International Journal of Sport Studies for Health

Journal Homepage

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

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Pantelis Theo Nikolaidis School of Health and Caring Sciences, University of West Attica, Athens, Greece l.youzbashi@znu.ac.ir	**Reviewer 1:** Masoud Mirmoezi Department of Physical Education and Sport Sciences, Islamic Azad University, Central Tehran Branch, Tehran, Iran. Email: massoudmirmoezi@live.com **Reviewer 2:** Mohammad Reza Khodabakhsh Department of Psychology, Neyshabour Branch, Islamic Azad University, Neyshabour, Iran. Email :hodabakhsh@ut.ac.ir

1. Round 1

1.1 Reviewer 1

Reviewer:

The sentence “Exercise increases metabolic rate, leading to a tenfold increase in calorie consumption compared to rest” seems exaggerated. Calorie consumption rarely increases tenfold during exercise—please provide a citation with corrected quantitative estimates.

In “Prolonged or intense exercise can also impact immune function,” clarify whether the impact is positive or negative—this will help maintain a consistent narrative on exercise outcomes.

The final acute session (5 minutes at 15 m/min) seems too short to elicit significant physiological responses. Justify its selection with literature or prior pilot data.

The elongation factor gene was used as a reference. Please provide justification or citation showing it remains stable under exercise conditions in rats.

The GMQE index is reported as 0.91. Indicate what this score implies in terms of model confidence, and state which template was used for homology modeling.

The phylogenetic tree (Figure 3) is informative but not discussed in the text. Briefly interpret why Mus musculus ACE has multiple clusters and how that informs the experimental model choice.

Author revised the manuscript and uploaded the updated document.

1.2 Reviewer 2

Reviewer:

The section header “The ACE Gene” is potentially misleading since the focus is on expression regulation. Consider revising to “Physiological Role and Genetic Regulation of the ACE Gene.”

Table 1 presents ACE gene characteristics for Homo sapiens, while the experimental work is conducted on rats. Consider adding a similar table for Rattus norvegicus to maintain biological relevance.

The manuscript mentions “three biological replicates per group.” Please specify whether this means three rats per time point or three independent measurements per organ.

While ACE polymorphisms (D and I alleles) are discussed in the introduction, they are not revisited in the discussion. Clarify whether exercise-induced changes may be influenced by genotype (even in rats).

You mention “lack of protein-level validation” as a limitation. Strengthen this by suggesting specific techniques such as ELISA or Western blotting for future studies.

The manuscript refers to “interactions with cell surface receptors and epigenetic regulation.” These are vague. Suggest specific receptors or epigenetic mechanisms potentially involved, even hypothetically.

Author revised the manuscript and uploaded the updated document.

2. Revised

Editor’s decision after revisions: Accepted.

Editor in Chief’s decision: Accepted.