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Effects of high-intensity interval training on chronic inflammation of lung tissue in healthy rats

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ABSTRACT

Background and Aims: Exercise training with various intensities has different effects on the immune system response. This study investigated the effect of a period of high intensity interval training on inflammation markers of lung tissue in healthy Wistar rats.

Methods: Twelve male Wistar rats were randomly divided into exercised (n=6) and control groups (n=6). The high intensity interval training program was carried out for 9 weeks. The training program was started with 25 m/min speed and ended with 70 m/min speed at the final stage. In lung tissue, expression of IL-17 was studied by the immunohistochemistry. Moreover, structural changes such as macrophage population, BALT, interstitial tissue and alveolar space volume were measured by stereological assessments using independent t-test. P $\leq$ 0.05 was considered statistically significant.

Results: The results showed that after the 9 weeks of the high intensity interval training, the protein IL-17, macrophage population, BALT volume and interstitial space volume significantly increased while alveolar space significantly decreased.

Conclusions: It seems that the 9 weeks of the high intensity interval training program induced chronic inflammation of the lung and defective remodeling of tissue.

1. Introduction

Various types of exercise training with different intensities exert different changes on the immune function (13, 38, 40). Athletes with increased volume and intensity of exercise training and reduced recovery time could be at risk of overtraining and impairment of the immune function (17). Severe exercise training can be a strong stimulus of the lung inflammation development (39). Exercise immunology studies suggest that training load beyond the normal ranges can elevate the risk of upper respiratory tract infection (URTI) (50).

Among the different exercise training protocols, interval exercise training usually has a high level of intensity. High intensity interval training (HIT) consists of short training sessions with relatively intense exercise and rest periods in between. Recently, it has been shown that HIT can cause great compatibility created by endurance training (35). The effects of endurance training and HIT have been studied in various tissues such as skeletal muscle (35), heart (25) and brain (47). These effects on respiratory system, generally, have been investigated in the upper airway and respiratory muscles (27) and the effects of these types of exercise training on the lower airway inflammation and pulmonary parenchyma are yet unclear.

Severe exercise training reinforces expression of pro-inflammatory interleukins with different mechanisms. These factors play an important role in pathological inflammation of tissues such as skeletal muscle and lung (31, 45). Interleukine-17 (IL-17) is a pro-inflammatory factor that has an important role in adaptive immunity dependent to T-cells and especially, to the innate immune system's inflammatory response. Moreover, by recruitment of neutrophils and stimulation of macrophages, endothelial cells, fibroblasts and epithelial cells of the adaptive immune response enhance inflammation (21, 33, 44). Growing evidences show that increased expression of IL-17 is related to inflammatory diseases of the airways such as chronic bronchitis, chronic obstructive pulmonary disease (COPD) and Asthma exacerbations (32, 34). Limited findings have been reported in relation to the effects of exercise training on the expression of IL-17, and so far, the level of this factor in the lung in response to exercise training has not been mentioned.

Additionally, macrophages are present in small amounts in lung parts such as the alveolar section. The interaction between pathogens and macrophages causes the release of small proteins that, in turn, create a state of inflammation in the tissue and recruit neutrophils and plasma proteins into the injury site (28). Function of macrophages in repairing and remodeling of lung tissue after injury is important (5). In this regard, it has been reported that long-term presence of macrophages in high number is clinically important sign for chronic inflammation (20). Since lung is a vital organ for gas exchange, its inflammation can be health threatening. In addition to macrophages, lymphoid tissue volume, surrounding the bronchi and bronchioles (BALT), is also the lung inflammatory marker, which has been more considered in recent years. The majority of BALT cells are IgM[a] and IgM

[a]. Immunoglobulin M

secreting B cells. However, immunoglobulin (Ig) G1 [b]IgA, plasma cells secreting [c]IgE, plasma cells and macrophages have been also detected in this tissue. BALT formation is associated with wide ranges of chronic inflammatory diseases, autoimmune diseases and infection (19). BALT is generally considered in chronic inflammatory and obstructive pulmonary diseases such as asthma and bronchitis (37, 36). Based on published evidences, researches on the effect of high intensity interval training on lung inflammatory changes are very limited. Therefore, in this research, we studied the chronic pulmonary inflammatory response to a period of high intensity interval training in healthy male rats.

2. Materials and Methods

2-1. Animals

This research was carried out in accordance with the national institute of health guide for the care and use of laboratory animals. All experiments that involved animals were conducted according to the policy of the Iranian convention for the protection of vertebrate animals used for experimental and other scientific purposes and the protocol was approved by the ethics committee of sciences, University of Mazandaran (UMZ) (number:2316813 date:18/2/2016). To conduct the experiments, 12 Wistar male rats (4 weeks old, 68±9 g weight) were acquired from the Pasteur Institute (Amol, Mazandaran, Iran) and maintained at the central animal house of the faculty of physical education and sports science of UMZ. During the experiments, all the animals were kept in standard polyester cage (46-L volume, 2 rats in each cage) in a room with standard temperature (22±1.4 °C) and humidity (%55±5) with 12-hour light/darkness cycle and free access to water and food. The animals were familiarized to environmental condition for one week and then, were randomly assigned into control (n = 6) and training (n = 6) groups. The control group remained sedentary whereas the training group participated in a HIT program.

2-2. Exercise training protocol

In the present study, we used high intensity interval training as a model of exercise intervention. At first, the animals were familiarized to rat treadmill apparatus, every day for 5 days. The training group was trained for 9 weeks using the same training protocol. In each training session, the rats completed 10 repetitions of 1-min activity, with 2 min active rest between the sessions. Work to rest ratio was 1:2 and Total exercise time was thirty minutes In each session, 5 d/w. Running velocity began with 25 m/min and gradually reached 70 m/min (43).

2-3. Sampling and tissue sectioning

For microscopical studies, the whole left lung was removed and fixed in neutral buffered 10% formalin. After tissue processing including dehydration in graded alcohol series, clearing in xylene and impregnation in paraffin wax, the lung was embedded in paraffin block according to an isotropic uniformly random (IUR) protocol. Each block was cut into four 20µm thick serial sections and four 5µm thin serial sections using a rotary microtome. Sections were collected on slides and stained with hematoxylin-eosin (H&E). Systematic random sampling

[b] . Immunoglobulin A

[c] . Immunoglobulin E

protocol was performed for sampling processes and the first section was chosen randomly. Finally, 20 to 25 thick sections and 5 thin sections were selected from each block for stereological and immunohistochemical analysis.

2-4. Immunohistochemical analysis

The selected 5 µm tissue sections were collected on Silanized slide (Dako, S3003), dewaxed in xylene, rehydrated in a series of graded ethanol solution and immunostained as previously described (26, 49). In brief, heat mediated antigen retrieval was performed in 10 mM citrate buffer (10 mM tri-sodium citrate, 10 mM citric acid, pH 6.0) with slides microwaved at 800W for 4 min, then at 160W for 10 min, then cool at room temperature for 20 min. The sections were delineated using a Dako pen (Dako, Glostrup, Denmark) rinsed in PBS and incubated with 3% hydrogen peroxide in methanol for 1 min to eliminate endogenous peroxidase activity. Subsequently, the slides were washed in PBS before being treated with 0.1% Triton X-100 in citrate buffer and then, were washed again and treated with 50% goat serum in PBS for 30 min. Following rinsing in PBS, the sections were incubated with IL-17 antibody (Abcam #9565, diluted 1:200 in 5% goat serum) in a humid chamber overnight at 4°C. Afterwards, the sections were washed three times for 5 min, each time with PBS, followed by incubation with secondary antibody biotinylated goat anti-rabbit IgG (Vector BA-1000, diluted 1:200) diluted in 5% goat serum and incubated for Thirty min at room temperature. The secondary antibody was washed off in PBS and avidin–biotin complex reagent prepared according to the manufacturer's directions was then applied to each section for 30 min followed by rinsing in PBS. Antibody was visualized with 3,3-Diaminobenzidine Tetrachloride (DAB) at room temperature for 2 min, washed in distilled water and lightly counter-stained with Haematoxylin for 2 min. The slides were then dehydrated and cleared in xylene before being mounted with a coverslip for microscopical analysis. Five fields from each section were photographed using an Olympus BX-60 microscope with a Olympus DP 12 digital camera (Olympus Corporation, Japan). Then, photomicrographs were analyzed by Image J software version 1.49 to quantify immunohistochemical reaction for IL-17 (15).

2-5. Stereological analysis

In this study, we aimed to develop an efficient and practical design-based combination of stereological tools for estimating various parameters of lung structure in rats. Stereological investigations provided information on volume, surface, length and, with some extended techniques (3D sample), number of structural components. It is a gold standard for lung morphometry. Stereological studies were carried out strictly under blind condition with the optical fractionator for estimating total number of alveolar macrophages (Dust cells) per lung and with Cavalieri's principle for estimating total volume of interstitial tissue, alveolar space and bronchial associated lymphoid tissue (BALT) using the stereo-investigator system version 9 (MBF Bioscience, MicroBrightField, Inc, Germany). This system consisted of a standard microscope, motorized stage, digital closed-circuit camera and software application.

To estimate the total volume of the interstitial tissue, alveolar space and BALT, volume density (V_v) of these structures was calculated using the following formula (41):

$$V_{v\,(structure/lung)} = P_{(structure)} / P_{(lung)}$$

where "$P_{(structure)}$" is the number of points hitting the profiles of the interstitial tissue/alveolar space and BALT and "$P_{(lung)}$" is the number of points hitting the lung. The total (absolute) volume was obtained through multiplying the density by the final lung volume:

$$V_{(structure)} = V_{v\,(structure/lung)} \times V_{(final)}$$

The final volume of the lung was estimated by Cavalieri's method, as in the following equation:

$$V_{(final)} := T \times (a/p) \times \sum_{i=1}^{n} Pi$$

where "*T*" is the distance between the sections, "*a/p*" is the area associated with each point and "P_i" is the number of points landing within the lung on the *i*th section.

To estimate the total number of alveolar macrophages, the 20 µm thick section and a high numerical aperture oil immersion lens were used. By means of the stereological software, an unbiased counting frame was superimposed on the lung sections. The number of macrophages was estimated using the optical dissector method as in the following formula:

$$N_{V\,(cell/ref)} := \frac{\sum Q^{-}}{\sum A \times h}$$

where "$\sum Q^{-}$" is the number of cells coming into focus in the disector height, "$\sum A$" is the total area of the unbiased counting frame in all microscopic fields and "*h*" is the height of dissector. The total number of cells was estimated through multiplying the numerical density (N_v) by the total volume of the lung.

2-6. Statistical analysis

The results were expressed as means ± SEM. To analyze the findings, independent t-test was used. $P \leq 0.05$ was considered statistically significant. All the statistical analyses were performed using SPSS version 21 (SPSS, Chicago, IL).

3. Results

Statistical analysis showed that the 9 weeks of the high intensity interval training significantly increased lung IL-17 protein levels (2.7-fold, $P \leq 0.05$) (Figure 1), pulmonary macrophage population (1.9-fold, $P \leq 0.05$) (Figure 3) and pulmonary BALT volume (74-fold, $P \leq 0.05$) (Figure 4). Another finding of this study was a significant increase in the volume of interstitial space in the lung parenchyma (14.10 percent, $P \leq 0.05$) and a significant decrease in alveolar space after the 9 weeks of the high intensity interval training program (46.45 percent, $P < 0.05$) (Figures 7 and 8).

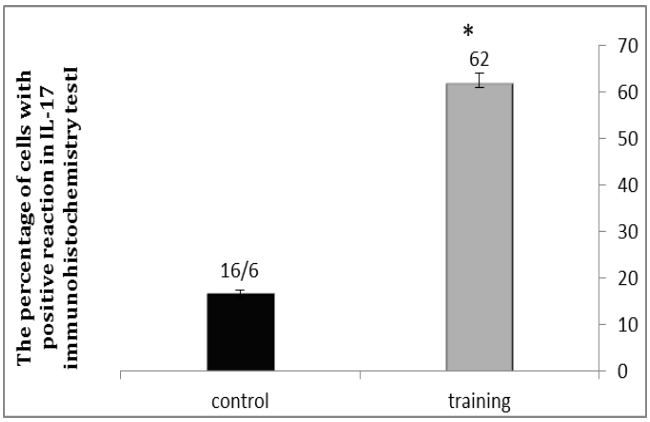


Figure 1. The effect of the 9-week high intensity interval training on IL-17 protein levels in the lungs of healthy Wistar rats; data were reported based on mean ± SEM. ***** Significant difference compared to the control group ($P \leq 0.05$)

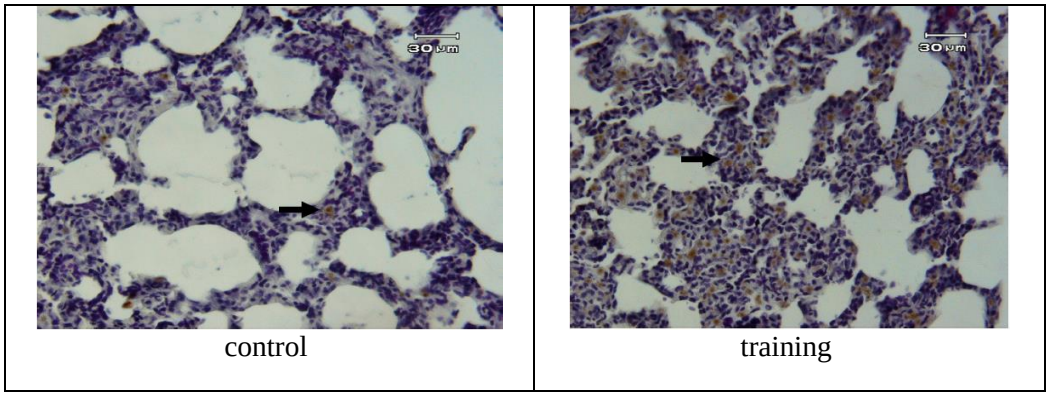


Figure 2. Immunohistochemical expression of IL-17 in lung tissue in the study groups (test chromogenic immunostaining with DAB, differential staining with hematoxylin and magnification 400x); brown color (arrows) in the context is the sign of IL-17 expression. The images show that after the 9 weeks of the high intensity interval training, expression levels of IL-17 in lung tissue in the animals increased remarkably.

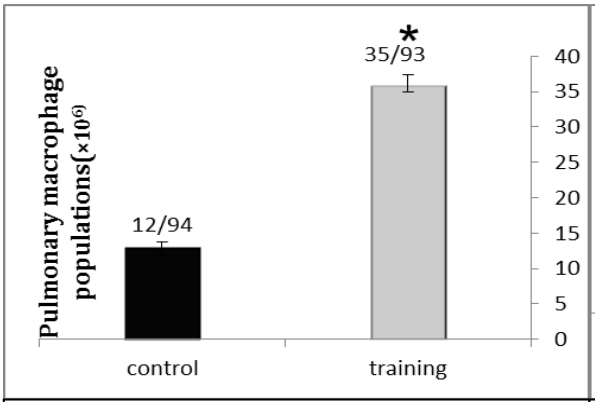


Figure 3. The effect of the 9 weeks of the high intensity interval training on lung macrophage population; data were reported based on mean ± SEM. * Significant difference compared to the control group (P≤0.05)

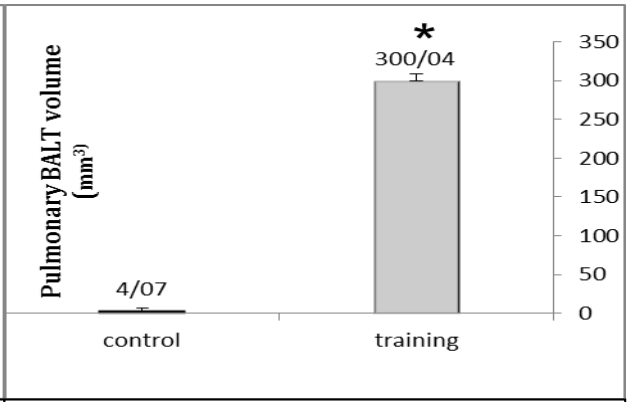


Figure 4. The effect of the 9 weeks of the high intensity interval training on volume of pulmonary BALT; data were reported based on mean ± SEM. *Significant difference compared to the control group (P≤0.05)

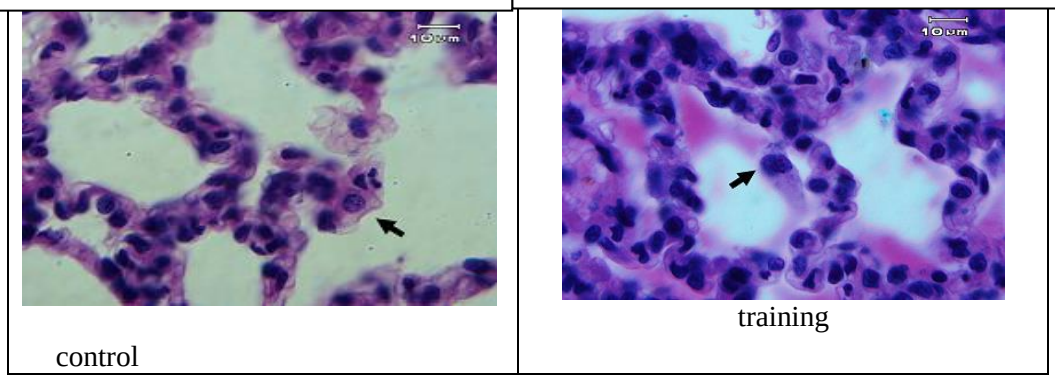
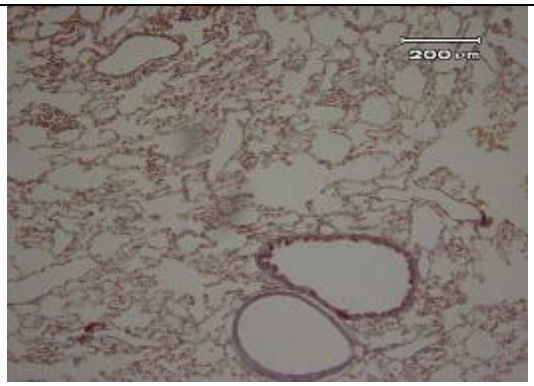


Figure 5. The distribution status and presence of pulmonary macrophages (Dust cells) in the training and control groups (stained with hematoxylin - eosin, magnification 1000 ×); the status and location of lung macrophages (arrows) were identified in the alveolar and interstitial lung in the groups. The presence of numerous macrophages is visible in the training group.



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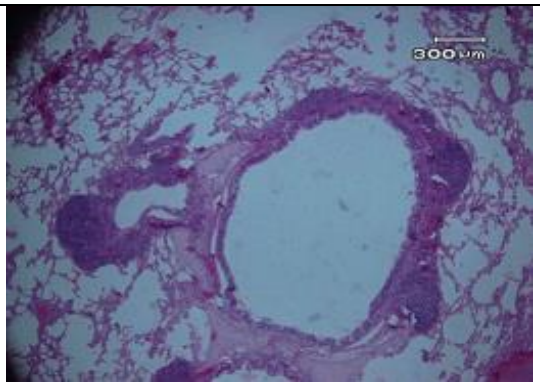


training

Figure 6. The situation of BALT (lymphoid tissue surrounding the pulmonary bronchi and bronchioles) groups with hematoxylin and eosin staining; stereological studies Been done by optical microscopy Frakshenitor, digital camera system and version 9 of Stereo – investigator Software in mm3 scale. Natural state of connective tissue around the bronchi and bronchioles in the control group as well as excessive accumulation of lymphoid tissue around the bronchi and bronchioles in the training group was visible.

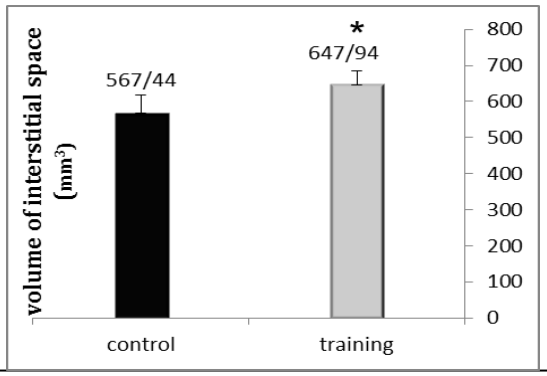


Figure 7. The effect of the 9-week high intensity interval training on the volume of interstitial space in the lungs; data were reported based on mean ± SD. *Significant difference compared to the control group (P<0.05).

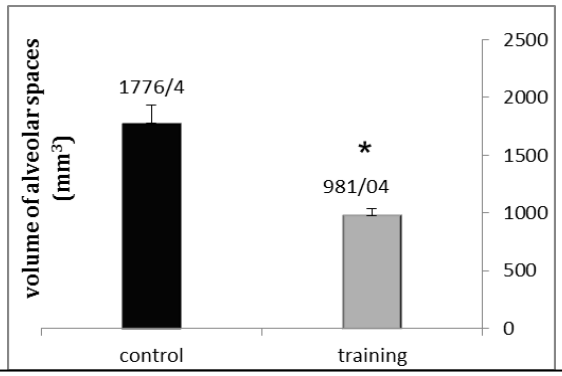
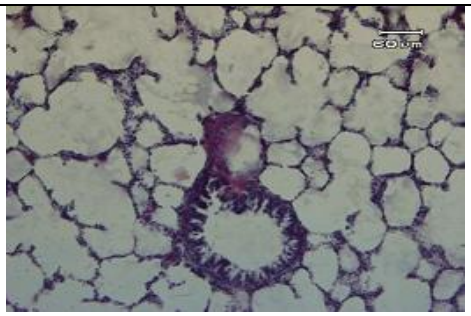
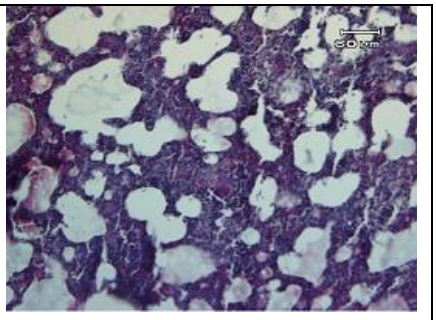


Figure 8. The effect of the 9-week high intensity interval training on the volume of alveolar spaces; data were reported based on mean ± SEM. *Significant difference compared to the control group (P≤0.05).



control



training

Figure 9: The histological structure of lung tissue in the training and control groups (stained with hematoxylin - eosin, magnification, 200 ×); Parenchyma structure of the control group was normal, while in the training group, increased emphysema and interstitial tissue was clearly visible. In the training group, interstitial tissue increased due to accumulation of inflammatory cells and alveolar space volume is affected (Decrease). Inflammation was evident in this group.

4. Discussion

The aim of this study was to evaluate the effect of a period of nine weeks of high intensity interval training on inflammatory lung condition in healthy Wistar rats. In summary, the findings of this study showed that after nine weeks of high intensity interval training, the protein IL-17, macrophage population and volume of pulmonary BALT significantly increased. Also, the alveolar interstitial space expanded and alveolar space volume decreased significantly. Limited researches have been done related to intense exercise training effect on pulmonary parenchyma and lower airways, and existent knowledge in this area does not provide answers to questions. In the present study, due to changes in the selected parameters, it seems that after the intense exercise training program, chronic inflammation state was induced in lung, which is likely a challenge to the respiratory system health.

Some studies have shown that in athletes, especially endurance athletes who are engaged in intense exercise, daily repeated physical exercise in the long run leads to epithelial damage and increased inflammation in the respiratory mucosa. Intense exercise by creating a temporary hypoxia and metabolic tissue reperfusion during rest provides the conditions of free radical formation (3). Oxidative stress damage caused by reactive oxygen species (ROS) production can cause irreversible damage to the lung tissue. The intensity and duration of physical activity have an important effect on the production of free radicals (18). Thus, severe exercise and poor opportunities for reconstruction and recovery of cells lead to excessive production of free radicals, weakened antioxidant defense system, damage to lipids, proteins, nucleic acids and DNA fragmentation, as well as to elevation of inflammatory markers in tissue. One of the most important indicators of acute and chronic inflammation in the lung tissue is high levels of IL-17 (29). In relation to the effect of exercise training on levels of IL-17, it was found that 8 weeks of submaximal exercise training significantly reduced plasma IL-17 and IFN-γ levels in patients suffering from multiple sclerosis (MS) (23). Compared to the results of this study, exercise intensity has an important role in inflammatory or anti-inflammatory outcomes of training. Supporting this interpretation, compared to the effect of moderate exercise and vigorous exercise on inflammatory responses and levels of IL-17, IL-6, IL-1ra in soleus muscle, increased levels of IL-17 and other inflammatory factors were observed only in those subjects who participated in high-intensity exercises (16). Also, it was reported that high-intensity exercise due to elevated levels of catecholamines, cortisol, free radicals and pulmonary epithelial tension increases expression of nuclear transcription factors such as NF-kB. NF-kB aggravates expression of pro-inflammatory interleukin such as IL-17, IL-13, IL-8 and IL-1 (31, 4, and 11). IL-17 by stimulating the expression of a variety of factors including proinflammatory cytokines, chemokines, acute phase proteins, antimicrobial peptides, mucin and metalloproteinases start-ups a cascade

of events that finally lead to recruitment of immune cells and inflammation (24).

In the present study, the macrophage population, BALT volume and pulmonary interstitial space significantly increased after performing high intensity interval training, which confirms the occurrence of chronic inflammation in the bronchi, bronchioles and lung parenchyma (5, 6). According to the theoretical basics, we can see that during stress, infection and inflammation, migration and homing of immune cells occur in different parts such as pulmonary interstitial space and connective tissue surrounding the bronchi and bronchioles and perivascular space as the main reasons for the increased BALT volume and interstitial spaces (19, 12).

In addition to the potential mechanisms that lead to the lung inflammation following a high intensity interval training, dehydration, decreased moisture and cool epithelial surface airways caused by intense and deep ventilation should be considered. Dehydration causes hiperosmosis and concentration of chlorine, sodium, calcium and potassium ions. Then, the flux of plasma intensifies in airway epithelial ion through channels and aquaporins. and local blood flow may lead to tissue edema and inflammation (10). airways adenosine triphosphate depletion is done at response to shear stress and metabolic needs of intense exercise and fluid osmolarity in epithelial surface. Epithelial surface dehydration is related to the incidence of eosinophilic inflammation and increased mucus production. Further, epithelial surface dehydrationis related to the release of epithelial histamines, leukotrienes, and prostaglandins and smooth muscle contraction to increase the lead edema (2). Also, the increment of osmolarity of epithelial airways is associated with increased expression of IL-8. IL-8 by recruiting neutrophils exacerbates neutrophilic inflammation (8).

Probably, the most important immune cells in chronic pulmonary inflammatory response induced by intense exercise are macrophages. Macrophages in the airways, alveoli and pulmonary interstitial space reside or migrate to the lung vessels (6, 7). Macrophages are a major source of cytokines, chemokines, and other inflammatory mediators. Looking for an invasion, macrophages and epithelial cells secrete chemokines and cytokines and develop their neutrophil accumulation and local inflammation (7). Previous studies showed that acute and chronic exercise were effective on the many macrophage functions including phagocytosis, anti-tumor activity, reactive oxygen and nitrogen metabolism and chemotactic. The rate of increased macrophage function is different according to the intensity and duration of exercise (14, 51). In this study, after interval training, the number of lung macrophages significantly increased, which indicated the presence of inflammation.

In athletes, especially endurance athletes, epithelial damage and increased inflammation in the respiratory mucosa have been seen.

Epithelial cells produce ROS, cytokines and platelet-activating factor for recruitment of inflammatory cells to the site of inflammation. Intense exercise training via increased ventilation leads to increased wear of the airways and tearing of the respiratory epithelium, resulting in inflammation and epithelial damage (9, 48, 1, and 30). Additionally, in this study, stereological indicators showed that after 9 weeks of the high intensity interval training, the alveolar space was deducted. The alveolar is the basic unit of the lung parenchyma and plays an important role in gas exchange between circulatory-respiratory systems. Therefore, morphological and physiological health of alveoli is very important (42). As the image of the histology shows, in the training group, due to the presence of inflammatory cells and edema, the volume of interstitial space increased. For this reason, alveolar space is impressed and dropped. This event leads to the reduction surface of the alveoli and gaseous exchange surface (respiratory surface), reducing the volume of parenchyma and possibly reducing the inspiratory capacity and vital capacity.

5. Conclusions

As a conclusion, it seems that high intensity interval training through various mechanisms such as changes in the expression of inflammatory interleukin, increased production of free radicals, dehydrated epithelial and antioxidant depletion causes potential inflammation of the upper and lower airways in lung. The inflammation is probably able to make a defective remodeling in parenchyma and reduces its efficiency in gas exchange. Thus, it seems that, in addition to the positive effects of high intensity interval training on performance and physiological indicators, paying attention to the inflammatory effects of this type of exercise training on the airways and prevention of such complications is necessary.

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