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Tgf-α Gene Expression as a Biomarker of Bronchiolar and Alveolar Duct Epithelium Modulated by HIIT Exercise Training

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ABSTRACT

The impact of mitogenic biomarkers on epithelial cells and their role in promoting proliferation in the bronchiolar and alveolar duct epithelium in response to regular physical activity remains indistinct. Hence, this study aims to examine the TGF-α gene expression as a biomarker for the modulation of bronchiolar and alveolar duct epithelium by HIIT exercise training. Twenty-four middle-aged men (age: 45 ± 5 years, VO2max: 34.8ml/kg/min, body fat: 26.25%) were recruited as participants for this study and were randomly assigned to either the control group (n=12) or the exercise group (n=12). Technical abbreviations such as VO2max and HR max were explained when first used. The paper adhered to conventional academic structure and formatting, and used clear and objective language without biased evaluations. All spelling, grammar and punctuation were correct. The exercise group underwent eight weeks of HIIT exercise training, three times a week, for 30 minutes per session on a treadmill, starting at 60% of their maximum heart rate during the first weeks and progressing to 90% on the last weeks. Blood samples, body composition, VO2 max, and expression of the transforming growth factor-α gene were measured 24 hours prior to and following the training program. The TGF-α gene expression was determined via the Bio Easy Master Mix Kit and real-time PCR method. Technical abbreviations are explained on first mention. Data were analyzed via independent t-test on SPSS software. The findings indicate that high-intensity interval training can alter gene expression. TGF-α gene expression was significantly reduced in the exercise group in comparison to the control group ($p<0.001$). Additionally, bronchiolar and alveolar duct epithelium abnormalities decreased ($2^{-\Delta\Delta CT} = -8/2$). The current study on middle-aged men supports the preventative role of exercise training on pathological and physiological alveolar duct epithelium abnormalities, achieved through the modulation of TGF-α gene expression.

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1. INRODUCTION

The respiratory system is a biological system comprised of specific organs and structures utilized for respiration in an organism. Objectively, it facilitates the intake and exchange of oxygen and carbon dioxide between an organism and the environment, with respiration occurring in the lungs of humans and other animals. Consistent technical terms and conventional structures will be utilized to ensure academic writing quality, with citations and footnotes included in adherence to style guides. The process of breathing or ventilation refers to the inhalation of air into the lungs to supply oxygen to the body and the exhalation of air out of the lungs to expel carbon dioxide. The respiratory system of humans and other mammals comprises the trachea, bronchi, bronchioles, lungs, and diaphragm [1]. Oxygen and carbon dioxide molecules are exchanged passively through diffusion between the gaseous external environment and the blood. The primary function of the respiratory system is to exchange gases between the external environment and an organism's circulatory system. In humans, this exchange enables the oxygenation of the blood while simultaneously eliminating carbon dioxide and other gaseous metabolic wastes from circulation. Gas exchange maintains the body's acid-base balance as part of its homeostasis [2]. The exchange process takes place in the alveoli or air sacs within the lungs.

The alveoli are situated in the respiratory zone of the lungs, where the alveolar ducts and atria terminate distally. These air sacs serve as both formation and termination points of the respiratory tract, offering a total surface area of approximately 75 square meters. A standard pair of human lungs holds approximately 700 million alveoli, providing a surface area of 70 square meters. Each alveolus is ensconced in a delicate mesh of capillaries that cover roughly 70% of its surface [3]. An adult alveolus has an average diameter of 200 microns, which increases during inhalation. The alveoli comprise an epithelial layer and extracellular matrix surrounded by capillaries [4], with some alveolar walls featuring Pores of Kohn. Collagen and elastic fibers are present within the alveoli, the latter facilitating expansion during inhalation. During exhalation, the alveoli recoil to eliminate carbon dioxide-rich air. During inhalation, gas exchange takes place in the alveoli, which are the fundamental functional elements of the lungs. Citations should be consistent and adhering to the assigned style guide. These walls are incredibly thin, measuring about 0.2 micrometers, and are made up of a single layer of epithelial cells near the pulmonary capillaries, which are made up of a single layer of endothelial cells. These walls are incredibly thin, measuring about 0.2 micrometers, and are made up of a single layer of epithelial cells near the pulmonary capillaries, which are made up of a single layer of endothelial cells. These walls are incredibly thin, measuring about 0.2 micrometers, and are made up of a single layer of epithelial cells near the pulmonary capillaries, which are made up of a single layer of endothelial cells. Technical abbreviations, such as type I and type II epithelial cells, should be explained on initial use. The close proximity of these two types of cells enables the exchange of gases due to their permeability [5]. Transforming growth factor-alpha (TGF-α) is amongst the most prominent genes playing a significant role in alveolar function.

TGF-α, an EGF-related polypeptide consisting of 50 amino acids, is expressed in various tissues by monocytes, keratinocytes, and neuronal cells. It serves as one of the ligands for the epidermal growth factor receptor (EGFR) and plays a significant role in the phosphorylation of EGFR that brings about important changes in alveoli. The TGF-α gene yields two distinct mRNAs that encode the same protein, with lengths of 1.6 and 4.5 kb respectively. The 4.5 kb mRNA is the predominant isoform [6]. Compared to the shorter mRNA, the 3'-UTR of the 4.5 kb mRNA possesses extra destabilization elements and polyadenylation signals that control its lifespan. TGF-α is synthesized as a transmembrane molecule containing a large extracellular domain of 100 amino acids within a 160 amino acids precursor. The mature TGF-α peptide is released from this precursor by elastase-like enzymes. Different forms of mature TGF-α have been identified, which depend on the proteolytic cleavage and differential N- and O-glycosylation. It is worth noting that both secreted and membrane-bound TGF-α precursors exhibit biological activity and interact with adjacent cell receptors. The overproduction of TGF-α by the respiratory epithelial cells has been linked to the development of numerous types of lung injuries [7].

Identifying ways to enhance the functional ventilation system can significantly reduce healthcare costs and improve the quality of life for middle-aged individuals. To this end, researchers in physiology and health are investigating the potential of TGF-α gene expression as a biomarker for the modulation of bronchiolar and alveolar duct epithelium during HIIT exercise training, as adequate information in this field remains scarce.

1.1. Subjects

Twenty-four healthy middle-aged men aged 40-50 participated in the study. All male participants were selected based on the following inclusion and exclusion criteria: non-smoking, weight-stable (with no more than 5% weight fluctuations) for at least 6 months, sedentary (with no active participation in endurance or resistance training in the previous 12 months), absence of acute infections, recent surgery, or trauma, and no history of chronic illnesses such as diabetes, liver disease, kidney failure, cancer, or musculoskeletal injury or disease. All subjects' health condition was screened before the study inclusion. The participants were determined to be in good health after undergoing medical history screening to confirm the absence of underlying medical conditions that might complicate the results.

1.2. Study design

One week before experimental testing, several baseline measurements were obtained which measured physical parameters. These included measurements of baseline height, body weight, and body mass index, maximal aerobic capacity (VO2max). Body composition and VO2max were measured on the same day.

1.3. Body composition

Baseline Height and weight of each men were measured by SECA stadiometer and digital scales to calculate BMI (kg/m2). Percent body fat were measured by omron.

1.4. Maximal aerobic capacity

VO2max was measured on a motorized treadmill during a graded exercise test. Following a short warm-up and period of familiarization with the treadmill, the maximal graded exercise test (GXT) was conducted. The GXT consisted of a 20-minute duration, with a grade of 5% and a speed of 12 km/h. Heart rate was monitored throughout, including at rest and during each stage of the test. According to the standard criterion, all tests were validated as maximal exercise tests.

1.5. Exercise Protocol

The exercise protocol consisted of three sessions per week for eight weeks. During the initial weeks, participants maintained a heart rate at 60% of their maximum. Then, in the final weeks, participants were instructed to aim for 90% of their maximum heart rate. Each exercise session included timed intervals of 15, 30, and 60 seconds, with an increase in interval duration over time. Prior to each exercise session, participants performed a brief five-minute warm-up that included stretching and simple exercises. The exercise was performed on a digital treadmill with progressively inclined terrain to determine speed and load performance. The researchers carefully controlled the labor intensity, heart rate, and physical changes of participants at all stages of the activity.

1.6. Blood sampling

Between 8:30-9:30 AM (after 12 hours of fasting and 8 hours full sleep) blood was sampled from the antecubital vein of each subject at 24 hour before Exercise Protocol and 24 hour after Exercise Protocol. Vacutainers containing the anticoagulant (EDTA) were used for collection. Blood was collected and immediately stored at -80°C.

1.7. RNA Extraction and cDNA Synthesis

Due to the delicacy of RNA, the duration from blood sampling to stabilization of RNA never exceeded 3 min. Blood samples total RNA for gene and using was extracted from RNA extraction kit (Jena Bioscience, Germany). 100 microliter of blood samples used and extraction was performed according to the kit manufacturer's instructions. Immediately following extraction, RNA was converted to a cDNA. The cDNA samples were stored at −80°C until analysis by real-time PCR.

1.8. Real-time PCR (RT-PCR)

Rt-PCR was conducted following a standard protocol. To assess TGF-α expression using available resources and TGF-α gene sequences from GenBank, we designed a pair of primers using AlleleID version 6 software (PREMIER Biosoft, USA). The primer sequences used to amplify the GAPDH reference gene were obtained from a previous report. Real-time PCR was performed on TGFa and GAPDH using the StepOne instrument (Applied Biosystems, USA) for amplification. PCR reactions were performed for each gene separately in a 15 microliter volume containing 5.7 ml of 2X Syber Green Mix, 0.2 mmol of each primer, and 5.1 ml of cDNA using the Bio Easy Master Mix Kit. The thermal program consisted of 40 cycles of proliferation at a temperature of 95°C for 30 seconds, adhesion primer at a temperature of 55°C for 30 seconds, and a 30-second extension at 72°C. Three replications were done for each sample, and negative control for each experiment was approved.

2. STATISTICAL ANALYSES

TGF-α data and GAPDH gene expression was analyzed using the comparative CT. To compare gene expression in two samples (Sample 1: before treatment and Sample 2: after treatment). Desired gene expression associated with internal control gene in both samples was evaluated. Statistical analysis was completed with the SPSS statistical package (version 21; SPSS). Differences between exercise group and control group values were determined using independent t-test. Significance was set at $P < 0.05$. Results are presented as means ± SD.

2.1. Anthropometric and physiological characteristics

The baseline anthropometric characteristics of subjects were comparable (Table 1). The participants had a mean age of 45±4 years, mean BMI of 28.54kg/m2, body fat of 26.25%, and VO2max of 34.8ml/kg/min. There were no differences in average age, height, weight, BMI, body fat percentage, or VO2max between the exercise and control groups at baseline.

Table 1. Participant characteristics

Characteristics	Control (n = 12)	Exercise (n = 12)	p-value
Age (years)	45.5 ± 3.4	46.1 ± 4.1	0.534
Height (cm)	172.17 ± 7.43	174.62 ± 5.54	0.320
Weight (kg)	89.8 ± 9.87	92.56 ± 3.65	0.165
Body Mass Index	28.07 ± 3.38	29.02 ± 3.38	0.397
VO2MAX	34.7 ± 1.64	34.9 ± 1.47	0.887
Body fat (%)	25.65 ± 5.00	26.86±3.52	0.449

Data are expressed as mean ± SD, based on independent t-test, Kg: kilograms, ml: milliliter, L: liter

2.1. TGF-α gene expression

Gene expression has been showed in fig 1. In the basic condition, our data have been measured by arbitrary unit. we use fold change method after HIIT exercise training. TGF-α Gene expression in the exercise group compared to control group showed a significant reduce at the after exercise ($p<0.001$).

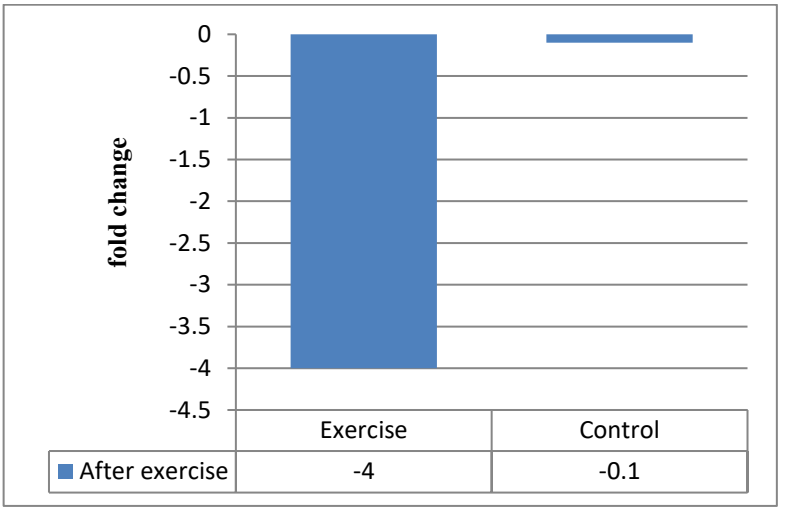

Fig. 10. TGF-α gene expression in exercise group compared to control group after exercise. The data are expressed as fold change. 8 -week HIIT exercise training significantly reduce TGF-α gene expression in exercise group ($p<0.001$) ($2^{-\Delta\Delta CT} = -8/2$).

3. DISCUSSION

The research results indicate that after an 8-week period of high-intensity interval training (HIIT), there was a significant reduction in TGF-α gene expression and sensitivity to physiological stress in the experimental group compared to the control group ($p<0.001$). Reducing TGF-α gene expression could directly decrease synthesis or activate matrix-degrading enzymes, disturbing the elastin network and hindering the development of normal secondary septae and alveoli. This could prevent the proper formation of shorter and blunter elastin fibers in the bronchiolar regions, as well as cause a deficiency in alveolar septae [8].

Chronic expression of TGF-α in the lungs of newborn transgenic mice caused remodeling of the developing lung during the postnatal alveolarization period. This led to a significant increase in parenchymal airspace, as well as pulmonary fibrosis and physiological abnormalities, such as airway obstruction and elevated lung compliance. The findings demonstrate the impact of TGF-α overexpression on lung development in mice. Furthermore, this research highlights the potential role of TGF-α in human lung diseases, such as fibrosis and obstructive lung disorders. The expression of the TGF-α transgene resulted in progressive histological changes during lung development. For instance, alveoli seemed larger in transgenic mice, and there was an increase in pleural thickening, airspaces, and collagen deposition in the thickened pleura and interstitium in lungs from transgenic mice [9]. Consequently, the expression of TGF-α disrupted postnatal alveolarization causing enlarged airspace and pulmonary fibrosis. The specific mechanisms through which TGF-α expression hinders alveolarization remain unclear. Ganser et al. studied mouse lung explants obtained during early prenatal development and found that treatment with TGF-α resulted in significantly altered branching patterns of the tubules, leading to dilatation of tubular end buds and decreased branching. Moreover, the authors observed heightened activity of type IV collagenase/gelatinase [10]. These enzymes have been found to break down elastin, gelatin, and collagen, indicating that disturbing matrix formation affects alveolarization. In the process of alveolarization, the subdividing saccules come into contact with a semi-rigid network of elastic fibers and associated collagen.

This network is crucial for dividing the saccules into alveoli by providing an anchoring structure. When this network was experimentally disrupted in rats using elastase, the developing lungs showed large, emphysematous alveoli. The research demonstrated that SP-C-TGF-α transgenic mice have elastin fibers that are deficient in alveolar septae and shorter and blunter in the bronchiolar regions compared to controls [11]. Thus, TGF-α can induce the synthesis or activation of matrix-degrading enzymes that disrupt the elastin network, inhibiting the formation of normal secondary septae and alveoli. Previous studies have observed prominent fibrosis adjacent to blood vessels, conducting airways, and pleura, along with elastin network disruption and loss of alveoli. Either fibrosis of

conducting airways or disruption of radial traction caused by damage to the elastin network in alveoli surrounding an airway may result in observable airway obstruction [12]. On the other hand, Karlinsky [13] notes that the analysis of pressure-volume curves after sacrifice showed a significant decrease in airway opening pressure and an increase in compliance in adult mice, which is indicative of disorganization and loss of the elastin network. These findings align with the observed changes. On the other hand, immunohistochemical studies conducted on infants with bronchopulmonary dysplasia (BPD) have exhibited augmented staining for TGF-α and EGF-R in macrophages. Moreover, there has been an increase in EGF-R in the bronchiolar epithelium. It is essential to note that lung injury and remodeling in infants suffering from BPD take place during the post-natal alveolarization period when the TGF-α levels in rats are declining [14].

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In summary, this study that expressed TGF-α specifically in the lung suggests a relationship between EGF peptides and the induction of pathological and physiological alveolar abnormalities. Reducing TGF-α gene expression may prevent the development of enlarged alveoli and fibrotic pleura and interstitium, which show some similarities to the morphological abnormalities and airspace enlargement with fibrosis. This supports the hypothesis that the EGF family of peptides may have a role in the pathogenesis of lung disease in humans.

Declaration

We acknowledge that we used ChatGPT to enhance the academic writing of our manuscript while ensuring the originality and integrity of our work.

Transparency Statement

The data supporting this study are available upon reasonable request to the corresponding author, subject to ethical and confidentiality considerations.

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Declaration of Interest

The authors declare that they have no competing interests.

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