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Effects of 6-Week Interval Aerobic Training (IAT) and Nano-Selenium Supplementation on Laminin α5 and Collagen IV Expression in the Extracellular Matrix of Alveolar Epithelial Cells in the Lungs of Healthy and Cigarette Smoke-Exposed Rats

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A B S T R A C T

Objective: This study aimed to investigate the effects of six weeks of interval aerobic training (IAT) and nano-selenium supplementation on laminin α5 and collagen IV expression in the extracellular matrix of alveolar epithelial cells in the lungs of cigarette smoke-exposed rats.

Methods and Materials: This experimental laboratory study used thirty-five male Wistar rats weighing 180-220 grams. The rats were randomly divided into seven groups: Healthy control, COPD control, COPD IAT, Healthy IAT, Healthy Selenium, Healthy Selenium-IAT, and COPD Selenium-IAT. Cigarette smoke extract was used to induce lung injury. Nano-selenium particles were administered via gavage. Interval aerobic training was performed for six weeks, five days per week. The expression of laminin α5 and collagen IV in the extracellular matrix of alveolar epithelial cells was evaluated. Data were analyzed using the Shapiro-Wilk test, two-way ANOVA, and Tukey's post hoc test.

Findings: Two-way ANOVA showed a significant difference in laminin α5 expression between the healthy control and COPD control groups (P=0.011), but no significant difference for collagen IV (P=0.971). There was also a significant difference in laminin α5 expression between the COPD control and COPD + IAT groups (P=0.011), but no significant difference for collagen IV (P=0.999). No significant differences in laminin α5 and collagen IV expression were found between the control and training or supplementation groups (P=0.999).

Conclusion: The results suggest that six weeks of interval aerobic training may be beneficial for mitigating the negative effects of cigarette smoke on laminin α5 expression in the lungs of rats with induced COPD.

Keywords: *Interval aerobic training, Nano-selenium supplementation, laminin α5, collagen IV, COPD*

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1. Introduction

The rising consumption of tobacco products and declining air quality due to modern lifestyles have turned chronic respiratory diseases into a global health crisis. Chronic Obstructive Pulmonary Disease (COPD) is a leading cause of death worldwide and ranks as the sixth most common cause of disability in developed nations (1). Various factors, including indoor air pollution from combustion, poor diets, and occupational exposures, contribute to COPD development, leading to airflow limitation, disability, and mortality (2). Cigarette smoking is a primary risk factor for COPD, as cigarette smoke contains a complex mixture of harmful gases such as carbon monoxide, nicotine, oxidants, particulate matter, and aldehydes. Research shows that inhaling cigarette smoke directly and resulting hypoxia causes inflammation in the lung's air sacs and damages airway walls, leading to the destruction of extracellular tissues and interstitial fibrosis (3). Smoking increases mucus gland secretion, causes obstructive bronchiolitis, and elevates the production of reactive oxygen species, which induce lung tissue damage and disrupt the oxidative and antioxidant balance (4). Overall, smoking or exposure to secondhand smoke is a primary cause of COPD (5).

A key feature of this disease is airway remodeling, which includes increased airway smooth muscle mass (ASM), neovascularization, epithelial shedding, cellular hyperplasia, and altered deposition of extracellular matrix (ECM) proteins, including basement membrane proteins (6). Basement membrane (BM) proteins influence cellular function, with laminins being a type of surface protein in the BM. The binding of laminins to cell membrane receptors initiates polymerization, forming a primary network of laminin fibers essential for BM formation (7). Laminin α5 is an ECM protein crucial for cellular changes associated with respiratory diseases in the lung. Collagens, another BM protein component, connect to the primary network of laminins (8). Collagen IV, the main component of BM collagen in many organs, including the lung and trachea, forms a network beneath epithelial and endothelial cells, creating barriers between tissue compartments. The expression of laminins and collagens may be altered in individuals with COPD (9). Studies by Shariat et al. (2012) and Karimfar et al. (2011) showed a significant increase in laminin α5 and collagen IV expression in newborn rats exposed to nicotine compared to controls (10, 11). Yurchenko et al. (2011) found that COPD alters BM protein expression, such as collagens and laminins, in the airways (12). However, Anoni et al. (2012) observed no change in collagen IV expression in COPD patients' large and small airways (13). Soltani et al. (2010) reported increased laminin α5 expression in COPD (14). Given the negative impacts of this disease, interventions like physical activity can positively affect disease management. COPD patients are less active daily, showing reduced activity levels and increased inactivity periods. Research indicates that targeted exercise interventions are crucial in controlling inflammation and promoting tissue repair, especially in the lungs (15). Studies have shown that smokers engaging in moderate or vigorous physical activity have a lower risk of developing COPD compared to those with low physical activity. Physical activity also reduces cigarette smoke-induced damage (16). A study showed that physical activity significantly modulated HIF-1α levels in the lung tissue of rat pups, suggesting a protective effect (17). Reactive oxygen species (ROS) production is linked to environmental factors, including smoking. Antioxidant mechanisms within cells, such as superoxide dismutase, catalase, ascorbic acid, glutathione, and selenium, counteract ROS effects (18). Selenium, which forms a group of selenoproteins, is an essential trace element, with deficiency linked to immunosuppression, viral infections, hypothyroidism, heart disease, and increased COPD susceptibility (19). Elsal et al. (2014) reported lower selenium levels in lung cancer groups and healthy smokers compared to non-smokers (18). Although some studies show no selenium effect on COPD (20), others suggest therapeutic benefits for COPD patients (18).

Cigarette smoke is a primary cause of COPD (21), leading to ECM protein alterations and immune cell infiltration. Physical activity and herbal supplements have been shown to have positive effects on lung health and exercise performance (15). However, limited research exists on the effects of Interval aerobic training (IAT) and selenium supplementation on cigarette smoke-induced COPD. Considering the importance of disease prevention, control, and management, this study aims to determine whether IAT and nano-selenium supplementation positively impact COPD treatment and management. By examining the effects of IAT and nano-selenium supplementation on laminin α5 and collagen IV expression in the ECM of alveolar epithelial cells in healthy and cigarette smoke-exposed rats, this study seeks to provide insights into this issue.

2. Methodology

This study was conducted using an experimental design with a simple random sampling method. Thirty-five adult male Wistar rats, aged 6 weeks and weighing approximately 180 grams, were obtained from the Pasteur Institute. They were housed in polycarbonate cages, with three rats per cage, in an environment maintained at a temperature of 22 ± 1.4°C and humidity of 55 ± 4%, under a 12:12-hour light-dark cycle. After a two-week acclimatization period, the rats, now weighing between 180 to 220 grams, were randomly assigned to one of seven groups: Healthy control, COPD control, COPD IAT, Healthy IAT, Healthy Selenium, Healthy Selenium- IAT & COPD Selenium- IAT. The animals were fed a standard diet following the guidelines of the Razi Serum and Vaccine Research Institute. Following the induction of disease in the appropriate groups, both supplementation and exercise interventions were initiated simultaneously and continued for six weeks. After the intervention period, the animals were anesthetized, and the target tissues were extracted for analysis using Real-Time PCR.

2.1 Interval aerobic training

Interval aerobic training was conducted over six weeks, with sessions held five days per week. Before starting the main exercise protocol, the rats underwent a one-week acclimatization period. During this time, they ran on a treadmill at a speed of 8-10 meters per minute with a 0-degree incline for five minutes per session. The main aerobic exercise regimen consisted of a 10-minute warm-up at 50-55% of VO2max, followed by seven intervals of four minutes at 80-90% VO2max. Each high-intensity interval was separated by three minutes at 60-75% VO2max, and the session concluded with a one-minute cool-down. To ensure the appropriate running speed and maintain relative exercise intensity, VO2max was assessed and evaluated every two weeks using an indirect method. This assessment utilized a treadmill with four incline levels, starting at a 25+ degree incline (22).

2.2 VO2max

VO2max was evaluated using a ramp protocol based on the study by Hoydal and colleagues (2007). The evaluation began with a warm-up at a speed of 2.0 m/s, after which the speed increased incrementally by 0.3 m/s every two minutes until the rats reached exhaustion and could no longer continue the test (23).

2.3 Preparation and Consumption of Nano-Selenium Particles

The preparation and administration of nano-selenium particles were conducted following established protocols from previous studies (24). A 2.5 mM solution of selenium dioxide was prepared and mixed with a 2.5 mM solution of ascorbic acid. The resulting mixture was centrifuged and washed using filter paper. The prepared stock solution of nano-selenium particles was administered via gavage at a dose of 100 mg, equivalent to 250 nanograms, every other day (25).

2.4 Induction of Lung Injury

To induce lung injury, cigarette smoke extract was used following established protocols (26). Briefly, three cigarettes were burned, and their extract was collected and mixed with 3 ml of phosphate-buffered saline using a syringe. This solution was freshly prepared for each induction session and filtered through a 0.22 µm HUNDAY MICRO filter to remove bacteria and impurities. The cigarette smoke extract was then stored at -80°C. This extract was considered to have a 100% purity level and was diluted to specific concentrations for use within 10 minutes of dilution. The healthy control group received 150 µl of normal saline intraperitoneally, while the diseased groups received the cigarette smoke extract intraperitoneally on days 7, 14, 21, 28, 35, and 42 (26).

2.5 Animal dissection

To avoid the acute effects of exercise, sample collection was conducted 48 hours after the last exercise session and following an 8-hour overnight fast. After completing the exercise period, the rats were deeply anesthetized with ketamine (50 mg/kg) and xylazine (5 mg/kg). Perfusion was performed to remove blood from the brain, after which the animals were euthanized. Five milliliters of blood were collected in tubes containing clotting gel, centrifuged at 2400 rpm for 15 minutes at room temperature, and stored at -20°C for subsequent analysis. Lung tissue samples were collected, washed with a 9% sodium chloride solution, and immersed in RNA later solution (Behnozhen Iran) before being transferred to tubes containing phosphate-buffered saline (PBS). The samples were then immediately homogenized in PBS (1:10 volume ratio) and transferred to

a liquid nitrogen tank. The tissue samples were stored at -80°C for further analysis.

2.6 RNA Isolation

Total RNA was isolated from the lung tissue with careful attention to prevent contamination. The RNA extraction area was designated and thoroughly cleaned with 70% ethanol. Sterile latex gloves were worn throughout the procedure, and RNase-free pipette tips and microtubes were used to eliminate RNase contamination. Equipment was washed with DEPC-treated water to further prevent contamination. Once these conditions were ensured, total RNA was extracted according to the specified protocol. The RNA extraction process involved several sequential steps. A total RNA extraction kit was prepared following the manufacturer's instructions. First, 20 mg of lung tissue was placed in a 2 ml microtube, and 750 µl of lysis solution (RL) was added. The mixture was homogenized and stored at 4°C for 5 hours. After this incubation, the contents were shaken for 30 seconds, three times, using a shaker. Next, 150 µl of chloroform was added, and the mixture was shaken for 15 seconds, then incubated at room temperature for 3 minutes. The microtubes were centrifuged at 13,000 rpm for 12 minutes at 4°C. In the subsequent step, 400 µl of the supernatant was transferred to a new RNase-free microtube, and 400 µl of 70% ethanol was added and mixed. This mixture was transferred to a column and centrifuged. The column was then placed on a collection microtube, and 700 µl of PW solution from the kit was added and centrifuged. To further purify the RNA, this step was repeated with 500 µl of PW solution. The microtube was then replaced, and a new RNase-free microtube was placed under the column. Fifty microliters of DEPC water were added to the column, and after a 3-minute incubation at room temperature, it was centrifuged again. Finally, the RNA was stored at -70°C for long-term preservation.

2.7 cDNA Synthesis from Total RNA

After confirming the extraction of total RNA with an appropriate concentration, cDNA synthesis was performed using a specialized kit. This step is crucial for preserving and amplifying the gene of interest. To ensure high-quality results, specific laboratory conditions were maintained, including reducing aerosol levels, using sterile nuclease-free microtubes and pipette tips, and employing a thermocycler with precise temperature control. The cDNA synthesis kit used in this experiment was manufactured by Pars Tous (Cat: A101161) and provided three methods for cDNA synthesis: Anchored-oligo(dT) primer, Sequence-Specific primer, and Random hexamer. The Anchored-oligo(dT) primer method was chosen for its simplicity and better sample yield. In this method, specific primers targeting poly(A) tails, present in all RNA molecules, were used. The reverse transcriptase enzyme, along with these primers, synthesized cDNA from RNA. Before starting the cDNA synthesis, preparatory steps included setting up an ice bath, thawing the kit solution, and placing all solutions, materials, and microtubes on the ice bath. A 20 microliter reaction was then prepared and executed in a thin-walled nuclease-free tube according to the specified protocol.

2.8 Annealing Step

During the annealing step, the sample was placed in a thermocycler at 65°C for 10 minutes. The thermocycler was equipped with a heated lid to minimize evaporation. After 10 minutes, the sample was immediately transferred to ice to maintain the denatured structure of the RNA. Subsequently, the 13-microliter microtube from the previous step was brought to a final volume of 20 microliters by adding the necessary reagents as specified. This step, which includes annealing and the addition of reagents to reach the final volume, is essential for preparing the sample for the subsequent steps of cDNA synthesis. After preparing the reaction mixture, the solutions were carefully mixed and spun several times to ensure thorough mixing and the removal of any potential bubbles. Depending on the type of primer used and the length of the target mRNA, the sample was placed in a thermocycler set at 55°C for 30 minutes. This step is critical for annealing the primers and initiating the activity of the reverse transcriptase enzyme. Following the annealing and extension phase, the reverse transcriptase enzyme was inactivated by placing the microtube at 85°C for 5 minutes. This temperature increase halts enzyme activity and concludes the synthesis process. Immediately after this step, the sample was transferred to ice to stabilize the reaction. Finally, the synthesized cDNA was stored at -20°C. This storage temperature is optimal for maintaining the stability and quality of the synthesized cDNA, ensuring its suitability for use in subsequent research stages.

2.9 Primer Design and Real-Time PCR for Gene Expression Analysis

Primer design and real-time PCR for gene expression analysis is a precise and sensitive process that was

meticulously conducted in this study. Initially, the OLIGO ANALYZER software was used to design suitable primers, allowing for the creation of specific and optimized sequences. Once designed, the desired sequences were synthesized by SinaClon and temperature-adjusted for optimal performance. For the real-time PCR reaction, a precise mixture of components was prepared: 1 microliter of synthesized cDNA, 10 microliters of CyberGreen master mix (prepared by Pars Tous), and an appropriate amount of the forward and reverse primer mixture. These materials were combined in a 20-microliter transparent microtube specifically designed for real-time PCR, with the final volume adjusted using DEPC water. The thermal cycling protocol was carefully calibrated, beginning with an initial cycle at 95°C for 10 minutes for primary denaturation. This was followed by 40 cycles, each consisting of three steps: 95°C for 30 seconds (denaturation), 60°C for 30 seconds (primer annealing), and 72°C for 45 seconds (extension). The reaction was conducted on a Corbett Rotor-Gene Q-6000 Real-Time PCR Analyzer, which allows for accurate real-time measurement of gene amplification. The expression of the target gene was then compared to both a control gene and a reference gene.

2.10 Data Analysis

In the descriptive statistics section, standard deviation, mean, and graphs were used as measures of dispersion. For inferential statistics, the Shapiro-Wilk test was employed to assess the normality of data distribution. A two-way analysis of variance (ANOVA) and Tukey's post-hoc test were used for further analysis. All statistical calculations were performed using SPSS version 26, with a significance level set at $p \leq 0.05$. The raw data was first transformed into an analyzable form using the 2^-ΔΔCt formula, followed by statistical analysis.

3. Results

Table 1 shows the mean and standard deviation of the seven groups.

Table 1. Descriptive Statistics (Mean±SD)

Group	Lamα5	Collagen IV
Healthy control	1.71±0.45	2.41±1.15
COPD control	5.41±3.84	0.64±0.45
IAT COPD	1.32±0.60	6.41±3.40
IAT Healthy	1.58±0.23	8.96±3.94
Healthy Selenium	1.63±0.46	6.00±3.56
Healthy Selenium- IAT	1.53±0.29	8.91±4.96
COPD Selenium- IAT	3.34±0.84	1.26±0.99

Table 2. Shows Two-way analysis of variance test results for laminin α5 and collagen IV. The results of the two-way ANOVA for laminin α5 and collagen IV indicated significant differences between the groups for both variables. The analysis revealed: Laminin α5: Tukey's post-hoc test showed significant differences between the healthy control and diseased control groups (P=0.011), and between the diseased control and exercise groups (P=0.004). These findings suggest that both the presence of disease and the introduction of exercise significantly affect laminin α5 expression levels. Collagen IV: Tukey's post-hoc test indicated significant differences between the healthy control and healthy exercise groups (P=0.036), and between the healthy control and healthy exercise-supplemented groups (P=0.038). This suggests that exercise and supplementation have a notable impact on collagen IV expression. Figure 1 and Figure 2 illustrate these pairwise comparisons, providing a visual representation of the differences in laminin α5 and collagen IV expression across the various groups. The statistical significance of these results underscores the effect of both disease and intervention on the expression of these proteins.

Table 2. Two-way analysis of variance test results for laminin α5 and collagen IV

Variable		Sum of Square	df	Mean Square	F	Sig
LAMα5	Intercept	195.69	1	195.69	83.20	0.000
	Groups	67.83	6	11.30	4.80	0.002
	Error	65.85	28	2.35	-	-
	Total	329.38	35	-	-	-
Collagen	Groups	855.90	1	855.90	87.96	0.000
	Error	367/81	6	61.30	6.30	0.000
	Total	272.43	28	9.73	-	-
	Groups	1496.15	35	-	-	-

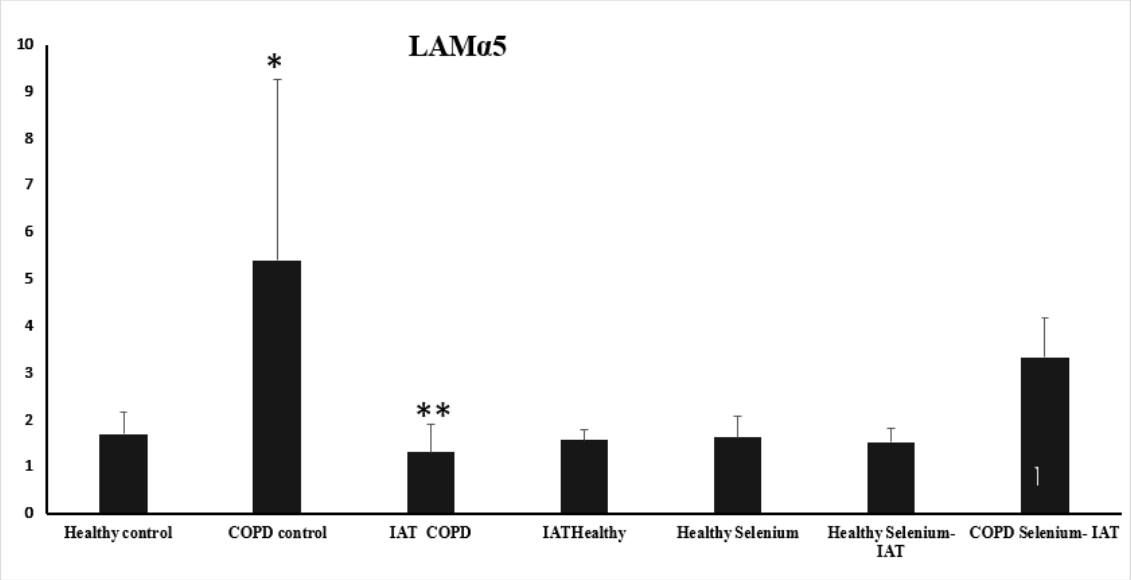

Figure 1. Pairwise Comparisons of Groups Using Tukey's Post Hoc Test for Laminin α5

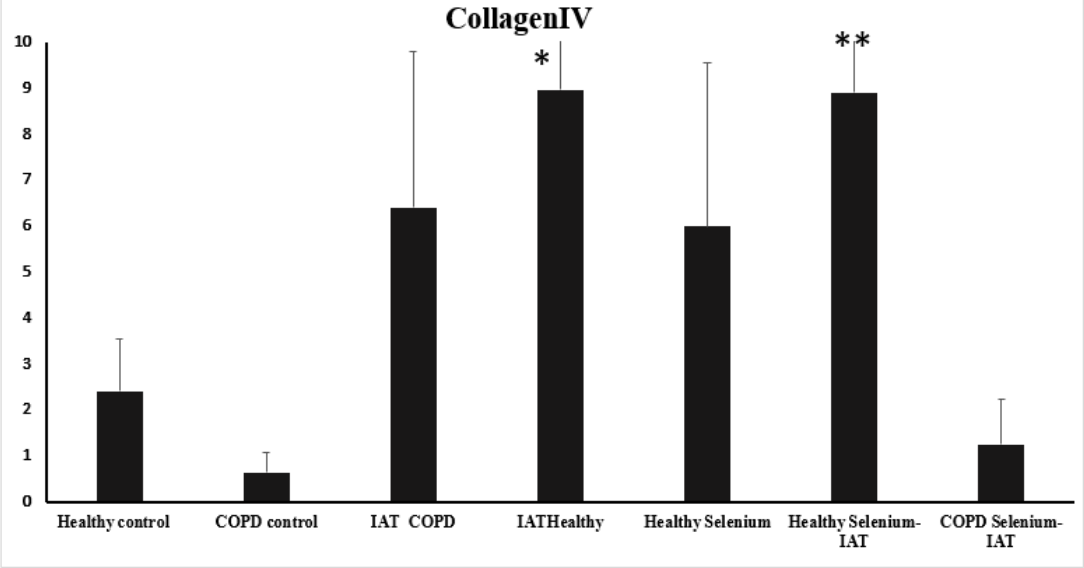

Figure 2. Pairwise Comparisons of Groups Using Tukey's Post Hoc Test for Collagen IV

*: Indicates a significant difference between the healthy control group and the healthy IAT group

**: Indicates a significant difference between the healthy control group and the Healthy Selenium- IAT group.

4. Discussion

The aim of this study was to investigate the effects of six weeks of Interval aerobic training (IAT) and nanoselenium supplementation on the expression of laminin α5 and collagen IV in the extracellular matrix of alveolar epithelial cells in the lungs of rats exposed to cigarette smoke. The results demonstrated that IAT significantly affected the expression of laminin α5 in the exercise-diseased group compared to the control-diseased group. However, the combination of IAT and nanoselenium supplementation did not produce a significant effect in the exercise-supplemented-diseased group compared to the control-diseased group. Additionally, this research showed that exposure to cigarette smoke increased the expression of laminin α5. Similarly, a study on the effects of cigarette smoke on alveolar epithelial cells found that laminin 5 was present in the extracellular matrix of these cells in mice exposed to cigarette smoke. This research supports the idea that laminin 5 in the extracellular matrix of alveolar epithelial cells in the lung increases in response to cigarette smoke exposure, which is consistent with the results of our study (27). Regarding the effectiveness of IAT in reducing laminin α5, previous research findings align with the results of our study. A study on high-intensity interval training showed that it significantly reduced the expression of laminin α5 in skeletal muscle after six weeks of training (28). Another study examining endurance exercise reported a decrease in laminin α5 mRNA levels in the skeletal muscle of trained individuals compared to untrained controls (29). However, other studies have yielded contrasting results. One study showed that 9 weeks of high-intensity interval training leads to lung inflammation and structural disorders. These effects are possibly associated with a reduction of maximum gas exchange in the lung blood-gas barrier during exercise performance and challenge overall health in the lung (29). Furthermore, a study on laminin expression in the central nervous system revealed that laminin α5 increased in response to ischemic injury, suggesting a role in wound healing and angiogenesis (28). A literature review also provided evidence of no significant effect of aerobic exercise (29), which contradicted the findings of our study. Overall, our study revealed that exposure to cigarette smoke significantly altered laminin α5 expression in the extracellular matrix of alveolar epithelial cells in the lung. This alteration is part of a broader mechanism involving the activation of various signaling pathways and the expression of profibrotic genes. The primary mechanism by which cigarette smoke upregulates laminin α5 expression involves the activation of the transforming growth factor beta (TGF-beta) signaling pathway. This increase in TGF-beta is facilitated by the activation of the integrin alpha and beta 6 (ITGαVβ6) complex, an epithelial-restricted integrin that interacts with latent TGF-beta complexes. This interaction activates TGF-beta, leading to an increase in the secretion of the active form of this growth factor. In the context of pulmonary fibrosis, laminin α5 plays a crucial role in the formation and maintenance of the extracellular matrix. Exposure to cigarette smoke results in increased laminin α5 expression, which enhances the formation and stability of the basement membrane (30-32).

Furthermore, the effectiveness of aerobic exercise and physical activity in reducing laminin α5 expression in the extracellular matrix involves multiple mechanisms. One mechanism is the inhibition of the TGF-β signaling pathway. This pathway transforms healthy lung cells into scar-forming cells, and exercise helps to halt this process. Additionally, exercise regulates the protein integrin alpha and beta 6, which is involved in activating TGF-β. By reducing this protein, exercise indirectly decreases TGF-β activity and laminin α5 production. Another pathway affected by exercise is the Wnt signaling pathway. Cigarette smoke activates this pathway, leading to increased laminin α5, but exercise can counteract this activation and ultimately reduce laminin α5 levels (33). Regarding the lack of effectiveness and contradictory results, factors such as the type of exercise, protocol, and intensity of physical activity can be considered. The type of tissue studied can also contribute to the observed discrepancies. As mentioned, the combination of Interval aerobic training and nanoselenium supplementation did not have a significant effect (34). To date, and based on the investigations conducted by the researchers of this study, there has been no comprehensive synthesis of evidence for the use of selenium in lung cancer. In the present study, changes in LMα5 in the selenium-receiving groups compared to the control group were increased in groups that received cigarette smoke. The chemopreventive effects of selenium have been demonstrated in several animal models of lung cancer using NNK or B(a)P as carcinogens. These compounds are only some of the carcinogens found in cigarette smoke, and they do not represent the full spectrum; the combined effect of hundreds of carcinogens in cigarette smoke is likely different from the effect of individual isolated carcinogens. In fact, in three trials using cigarette smoke as a carcinogen, selenium

failed to show significant protective effects (30, 35). One study directly compared the effect of selenium on lung cancer induced by cigarette smoke and found effectiveness in an animal model but not in a cigarette model (36). Furthermore, the results of this study demonstrated that Interval aerobic training significantly impacted collagen IV expression in the extracellular matrix of alveolar epithelial cells in the lungs of healthy rats exposed to cigarette smoke. However, no significant difference was observed between the diseased exercise group exposed to cigarette smoke and the diseased control group. Additionally, the results showed that exposure to cigarette smoke had no significant effect on collagen IV expression. Numerous studies have provided evidence for increased collagen IV degradation in idiopathic pulmonary fibrosis (IPF), chronic obstructive pulmonary disease (COPD), and bronchopulmonary dysplasia (BPD) (37, 38), which is inconsistent with the results of the present study. Generally, it seems that there is no study indicating a lack of effect of cigarette smoke on collagen IV. Regarding Interval aerobic training, the results showed a significant difference only between the healthy exercise group and the control group, while no difference was observed between the diseased exercise group and the diseased control group. Cigarette smoke can damage collagen IV and lead to respiratory problems. Since exposure to cigarette smoke in the diseased control group compared to the healthy control group led to a decrease in collagen IV levels, although this change was not significant, it is consistent with the results of our study in diseased rats. Another study found no significant effect of aerobic exercise on collagen IV expression in the alveolar ECM of mice exposed to cigarette smoke, which is consistent with our results (9). However, a study showed that aerobic exercise prevents the decrease in collagen IV expression caused by exposure to cigarette smoke. This suggests that exercise may help protect the lung extracellular matrix (ECM) from the harmful effects of smoke (39), which is inconsistent with our results. Aerobic exercise is known to stimulate collagen synthesis through various pathways. It increases the activity of mechanosensitive factors such as TGF-β (transforming growth factor beta) (39). TGF-β regulates genes responsible for collagen production. This may explain the increased collagen IV observed in healthy mice. Cigarette smoke contains harmful chemicals that can degrade collagen (40). It may decrease the same pathways (TGF-β) that aerobic exercise activates for collagen synthesis. This could explain why mice exposed to smoke do not show a change in collagen IV. Additionally, aerobic exercise has well-documented antioxidant effects. Cigarette smoke produces free radicals that damage tissues, including the extracellular matrix where collagen is located (9). By increasing the antioxidant response, exercise may protect the extracellular matrix and collagen IV from smoke-induced damage. On the other hand, the results of the study showed no significant difference between the diseased exercise-supplemented group and the diseased control group, while there was a significant difference between the healthy exercise-supplemented group and the healthy control group. Amorphous nanoselenium quantum dots (A-SeQDs) can distribute in the lungs and positively regulate the BH4 salvage pathway, which is involved in the coupling of endothelial nitric oxide synthase and the potential modulation of extracellular matrix components such as collagen. While this study did not specifically measure collagen IV levels, it suggests that A-SeQDs may have a protective effect on the pulmonary extracellular matrix by improving endothelial function and reducing oxidative stress (41). In any case, it appears that nanoselenium supplementation, with its increased bioavailability, can provide better antioxidant protection compared to conventional selenium (42). This may counteract smoke-induced oxidative damage to the ECM and potentially affect collagen IV expression. While selenium may be involved in collagen synthesis through selenoproteins, the exact mechanism is unclear, and further research is needed to understand how nanoselenium specifically affects collagen IV synthesis in alveolar epithelial cells (42).

One important limitation of this study is that, while comprehensive, it only assesses the efficacy and risk of using selenium as an individual agent rather than as part of a combined therapeutic strategy for preventing conditions related to cigarette smoke exposure. More extensive and long-term research on the effects of cigarette smoke on the lung and the use of selenium and other exercise treatments is necessary to fully evaluate the role of selenium and exercise in lung prevention and improvement.

5. Conclusion

The findings of this study indicate that six weeks of Interval aerobic training (IAT) significantly impacted the expression of laminin α5 (LMα5) in the extracellular matrix of alveolar epithelial cells in the lungs of rats exposed to cigarette smoke. However, there was no significant effect observed on collagen IV expression. Additionally, the combination of IAT and nanoselenium supplementation

over six weeks did not significantly affect the expression of either LMα5 or collagen IV. These results suggest that IAT alone may be beneficial for improving lung health in rats exposed to cigarette smoke.

Authors' Contributions

M. G. and S. M. collaborated on this experimental laboratory study investigating the effects of interval aerobic training (IAT) and nano-selenium supplementation on laminin α5 and collagen IV expression in the extracellular matrix of alveolar epithelial cells in the lungs of rats exposed to cigarette smoke. M. G. contributed to the conceptual framework, research design, and data collection, including overseeing the animal model and intervention protocols. S. M. was responsible for conducting the biochemical analysis, assessing protein expression levels, and interpreting the data related to laminin α5 and collagen IV. Both authors were involved in the statistical analysis, the interpretation of the results, and the writing of the manuscript.

Declaration

In order to correct and improve the academic writing of our paper, we have used the language model ChatGPT.

Transparency Statement

Data are available for research purposes upon reasonable request to the corresponding author.

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Declaration of Interest

The authors report no conflict of interest.

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Ethics Considerations

The study protocol adhered to the principles outlined in the Helsinki Declaration, which provides guidelines for ethical research involving human participants.

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