

The Anti-inflammatory Effects of Aerobic Training and Octopamine Consumption in the Heart Tissue of Rats Fed Deep-Fried Oil

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Abstract

Background and objectives: It has been reported that deep-fried oils (DFOs) used in the preparation of fast foods can affect the immune system. On the other hand, regular physical activity and proper nutrition, in addition to affecting physical function, have a tremendous impact on the immune system. The aim of the present study was to investigate the anti-inflammatory effects of aerobic training (T) and octopamine (O) consumption in the heart tissue of rats fed DFO.

Methods: In this experimental study, 25 male Wistar rats with mean age of 20 weeks old and weighing 300- 350 were purchased and divided into 5 groups of 5 rats, including: 1) control, 2) DFO, 3) DFO+T, 4) DFO+O and 5) DFO+T+O. During four weeks, groups 2- 5 received DFO by gavage; groups 3- 5 ran on treadmill (with speed of 50% Vo_{2max} in the first week which reached 65% Vo_{2max} in the last week) five sessions per week and 20 minutes per session and groups 4-5 received 81 $\mu\text{mol/kg}$ octopamine supplement peritoneally for 5 days per week. Nuclear factor kappa B (NF- κ B) and tumor necrosis factor-alpha (TNF- α) gene expression were measured in heart tissue.

Results: DFO significantly increased NF- κ B ($P=0.005$) and TNF- α ($P=0.001$) gene expression levels; T significantly on decreased NF- κ B ($P=0.01$) and TNF- α ($P=0.007$) gene expression levels; O consumption significantly decreased NF- κ B ($P=0.001$) and TNF- α ($P=0.001$) gene expression levels; however, the interactive effects of T and O consumption on NF- κ B ($P=0.57$) and TNF- α ($P=0.20$) gene expression levels was not significant.

Conclusion: It seems that T and O consumption alone have anti-inflammatory effects on the heart tissue of rats fed DFO, however T and O do not have anti-inflammatory interactive effects.

Keywords: Training, Octopamine, Deep-Fried Oil, TNF- α , NF- κ B

Introduction

The change in lifestyle has been one of the achievements of modernity that society, especially urban society, has experienced. One of the implications of lifestyle changes is the change in diet. An obvious example is the over-consumption of fast foods among families and individuals in the community (1). One of the important factors in the process of fast food preparation is the process of cooking these foods, including the oils used. These oils are known as deep-fried oils (DFOs) and contain toxic substances such as acrylamide (released from roasted starch) and heterocyclic amine (which are created by the heating and cooking process of protein materials when amino acids and creatine react at high annealing temperature (barbecue or frying) (3). Seventeen different types of heterocyclic amines are caused by cooking muscle meat such as beef, chicken and fish, which may increase cancer risk (such as gastric cancer, colorectal cancer, pancreatic cancer, and breast cancer) by increasing free radicals (4).

Oxygen free radicals cause deoxyribonucleic acid (DNA) fragmentation and by damaging DNA and decomposing the polyadenosine diphosphate ribose polymerase (PARP) enzyme into two fragments of 89 and 24 kDa, the cell can no longer enter the DNA repair pathway and thus follow the apoptosis pathway (5). Reactive oxygen species (ROSs) are highly inactive molecules that can destroy cell structures, including carbohydrates, nucleic acids, fats and proteins, affect their function and lead to inflammatory factors; however, antioxidants can prevent these degradations (6). Mild chronic inflammation is associated with higher than normal levels of several cytokines including tumor necrosis factor-alpha (TNF- α), interleukin-6 (IL-6) and

C-reactive protein (CRP). TNF- α is a multifunctional cytokine that induces a wide range of cellular responses. There is little TNF- α in the normal brain; however, pathological stimuli such as injury, ischemia, and infection can dramatically increase TNF- α expression (7).

Low to moderate intensity aerobic training has been reported to improve the physiological function of skeletal and cardiac muscles, and to reduce the incidence of a wide range of diseases, including cardiovascular disease, as well as to improve inflammatory factors (8). Over the past decade, regular aerobic training (T) has been shown to have beneficial effects on the immune system. Consequently, regular exercise can improve the immune system by increasing muscle mass and decreasing fat mass (9). Also, proper nutrition in addition to affecting the physical performance, has a tremendous impact on the immune system, so that nutritional deficiencies can cause or aggravate immune-related diseases (10).

In this regard, it has been reported that octopamine (O) as an endogenous biogenic amine is closely related to norepinephrine, acts as a neurotransmitter in invertebrates, and is a trace amine of unspecified properties in vertebrates and is effective on adrenergic and dopaminergic systems. O is present in a variety of plants and fruits, but its richest sources can be referred to as the orange blossom (11), and various studies have indicated the anti-inflammatory effects of O (12). Considering the importance and urgency of the issue of vital organ damage to the human body in the consumption of fast food and frequently-used DFO, as well as anti-inflammatory effects of exercise and O supplementation, this study aimed to investigate the effect of aerobic training (T)

and O supplementation on TNF- α and nuclear factor kappa B (NF- κ B) gene expression in the heart tissue of rats fed DFO.

Materials and Methods

In this experimental study, 25 male Wistar rats, approximately 20 weeks old, weighing 300- 350 g were purchased from the Histogen Research Center and transferred to the laboratory and were kept in the lab under standard conditions (temperature: 23 \pm 2 °C; humidity: 45 to 55 %; light/dark cycle: 12:12) for adaptation to the environment for one week. On the 8th day, according to body weight, they were divided into five groups of five rats, including: 1) control, 2) DFO, 3) DFO+T, 4) DFO+O and 5) DFO+T+O. During four weeks, groups 2- 5 received DFO by gavage; groups 3 and 5 ran on treadmill (Danesh Salar Iranian Company; Iran) for five sessions per week at moderate intensity, and groups 4 and 5 received 81 μ mol/kg O supplement peritoneally for five days per week. Forty eight hours after the last training session and O supplementation, the rats were anesthetized by peritoneal injection of ketamine and xylazin. To measure NF- κ B and TNF- α gene expression, the heart tissue of the rats was extracted and inserted into specific microtubes. The microtubes were transferred into a nitrogen tank and stored at -80 ° C until cell analysis. Measurements of NF- κ B and TNF- α were performed by Real Time PCR. Researchers received introduction letters from Islamic Azad Tehran Medical Sciences University with code IR.IAU.PS.REC.1398.321.

O administration

In the present study, O supplement was prepared by Sigma Aldrich. It should be noted

that to prepare for injection, octopamine was dissolved in 9% normal saline solution (13).

DFO Preparation

According to previous studies, 8 liters of sunflower oil was used to prepare DFO. The oil was heated for 4 consecutive days, 8 hours a day, at a temperature of 190 to 200 ° C and based on the available sources, the food (chicken nuggets, potatoes, chicken and protein products such as sausages and cold-cuts) was immersed in the oil every 30 minutes, and at the end of the fourth day the oil was fed to rats by gavage (14).

Determine the aerobic power

In order to determine the aerobic power (Vo₂max) or maximum speed in rats, first, rats ran at speed of 8m/min for 5 minutes. In the next step, they ran at speeds of 10 to 15 m/min for 8 minutes; in the third step, they ran at speed of 20 m/min for 5 minutes; and at the fourth step, at a speed of 25 m/min for 10 minutes, and afterwards they ran at speed of 30 m/min for 20 minutes. At the final step, the speed of 35 m/min was regarded as exhaustive (VO₂max) until the rats were perpetually exposed to the end of the treadmill three times per one minute.

T protocol

The protocol of aerobic training was performed at moderate-intensity level, so that the rats ran at 16 /min (50% of Vo₂max) in the first week which reached 26 m/min (65% of Vo₂max) in the last week. The running time for the whole research period was 20 minutes. It is noteworthy that in order to adapt the rats to run on the treadmill, rats walked at a speed of 9 m/min for 20 min before the start of the main training program for one week; also, at each training session

before starting the training, rats warmed up at speed of 7 m/min for 5 min and cooled down at speed of 5 m/min for 5 min after the main training (15).

Real Time PCR method

For molecular analysis at gene expression level, RNA was extracted from the heart tissue by FavorPrep™ Tissue Total RNA Mini Kit (FavorPrep, Taiwan), then the purity and concentration of RNA were evaluated by optical density measurements on a NanoDrop Lite Spectrophotometer (Thermo Fisher Scientific, USA). After extraction of high purity and high concentration RNA from all studied samples, cDNA synthesis was performed by RevertAid™ First Strand cDNA Synthesis kit (Thermo Fisher Scientific, USA). Reverse transcription quantitative polymerase chain reaction (RT qPCR) was performed using the ABI Biosystems StepOne and the RealQ Plus 2x

Master Mix Green (Ampliqon, Denmark). The Gap housekeeping gene was also used as internal control of qPCR reactions. The qPCR conditions were set for 10 min at 94°C

followed by 40 cycles of 15 sec at 94°C, 60 sec at 60°C and final melt curve stage for product specificity analysis. The amplification signals of different samples were normalized to Gap cycle threshold (Ct), and then Livak method (2- $\Delta\Delta$ Ct) was applied for comparing mRNA levels of different groups, which represented as fold change in data analysis. The primers sequences are reported in Table 1.

Statistical analysis

Kolmogorov-Smirnov test was used for review the normal distribution of data as well as independent samples t-test and two-way ANOVA tests were used for statistical analysis of data (P<0.05).

Table 1. Primers sequence used in the study

Gene	Forward (5'-3')	Reverse (5'-3')
NF- κ B	CATACGCTGACCCTAGCCTG	TTTCTTCAATCCGGTGGCGA
TNF- α	GAGATGTGGAAATGGCAGAGGA	GAGAAGATGATGTGAGTGTGAGG
Gap	AAG TTC AAC GGC ACA GTC AAG G	CAT ACT CAG CAC CAG CAT CAC C

Results

The NF- κ B and TNF- α gene expression levels in the five research groups are presented in Figures 1 and 2 respectively. The results of independent samples t-test showed that NF- κ B (P=0.005) and TNF- α (P=0.001) gene expressions levels in DFO group were significantly higher than control group.

The results of two-way ANOVA showed that T (P=0.01, μ =0.29) and O (P=0.001, μ =0.51) had significant effect on decrease of NF- κ B, however the effect of T and O was not significant on decrease of NF- κ B (P=0.57, μ =0.02) (Figure 1); also, T (P=0.007, μ =0.37) and O (P=0.001, μ =0.55) had significant effect on decrease of TNF- α , however the effect of T and O on TNF- α was not significant (P=0.20, μ =0.02) (Figure 2).

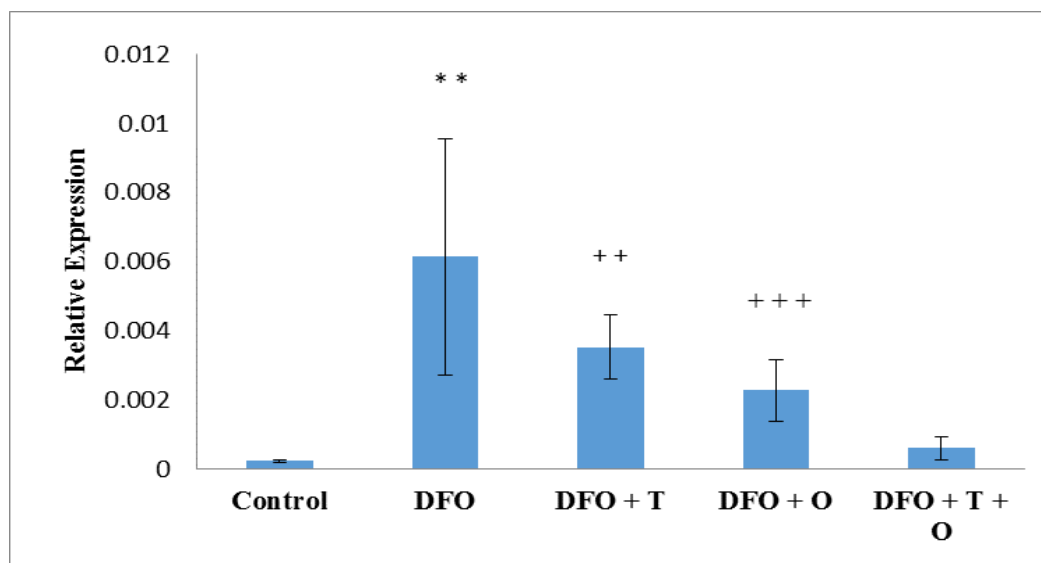


Figure 1. NF- κ B gene expression levels in the five groups of study
** P<0.01 Significant increase compared to the control group
+++ P<0.001; ++ P<0.01 Significant effect on decrease of NF- K β
(DFO: deep- fried oil; T: training; O: octopamine)

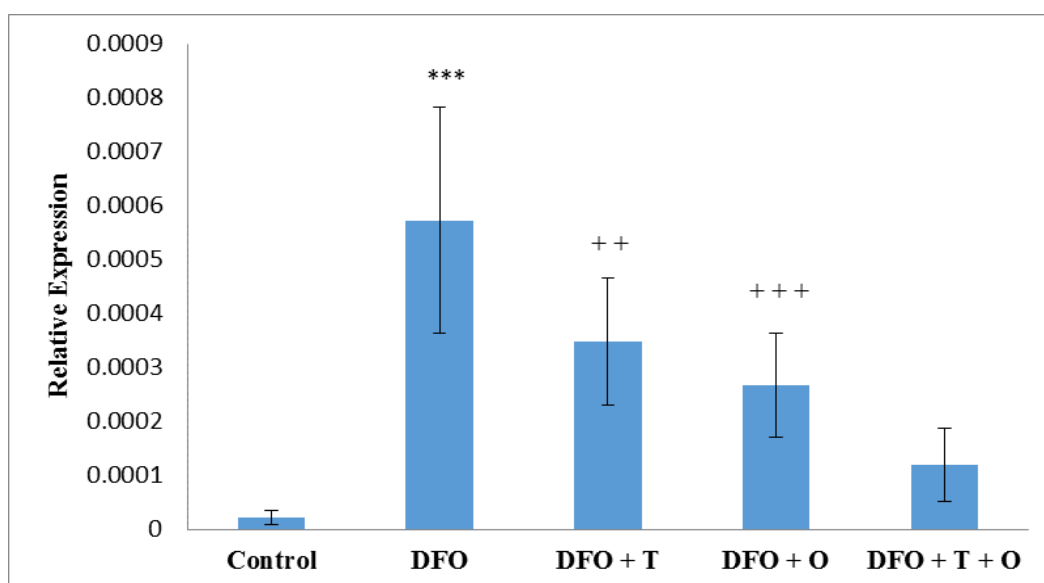


Figure 2. TNF- α gene expression levels in the five groups of study
** P<0.01 Significant increase compared to the control group
+++ P<0.001; ++ P<0.01 Significant effect on decrease of TNF- α
(DFO: deep- fried oil; T: training; O: octopamine)

Discussion

The results of the present study showed that DFO significantly increased NF- κ B and TNF- α gene expression levels in the heart tissue of rats. Consistent with the findings of the present study, a study on the effect of DFO on impaired hepatic balance and increased adipose tissue and fatty liver injury in Wistar rats showed that DFO consumption decreased antioxidant enzymes of the liver and increased oxidative stress factors in the Wistar rats (16), so that DFO caused liver damage (16). Also, Wang *et al.* (2016) showed that DFO consumption leads to liver injury in rats (14).

The results of this study also showed that T significantly decreased NF- κ B and TNF- α gene expression levels in the heart tissue of rats fed DFO. Long-term and short-term exercise activities by different intensity and duration have been reported to play an important role in the prevention and control of cardiovascular diseases (17). Consistent with the findings of the present study, 16 weeks of aerobic training resulted in improved TNF- α levels in rats (18) however, inconsistent with findings of the present study, 16 weeks of resistance training led to increase of TNF- α levels in rats (18); this contradiction in findings may be due to the type of training as well as the contractions in the aerobic and resistance training. Eight weeks of moderate-intensity aerobic training resulted in a significant decrease in TNF- α levels as well as a decrease in activation of the I κ B α /NF- κ B pathway (19). Concerning the effects of exercise activities, it has been reported that performing regular aerobic training results in increased antioxidant defense, and decreased lipid and protein peroxidation. In fact, reinforcing antioxidant defenses further neutralizes free radicals; with these

adaptations, oxidative stress is expected to reduce after aerobic training. Regular training increases the ability of the body's antioxidant systems and protects the body against the degenerative property of the increased oxidative stress caused by exercise (20).

Today, behavioral changes and lifestyle modifications such as increased physical activity along with taking natural and pharmacological supplements are among the most common strategies to reduce and control diseases, so that we are witnessing a growing trend in the use of herbal and natural supplements for a variety of therapeutic and dietary purposes that have very high antioxidant effects (21). The results of present study showed that O supplementation significantly decreased NF- κ B and TNF- α gene expression levels in the heart tissue of rats fed DFO. Various therapeutic effects have been reported for octopamine. For example, Brial *et al.* (2019) reported that O consumption had metabolic and histopathological enhancing effects in rats with nonalcoholic fatty liver (22). In the above study, it was pointed out that O is a norepinephrine analog biosynthesised from tyrosine and a sympatomimetic drug. It can be found in many plant products and seafood. O stimulates lipolysis in adipocytes and inhibits glucose uptake. It also stimulates hepatic oxidation of fatty acids. A molecule related to octopamine (N-trans-feruloyloctopamine) shows anti-steatohepatitis properties in a model of non-alcoholic steatohepatitis (22). It has been reported that octopamine increases many hemocyte functions, such as phagocytosis, and these changes would tend to mitigate the decline in immunity due to the loss of molecular resources (23).

Concerning the interactive effects, the results of the present study showed that T

simultaneously with O consumption had not interactive effects on NF- κ B and TNF- α gene expression levels in the heart tissue of rats fed DFO. Lack of sufficient information on the effect of T and O consumption on NF- κ B and TNF- α gene expression levels in the heart tissue of rats fed DFO and lack of access to NF- κ B and TNF- α assay methods are among the limitations of the present study. Therefore, it is recommended in future studies to investigate the effect of T at different intensities with different doses of O on inflammatory factors and antioxidant markers such as superoxide dismutase and catalase as well as use ELISA and western blot techniques to measure NF- κ B and TNF- α .

Conclusion

According to the results of the present study, it seems that aerobic training and octopamine consumption alone can improve NF- κ B and TNF- α in the heart tissue of rats fed DFO, however they do not have interactive effects also octopamine have favorable effects on decrease of NF- κ B and TNF- α compare to training

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Conflict of interest

The authors declare that they have no conflict of interest.

Authors' contributions

Laboratory studies and tests: FJA; study and review: SAH; analysis and interpretation of data: MAA and PFA.

Ethical Approval Considerations

Researchers received introduction letters from Islamic Azad Tehran Medical Sciences University with code IR.IAU.PS.REC.1398.321

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