

Original Article

The Effects of Aerobic Exercise on NF- κ B and TNF- α in Lung Tissue of Male Rat

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Abstract

Background: Regular aerobic exercise improves the inflammatory status in different lung diseases. However, the effects of long-term aerobic exercise on the lung response have not been investigated. The present study evaluated the effect of aerobic exercise on the lung inflammatory.

Materials and Methods: 12 adult male Wistar rats were divided to 2 groups: A: control (n=6), B: aerobic exercise (five times per week for 4 week; n=6). The gene expression of NF- κ B and TNF- α were analyzed in lung tissue by Real time-PCR. In order to determine the significant differences between groups independent t-test were used.

Results: Aerobic exercise inhibited the gene expression of NF- κ B and TNF- α . But there was no significant difference between A and B groups for TNF- α and NF- κ B.

Conclusion: We conclude that four week aerobic exercise decrease inflammatory status in lung tissue. Our results indicate a need for human studies that evaluate the lung responses to aerobic exercise.

Keywords: aerobic exercise, inflammation, NF- κ B

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Introduction

Increased expression of inflammatory proteins including cytokines in air way is associated with asthma, chronic obstructive pulmonary disease, and lung cancer. Inflammation appears to be etiologically linked to the pathogenesis of all of these conditions¹⁻⁴, and the development of a chronic low-grade inflammatory state has been established as a predictor of risk for several of them⁵. However, when the inflammatory conditions are persistent, or when resolution mechanisms fail, a state of chronic inflammation ensues that can lead to loss of normal physiological functions. A central role in the

induction of chronic inflammation is played by a set of genes encoding proinflammatory cytokines such as IL-1, IL-2, IL-6, and TNF- α (Tumor necrosis factor- α) and monocyte chemotactic protein 1. What is common to all these molecules is that they are regulated by the NF- κ B (nuclear factor- κ B)⁶⁻⁸. Some NF- κ B-regulated cytokines, such as TNF- α and IL-1 β , in turn regulate NF- κ B itself⁹. Physical inactivity and sedentary behavior also increase the risk of these conditions.

Exercise has anti-inflammatory effects, and therefore, in the long term, regular physical activity can protect against the development of chronic diseases¹⁰⁻¹³. In the present study, we hypothesized that a decrease in NF- κ B and TNF- α expression could be the basis for

decrease the chronic inflammation and in above diseases.

Methods

In all experiments, the Tarbiat Modares University guidelines for animal care were followed. This study was approved by the Tarbiat Modares University of Tehran (Cod number: 62/2987).

Animals

Twelve adult male Wistar rats aged 8 weeks were obtained from Pasteur Institute of Iran and randomly divided into the 2 groups: A; control (n=6), B; aerobic exercise (five times per week for 4 week; n=6), Rats were housed in cages under controlled environment (23°C and 12h light–dark cycle) with free access to normal chow and tap water.

Exercise treadmill test and training

Animals in B group were adapted to the treadmill for rat (will running treadmill, Lafayette American) training for 3 days (15min, 20m/min). On the fourth day, the individual maximal exercise capacity test was performed with a 5min warm-up (6m/min) and followed by an increase in treadmill speed (3m/min every 3min) until animal exhaustion (i.e., when they were not able to run voluntarily after 3 mechanical stimuli)¹⁴⁻¹⁶. The maximal exercise capacity (100%) was defined as the maximum speed reached by each animal. The physical test was performed for each rat individually. The speed average of group was calculated, and then the rats were submitted to treadmill training as a mean speed of the group workload. rat were trained at low intensity, corresponding to 50% of the initial maximal speed obtained in the exercise test, for 60 min, 5 five times per week, as previously described¹⁴⁻¹⁶.

Analysis of mRNA Expression NF- κ B and TNF- α by RT-PCR

After scarified rat and Total lung tissue RNA was isolated using Trizole reagent (Qiagen, Germany), according to the manufacturer's instructions. The RNA samples were subjected to reverse transcription using Thermo Scientific Revert Aid First Strand cDNA Synthesis Kit (Ferementase). In the subsequent step, the cDNAs were used as templates to perform real-time PCR using SYBR green PCR master mix (SYBR green I,) by step one ABI system (Applied Bio system).The crossing threshold values

assessed by the real-time PCR were evaluated for the transcripts and normalized to the results for GAPDH mRNA. The corresponding primer pairs for NF- κ B, and TNF- α and GAPDH (housekeeping gene) were listed in table 1.

Real-Time PCR

All the tests were done duplicated in each group. The threshold cycle (Ct) for each specific gene, corresponding housekeeping gene (GAPDH) and their differences (Δ Ct) were determined and then evaluated gene expression changes using $\Delta\Delta$ CT formula.

Statistical Analysis

Results are expressed as Mean \pm SD. Differences in NF- κ B, and TNF- α expression between control and exercise groups were examined by independent t-test. P<0.05 were considered statistically significant

Results

Data presented in figure 2 demonstrate that aerobic exercise decreases the gene expression of NF- κ B and TNF- α in lung tissue compared with control group. No significant effect was observed on the gene expression TNF- α (P=0.669) and NF- κ B (P=0.079) by independent t-test.

Discussion

In the present study, we demonstrated that aerobic exercise inhibits lung inflammation and proinflammatory cytokine release in lung tissue in an experimental model. A central role in the induction of chronic inflammation is played by a set of genes encoding proinflammatory cytokines such as IL-1, IL-

Table 1: Primer Sequences Used in Real-Time PCR.

Gene	Primer sequence
NF- κ B	FOR:5'-
	AACACTGCCGAGCTCAAGAT -3'
	REV:5'-
	CATCGGCTTGAGAAAAGGAG -3'
TNF- α	FOR:5'-
	GACCCTCACACTCAGATCATCTTC
	-3'
	REV:5'-
	TGCTACGACGTGGGCTACG -3'
GAPDH	FOR:5'-
	GACATGCCGCTGGAGAAAC -3'
	REV:5'-
	AGCCAGGATGCCCTTTAGT -3'

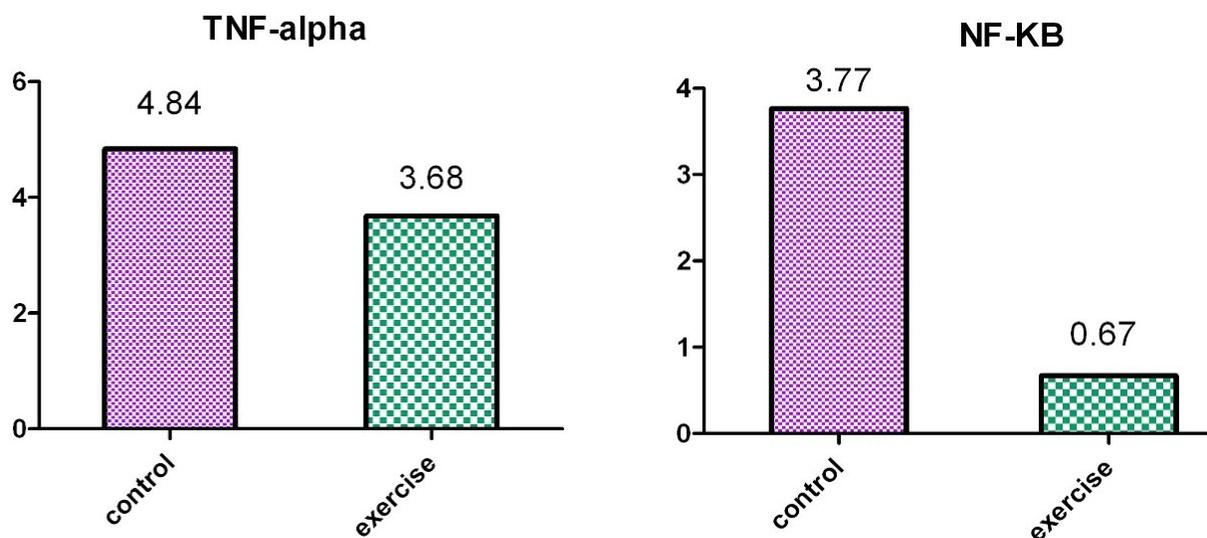


Figure 1. Effects of aerobic exercise on of NF- κ B and TNF- α expression in lung. NF- κ B and TNF- α expression decreased after 4 weeks aerobic exercise

2, IL-6, and TNF- α and monocyte chemoattractant protein 1⁶⁻⁸ that are regulated by the transcription factor (NF- κ B)⁶⁻⁸. Increased expression of NF- κ B associated with asthma, chronic obstructive pulmonary disease¹⁻⁴.

The mechanisms responsible for the effects of exercise on immune responses in the lung remain ill defined: however, data presented suggest that such mechanisms may involve exercise-induced changes in NF- κ B activation. Pastva et al. have reported that aerobic exercise attenuates airway inflammation in a mouse model of atopic asthma via modulation of NF- κ B activation¹⁷. Similarly, Henriksen and Nielsen demonstrated a beneficial effect of endurance training on exercise-induced bronchoconstriction and working capacity¹⁸. Exercise decreased the numbers of each of the observed infiltrating cell types. In general, exercise appeared to decrease cellular infiltration by improving the integrity of the epithelial layer¹⁹. Catecholamine's, such as epinephrine, may exert bronchodilatory effects that lessen compressive pressures and thereby decrease stress-induced NF- κ B activation in the airways^{20,21}. Catecholamine's increased during exercise.

Specifically, we showed that aerobic exercise decreased NF- κ B and TNF- α expression in lung tissue. Alternatively, improved physical fitness and decreased inflammatory load as a consequence of

moderate intensity training can be reason for this result.

Conclusion

We conclude that four week aerobic exercise decrease inflammatory status in lung tissue. Although change in gene expression in present study was slight, however this result can be clinically important. Our results indicate a need for human studies that evaluate the lung responses to aerobic exercise.

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