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Bioinformatics Analysis and Expression Dynamics of the ACE Gene in Rats Following Exercise Interventions

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A B S T R A C T

Objective: In this study, we combined bioinformatics analyses with experimental approaches to investigate the effects of exercise on ACE gene expression in rats.

Methods and Materials: Bioinformatics tools were used to analyze the structure, stability, and phylogeny of the ACE protein. Experimentally, rats underwent a treadmill exercise protocol, and ACE gene expression was measured in cardiovascular, lung, kidney, and other tissues at 6, 12, and 24 hours post-exercise using real-time PCR.

Findings: Results showed that ACE gene expression increased in the heart, lung, and kidney at 12 hours post-exercise, followed by a significant decrease at 24 hours. Notably, the highest ACE expression was observed in the small intestine and duodenum, while the lowest expression was detected in the cerebral cortex and skin.

Conclusion: These findings suggest that exercise induces dynamic, tissue-specific changes in ACE gene expression, which may contribute to cardiovascular adaptation and health. This study provides new insights into the molecular mechanisms underlying exercise-induced physiological changes.

Keywords: *Exercise, angiotensin-II, protein kinase, ACE, Gene Expression.*

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1. Introduction

1.1 Effects of Exercise on Body Organs

Exercise is widely regarded as a powerful intervention for promoting the health of various body organs (1). Regular physical activity is associated with a reduced risk of cardiovascular diseases (2, 3), and it plays a critical role in maintaining heart health (4, 5). Endurance activities such as running not only support cardiovascular function but also decrease the incidence of diabetes, cancer, fractures, and osteoporosis (6-10). Exercise increases metabolic rate, leading to a tenfold increase in calorie consumption compared to rest (6, 11-13), and elevates oxygen consumption by 10- to 20-fold, which in turn increases free radical production. Therefore, post-exercise consumption of antioxidant-rich foods is recommended to mitigate oxidative stress (14-17).

1.2 Gene Networks and Sports Activities

Emerging evidence demonstrates a strong connection between exercise and gene regulation. Endurance and resistance training trigger inflammatory responses and activate intracellular signaling pathways, resulting in widespread changes in the proteome and gene expression profiles (18-20). Prolonged or intense exercise can also impact immune function and contribute to chronic disease risk (21-24). Of particular interest are genes related to cardiovascular function, which are involved in the regulation of vascular cell walls, capillaries, and smooth muscle cells, and are critical for myogenesis and cardiovascular adaptation (25-28). This study hypothesizes a direct relationship between exercise and ACE gene expression, aiming to elucidate the effects of physical activity on this gene using both bioinformatics and experimental approaches.

1.3 The ACE Gene

The angiotensin I-converting enzyme (ACE) gene encodes a potent protease that hydrolyzes peptide bonds (Table 1). ACE gene polymorphisms, particularly the D and I alleles, have been linked to variations in athletic performance, including endurance and strength. ACE catalyzes the conversion of angiotensin I to angiotensin II, a process that increases muscle contraction and blood pressure, thereby elevating the risk of cardiovascular events (29). However, regular exercise may counteract these effects by promoting vasodilation and reducing angiotensin II levels, ultimately supporting cardiovascular health.

Table 1. Characteristics associated with the ACE gene.

Name	ACE
ORGANISM	Homo sapiens (Human)
Accession number nucleotide	NM_000789.4
Accession number protein	NP_000780.1
Gene ID	1636
Chromosome	17
Cytogenetic location	17q23.3
Chromosome location bp	63477061-63498373
nucleotide length	4962 bp
protein length	1306 aa
Molecular weight (Da)	149714.86
Isoelectric point	5.95
Total Exon	25

2. Methods and Materials

2.1 Bioinformatic Analysis of the ACE Gene

The ACE gene sequence (accession number NM_000789.4) was retrieved from the NCBI database. Protein sequences were identified using BLAST, and the three-dimensional structure and Ramachandran plot of the ACE protein were analyzed using ProtScale. Phylogenetic relationships were assessed with MEGA5 software and visualized using the iTOL platform. ACE gene expression patterns were further examined using the OMIM database.

2.2 Animal Model and Experimental Design

Thirty-six male rats (average weight: 238 g) were randomly assigned to control and experimental groups (n=18 per group), with three biological replicates per group. Animals were housed under controlled conditions (22°C, 50% humidity, 12-hour light/dark cycle). All procedures

were conducted in accordance with institutional animal care guidelines.

2.3 Exercise Protocol

Rats in the experimental group underwent a treadmill training protocol for 6 weeks, three sessions per week, running for 2 minutes at a speed of 15 m/min. After the training period, experimental rats completed a final exercise session: 5 minutes at 15 m/min. Control rats did not undergo exercise.

2.4 Tissue Collection

At 6, 12, and 24 hours after the final exercise session, rats were anesthetized with ketamine/xylazine. Tissues (heart, lung, kidney, small intestine, duodenum, cerebral cortex, and skin) were rapidly excised, flash-frozen in liquid nitrogen, and stored at -80°C for subsequent analysis.

2.5 Real-Time PCR Analysis

Total RNA was extracted from tissue samples using the DENA ZIST ASIA kit, and cDNA was synthesized according to the manufacturer's protocol. Real-time PCR was performed using a BIO-RAD system, with the elongation factor gene as an internal control. Relative ACE gene expression was calculated using the $2^{\wedge-\Delta\Delta Ct}$ method (30).

2.6 Statistical Analysis

Data were analyzed using SAS software. Results are presented as mean ± standard deviation. Statistical significance was determined using ANOVA, with $p < 0.05$ considered significant.

3. Results

3.1 Structural and Stability Analysis of the ACE Protein

The predicted three-dimensional structure of the ACE protein, assessed by the GMQE index (0.91), indicated high model reliability (Figure 1). Ramachandran plot analysis revealed that 86.5% of residues were in favored regions, supporting the structural stability of the ACE protein (Figure 2).

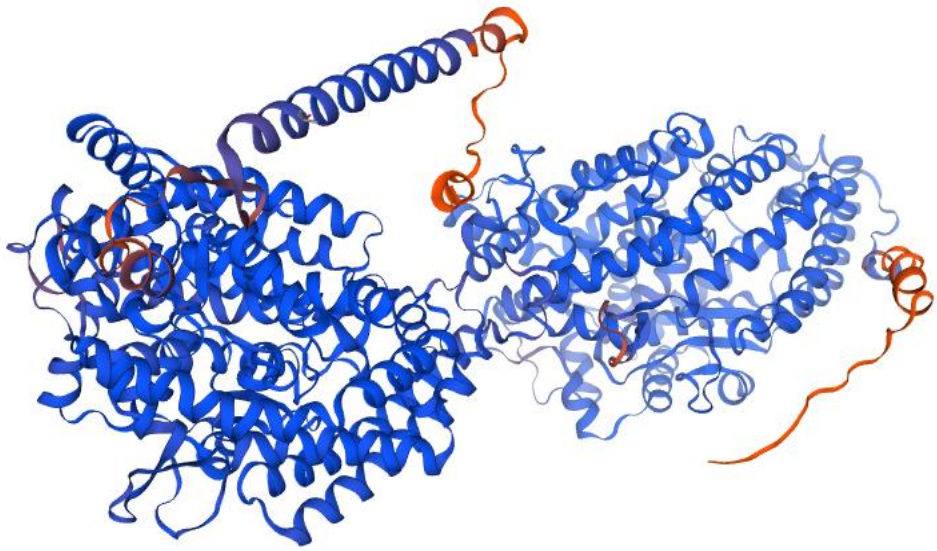

Figure 1. Estimation of the three-dimensional structure of the ACE protein.

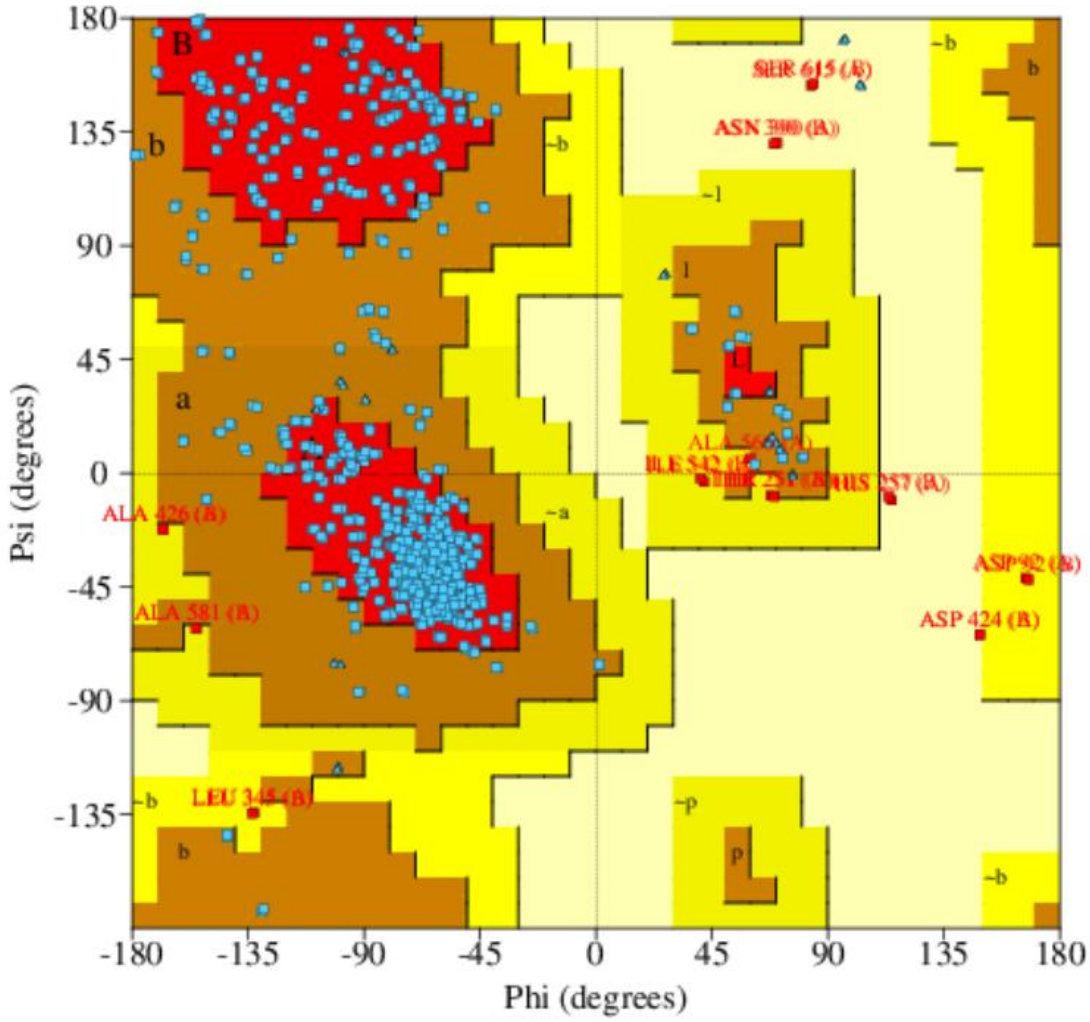

Figure 2. Ramachandran plot of the ACE protein. This diagram is obtained by considering the amount of φ and ψ angles.

3.2 Phylogenetic Analysis

Phylogenetic analysis of ACE protein sequences from Homo sapiens and Mus musculus revealed high conservation, with the Mus musculus sequence NP_001123985.1 showing the greatest similarity to the human ACE protein. Human ACE sequences clustered into two distinct groups, while mouse sequences formed four groups (Figure 3).

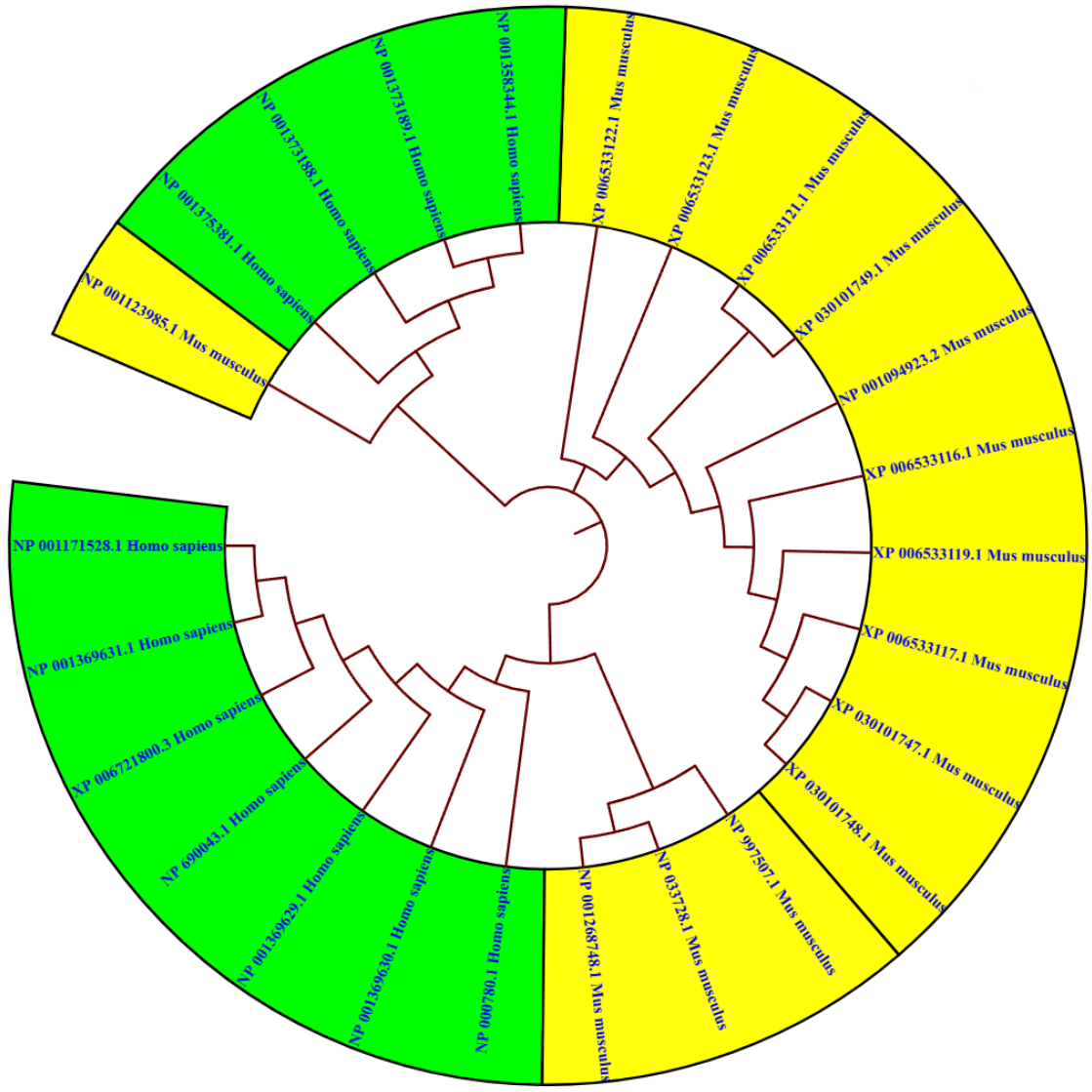

Figure 3. Phylogenetic tree related to the ACE protein in Homo sapiens and Mus musculus. The analyses related to this graph were drawn by MEGA5 software and the iTOL database.

3.3 ACE Gene Expression in Human Tissues (Bioinformatics)

Bioinformatic analysis using the OMIM database indicated that ACE gene expression is highest in the small intestine and duodenum, and lowest in the cerebral cortex and skin (Figure 4, Figure 5).

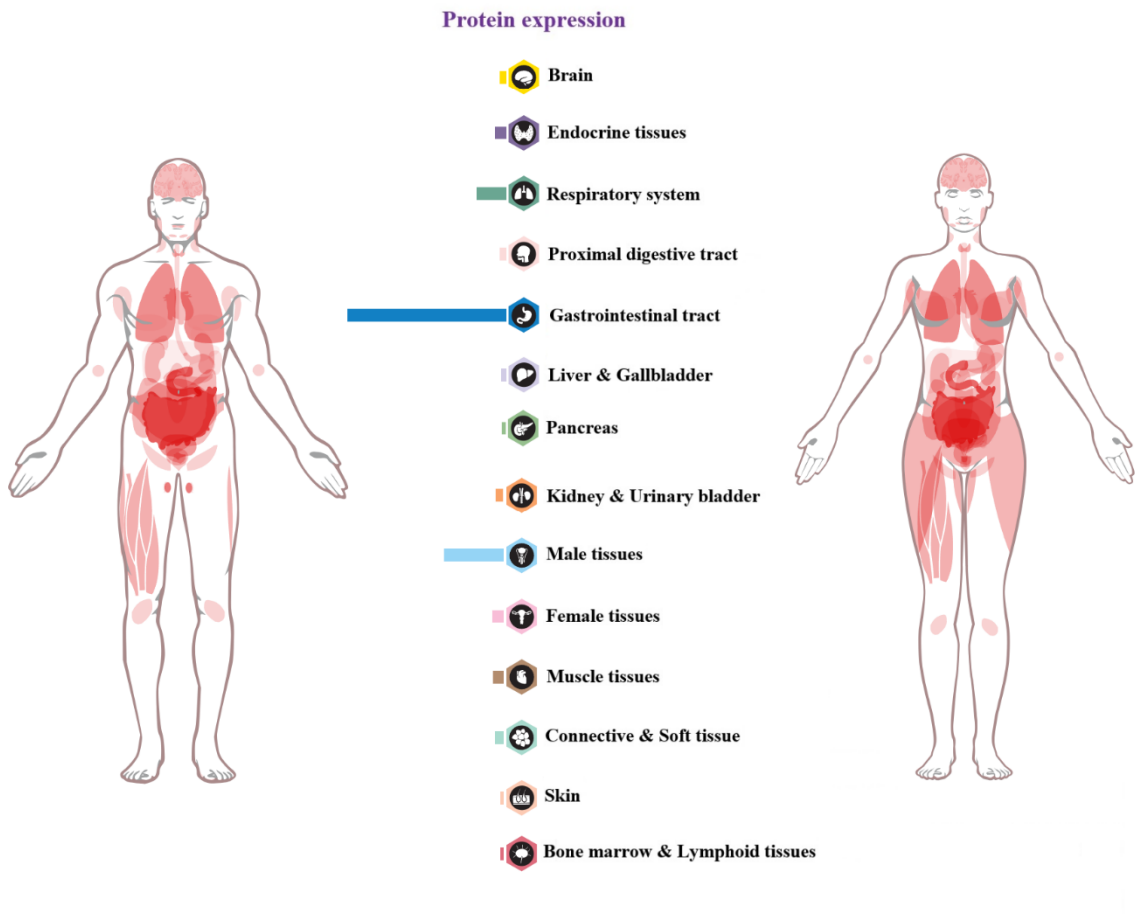

Figure 4. Results of ACE gene expression analysis in different organs of the human body.

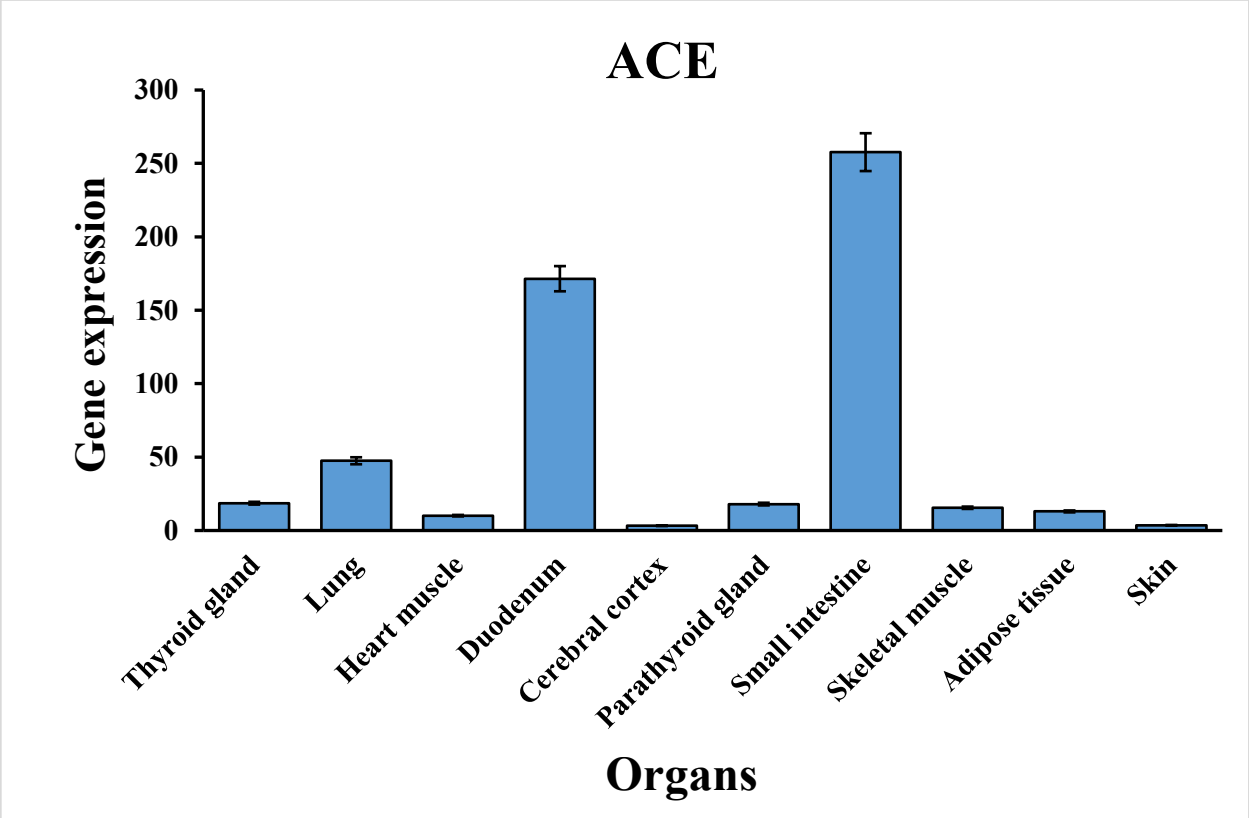

Figure 5. Changes in ACE gene expression extracted from the OMIM database in different organs of the body.

3.4 Exercise-Induced Changes in ACE Gene Expression (Experimental)

Real-time PCR analysis demonstrated that ACE gene expression in rat heart, lung, and kidney tissues increased at 6 hours post-exercise, peaked at 12 hours, and significantly decreased at 24 hours compared to controls (Diagrams below). The magnitude and timing of expression changes were tissue-specific, with the most pronounced effects observed in the heart.

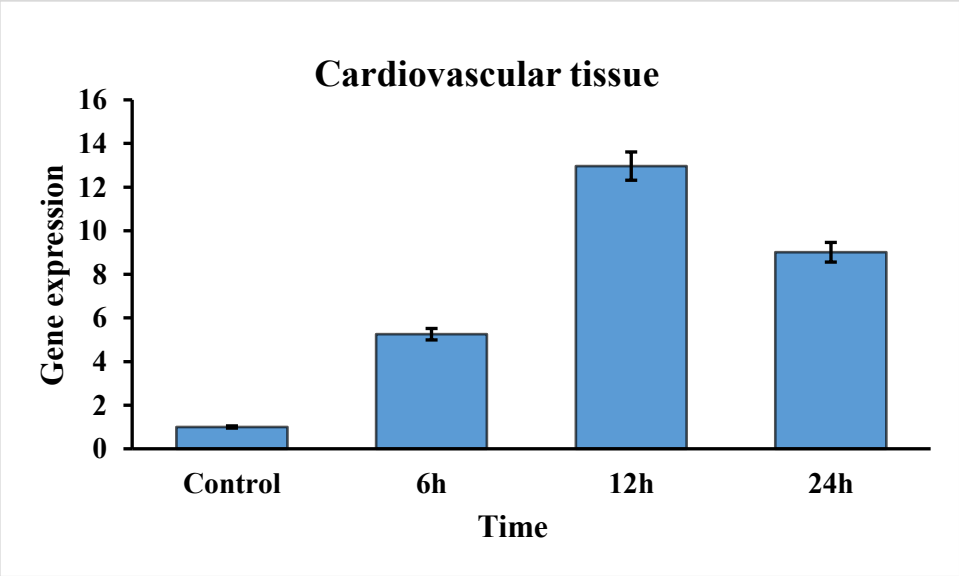

Figure 6. Changes in the expression level of the ACE gene in the cardiovascular tissue of rats.

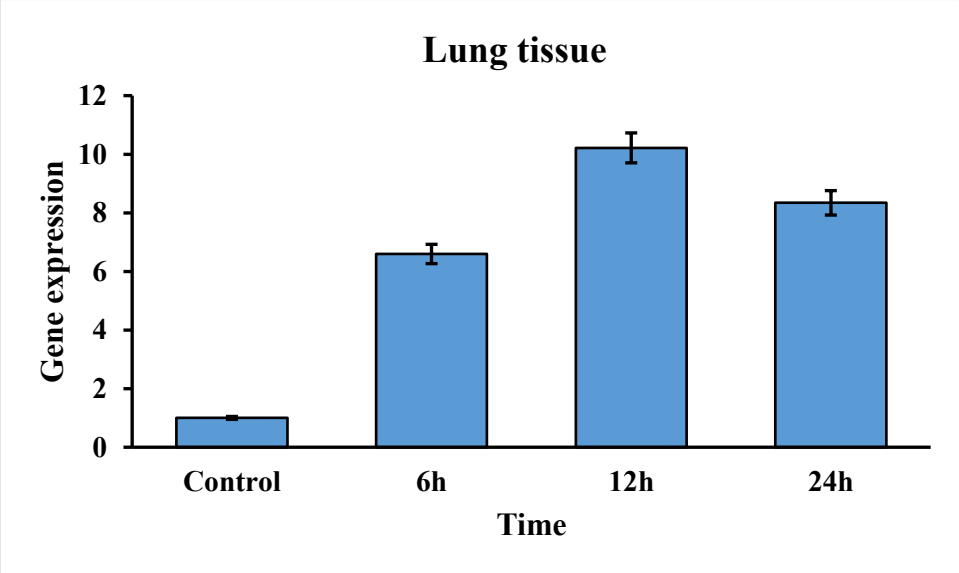

Figure 7. Changes in ACE gene expression in the lung tissue of rats.

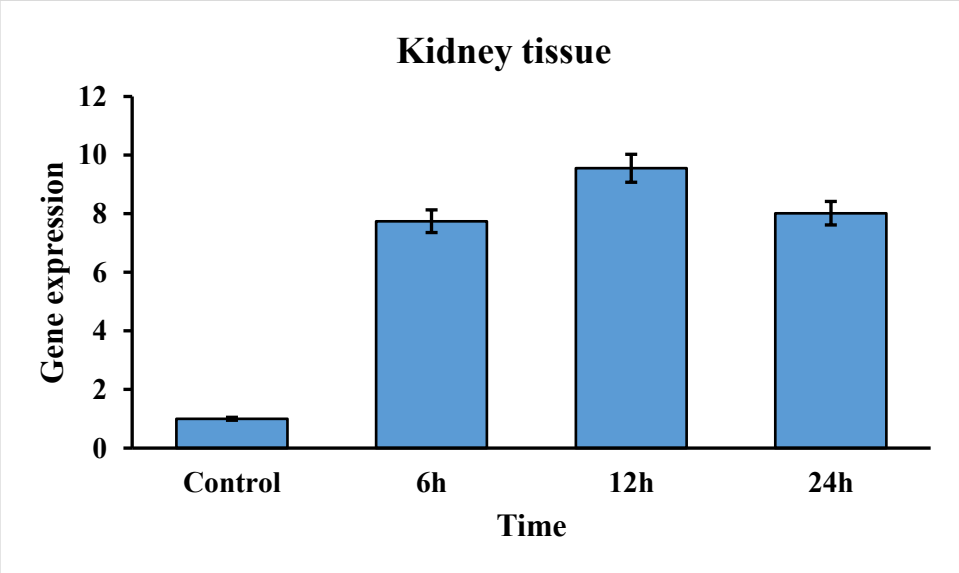

Figure 8. Changes in the expression level of the ACE gene in kidney tissue of rats.

4. Discussion and Conclusion

This study demonstrates that exercise induces dynamic, time-dependent changes in ACE gene expression in rat tissues. The initial increase in ACE expression observed at 6 and 12 hours post-exercise may reflect an acute physiological response to increased cardiovascular demand and metabolic stress. The subsequent decrease at 24 hours suggests a return toward homeostasis and adaptation to exercise-induced stress.

Our findings align with previous research indicating that physical activity modulates gene expression through both genetic and epigenetic mechanisms, contributing to improved muscle adaptation, cardiovascular health, and metabolic function. The observed tissue-specific expression patterns, with the highest ACE expression in digestive organs and lowest in the brain and skin, are consistent with

the known physiological roles of ACE in regulating blood pressure, electrolyte balance, and fluid homeostasis.

A key limitation of this study is the lack of protein-level validation and functional assays to directly link ACE expression changes to physiological outcomes. Future studies should address these aspects and explore the molecular pathways underlying ACE regulation during exercise.

This study provides evidence that exercise modulates ACE gene expression in a time- and tissue-dependent manner in rats. The ACE gene plays a central role in cardiovascular adaptation to physical activity, likely through interactions with cell surface receptors and epigenetic regulation. Further research on the molecular mechanisms linking exercise, ACE gene expression, and cardiovascular health may inform strategies for disease prevention and performance optimization.

Authors' Contributions

All authors equally contributed to this study.

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.

Acknowledgments

Not applicable.

Declaration of Interest

The authors report no conflict of interest.

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Ethical Considerations

Not applicable.

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