but one of its richest sources is orange blossom (16).

2.4 Aerobic exercise program

The subjects in the exercise groups exercised on the treadmill for 20 minutes at a speed of 9 m/min (meters per minute) for five days before starting the exercise.

The exercise program started with 4 weeks (5 days a week with 2 rest days) of moderate intensity and a speed of 16 m/min and at the end of the fourth week, it reached a speed of 26 m/min. A daily exercise session consisted of 5 minutes of warming up, 20 minutes of running and 5 minutes of cooling down.

2.5 Biopsy from animals

Forty-eight hours after the last intervention, all the rats were fasted for 8 to 10 hours and were weighed before biopsy. Anesthesia was done by inhalation with chloroform. After general anesthesia and pain test, making sure that there is no consciousness, blood was taken from the left ventricle of the heart. Then a visceral white adipose tissue was quickly removed from the body and the mucus, blood and extra substances were cleaned by washing with phosphate buffered saline (PBS) and then the tissue was placed inside the coded 2ml microtube. The microtube was transferred into a nitrogen tank and then kept in a freezer at -80°C until cell analysis.

3 How to measure HIF protein gene expression

Quantification of the protein content of a sample is an important process and has wide applications in medical diagnosis and research laboratories. Accurate quantification of the protein sample is a vital step in protein analysis. During the last two decades, different techniques have been developed to measure the concentration of protein samples. Spectroscopic methods are among the most common methods for quantifying the concentration of protein samples. The common methods used for protein quantification include various methods such as Lowry, Bivert, Bradford and BCA, of which Bradford and BCA methods are the most used today.

In order to check the protein content of the protein extracts obtained from the cells, the Bradford method was used, which is one of the most accurate methods for measuring the amount of protein. This method is based on the binding of Coomassie Brilliant Blue G-250 dye to protein. At acidic pH, the color of Bradford solution is red, which in this case is a strong absorber at the wavelength of 465 nm, but when the dye is attached to the protein, it becomes blue by taking two protons and becomes stable. In this case, the absorption maximum changes from 465 nm (red color) to 595 nm (blue color).

BSA was used as the standard protein to draw the Bradford standard curve, so that 2 mg of BSA was dissolved in 1 ml of distilled water at first. Then, from this stock, protein solutions with concentrations of 0, 200, 400, 600, 800, 1000, 1200, 1400, 1800 and 2000 µg/ml were prepared with distilled water. After preparing BSA standards, 2 ml of Bradford reagent (0.01% Coomassie Brilliant Blue G-250, 5% ethanol 95%, 10% phosphoric acid 85% in distilled water) was poured into test tubes. 40 µl of prepared BSA standard solutions were added to each tube. Distilled water was added to Blank sample instead of protein. The samples were vortexed and after keeping at ambient temperature for 10 minutes, their optical absorption was measured at 595 nm and the standard curve was drawn.

In order to determine the concentration of protein samples for taking equal amounts of them for SDS-PAGE electrophoresis, 40 µl of each protein extract that was previously extracted from the cells was added to 2 ml of Bradford's reagent, and distilled water was added to the Blank sample instead of protein. Then, using the line graph obtained from the standard curve as well as the absorbances obtained from cell extracts, the concentration of protein samples was calculated in mg/ml.

3. Results

I) Write the effect of receiving heated oil on the amount of this protein, then write the following items.

Aerobic exercise significantly reduced HIF-1 protein concentration in visceral fat (F=121.57, P=0.001, η=0.865). Octopamine supplementation also caused a significant decrease in visceral fat HIF-1 protein concentration (F=69.27, P=0.001, η=0.879). The interaction of exercise and Octopamine supplementation also had a significant effect on the HIF-1 protein concentration of visceral fat, and the combination of two interventions of exercise and Octopamine supplementation decreased the HIF-1 protein concentration of visceral fat (F=27.66, P=0.001, η=0.593) (Figure 1).

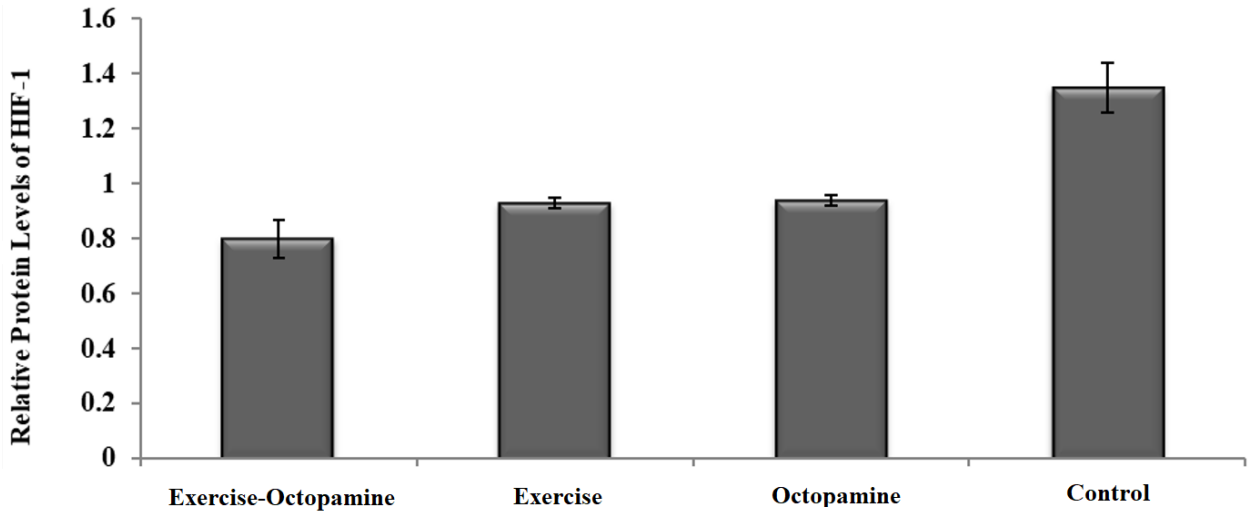

Figure 1. HIF-1 protein concentration of visceral fat in the studied groups. Data are reported based on mean and standard deviation.

II) comparing the number of white cells in the healthy control group and the control poisoned with deeply-heated oil

Aerobic exercise had a significant effect on the number of white cells (F=138.52, P=0.001, η=0.879). Octopamine supplementation also had a significant effect on the number of white cells (F=145.55, P=0.001, η=0.939). The interaction of aerobic exercise and octopamine supplementation also had a significant effect on the number of white cells (F=109.08, P=0.001, η=0.852) (Figure 2).

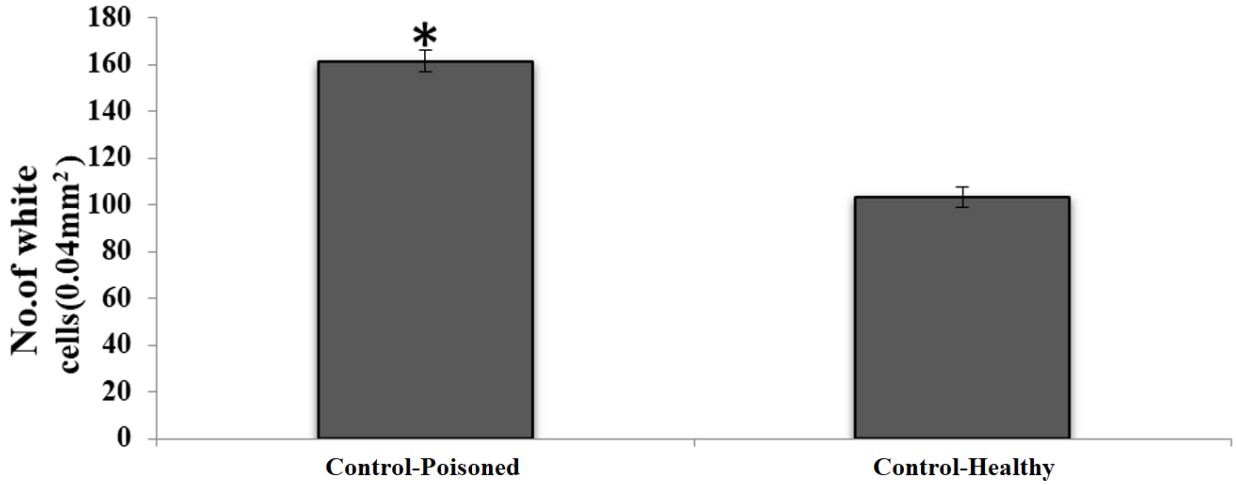

Figure 2. Comparison of the number of white cells in the healthy control group and the control poisoned with deeply-heated oil. sign of significant difference compared to the healthy control group

III) Comparison of gene expression

Based on the results obtained from the two-way analysis of variance test, it was found that exercise had a significant effect on HIF-1 gene expression in visceral fat (F=8.17, P=0.010, η=0.301). Octopamine supplementation had a significant effect on HIF-1 gene expression in visceral fat (F=8.39, P=0.002, η=0.469). However, the interaction of exercise and octopamine supplementation had no significant effect on HIF-1 gene expression in visceral fat (F=2.20, P=0.154, η=0.104) (Figure 3).

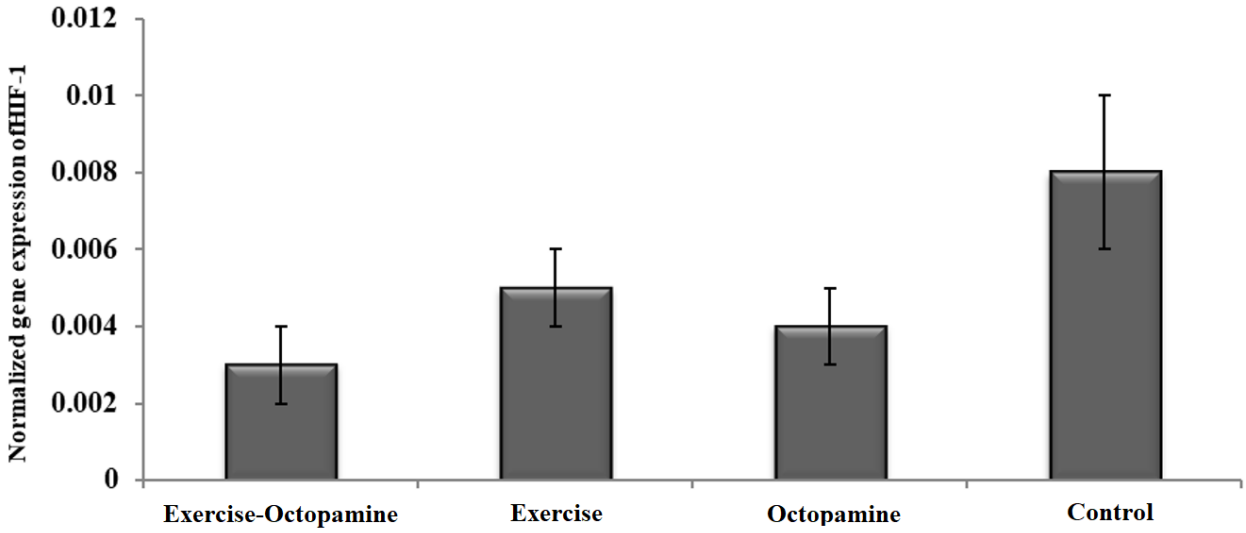

Figure 3.

4. Discussion

In this part of the article, the mechanism of the findings obtained from the study will be discussed and interpreted according to the previous studies.

The use of heated oils has become an integral part of today's diet. Studies show that with the development of obesity, capillarization in adipose tissue does not occur in line with changes in cell size. Therefore, adipose tissue in obese people is always associated with lack of oxygen and by releasing inflammatory mediators, it causes systemic inflammation. Based on this, the aim of this study was to determine the effect of aerobic exercise and octopamine on HIF-1 gene expression, HIF-1 protein concentration, and the number of visceral fat white cells of rats poisoned with deep heated oil. In an experimental trial, 30 male Wistar rats were divided into five groups: healthy control, control-heated oil, aerobic exercise-heated oil, octopamine-heated oil and exercise, and octopamine-heated oil. Octopamine was given to rats by intraperitoneal injection daily for 4 weeks and 5 days a week. Aerobic exercise was also performed for four weeks and five days a week with moderate intensity on the treadmill. Forty-eight hours after the last intervention, the rats were anesthetized and visceral adipose tissue was removed from the body to measure HIF-1 gene expression. As a result of receiving deeply-heated oil, the expression of HIF-1 gene and protein in visceral fat increased significantly ($p=0.001$), but the number of white cells in visceral adipose tissue increased significantly ($p=0.001$). Aerobic exercise caused a significant decrease in HIF-1 gene and protein expression ($p=0.01$). After the end of the exercise period, it increased significantly ($0=0.003$). Receiving octopamine also decreased HIF-1 gene and protein expression ($p=0.002$).

Octopamine could significantly reduce the expression of HIF-1 protein and the number of white cells. The interaction of exercise and octopamine was significant for the expression of HIF-1 protein and the number of white cells. The results of this study showed that aerobic exercise and octopamine improve the process of angiogenesis of visceral adipose tissue that has been disturbed by heated oils and reduce the damage caused by feeding with deeply-heated oils. The first finding of this study showed that the HIF-1 gene expression of visceral fat increases significantly as a result of receiving deeply-heated oil. Fujisaka et al. (2013) reported an increase in HIF-1 in the epididymal fraction after twelve weeks of high-fat diet (17). In the condition of obesity, the adipose tissue is in a hypoxic state. Several possible mechanisms have been proposed to explain this phenomenon. It seems that one of the reasons for the hypoxia of adipose tissue in obesity is the reduction of capillarization in this tissue (18). In this condition, the adipose tissue will have less blood circulation (19). It seems that the reason for the increase of HIF-1 in this study is the increase of hypoxia in the visceral adipose tissue. Since these cells become hypertrophied as a result of receiving heated oil and the speed of this increase in cell size exceeds the speed of angiogenesis in the visceral tissue, the blood flow does not reach these cells sufficiently and the need for visceral adipose tissue oxygen is greater than the amount provided by the existing capillaries. As a result, hypoxia is created and, in this condition, HIF-1 gene expression is activated. In this study, the changes made in the studied outcomes due to receiving deeply-heated oil were in line with each other. Capillarization indicators such as HIF-1 have increased prominently.

On the other hand, the penetration rate of immune cells into the white fat tissue also increased, and all of these changes show the development of hypoxia, increased sensitivity to insulin, and the development of inflammation caused by obesity. But on the other hand, in this study, a decrease in HIF-1a gene and protein expression was observed after performing aerobic exercises for four weeks. It has been reported that aerobic exercise in the bioavailability animal model increases the blood required by the adipose tissue by increasing blood circulation in both subcutaneous and visceral adipose tissue (20). In the present study, as a result of exercise, the expression of HIF-1a gene and protein - which was increased after receiving deeply-heated oil – was decreased, which can be considered as the mechanism of reducing hypoxia in visceral adipose tissue in this study (21). On the other hand, it was found that Octopamine was able to decrease the expression of HIF-1a gene and protein in visceral adipose tissue. Octopamine causes the activation of beta 3 adrenergic receptors and as a result of the activation of these receptors in white fat cells, lipolysis is stimulated and oxygen consumption increases in brown fat cells, which is a sign of increased oxidation of white fats (22). However, it seems that the increase in lipolysis in white adipose tissue and oxygen consumption in brown fat cells caused by receiving octopamine can cause the size of visceral fat cells to shrink. In this condition, as the size of the adipose cells decreases, the amount of oxygenation increases and as a result, cellular hypoxia decreases. In fact, a reduction in the stimulation of HIF-1a gene and protein expression can be expected. In this study, the group receiving octopamine showed a decrease in HIF-1a gene and protein expression, which can be attributed to the slimming effects of octopamine and hypoxia reduction in visceral adipose tissue.

In general, it seems that the mechanisms justifying the decrease in HIF-1a gene expression due to octopamine and aerobic exercise can be considered common and it can be justified based on the changes in the structure and metabolic function of visceral adipocytes, because both aerobic exercise and octopamine, by stimulating lipolysis and then moving free fatty acids to muscle cells and oxidizing fatty acids, create conditions for reducing the size of adipose cells, which results in better oxygenation of adipose tissue, followed by development of angiogenesis processes and reduction of hypoxia in adipose tissue. In these conditions, not only the metabolism of adipose tissue is developed, but also with the reduction of hypoxia, the level of inflammation also decreases (23,24), which itself can be one of the positive effects of aerobic exercise and receiving octopamine in the conditions of receiving deeply-heated oils. In the view of above, it seems that as a result of aerobic exercise, octopamine and the combination of these two interventions, the size of visceral white fat cells has been decreased; as a result of decreased cell size, hypoxia at the tissue level is reduced; and therefore, HIF-1a is reduced.

Conclusion

The study of the effect of obesity on the process of capillarization of adipose tissue shows that the balance between adipose tissue and capillaries needed to provide blood flow is disrupted due to obesity, and capillaries do not develop as much as fat cells grow. In this condition, the cell is placed in a hypoxic condition and the process of inflammation increases incrementally. Physical activity in various forms can develop the imbalance of capillaries in adipose tissue and prevent the occurrence of hypoxia in adipose tissue through several mechanisms.

It seems that exercise exerts its positive effects by causing weight loss and then by stimulating angiogenesis pathways in white adipose tissue, while octopamine can only play its role by reducing the size of fat cells. Considering the lack of studies regarding the simultaneous effect of aerobic exercise and octopamine on angiogenesis process in white adipose tissue, the present study was designed and implemented.

The results of this study showed that the expression of HIF-1a gene and protein of visceral fat increases as a result of receiving deeply-heated oils. On the other hand, aerobic exercise and octopamine can return the conditions to normal by changing the expression of genes affecting the process of angiogenesis. The important point in this study is that exercise: improved the process of capillarization in fat cells exposed to deeply-heated oils; developed the process of transferring glucose into the white fat cell; and prevented the penetration of leukocytes into the white adipose tissue, which itself is a cause of inflammation in obesity. Based on this, it is concluded that in the conditions of feeding with deeply-heated oils, aerobic exercise and octopamine alone have a protective effect on visceral adipose tissue through the development of capillarization process, reduction of insulin resistance and reduction of mechanisms of development of inflammatory mediators. Therefore, in the conditions of feeding with these types of oils, performing aerobic exercise and taking octopamine will be an efficient measure to curb the negative effects of these oils. However, there is a need for more studies in this field.

Acknowledgements

Hereby, from all the patients and people participating in the present research and their loved ones We are grateful to those who have helped us in this research.

Funding

This study did not have any funds.

Compliance with ethical standards

Conflict of interest None declared.

Ethical approval the research was conducted with regard to the ethical principles.

Author contributions

Conceptualization: F.S., M.A.A., M.P., F.GH.; Methodology: F.S., M.A.A., M.P., F.GH.; Software: F.S., M.A.A., M.P., F.GH.; Validation: F.S., M.A.A., M.P., F.GH.; Formal analysis: F.S., M.A.A., M.P., F.GH.; Investigation: F.S., M.A.A., M.P., F.GH.; Resources: F.S., M.A.A., M.P., F.GH.; Data curation: F.S., M.A.A., M.P., F.GH.; Writing - original draft: F.S., M.A.A., M.P., F.GH.; Writing - review & editing: F.S., M.A.A., M.P., F.GH.; Visualization: M.H., F.S., M.A.A., M.P., F.GH.; Supervision: F.S., M.A.A., M.P., F.GH.; Project administration: F.S., M.A.A., M.P., F.GH.; Funding acquisition: F.S., M.A.A., M.P., F.GH.

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