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The Effect of Aerobic and Resistance Training on Gut Microbiome Composition and Its Association with Irisin Protein Levels in Aged Mice: The Role of *Faecalibacterium prausnitzii*, *Clostridium difficile*, and *Enterococcus faecalis*

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1. Round 1

1.1 Reviewer 1

Reviewer:

In paragraph 4 of the Introduction, the manuscript notes that “these age-related changes… are tightly linked to remodeling of the intestinal ecosystem—the gut microbiota…”. This is a major claim. The paper should integrate a brief mechanistic explanation (e.g., SCFA-mediated mitochondrial signaling) to make the argument more explicit.

In paragraph 14 of the Introduction, the authors write: “the specific role of F. prausnitzii… has attracted growing interest…”. Since the Methods focus only on three species, the authors should justify why no additional SCFA producers (e.g., Roseburia spp., Akkermansia muciniphila) were assessed. This is especially relevant because paragraph 3 earlier mentions “reduced microbial diversity” as central to aging.

No information is given about how sample size (n = 8 per group) was determined. Please add justification or power analysis following the sentence in Methods: “A total of 40 male Wistar rats were used.”

In the qPCR section, the manuscript states: “specific primers were used…” but does not provide primer sequences or references. These are essential for reproducibility. Please add them in Methods or as supplementary material.

In Methods: “Data were analyzed using two-way ANOVA…”. Please specify whether interaction effects (training × age/group) were tested and report corresponding F-values.

In Findings, paragraph 1: “there were no significant differences… in final body weight…”. Given that aging normally leads to increased adiposity but decreased lean mass, authors should discuss why weights between sham and aged groups remain identical. Potential explanations (e.g., diet control) should be included.

Authors revised the manuscript and uploaded the updated document.

1.2 Reviewer 2

Reviewer:

In the Abstract and throughout the paper, “aged mice” is used, but the Methods state explicitly that 40 male Wistar rats were used. For example, in Methods paragraph 1: “40 male Wistar rats were used…”. Please correct this inconsistency across all sections.

In Methods paragraph 2: “The rats were randomly divided into five groups…”. Please specify how randomization was conducted (e.g., random number generator, cage-based assignment) to satisfy ARRIVE guidelines.

In paragraph 3 of Methods: “housed in polycarbonate cages… with free access to standard chow…”. Please specify whether animals received any form of enrichment (e.g., tunnels, nesting material), as such environmental factors significantly influence gut microbial variability.

In Methods paragraph 5, the text cites a speed of “12 m/min… increasing to 56 minutes”. However, there is no explanation of how this speed corresponds to moderate intensity. Please include VO2max equivalents or reference physiological benchmarks for rats.

In paragraph 6: “Each session consisted of eight climbs with one minute of rest…”. Please clarify if “eight climbs” represents one set or multiple sets, and whether animals failed or completed the climb consistently. Ladder-climbing studies usually report “successful climbs”.

The Methods state: “rats were placed on an operating treadmill at 3 m/min without incline…”. To validate that the sham group mimics stress exposure, please report biomarkers of handling stress or cite empirical studies confirming that low-speed treadmill exposure induces equivalent environmental stress.

Authors revised the manuscript and uploaded the updated document.

2. Revised

Editor’s decision after revisions: Accepted.

Editor in Chief’s decision: Accepted.

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