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The Effects of Octopamine and Aerobic Exercise on the Genes Affecting the Angiogenesis of the Visceral Adipose Tissue in Rats Fed with Deep-fried Oil

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ABSTRACT

Introduction: Today, changes in dietary patterns have increased the share of deep-fried oils (DFOs) in the daily diet. DFOs adversely affect the angiogenesis of the visceral adipose tissue. Aerobic training (AT) and the intake of sympathetic mimicry herbal materials may reduce the adverse changes caused by DFO use through stimulating the lipolysis process and increasing lipid utilization. The present study aimed to review the effects of octopamine (O) and AT on the *VEGF* and *HIF-1a* gene expression levels in the visceral adipose tissue of rats fed with DFO.

Methods: This experimental study was conducted on the rats divided into the groups of HFO+O, HFO+AT, HFO+O+AT, DFO control, and healthy control. For four weeks, the rats received the intraperitoneal injection of O (81 µmol/kg/day) five days per week. In addition, AT was implemented five days per week with moderate intensity on a treadmill. Data analysis was performed using the two-way analysis of variance (ANOVA) and independent samples t-test (P≤0.05).

Results: DFO intake significantly increased the HIF-1 visceral fat (P=0.001), while significantly reducing the *VEGF* expression (P=0.021). AT significantly reduced the *HIF-1* expression (P=0.01), while increasing the *VEGF* expression (P=0.003). Moreover, O administration decreased the *HIF-1* expression (P=0.002) and increased the *VEGF* expression (P=0.002). Simultaneous with AT, O administration had no significant interactive effects on the *HIF-1* and *VEGF* expression (P≥0.05).

Conclusion: According to the results, O and AT could improve the angiogenesis of the visceral adipose tissue, which was impaired by DFO, and reduce the damage caused by DFO feeding.

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Introduction

Increased body fat percentage, which is also known as obesity, is a major health- threatening issue in today's world (1). Adipocyte hypertrophy disrupts lipid metabolism, reduces the adipose tissue blood flow, and causes mild inflammation, all of which of the underpinnings of other widespread disorders associated with obesity (2, 3). Although angiogenesis is induced by the hypertrophy of the adipocytes (4), evidence suggests that in severe obesity, the balance between the adipocyte oxygen requirement and oxygen availability is disrupted since angiogenesis does not occur to the extent that is required by the adipose cells to grow (5, 6). Consequently, hypoxia occurs in the adipose tissue in obesity (7), which may justify some of the disorders associated with obesity (2, 8).

Hypoxia markers such as the vascular endothelial growth factor (VEGF) and hypoxia-inducible factor 1-alpha (HIF-1α) play a key role in hypoxia. HIF-1α could be an indicator of the adipose tissue hypoxia (9, 10). It has been reported that in obesity, the angiogenesis of the adipose tissue decreases (11), and both the protein and gene expression of VEGF in the adipose tissue are altered, thereby decreasing the capillary density of the tissue (12, 13).

The overexpression of VEGF and increased angiogenesis protect the body against insulin resistance and hypoxia (14, 15). On the other hand, obesity increases the *HIF-1α* gene expression (16), leading to the entry of the macrophages of the M1 family and inflammation (17). Recent evidence suggests that physical exercises such as aerobic training (AT) could improve the chronic inflammation markers in

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obese patients (18, 19). Octopamine (O) is an endogenous amine, which could stimulate the lipolysis process in the white adipocytes by stimulating the beta-3 adrenergic receptors and induce thinning (20, 21).

Considering the role of AT in the stimulation of angiogenesis and reduction of the adipocyte size, the oxygen supply to the white adipose tissue increases, thereby decreasing the subsequent hypoxia and inflammation.

The present study aimed to investigate the simultaneous effects of AT and O on the *HIF-1a* and *VEGF* gene expression levels in the visceral adipocytes after feeding with deep-fried oil (DFO). The main hypothesis of the research was that AT and O have interactive effects on the *VEGF* and *HIF-1a* gene expression in the visceral adipocytes of rats after DFO consumption.

Materials and Methods

Experimental Animals

This experimental study was conducted on 30 male rats, which were purchased and transferred to the animal laboratory. After one week of adaptation to their new environment, the rats were divided into five groups of six, including HFO+O, HFO+AT, HFO+O+AT, DFO-control, and healthy control. For four weeks, the rats received the intraperitoneal injection of O (81 µmol/kg/day) five days per week. In addition, AT was implemented five days per week with moderate intensity on a treadmill.

DFO

To prepare the HFO, sunflower oil (8 kg) was heated for eight hours at the temperature of 190-200°C for four consecutive days, and every 30 minutes, protein products (sausages), potatoes, chicken nuggets, and poultry were dipped into the oil (22).

O Consumption

For four weeks, the rats in the O consumption groups received the intraperitoneal injection of O (81 µmol/kg/day; Sigma-Aldrich, USA) five days per week. Notably, O was dissolved in 9% normal saline (21).

AT Protocol

Before the implementation of the AT protocol and for the adaptation of the rats to the training protocol, the rats ran on the treadmill with the speed of 9 m/min for 20 minutes for one week. After the adaptation, the AT protocol started in the first week with the speed of 16 m/min, which reached 26 m/min in the last week. Notably, the duration of the training was 20 minutes in addition to five minutes of warm-up (speed: 7 m/min) and five minutes of cool-down (speed: 7 m/min).

Animal Sacrifice and Tissue Sampling

After the last day of the intervention, the rats were given free access to water and food for 48 hours to neutralize the acute effects of the AT and O consumption. Afterwards, the rats were weighed on the day of sacrifice after 8-10 hours of fasting and prior to sacrificing. Finally, the animals were anesthetized via chloroform inhalation, and pain and consciousness tests were performed to ensure anesthesia. After washing the visceral adipose tissue with saline, the tissues were encoded in a two-milliliter encoded microtube, stored at the temperature of -80°C, and transferred to the laboratory for the measurement of the *VEGF* and *HIF-1* gene expression levels using the real-time polymerase chain reaction (PCR). In addition, glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*) was selected as the housekeeping gene. The primer sequences of *VEGF*, *HIF-1*, and *GAPDH* are presented in Table 1.

Table 1. Primer Sequences Used for RT-PCR

Gene	Sequence 5'-3'	Accession Number
HIF-1 alpha	F 5' CAGGTGACCGTGCCCCTACTATG 3' R 5' CACAATCGTAACTGGTCAGCTGTG 3'	NM_024359.1
VEGF	F 5'AGATGGTGAGAGAGATGGTGT 3' R 5' AGATGGTTGATGGCTTAGATTAG 3'	NM_053549.1
GAPDH	F 5' CATACTCAGCACCAGCATCACC 3' R 5' AAGTTCAACGGCACAGTCAAGG 3'	NM_017008.4

Histological Analysis

The samples were soaked in a 30% sucrose solution in phosphate buffered saline (PBS) at the temperature of 4°C for three days. The sucrose solution was removed from the samples, and the samples were directly placed into a cryomold with adequate optimal cutting temperature compound (OCT) embedding media in order to folate the tissues. The cryomold was fixated onto the cryobar in the cryostat.

Afterwards, the frozen tissues were visualized from the cryomold and attached to a textured cryosectioning cold surface using the OCT as the adhesive. In addition, eight-micrometer sections were cut and separately placed on dry microscope slides. Each section was dissolved into PBS directly and rapidly to remove the residual OCT and ensure the hydration of the tissue sections. H&E staining was also prepared, and images were captured and investigated using the Image J software to calculate the adipocyte cells.

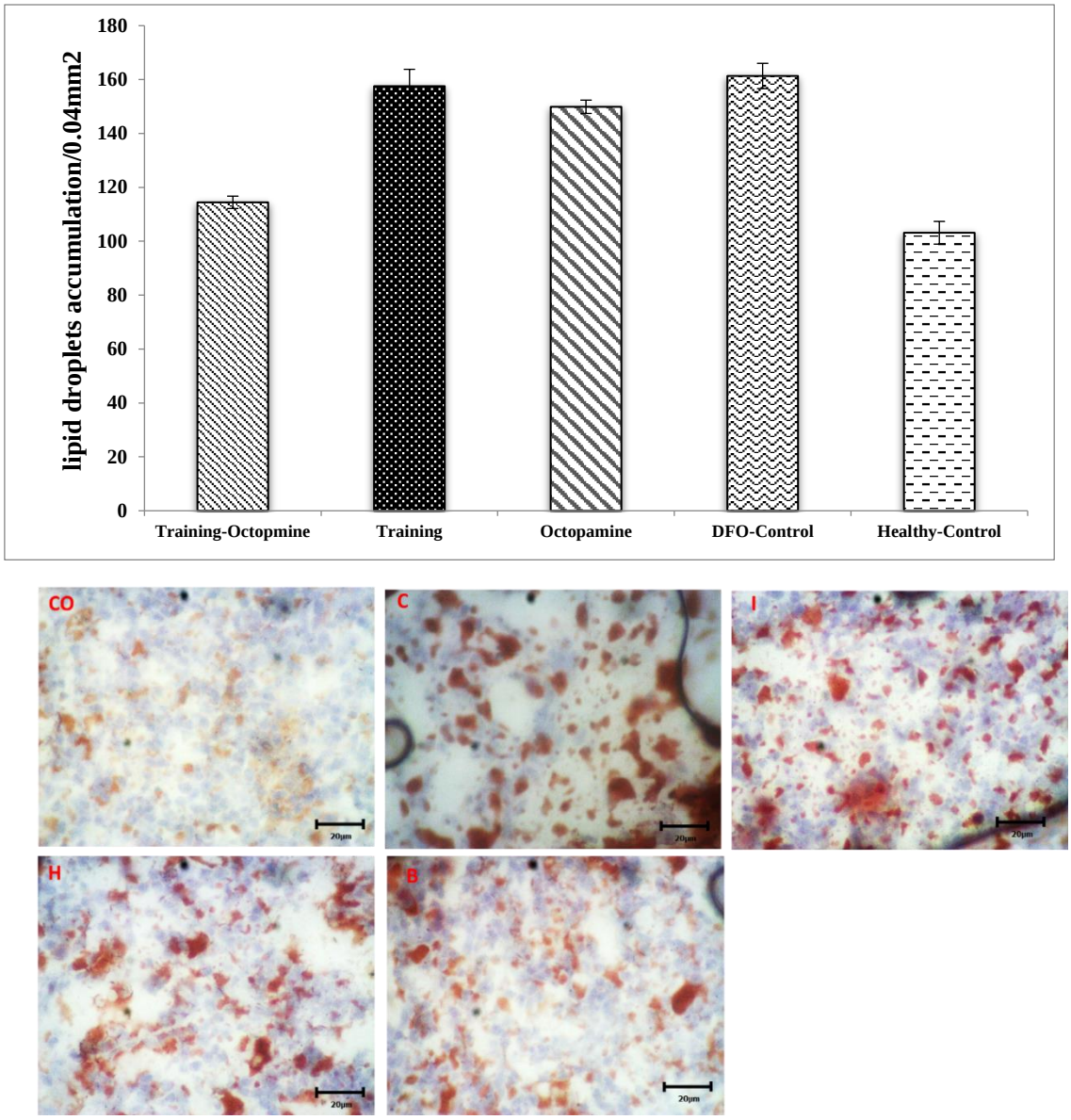

Figure 1. Oil red O staining in the visceral adipose tissue. Red color shows cytoplasmic lipid droplet accumulation in fatty in experimental groups including: Co: healthy control. C: Training-Octopamine. I: DFO control. H: Training. B; Octopamine. Abnormal distribution of lipid droplets was observed in DFO treated groups in comparison with control group. The number of droplet decreased significantly in Training-Octapamine group compared to other experimental groups.

Statistical Analyses

Data analysis was performed in SPSS version 22 using independent samples t-test to compare the *VEGF* and *HIF-1* gene expression levels between the healthy control and DFO-control groups. In addition, two-way analysis of variance (ANOVA)

was applied to investigate the effects of O and AT on the *VEGF* and *HIF-1* gene expression (P≤0.05).

Results

The visceral adipose *VEGF* gene expression significantly reduced by DFO (P=0.021) (Figure 2-A). On the other hand, DFO administration significantly increased the visceral adipose *HIF-1* gene expression (P=0.001) (Figure 1-B).

According to the obtained results, AT significantly increased the visceral adipose *VEGF* gene expression (P=0.003), and O also significantly increased the visceral adipose *VEGF* gene expression (P=0.002). Although the lowest visceral adipose *VEGF* gene expression was observed in the AT+O group, the interactive effect of AT with O on the visceral adipose *VEGF* gene expression was not considered significant (P=0.409) (Figure 3-A).

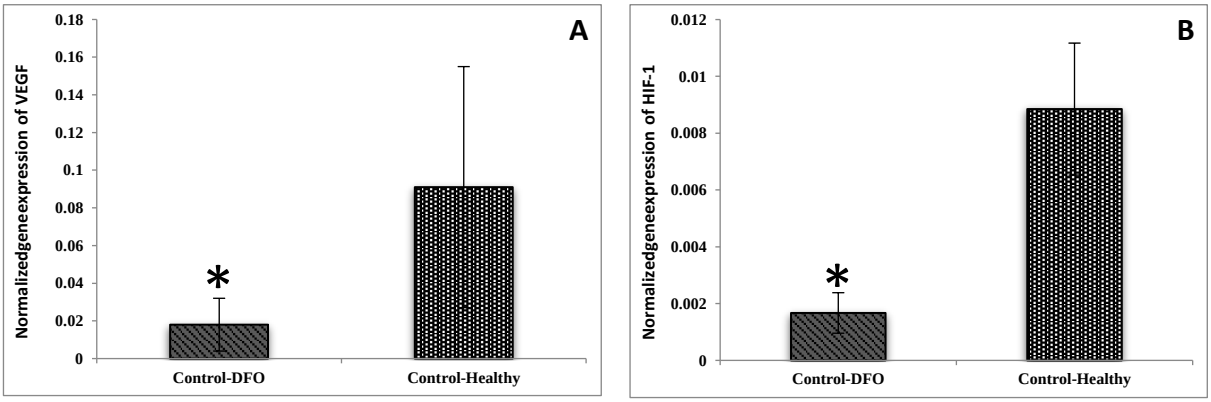

Figure 2. Visceral Adipose A) *VEGF* and B) *HIF-1* Gene Expression in Healthy Control and DFO-control Groups (*Significant difference with healthy control).

The findings indicated that AT significantly decreased the visceral adipose *HIF-1* gene expression (P=0.010), while O supplementation significantly decreased the *HIF-1* gene expression (P=0.002). Although the visceral adipose *HIF-1* gene expression in the AT+O group was lower than the other groups, the interactive effect of AT and O on the visceral adipose *HIF-1* gene expression was not considered significant (P=0.154) (Figure 3-B).

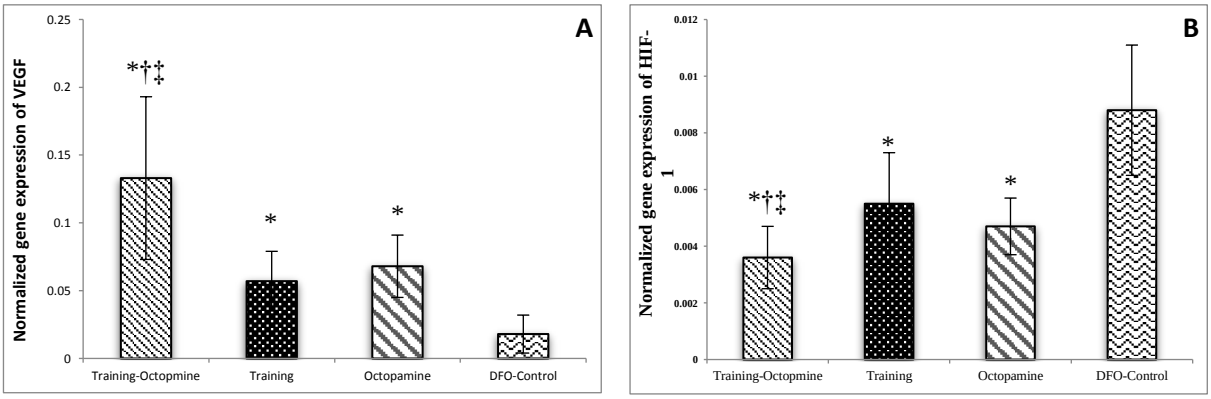

Figure 3. Visceral adipose A) *VEGF* and B) *HIF-1* Gene Expression in Study Groups. *Denote significantly difference to DFO-control group.† Denote significantly difference to training group.‡ Denote significantly difference to Octopamine group (Data expressed as mean and standard deviation)

Discussion

The findings of the current research indicated that the visceral adipose *HIF-1a* gene expression significantly increased by DFO, while the visceral adipose *VEGF* gene expression significantly decreased. In another study, Fujisaka et al. (2013) reported increased *HIF-a* in the epididymal segment after 12 weeks of adherence to a high-fat diet (17). In obesity, the adipose tissue is in a hypoxic state, and several possible

mechanisms have been proposed to justify this phenomenon.

One of the causes of adipose tissue hypoxia could be the reduced angiogenesis in the adipose tissue (12); in this condition, the adipose tissue has lower circulation (23), and the *VEGF* gene expression, which is the main factor involved in angiogenesis, decreases, while the other genes affecting hypoxia increase (8). However, a significant increase in the *VEGF* expression in transgenic mice with a high-fat diet has been shown to increase the number and size of the blood vessels in the white and brown adipose tissues (14), which confirms the key role of *VEGF* in hypoxia (hypertrophy in the adipose tissue).

In the present study, the increased *HIF* could be due to the increased hypoxia in the visceral adipose tissue as these cells became hypertrophied following DFO consumption, and their growth rate was higher compared to the angiogenesis rate in the visceral tissue. As a result, the blood supply to these cells was insufficient, and the demand for the visceral adipose tissue oxygen is greater than it was provided by the capillaries.

As a result of hypoxia, the *HIF-1* gene expression is activated. In the current research, the *HIF-1a* gene expression decreased, while the *VEGF* gene expression increased after four weeks of AT. In the previous studies in this regard, AT in animal models has been reported to increase the blood supply to the adipose tissue by increasing circulation in both the subcutaneous and visceral adipose tissues (19). On the other hand, increased VEGF mRNA expression has been observed in the subcutaneous adipose tissue in the adults exercising four sessions per week (24). Accordingly, the increased *VEGF* gene expression after each exercise session might have resulted from the steady and permanent increase in angiogenesis. Similarly, 12 weeks of AT have been reported to increase the capillary density in the subcutaneous adipose tissue without weight loss in insulin-sensitive, obese individuals, while no such increase has been reported in insulin-resistant individuals (23).

Angiogenic processes could be inhibited by increased inflammation, fibrosis, and the other metabolic disorders caused by obesity, along with insulin resistance; this in turn diminishes the effects of AT on angiogenesis. Other factors may also be proposed to justify the increased VEGF mRNA in the adipose tissue through AT. The changes in free fatty acid and interleukin-6, which are induced by a single physical activity session, may be one of the mechanisms explaining the increased adipose tissue VEGF during exercise (25-28). Under such circumstances, increased *VEGF* gene expression could increase angiogenesis and the oxygen delivery to the adipose tissue, which in turn decrease the hypoxia levels.

In the present study, DFO administration significantly decreased the *HIF-1a* gene expression, which may be due to the decreased hypoxia in the visceral adipose tissue (29). On the other hand, the gene expression levels of *VEGF* and *HIF-1a* in the visceral adipose tissue significantly decreased after the O administration. O activates the beta-3 adrenergic receptors, and lipolysis is stimulated by the activation of these receptors in the white and brown adipocytes, thereby increasing oxygen consumption and the oxidation of the white adipose tissue (20). However, the increased lipolysis in the white adipose tissue and oxygen consumption in the brown adipocytes induced by O administration may decrease the size of the visceral adipocytes. As the size of the adipose cells reduces, the oxygen uptake level increases, and cellular hypoxia reduces (8), which is in turn expected to decrease the stimulation of the *HIF-1* gene expression.

According to the results of the present study, the *HIF-1a* gene expression decreased in the O treatment group, which could be attributed to the thinning effects of O and reduced hypoxia in the visceral adipose tissue. In general, it seems that the mechanisms underlying the reduction of the *HIF-1α* and *VEGF* gene expression in the visceral adipose tissue due to O administration and AT is common and could be justified based on the changes in the structure and metabolic function of the visceral adipocytes.

Since both AT and O could stimulate lipolysis and subsequently move the free fatty acids into the muscle cells and fatty acid oxidation creates the conditions for the reduced size of the adipose cells, the better oxygenation of the adipose tissue occurs, which in turn leads to the development of angiogenesis and hypoxia in the adipose tissue. This condition not only increases the metabolism of the adipose tissue, but it also decreases the hypoxia level by reducing inflammation (12), which may be due to the positive effects of AT and O intake through DFO feeding.

One of the limitations of the present study was the inability to measure the protein levels of

VEGF and HIF-1a using the Western blot and ELISA assay. Therefore, it is suggested that Western blotting and ELISA assay be used in the further investigations in order to confirm the findings of the current research.

Conclusion

According to the results, DFO feeding, AT, and O supplementation each exerted protective effects on the visceral adipose tissue alone through stimulating angiogenesis. Therefore, in the case of DFO feeding, AT and O administration could effectively control the adverse effects of these oils. However, further investigations are required in this regard.

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