Research Article

Translational research from bioinformatics to animal studies: Exploring gene expression and muscle health in COPD through selenium nanoparticles and exercise

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Abstract

Recent studies highlight the role of molecular pathways, such as oxidative stress response and mitochondrial function, in COPD. This study explores the role of the PGC-1α gene, a key regulator of mitochondrial biogenesis and energy metabolism, using a rat model and bioinformatics analysis of human lung tissue samples. This study utilized a combined approach, analyzing gene expression in rat lung tissue alongside bioinformatics analysis of public human datasets. A total of 42 male Wistar rats were divided into seven groups, receiving treatments including cigarette smoke extract (CSE), nano-selenium (SeNPs), and aerobic interval training (AIT). PGC-1α expression levels were evaluated using quantitative Real-Time PCR (qRT-PCR) and analyzed using one-way ANOVA, followed by Dunnett's post hoc test for multiple comparisons to determine significance across groups. The CSE+SeNPs+AIT group exhibited significantly higher PGC-1α expression compared to controls ($p = 0.0289$), indicating a potential protective role of SeNPs and exercise against oxidative stress. Bioinformatics analysis identified 250 differentially expressed genes (DEGs), with PGC-1α emerging as a critical hub gene associated with pathways like oxidative stress response and mitochondrial regulation. Protein-protein interaction (PPI) analysis further highlighted the centrality of PGC-1α in COPD pathophysiology. This study underscores the importance of PGC-1α in regulating mitochondrial function and oxidative stress in COPD. PGC-1α could serve as a potential therapeutic target, offering insights into the development of interventions aimed at improving respiratory health in COPD patients.

Key Words: **PGC-1α, COPD, Gene expression, Oxidative stress, Bioinformatics**

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Received 24 March 2024; Accepted 12 June 2024

Introduction

Chronic Obstructive Pulmonary Disease (COPD) is a leading cause of mortality worldwide, accounting for over 3 million deaths annually, or approximately 5.6% of total global deaths (Agusti et al., 2020). This progressive disease is characterized by irreversible airflow obstruction, primarily due to chronic lung inflammation and structural damage to alveolar tissue (Cavailles et al., 2013). The most common risk factors for COPD include long-term exposure to tobacco smoke, environmental pollutants, and genetic predispositions (Sin ., 2006). COPD manifests with debilitating symptoms such as persistent cough, sputum production, and shortness of breath, which significantly impair the quality of life for affected individuals (Barnes et al., 2009).

Traditionally, COPD is diagnosed only after significant lung function has been lost, often leaving limited options for effective intervention (Pauwels et al., 2004). Biomarkers like C-reactive protein (CRP), interleukin-6 (IL-6), and tumor necrosis factor-alpha (TNF-α) are commonly used to gauge the inflammatory response in COPD. However, these biomarkers show limited sensitivity and specificity in accurately diagnosing and predicting disease progression (Agusti et al.,2010). This limitation underscores the need for research focused on identifying new biomarkers and molecular targets, which may improve both diagnostic accuracy and treatment efficacy (Wouters ., 2002).

Recent advancements in bioinformatics and molecular biology have enabled comprehensive analyses of gene expression profiles in COPD. High-throughput methods like microarray analysis allow researchers to identify differentially expressed genes (DEGs) between healthy individuals and COPD patients, offering insights into key molecular changes driving COPD pathophysiology (Barbera & Blanco 2016, Viegi G et al., 2001). In this study, we identified and analyzed DEGs using public datasets, alongside network analyses of gene-gene and protein-protein interactions to highlight hub genes involved in COPD. Hub genes are those with high connectivity within

biological networks, often acting as central regulators in disease-relevant pathways (Castaldi PJ et al.,2010, Wan et al., 2009). One such hub gene identified in our study is PGC-1α (Peroxisome proliferator-activated receptor gamma coactivator 1-alpha), which plays a pivotal role in mitochondrial biogenesis and energy metabolism (Dahl M et al., 2009).

Aerobic interval training (AIT). Selenium is a crucial micronutrient known for its potent Beyond genetic insights, this study explores an innovative therapeutic approach that combines selenium nanoparticle (SeNP) supplementation with aerobic interval antioxidant properties, largely due to its incorporation into selenoproteins that counteract oxidative damage. COPD is associated with heightened oxidative stress and mitochondrial dysfunction, making antioxidant interventions particularly relevant (Rehman et al,. 2021). Nanotechnology has enabled the development of selenium nanoparticles, which enhance selenium's bioavailability and antioxidant efficacy in biological systems (Pouri et al,. 2017).

On the other hand, AIT is a well-recognized method for promoting mitochondrial health and enhancing the body's antioxidant defenses. Exercise has been shown to upregulate genes involved in mitochondrial biogenesis and oxidative phosphorylation, thereby increasing the capacity to combat oxidative stress. Exercise-induced oxidative stress, though seemingly paradoxical, may trigger adaptive responses that bolster endogenous antioxidant pathways (Sorriento et al,. 2021). When combined with SeNPs, AIT may further amplify these effects by enhancing antioxidant activity and reducing oxidative damage in lung and muscle tissues. Preliminary evidence suggests that such combined approaches could exert additive or even synergistic benefits, particularly in chronic inflammatory conditions like COPD (Barchielli et al,. 2022)

This study hypothesizes that AIT could potentiate the bioactivity of SeNPs, resulting in enhanced protective effects against COPD-related oxidative stress and mitochondrial dysfunction. Through a combination of bioinformatics analysis and in vivo experiments in a rat model, this study aims to uncover how SeNPs and AIT might act together to mitigate key pathological features of COPD, potentially providing a novel therapeutic approach for patients.

Materials and Methods

Study design overview

Chronic Obstructive Pulmonary Disease (COPD) is a major global cause of mortality, characterized by chronic obstruction of airflow in the lungs. This study utilized next-generation sequencing data available for free to investigate the molecular mechanisms related to COPD. The GEO database with accession code GSE148004 was used to obtain the gene expression profile. Experiments were conducted using selected data on the Affymetrix microarray platform. To prevent false-positive results, criteria such as |logFC| < 1 and P > 0.01 were applied, and the adjusted p-value was considered.

Analysis

Results were presented in tables of genes sorted by significance. GO pathway analysis panels were used with the help of the Genemania and NetworkAnalyst databases to identify DEGs (differentially expressed genes). A threshold of P > 0.05 was used to indicate significance. Additionally, a protein-protein interaction (PPI) network was constructed using the Genemania search tool from the identified DEGs. A confidence score of ≥0.4 was set as the threshold for reliability in this process.

Identification of differentially expressed genes (DEGs)

Analysis of the GSE148004 dataset, which included nine normal and seven COPD samples, revealed 250 differentially expressed genes (DEGs) with significant expression changes (logFC > ±1, p < 0.01). Among these, 187 genes were downregulated, and 47 were upregulated in COPD patients. Figure 1 provides an overview of these expression changes, with upregulated and downregulated genes highlighted. This figure helps visualize the overall gene expression pattern differences between COPD and healthy samples, illustrating the broad impact of COPD on gene expression.

Gene-gene interaction analysis

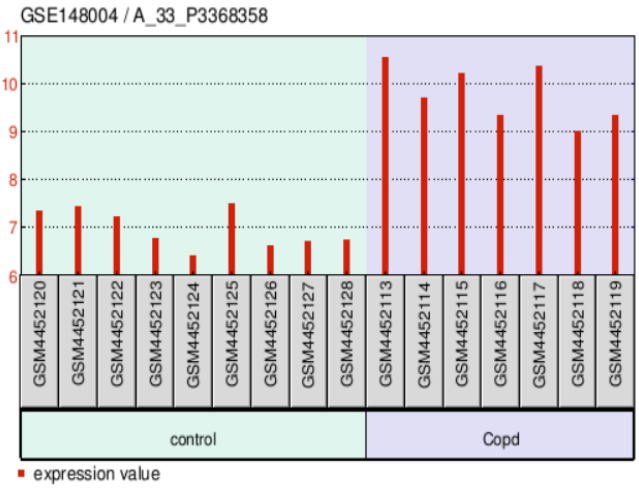

Figure 1 (Volcano Plot). The volcano plot in Figure 1 displays the upregulated and downregulated genes with their respective expression levels and statistical significance. This plot helps pinpoint the most differentially expressed genes, providing a foundation for further analysis of COPD pathogenesis.

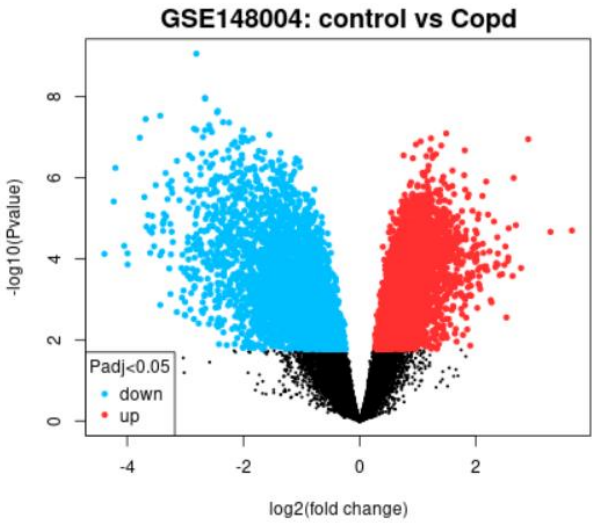

Figure 2 (Gene Interaction Network). Figure 2 visualizes the interactions among DEGs, where hub genes, particularly PGC-1α, are emphasized by their central network positions. This figure illustrates the regulatory influence these genes may exert over COPD-related biological pathways.

To further explore the functional significance of the identified DEGs, we conducted a gene-gene interaction analysis using the Genemania platform. This analysis highlighted key interactions and connections among DEGs, emphasizing the interconnectedness of certain genes within COPD-related pathways. Figure 2 illustrates these interactions, with hub genes positioned at central nodes. The network provides a visual repre-sentation of the regulatory roles these genes may play within the COPD disease framework, highlighting potential targets for therapeutic interventions.

Protein-protein interaction (PPI) network and pathway enrichment analysis

Utilizing the NetworkAnalyst tool, we analyzed the DEGs to construct a protein-protein interaction (PPI) network and identify hub genes with significant interaction scores. PGC-1α emerged as a central hub within the network, linked to key pathways such as oxidative stress response and mitochondrial function. Table 1 presents the identified hub genes and their interaction scores. The PPI network underscores PGC-1α's centrality in COPD pathophysiology, suggesting it as a potential therapeutic target.

Gene-gene interaction analysis

With the help of the Genemania software, many gene interactions can be examined. Therefore, we used this software to analyze the interactions between the selected genes.

a- Analysis of gene-gene interactions for downregulated genes (Fig. 3).

b- Examination of gene-gene interaction in genes with increased expression (Fig. 4)

The aim of examining the above genes in the Genemania database is to identify the existing interactions between the genes. Additionally, considering that in these interactions, the co-

Table 1. List of genes with expression changes greater than or less than 3.

No.	Genes	Expression	Number of genes
1	PNPLA3, TEX14, TLR7, BACE1, HDDC2, SPRY1, EPHX1, ANO5, TMEM229B, CDCA7L, LGALS9, TRAPPC6A, SMAD1, RDH12, PRDX4, LSM6, DNAJC5B, LYPLAL1, PCCA, SIN3B, ZNF616, GSTP1, LSM2, LRRC20, GSTZ1, ATP5MC1, ASB2, SRSF7, TMED3, AKR7A3, DHAK2, PPIA, NUDCD3, EFCAB11, STAU2, ACOT8, AKR7A2, TULP4, NDUFB5, HMOX1, NTHL1, ARSD, AKR7A2P1, ZNF814, AKR7L, IL7, DENND2D	Up regulate	**47**
2	PHC1, HIST1H3H, USP6, RFX2, GRK6, WAC, AFTPH, TOMM40L, NR2C2, STK4, ANKRD33, ASAP1, TFBIGEM, BRD4, CHD2, FAM27A, PRAF2, CALCRL, NOTCH2, UHRF1BP1L, SLC6A6, ITGA5, SAT1, ATXN1, PPP4R1, DOT1L, MTMR3, GRB10, STK4, ARHGAP26, AKIRIN1, MGAT1, SLC38A1, AFF4, CABP22P, ZMIZ1, MAP3K2, WAC, C16orf7, MECP2, SEMA6B, HIP1, RNF145, USP6, PPP4R2, FYN, NECAP1, FAM100B, PDLIM7, PADI2, RUNX3, CSGALNACT2, CDC-like1, TLN1, PDLIM7, KIAA1683, MND5, MAN2A2, TBC1D3G, PHF20L1, KCNIP2, ERN1, CSGALNACT2, VSIG8, ATP2B1, ITPRIP, VDR, ZNF493, SPAG9, SLC2A14, MLL5, DNAJB5, GGTLC2, TSC22D3, TANK, FAM27A, PNRC1, TRIB1, SEMA6B, BCL6, LOC401149, LINC0152, C5orf32, ATP13A3, REC8, CHRNA10, CHD3, SKIL, PDLIM7, TMEM88, GGTLC1, LOC401149, PTPN7, PHACTR1, RLF, LOC729737, IRAK3, LINC0265, MYBPC3, ANTXR2, LOC401357, MND5, EIF2C2, PLK3, GK, JMJD1C, LINC0085, LINC0426, SLC16A10, NFE4, PKN2, Sipa1l1, C12orf35, DUSP13, LOC100506459, CXORF65, LOC100130357, CST7, JARID2, LOC401149, NEDD9, LOC100508384, CD55, LOC399844, DKFZp434F142, LOC286058, PLEKHG2, VCAN, IL1R1, C20orf106, TECPR2, PLIN4, SMCFH1, MAFF, PRDM8, ATG2A, DUSP4, FBXL13, IL18R1, ZNF165, GK, TIAM2, LOC399844, PHACTR1, ZEB1, FAM101B, ZNF267, LOC100129322, NEDD9, ITGAX, IL18R1, ECE1, DOCK5, LOC284751, IL1RAP, EPHB1, TPST1, ANTXR2, IL6R, EDN1, IBA57, BMP6, LRRC70, LIPN, PRR7, IL1R1, IL18R1, TNFSF14, PGC-1α	Down regulate	**187**

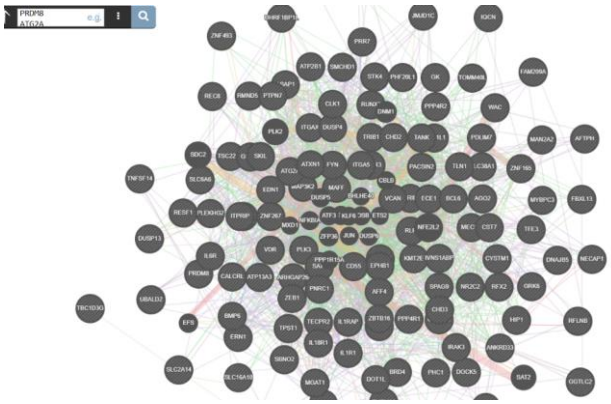

Figure 3. Examination of the relationship between downregulated genes and the Genemania database.

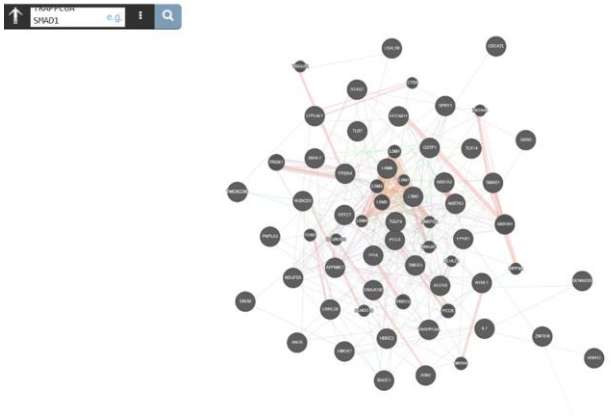

Figure 4. Examination of the relationship between the genes that have increased expression and the Genemania database.

Examination of protein-protein interaction

a. Examination of Protein-Protein Interactions of Genes with Decreased Expression Using the NetworkAnalyst Database:

The analyses conducted in the NetworkAnalyst database revealed that among the 187 genes with decreased expression, 7 genes—AFF4, EDN1, GK, SAT1, ECE1, ATXN1, and PGC-1α—have the highest scores.

b. Analysis of Protein-Protein Interaction of Genes with Increased Expression Using the Database: Networkanalyst

It was determined that among the 47 genes with increased expression, two genes, PCCA and NTHL1, have the highest scores.

Animals and experimental methodology

A total of forty-two male Wistar rats, aged eight weeks and weighing approximately 180–220 g, were utilized in the experiments in accordance with the NIH Guidelines for animal research. These rats were produced in the Pasteur Institute of Iran animal laboratory and kept in a standard laboratory environment (ethical code: IR.BMSU.REC.1400.117). The tested animals were housed in cages. The ambient temperature was 22±4.1°C, the light-dark cycle was 12:12 hours, and the humidity was 55.6±4%. All animals had free access to water and exceptional mouse food. Rats' weights were recorded on a monthly basis using a miniature electronic scale (HL-DDC, Beijing Heli Science and Technology Company, Beijing, China)

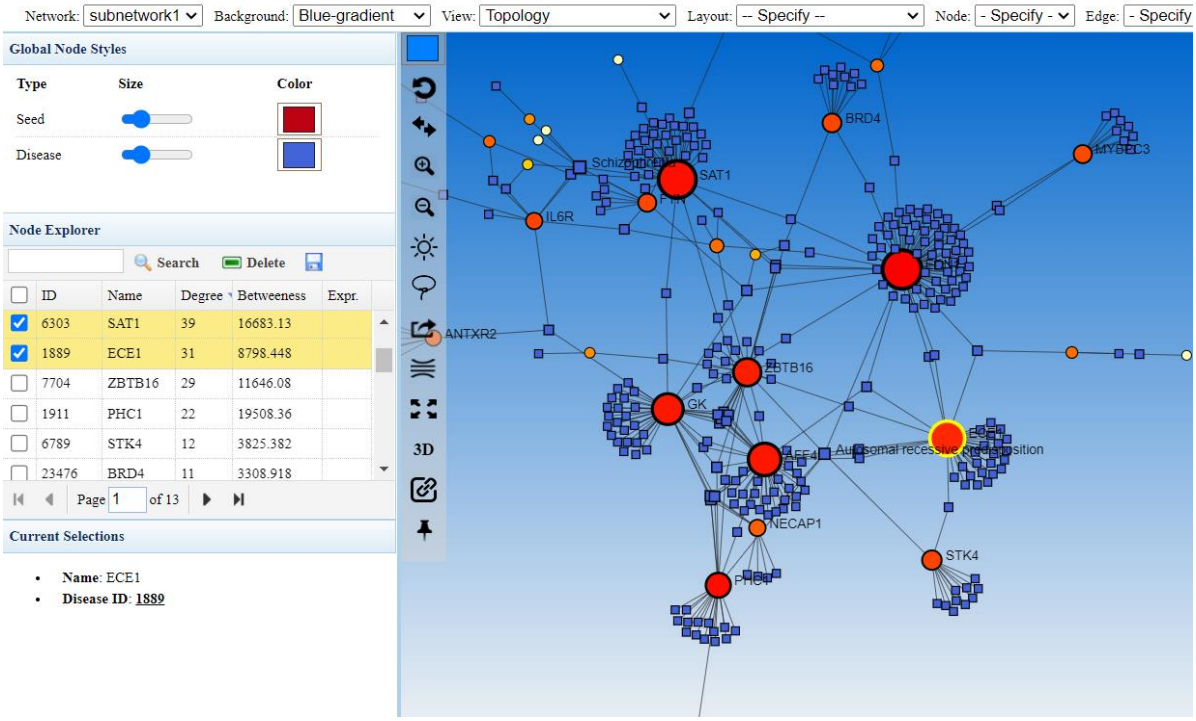

Figure 5. Assessment of the level of interaction between candidate proteins and valuation using the Networkanalyst database for genes that have decreased expression.

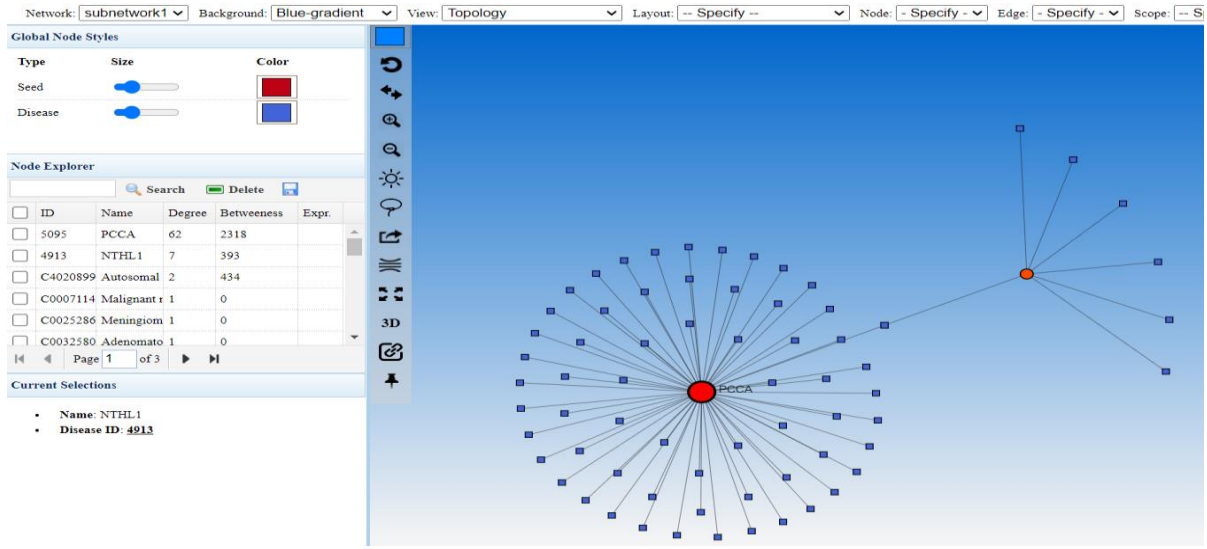

Figure 6. Assessment of the level of interaction between candidate proteins and valuation using the Networkanalyst database for genes that have increased expression.

designed for use with small animals. The inclusion criteria for the current study included the complete health of the mice and the absence of any drug use. Injury sustained during exercise and failure to adhere to the exercise protocol constituted exclusion criteria. There were seven groups comprised of 42 male Wistar rats that were randomly assigned:

1. Control group: administered 150 µL of normal saline (the vehicle) intraperitoneally.

2. CSE group: administered 150 µL of CSE intravenously once weekly for a duration of six weeks.

3. CSE+AIT: administered CSE and carried out AIT.

4. AIT group: The treadmill aerobic interval training for six weeks consisted of seven sets of four minutes at 80-90% VO2max followed by three minutes at 65-75% VO2max.

5. SeNPs group: administered 2.5 mg/kg b.w. via gastric gavage, three times per week, for a duration of six weeks.

6. SeNP+AIT group: administered SeNP and carried out AIT.

7. CSE+SeNPs+AIT group: administered CSE, SeNPs, and carried out AIT.

Preparation and administration of cigarette smoke extract (CSE)

Cigarette smoke extract (CSE) was prepared following established protocols to ensure consistency and reliability in inducing COPD-like symptoms in animal models. Briefly, three Winston cigarettes (12 mg tar and 0.9 mg nicotine each) were smoked using a vacuum pump, drawing smoke into a container filled with 10 mL of phosphate-buffered saline (PBS). The solution was filtered through a 0.22 µm filter to remove particulate matter, and fresh CSE was prepared before each administration to maintain potency.

CSE exposure protocol

Each rat in the CSE groups received an intraperitoneal injection of 150 µL of CSE-PBS solution once weekly for a period of six weeks. This frequency and dosage were chosen to approximate the chronic, low-dose exposure seen in humans who develop COPD due to long-term smoking. Weekly exposure is intended to provide a balance between physiological relevance and the need to induce detectable changes within the study timeframe. This protocol has been validated in previous studies to effectively mimic the lung inflammation, oxidative stress, and tissue damage observed in COPD patients, thus serving as a suitable model for studying COPD-related pathology and testing therapeutic interventions.

Nano-selenium supplementation

The nano-selenium utilized in this investigation was produced by ARMINANO (Armina Engineering Co., Tehran, Iran). In order to formulate the concoction, the aqueous extract of ginger that was acquired was initially employed as a precursor for the synthesis of nano-selenium, as specified in the company description. Ginger extract (2 mL) was introduced dropwise into a 20 mL solution containing 10 mM SeO3 while agitating vigorously. For a period of 72 hours under dark conditions, the solution was incubated on a rotatory orbital agitator set at a temperature of 30 °C and 200 rpm. The monitoring of selenium ion reduction involved sampling 3 mL aliquots of the solution at 24-hour intervals, after which the absorption maximum was determined. The absorption maximum was ascertained by employing a UV–Vis spectrophotometer to measure the optical density of the subs-

substance within the wavelength range of 350–700 nm (20). The supplementation group received 2.5 mg/kg b.w. of SeNPs orally three times weekly.

Aerobic interval training

the rats ran on a treadmill at a speed of 8-10 m/min with no slope for 5 minutes in five sessions over a week to become used to the surface and prepare for the primary training routine. After a 10-minute warmup at 50-55% VO_{2max}, the aerobic interval training (AIT) protocol called for seven sets of interval training, with four minutes at 80-90% VO_{2max} and three minutes at 65-75% VO_{2max}, followed by a 5-minute rest.

Selection of the critical gene from the identified eight HUB genes

Among the eight identified HUB genes, PGC-1α (Peroxisome proliferator-activated receptor gamma coactivator 1-alpha) has been selected as the critical gene for further analysis based on several scientific reasons:

1. Key role in metabolism and energy production: PGC-1α is a master regulator of metabolic processes in cells, particularly in energy-demanding tissues like muscle, heart, and lung. By regulating mitochondrial activity and energy-related processes, PGC-1α is likely to play a fundamental role in the metabolic changes observed in Chronic Obstructive Pulmonary Disease (COPD).

2. Association with oxidative stress response: In patients with COPD, increased oxidative stress and tissue damage in the lungs are significant contributors to disease progression. PGC-1α has been shown to regulate antioxidant responses, reducing oxidative stress and protecting lung tissue, making it a potential biomarker for COPD.

3. Impact on mitochondrial function and respiratory efficiency: PGC-1α directly influences mitochondrial function and ATP production. Mitochondrial dysfunction is a hallmark of COPD and is associated with reduced respiratory capacity and lung tissue degeneration, highlighting the importance of this gene in COPD pathology.

4. Modulation of inflammatory processes: Chronic inflammation is a key characteristic of COPD. PGC-1α plays a regulatory role in modulating inflammatory pathways, potentially reducing inflammation and providing protective effects on lung tissue.

Given these factors, PGC-1α stands out as a critical gene in COPD research. Its selection as a primary focus for further investigation could facilitate the discovery of new diagnostic biomarkers and advance our understanding of COPD pathophysiology.

cDNA synthesis and quantitative real time PCR

The total RNA was extracted from gastrocnemius muscle tissue using Qiazol (Qiazol lysis reagent, USA) in a sterile RNase-free tube. Using a Nanodrop ND-100 spectrophotometer (Thermo Scientific, USA), the absorbance at 260 and 280 nm ratio (the A260/280 ratio) was used to evaluate the concentration and purity of the RNA. Following the instructions provided by the manufacturer, a 25 µL amount of RNA was transformed into cDNA using the RevertAid cDNA synthesis kit from Fermentas, Germany. The following components were used in the polymerase chain reaction (PCR) amplification procedure: 2 microliters of the cDNA synthesis reaction, 12.5 microliters of AccuPrime SuperMix I (Germany, Fermentas), 10.1 microliters of distilled water, and 0.2 microliters of each forward and reverse primer (100 micromoles/L). Primers were designed and confirmed using the NCBI BLAST Instrument and Primer3 software.

When evaluating relative gene expression using real-time PCR, 500 ng of the freshly generated cDNA was used. 12.5 µL of SYBR Green Premix 2X from Takara, Japan, and 25 µL of mixed primers (10 p-molar) were used in the PCR reactions. A thermal cycling procedure was used, which consisted of a 10-second heating step at 95°C, 40 denaturation cycles at 94°C for 5 seconds each, and a 34-second annealing and extension step at 60°C. The relative expressions of lung genes were quantified using the ΔΔCT method. The Ct samples were standardized by employing Gapdh samples, which served as the internal control and housekeeping gene. We used the Qiagen detection system (Qiagen, USA) for real-time PCR.

The primer sequence utilized in this study were selected based on their availability in the PRIMERBANK database (http://pga.mgh.harvard.edu/primerbank/index.html). Each reaction was double-checked. The specificity of the polymerase chain reaction was verified using melting curve analysis and electrophoresis. For gene analysis, Graph Pad Prism 8.3.0 was utilized. The specific primer sequences may be found in Table 1. This choice was made based on previous evidence demonstrating the stability of GAPDH expression within skeletal muscle during exercise. Additionally, our microarray study confirmed the stability of GAPDH expression across different periods.

Statistical analysis

Data are expressed as mean ± SD. To verify the normality of the data, a Shapiro–Wilk test was performed. Following a one-way analysis of variance, the Dunnett post hoc test was utilized to determine statistical significance (Graph Pad Prism 8.3.0).

Results

Table 1. The qPCR primer sequences.

Gene name	Primer sequence
PGC-1α	F: 5 -TGACTGGCGTCATTCAGGAG-3
	R: 3 -GAGGACTTACTGCGGTCAGT-5
GAPDH	F: 5' -ATCAAGAAGGTGGTGAAGCAGG- 3'
	R: 5' -TGGGAGTTGCTGTTGAAGTCAC- 3'

Lung PGC-1α gene expression

PGC-1α gene expressions in the lung tissue of various groups are depicted in Figure7.

As shown in Fig.2A, the CSE+SeNPs+AIT group showed a significantly higher expression of the PGC-1α gene ($p = 0.0289$) compared to the healthy control group. In contrast, other groups exhibited no significant difference compared to the control group ($p > 0.05$). Compared to the control group.

Discussion

Chronic Obstructive Pulmonary Disease (COPD) is a complex disease characterized by persistent respiratory symptoms and airflow limitation. The identification of differentially expressed genes (DEGs) using next-generation sequencing data provides valuable insights into the underlying molecular mechanisms of COPD. In this study, a comprehensive analysis of gene expression profiles from the GEO database (GSE148004) revealed a total of 250 DEGs, with 187 genes downregulated and 47 upregulated in patients with COPD. This finding aligns with previous studies suggesting that alterations in gene expression play a critical role in the pathophysiology of COPD.

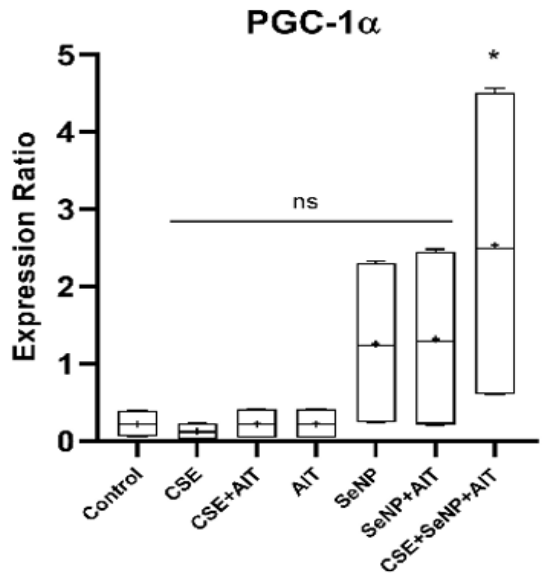

Figure 7. The PGC-1α gene displayed higher expression in CSE+SeNPs+AIT group.

Differentially expressed genes (DEGs)

Among the identified DEGs, the downregulated genes predominantly include those involved in metabolic processes and oxidative stress response, such as PGC-1α. This gene is particularly notable as it regulates mitochondrial biogenesis and energy metabolism, which are crucial in the context of COPD where oxidative stress and mitochondrial dysfunction are prevalent. Conversely, upregulated genes such as TLR7 and SMAD1 may be implicated in inflammatory responses, reflecting the chronic inflammation characteristic of COPD. The inclusion of GO pathway analysis further emphasizes the biological processes affected in COPD, including inflammation, cellular signaling, and stress responses.

Gene-gene and protein-protein interaction networks

The analysis of gene-gene interactions via Genemania revealed significant relationships among the DEGs, with certain genes scoring higher based on their interaction significance. The identification of hub genes allows for a targeted approach in understanding disease mechanisms, as these genes may serve as central points in the regulatory networks influencing COPD progression. The protein-protein interaction (PPI) analysis using NetworkAnalyst highlighted key proteins, such as PGC-1α, that may play a pivotal role in the disease's pathophysiology, particularly regarding energy metabolism and oxidative stress regulation.

Nano-selenium and aerobic interval training (AIT)

The experimental aspect of the study further evaluated the effects of nano-selenium supplementation and aerobic interval training on lung function and gene expression. The results indicated that the combination of CSE, SeNPs, and AIT significantly increased PGC-1α expression in the CSE+SeNPs+AIT group compared to controls. This finding suggests that both SeNPs and AIT may exert protective effects on lung tissue through the modulation of PGC-1α, enhancing mitochondrial function and reducing oxidative stress. This aligns with previous research highlighting the benefits of exercise and antioxidants in improving pulmonary health.

PGC-1α (Peroxisome proliferator-activated receptor gamma coactivator 1-alpha) was prioritized as a key hub gene due to its multifaceted role in COPD pathophysiology, particularly in metabolic regulation, mitochondrial biogenesis, and oxidative stress response. While several other hub genes were identified through protein-protein interaction (PPI) and gene-gene interaction analyses, PGC-1α stood out because it integrates multiple pathways directly relevant to the interventions tested in this study, namely selenium nanoparticles and aerobic interval training (AIT).

PGC-1α is a master regulator of mitochondrial biogenesis and energy metabolism, crucial processes that are often compromised in COPD due to chronic oxidative damage and impaired mitochondrial function. Given that mitochondrial dysfunction is a hallmark of COPD and contributes to muscle wasting and reduced respiratory efficiency, enhancing PGC-1α activity through targeted interventions could help restore mitochondrial health and improve energy production in lung and muscle tissues. In this study, both SeNPs and AIT were chosen for their potential to support mitochondrial function, making PGC-1α an ideal focal point for examining combined therapeutic effects.

COPD is characterized by chronic oxidative stress, leading to cellular damage in lung tissues. PGC-1α plays a crucial role in regulating antioxidant responses, activating pathways that help neutralize reactive oxygen species (ROS) and reduce oxidative stress. By prioritizing PGC-1α, this study aligns with SeNPs' known antioxidant properties and AIT's capacity to enhance endogenous antioxidant defenses, thereby providing a targeted approach to mitigate oxidative damage in COPD. Other hub genes, while important, do not exhibit the same direct influence on antioxidant response and mitochondrial health, key areas impacted by both SeNPs and AIT.

The dual approach of SeNP supplementation and AIT targets both mitochondrial function and antioxidant capacity, areas where PGC-1α has demonstrated regulatory influence. Research indicates that PGC-1α activation may be further enhanced by the presence of selenium, which is essential for selenoproteins that counter oxidative stress. Moreover, exercise is known to upregulate PGC-1α, suggesting that AIT could act synergistically with SeNPs to amplify PGC-1α's activity. Thus, prioritizing PGC-1α allows this study to explore how these interventions might jointly promote mitochondrial resilience and antioxidant defenses in COPD patients.

Although other genes like HMOX1 and SOD2 are implicated in antioxidant responses, PGC-1α's role as an upstream regulator provides broader control over multiple pathways, including mitochondrial biogenesis and metabolic adaptation. This broader regulatory capacity makes PGC-1α especially relevant to COPD pathophysiology, where both energy metabolism and oxidative stress are disrupted. By focusing on PGC-1α, the study addresses central aspects of COPD progression and the potential to modulate these processes through combined therapeutic interventions.

Conclusion

In conclusion, this study underscores the importance of identify and analyzing differentially expressed genes in understanding the molecular mechanisms underlying Chronic Obstructive Pulmonary Disease (COPD). The comprehensive examination of gene expression profiles, coupled with gene interaction analyses, revealed critical insights into the pathways affected in COPD. Notably, PGC-1α emerged as a key gene influencing mitochondrial function, oxidative stress response, and inflammatory processes.

The experimental findings highlight the potential therapeutic benefits of nano-selenium supplementation and aerobic interval training in enhancing PGC-1α expression, which could have significant implications for COPD management. These results suggest that interventions targeting metabolic pathways and oxidative stress may offer novel strategies for treating or alleviating symptoms of COPD. Future studies should focus on the functional validation of these findings and the exploration of targeted therapies aimed at modulating the expression and activity of critical genes like PGC-1α in COPD patients.

While PGC-1α is indeed a critical hub gene in this study, its activity does not occur in isolation but rather within a network of interconnected molecular pathways that are highly relevant to COPD pathophysiology. Expanding on PGC-1α's interactions with other pathways can provide a more comprehensive understanding of its role in COPD, particularly in managing muscle health, oxidative stress, and inflammation.

One significant pathway to consider is the NF-κB (Nuclear Factor kappa-light-chain-enhancer of activated B cells) pathway, which plays a central role in regulating immune response and inflammation. In COPD, chronic activation of NF-κB contributes to sustained lung inflammation and oxidative stress. PGC-1α has been shown to interact with the NF-κB pathway by modulating its transcriptional activity. This interaction is essential because, under conditions of oxidative stress, NF-κB can amplify inflammatory responses, which exacerbate muscle wasting and impair mitochondrial function in COPD patients. The potential for PGC-1α to mitigate NF-κB-driven inflammation highlights a critical avenue by which PGC-1α might protect muscle health and reduce oxidative stress in COPD, suggesting that interventions targeting both PGC-1α and NF-κB could provide synergistic benefits.

Additionally, the Nrf2 (Nuclear factor erythroid 2–related factor 2) pathway is a key player in cellular defense against oxidative stress, often dysregulated in COPD. Nrf2 controls the expression of various antioxidant genes, such as HO-1 and NQO1, that protect against oxidative damage. Studies suggest that PGC-1α can activate Nrf2 under certain conditions, enhancing cellular antioxidant capacity. This interaction between PGC-1α and Nrf2

may be particularly relevant in COPD, where oxidative stress is a hallmark of disease progression. A coordinated activation of PGC-1α and Nrf2 could help bolster cellular defenses in lung and muscle tissues, potentially offsetting the oxidative damage seen in COPD. Future studies could explore this interaction further to determine if enhancing Nrf2 activity via PGC-1α activation provides tangible therapeutic benefits.

The AMPK (AMP-activated protein kinase) pathway is another pathway deeply involved in energy regulation and mitochondrial biogenesis. AMPK acts as an upstream regulator of PGC-1α, and its activation has been shown to upregulate PGC-1α expression, promoting mitochondrial biogenesis and energy efficiency. This relationship is particularly pertinent in the context of aerobic exercise, where AMPK activation likely plays a role in upregulating PGC-1α as part of the adaptive response. Since energy dysregulation and mitochondrial dysfunction are characteristic of COPD, understanding the AMPK-PGC-1α interaction could inform strategies for using exercise or AMPK activators as treatments to improve muscle function and endurance in COPD patients. This highlights the potential of a therapeutic approach that combines exercise interventions with AMPK-activating compounds to enhance PGC-1α's beneficial effects on mitochondrial health and muscle maintenance.

Lastly, SIRT1 (Sirtuin 1), a NAD+-dependent deacetylase, is a well-known partner of PGC-1α. SIRT1 deacetylates PGC-1α, increasing its activity, particularly under conditions of cellular stress. SIRT1 itself is regulated by metabolic and stress-related signals and is known to play protective roles in inflammation and oxidative stress responses. In COPD, where SIRT1 levels are often decreased, restoring SIRT1 activity might indirectly boost PGC-1α function, thereby supporting mitochondrial biogenesis and reducing oxidative damage. Investigating ways to activate SIRT1 in conjunction with PGC-1α upregulation could offer new approaches for enhancing muscle health and resilience against COPD-related oxidative damage.

By expanding this discussion, the study can better contextualize PGC-1α within a broader network of molecular pathways involved in COPD, including NF-κB, Nrf2, AMPK, and SIRT1. These pathways collectively influence inflammation, oxidative stress, and mitochondrial health—key factors in COPD pathology and muscle health deterioration. Further research on these interactions may uncover potential multi-target therapeutic strategies that leverage PGC-1α alongside other pathways to combat COPD symptoms more effectively.

What is already known on this subject?

Chronic Obstructive Pulmonary Disease (COPD) is associated with persistent inflammation, oxidative stress, and mitochondrial dysfunction, which severely impacts respiratory health.

PGC-1α (Peroxisome proliferator-activated receptor gamma coactivator 1-alpha) is recognized as a critical gene involved in mitochondrial biogenesis and energy metabolism, making it a potential therapeutic target in COPD.

Previous studies have shown that selenium, a powerful antioxidant, and aerobic interval training (AIT) can independently provide protective effects against oxidative stress and improve mitochondrial health in lung tissue.

What this study adds?

This study identifies *PGC-1α* as a central hub gene that can be modulated by the combined effects of selenium nanoparticles (SeNPs) and AIT, which potentially synergize to counteract COPD-induced oxidative stress and mitochondrial dysfunction.

-By employing both bioinformatics and animal model analyses, the research suggests that targeting *PGC-1α* through SeNP supplementation and AIT may offer a promising therapeutic strategy for managing COPD symptoms and preserving respiratory function.

Organ Cross-Talk Tips:

- Consider interactions between the lung and muscle tissues in COPD, as oxidative stress affects both tissues, leading to potential systemic benefits from targeted mitochondrial support.
- Explore the role of inflammation reduction in multi-organ health, as lowering oxidative stress in the lungs could reduce systemic inflammatory markers that affect other organs, particularly the cardiovascular system.

Acknowledgements

We would like to express our sincere gratitude to the research staff at Sport Physiology Research Center and the laboratory team for their invaluable contributions and technical support in carrying out this study.

Funding

This study was funded by the Sport Physiology Research Center.

Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

Ethical approval This study adhered to ethical guidelines for animal research and was approved by the relevant institutional ethics committee (approval code: IR.BMSU.REC.1400.117)

Informed consent Not applicable

Author contributions

Conceptualization: M.B, B.B, A.B; Methodology: M.B, B.B A.B; Software: M.B; Validation: M.B, A.B, B.B; Formal analysis: M.B, S.A, A.B; Investigation: M.B, B.B, A.B; Resources: M.B; Data curation: M.B, B.B; Writing - original draft: M.B, B.B, A.B; Writing - review & editing: M.B, B.B.; Visualization: M.B, S.A, A.B; Supervision: B.B, A.B, S.A; Project administration: M.B, B.B; Funding acquisition: M.B, B.B, A.B.

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