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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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A B S T R A C T

In the aging period, alterations in gut microbiome composition and the decline of myokine proteins—such as irisin—are considered major contributors to metabolic disorders, inflammatory conditions, and muscle weakness. In this context, the role of exercise interventions in simultaneously modulating these two biological indicators has gained increasing attention. The present study aimed to examine the effects of aerobic and resistance training on gut microbiome composition and plasma irisin levels in aged mice, with a focus on three key species: *Faecalibacterium prausnitzii*, *Clostridium difficile*, and *Enterococcus faecalis*. This experimental study was conducted over eight weeks on 40 male Wistar rats assigned to five groups: young control, aged without training, sham, aerobic training, and resistance training. Plasma irisin levels were measured using the ELISA method, and the relative abundance of bacteria was assessed using real-time polymerase chain reaction (qPCR). Data were analyzed using multifactorial ANOVA and Tukey's post hoc test. The results indicated that aerobic and resistance training led to a significant increase in plasma irisin levels in aged mice compared with the inactive group. Resistance training showed the greatest increase. Additionally, the abundance of *F. prausnitzii* increased in the trained groups, while the abundance of *C. difficile* and *E. faecalis* decreased. Composite microbial health indices (F/BAD, F/Cd, F/Ef) also improved significantly. A positive association between irisin levels and microbial ratios was confirmed. The findings suggest that physical exercise—particularly resistance training—can simultaneously enhance gut microbiome composition and myokine status in the elderly. These results support the central role of the "gut–muscle axis" in the physiological response to exercise and provide a basis for designing personalized training interventions in geriatric medicine.

Keywords: *Gut microbiome; Irisin; Resistance training; Aerobic training; Aging.*

1. Introduction

Aging is accompanied by profound alterations in body composition, immune function, and metabolic homeostasis that together promote a state of chronic low-grade inflammation, increased vulnerability to disease, and functional decline (1, 2). Among the most clinically relevant

manifestations of aging is sarcopenia, characterized by the progressive loss of skeletal muscle mass and strength, which increases the risk of frailty, disability, and mortality in older adults (2, 3). Accumulating evidence indicates that these age-related changes are not driven solely by intrinsic muscle or immune alterations but are tightly linked to remodeling of the intestinal ecosystem—the gut microbiota—and its metabolites (1, 4). In community-dwelling older men, objectively measured physical activity associates with distinct gut microbial profiles, suggesting that both lifestyle and aging jointly shape the "gut–muscle axis" (5). Understanding how exercise interventions can modulate this axis in aging therefore has major implications for geroscience and preventive strategies targeting functional decline (3).

The aging gut is typically characterized by dysbiosis, reduced microbial diversity, and a selective decline in short-chain fatty acid (SCFA)–producing bacteria, particularly taxa involved in butyrate synthesis (1, 6). Butyrate, a key SCFA, plays a central role in maintaining epithelial integrity, regulating immune responses, and fueling colonocytes, such that its depletion contributes to low-grade systemic inflammation and metabolic dysregulation in older adults (6, 7). *Faecalibacterium prausnitzii* is one of the most abundant butyrate-producing commensals in healthy individuals and has been repeatedly identified as a keystone anti-inflammatory species whose abundance declines in a variety of chronic inflammatory and age-related conditions (8, 9). Recent geroscience-oriented work further highlights that the loss of SCFA-producing bacteria, including *F. prausnitzii*, is a hallmark of age-related dysbiosis with direct implications for metabolic and musculoskeletal health (3, 6).

Conversely, age-related dysbiosis is frequently accompanied by an expansion of opportunistic and pathogenic taxa that compromise barrier function and promote mucosal inflammation (1, 7). *Clostridium difficile* is a well-known enteric pathogen whose overgrowth is associated with antibiotic exposure, immunosenescence, and increased risk of severe colitis in the elderly (10). *Enterococcus faecalis* has also been implicated in the pathophysiology of inflammatory bowel disease and other chronic inflammatory conditions, acting through virulence factors, biofilm formation, and epithelial disruption (11). The combined shift toward depletion of beneficial SCFA producers such as *F. prausnitzii* and enrichment of potentially harmful species including *C. difficile* and *E. faecalis* creates a pro-inflammatory milieu with systemic consequences for musculoskeletal and metabolic health in older adults (8-11).

These observations converge in the emerging concept of a bidirectional "gut–muscle axis," whereby gut-derived metabolites and inflammatory mediators influence muscle protein turnover, mitochondrial function, and neuromuscular performance, while muscle-derived myokines and physical activity patterns, in turn, shape gut microbial composition (2, 12). Experimental and clinical data indicate that gut dysbiosis contributes to sarcopenia by promoting anabolic resistance, oxidative stress, and chronic inflammation (2, 3). At the same time, exercise and muscle contraction induce systemic signals that can remodel the intestinal ecosystem, suggesting that targeted training programs may represent a feasible strategy to restore a more "youthful" microbiome in aging (4, 12).

Over the past decade, several human studies and systematic reviews have investigated the relationship between physical activity and the gut microbiota. Cross-sectional and intervention studies in adults indicate that exercise can increase microbial diversity, enrich SCFA-producing taxa, and shift functional pathways toward improved metabolic and immune profiles (13-15). Systematic reviews suggest that regular physical activity is associated with favorable changes in microbiome composition, although the magnitude and direction of effects may depend on baseline fitness level, training modality, and dietary context (16-19). For example, in older individuals, higher exercise frequency has been linked to increased microbial diversity and the enrichment of health-associated taxa (20, 21). However, other reviews emphasize substantial heterogeneity in study designs, small sample sizes, and limited mechanistic endpoints, particularly in older populations (13, 18).

Evidence specifically focused on older adults underscores both the potential and the complexity of exercise–microbiome interactions in aging. Community-dwelling older men with higher objectively measured activity show distinct microbial signatures compared with less active peers, even after adjusting for diet and comorbidities (5). In another cohort of older adults, physical

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fitness indexes correlate with dietary intake, gut microbiota composition, and metabolomic profiles, highlighting a triadic relationship linking lifestyle, microbial metabolism, and functional capacity (22). Endurance-oriented interventions in elderly men have been shown to reduce *C. difficile* abundance and improve markers of gut health, suggesting that exercise can counteract specific pathogenic shifts associated with aging (10). At the same time, systematic reviews note that many human studies cannot fully disentangle the effects of exercise from those of diet and other lifestyle factors, making animal models particularly valuable for mechanistic exploration (16, 17, 21).

Within this context, the specific role of *F. prausnitzii* has attracted growing interest. This species has been identified as a key anti-inflammatory commensal whose depletion is associated with Crohn's disease and other chronic disorders, and whose presence appears to promote mucosal tolerance and barrier integrity (8, 9). Recent geroscience-oriented work shows that older adults with higher adherence to physical activity display greater abundance of *F. prausnitzii*, supporting a link between exercise behavior and preservation of beneficial SCFA-producing taxa (23). These findings are consistent with broader evidence that age-related loss of SCFA producers contributes to metabolic and inflammatory vulnerability and may represent a modifiable target through lifestyle interventions (3, 6). However, the degree to which different training modalities—such as aerobic versus resistance exercise—differentially affect *F. prausnitzii* and its balance with potentially harmful bacteria like *C. difficile* and *E. faecalis* has not been fully clarified.

Parallel to microbiome research, irisin has emerged as a prominent exercise-induced myokine linking skeletal muscle activity to systemic metabolic and neuroimmune adaptations. Irisin is cleaved from the membrane precursor FNDC5 and is released into the circulation in response to muscular contractions, where it promotes browning of white adipose tissue, enhances energy expenditure, and exerts anti-inflammatory and neuroprotective effects (12, 16). Aging is associated with reduced FNDC5/irisin expression in skeletal muscle, which has been implicated in the development of sarcopenia and diminished responsiveness to exercise interventions (24). Experimental data indicate that FNDC5/irisin deficiency induces gut dysbiosis and depressive-like behavior through the gut–brain axis, underscoring its integrative role at the interface of muscle, microbiota, and central nervous system (25). Furthermore, irisin has been shown to ameliorate osteoporosis and gut dysbiosis by strengthening intestinal barrier function and modulating microbial composition in ovariectomized mice, directly supporting a protective "irisin–microbiome" axis (26).

Importantly, exercise modality and intensity appear to differentially regulate both irisin dynamics and microbiome features. Human interventions comparing aerobic and resistance exercise report distinct patterns in gut microbial diversity and SCFA production, with some evidence that combined or higher-intensity regimens may elicit more pronounced shifts in beneficial taxa (27). In aged mice, aerobic and resistance training have been shown to exert differential effects on oxidative stress markers and TGF-β signaling in cardiac tissue, suggesting that training mode may selectively modulate molecular pathways relevant to aging (28). Recent work in aged mice further demonstrates that aerobic versus resistance training produces distinct changes in irisin levels and in the expression of inflammatory genes such as TGF-β, NF-κB, and SIRT1 in intestinal lymphocytes, directly linking exercise mode to both myokine signaling and gut-associated immune regulation (29). Complementary animal studies indicate that high- and moderate-intensity aerobic interventions differentially alter the abundance of key genera such as *Lactobacillus*, *Bifidobacterium*, and *Escherichia coli* in aged mice, reinforcing the notion that specific training prescriptions can be used to fine-tune gut microbiome composition (30).

Despite these advances, several important gaps remain. Most human studies rely on heterogeneous exercise protocols, focus on global diversity rather than targeted quantification of key bacterial species, and rarely integrate detailed myokine measurements such as irisin (13, 18, 19). Animal models, by contrast, allow for precise control of age, diet, training load, and environmental conditions, making them well suited for disentangling the relative contributions of aerobic versus resistance exercise to gut microbial remodeling and myokine responses in aging (28, 30). Classical exercise models such as treadmill running and ladder climbing have been extensively used to examine

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musculoskeletal, cardiac, and skeletal adaptations to training and detraining in rodents and humans, providing a robust methodological foundation for designing age-appropriate protocols (31, 32). Yet, to date, few studies have simultaneously characterized gut microbiome composition—at the level of both beneficial and harmful taxa—and circulating irisin levels in aged organisms undergoing modality-specific training.

In particular, the triad comprising *F. prausnitzii* (as a prototypical SCFA-producing, anti-inflammatory commensal), *C. difficile* (as an age-sensitive pathogen), and *E. faecalis* (as an opportunistic species linked to inflammatory bowel disease) offers a biologically meaningful framework for assessing whether exercise can rebalance the aged microbiome toward a more favorable, health-promoting configuration (8-11). Moreover, examining composite indices that relate the abundance of *F. prausnitzii* to these potentially deleterious taxa may improve the sensitivity of microbiome-based markers of gut health in the context of exercise interventions in aging (6, 23). By coupling these microbiological endpoints with precise quantification of plasma irisin, it becomes possible to probe the coordinated response of the "gut–muscle axis" to distinct training modalities in a controlled aging model (4, 12).

Against this background, and building on previous evidence that aerobic and resistance training differentially modulate inflammatory pathways, gut microbiota, and irisin signaling in aging (26-30), the present experimental study aimed to compare the effects of structured aerobic and resistance training on gut microbiome composition and plasma irisin levels in aged mice, with a specific focus on the key bacterial species *Faecalibacterium prausnitzii*, *Clostridium difficile*, and *Enterococcus faecalis*.

2. Methods and Materials

This study was conducted in a laboratory-based experimental design using a post-test with control and sham groups to examine the effects of aerobic and resistance training on gut microbiome composition and plasma irisin protein levels in aged rats. The study was carried out from March 2025 to June 2025 and all procedures were approved by the University Ethics Committee (Ethics Code: IR.UI.REC.1403.121), in accordance with the international guidelines for the care and use of laboratory animals (NIH Guide for the Care and Use of Laboratory Animals). A total of 40 male Wistar rats were used. The rats were randomly divided into five groups of eight animals each:

1. Young Control Group (Ct): Eight-week-old rats weighing approximately 220–250 g, without training.
2. Aged Without Training Group (Aged): Fourteen-month-old rats weighing 450–500 g, maintained under normal conditions only.
3. Sham Group (Sham): Aged rats exposed to treadmill or ladder conditions similar to the training groups, but without any effective training load (exposure without exercise).
4. Aerobic Training Group (Aero): Aged rats undergoing moderate-intensity aerobic training.
5. Resistance Training Group (Res): Aged rats undergoing resistance training with progressive loading.

The selection of aged rats was due to the physiological similarities between aging processes and gut microbiome alterations in humans. The rats were housed in polycarbonate cages (four per cage) under controlled conditions with a 12-hour light/dark cycle (07:00 to 19:00), temperature of 22 ± 2 °C, 55% humidity, and free access to standard chow and water. All groups underwent one week of acclimatization in the laboratory environment prior to the start of training.

Aerobic training was performed using an animal treadmill. The familiarization period lasted one week, with a speed of 5 m/min for 10 minutes per session. The main protocol continued for eight weeks, five days per week. Each session consisted of a 5-minute warm-up (speed 8–10 m/min), a main training phase at a constant speed of 12 m/min that increased from 10 minutes in week 1 to 56 minutes in week 8, and a 5-minute cool-down (8 m/min) (Iwamoto et al., 2001).

Resistance training was performed according to the standard ladder-climbing model with weights (Goudarzi, 2020; Horn, 2016). The rats climbed a 1-meter ladder at an 80-degree incline. During the first week, five familiarization sessions without weights were performed. From week 2, a weight equivalent to 5% of body weight was attached to the tail, progressively increasing to 40% by week 8. Each

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session consisted of eight climbs with one minute of rest between climbs.

The sham group was included to control for stress related to equipment exposure and researcher handling. These rats were placed on an operating treadmill at a constant speed of 3 m/min without incline or speed increase for 15 minutes. In the resistance-related sham protocol, rats climbed the ladder without weights or with a very minimal load equivalent to 1% of body weight. The duration and frequency of exposure were identical to those of the training groups to ensure environmental stress control.

At 48 hours after the completion of the training period, the rats were anesthetized via intraperitoneal injection of ketamine (50 mg/kg) and xylazine (10 mg/kg). Blood samples, as well as colon and ileum contents, were collected. Blood was drawn from the retro-orbital vein and stored in EDTA tubes. After centrifugation at 3000 rpm for 10 minutes at 4 °C, plasma was separated and stored at −80 °C. Colon and ileum contents were immediately frozen in liquid nitrogen and stored at low temperature for microbiological analyses.

Variable assessment in this study included two main components. In the first component, plasma irisin concentration was measured using a rat-specific ELISA kit (MyBioSource, USA) following the manufacturer's instructions. Prepared plasma samples were loaded into designated wells, and optical density was read at 450 nm using a Biotek ELx800 microplate reader to determine irisin concentration in ng/mL. In the second component, bacterial genomic DNA was extracted from colon contents using the QIAamp DNA Stool Mini Kit (Qiagen, Germany), and DNA quality and purity were assessed using a NanoDrop system. Real-time polymerase chain reaction (qPCR) with specific primers was used to identify and quantify the relative abundance of *Faecalibacterium prausnitzii*, *Clostridium difficile*, and *Enterococcus faecalis*. Each reaction consisted of a final volume of 20 µL containing 1 µL of template DNA, 10 µL of SYBR Green Master Mix, 1 µL of forward and reverse primers, and nuclease-free water. Data were analyzed using the 2^−ΔΔCt method and normalized to the reference 16S rRNA gene to determine the relative abundance of each bacterial species in different groups.

Data were analyzed using SPSS version 26. The effects of exercise type (aerobic and resistance) and group (control, sham, aged) on irisin and microbiome composition were examined using two-way ANOVA. When significant effects were observed, Tukey's HSD post hoc test was used for between-group comparisons. All results were reported as Mean ± SD, and a significance level of $p < 0.05$ was considered. All research procedures—including analgesia, anesthesia, and animal care—were performed in accordance with ARRIVE Guidelines. No animal was euthanized without scientific necessity, and all samples were disposed of following environmental safety regulations.

3. Findings and Results

To accurately examine the effects of aerobic and resistance training on physiological and microbial indices in aged mice, it was first necessary to ensure group homogeneity and the validity of biological data. A comparison of baseline characteristics among the experimental groups showed that the mean initial body weight of the mice ranged from 243.6 g in the young control group to approximately 467 g in the aged groups; however, there were no statistically significant differences among the aged groups (Aged, Sham, Aero, and Res) ($P > 0.05$). Similarly, final body weight, daily food intake (approximately 20–21 g), and survival rate (100% in all groups) showed no significant differences. Laboratory quality control indicators—including DNA purity ratio (A260/A280 between 1.78 and 1.85), accuracy of qPCR melt curves (single peak, no contamination), and intra-plate coefficient of variation for irisin measurement (below 9% in all groups)—confirmed the high quality of generated data. These results indicate that the groups were homogeneous in baseline biological characteristics and that technical data quality was sufficient to support comparative statistical analyses.

Next, changes in plasma irisin levels among the five groups were reported. These data were measured using an ELISA kit and expressed as ng/mL. Table 1 presents the mean and standard deviation of irisin concentrations. Statistical differences among the groups were examined using two-way ANOVA, and significant differences were marked with a star.

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Table 1

Plasma Irisin Levels in Experimental Groups (ng/mL)

Group	Irisin Concentration (Mean ± SD)
Young Control (Ct)	8.32 ± 0.47 *
Aged Without Training (Aged)	5.71 ± 0.39
Sham Group (Sham)	5.85 ± 0.42
Aerobic Training (Aero)	7.26 ± 0.51 *
Resistance Training (Res)	7.91 ± 0.44 *

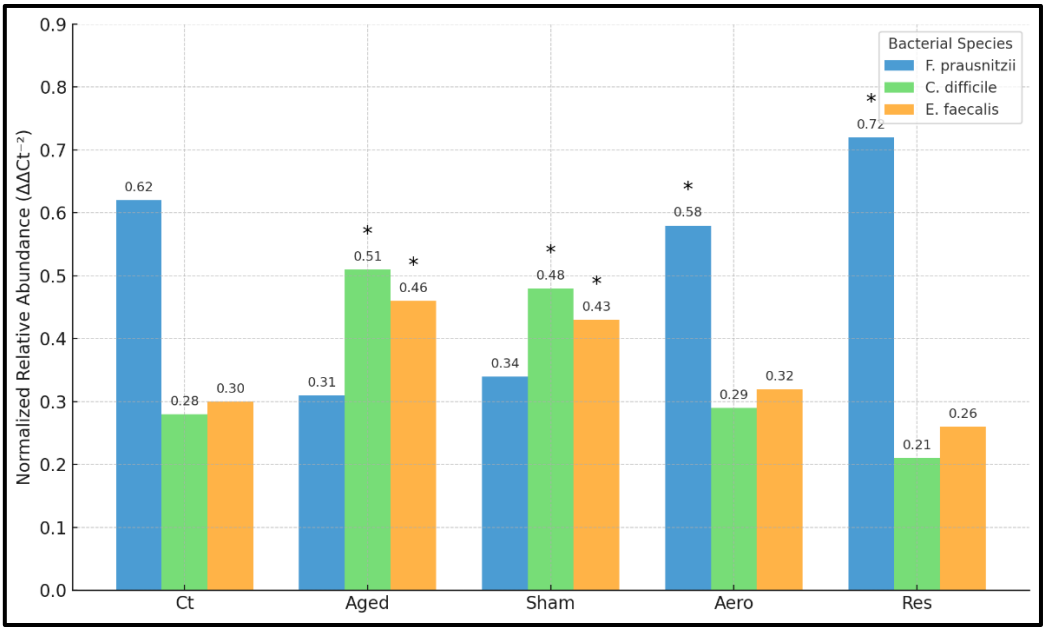
*indicates statistically significant difference compared with the Aged group at $p < 0.05$ according to Tukey's post hoc test.

The results showed that plasma irisin concentration in the trained groups (Aero and Res) was significantly higher than in the aged without training group (Aged) ($p < 0.05$). Additionally, irisin levels in the young control group (Ct) were higher than in aged animals, indicating a decline attributable to aging. The subsequent elevation in trained groups demonstrates the beneficial role of exercise—particularly resistance training—in improving myokine status in elderly subjects.

Figure 1 displays the comparison of normalized relative abundance of three key bacterial species—*Faecalibacterium prausnitzii* (anti-inflammatory), *Clostridium difficile* (pathogenic), and *Enterococcus faecalis* (opportunistic)—across the experimental groups (Ct, Aged, Sham, Aero, Res). These data were obtained from qPCR analysis using the 2^–ΔΔCt method, and between-group differences were assessed using ANOVA followed by Tukey's post hoc test.

Figure 1

Comparison of Normalized Relative Abundance of Faecalibacterium prausnitzii, Clostridium difficile, and Enterococcus faecalis Across Experimental Groups



* indicate statistically significant differences between groups (ANOVA + Tukey HSD, $p < 0.05$).

The results show that the resistance training group (Res) exhibited the greatest increase in *F. prausnitzii* abundance, while the aerobic training group (Aero) also showed significant improvement compared with non-exercising groups. In contrast, both inflammatory bacteria (*C. difficile* and *E. faecalis*) were reduced in the training groups, particularly in the Res group. These findings confirm that regular physical training—especially resistance training—

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can shift gut microbial balance toward beneficial species. Multi-group ANOVA with Tukey's test also confirmed that these differences were statistically significant ($p < 0.05$).

In the next section of the findings, composite ratios between beneficial and harmful bacterial species were examined as indicators of gut microbiome health across exercising and non-exercising conditions. Table 2 presents the ratio of *Faecalibacterium prausnitzii* (beneficial bacterium) to the sum of *Clostridium difficile* and *Enterococcus faecalis* (two harmful species), referred to as the F/BAD index. Additionally, the separate ratios F/Cd and F/Ef were included for more precise interpretation. These indices were compared among young (Ct), aged (Aged), sham (Sham), aerobic (Aero), and resistance (Res) groups.

Table 2

Composite Microbial Health Indices (F/BAD, F/Cd, F/Ef) in Experimental Groups (Mean ± SD)

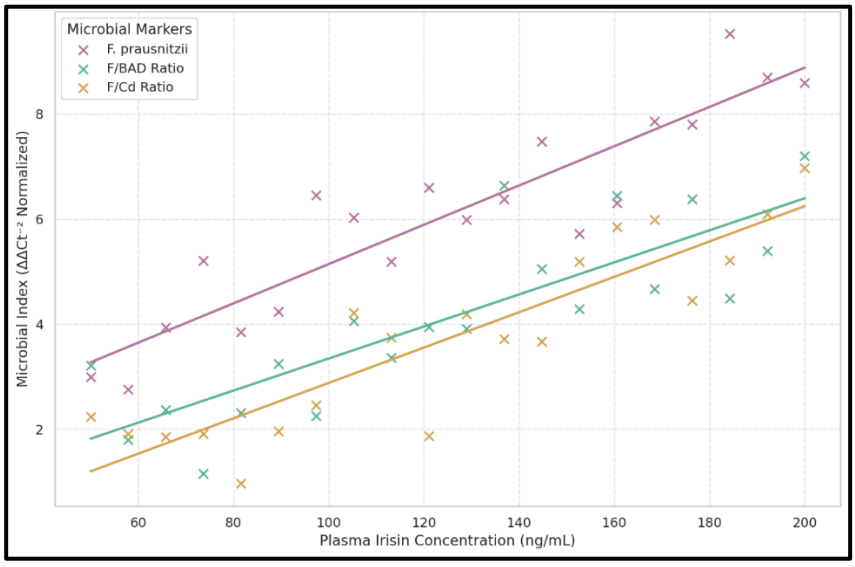
Index	Ct (Young)	Aged	Sham	Aero	Res	P-value
F/BAD	1.72 ± 0.28	0.94 ± 0.21	1.01 ± 0.19	1.48 ± 0.23	1.61 ± 0.25	0.004
F/Cd	3.21 ± 0.54	1.68 ± 0.39	1.75 ± 0.32	2.95 ± 0.48	3.28 ± 0.41	0.002
F/Ef	2.36 ± 0.47	1.03 ± 0.24	1.09 ± 0.28	2.07 ± 0.31	2.41 ± 0.36	0.006

The findings in Table 2 show that the F/BAD ratio in trained groups (Aero and Res) was significantly higher than in the aged (Aged) and sham groups ($p < 0.01$), indicating improved microbial balance and dominance of beneficial bacteria over inflammatory and pathogenic species due to exercise. The Res group also exhibited the highest F/Cd and F/Ef ratios, demonstrating the superior effectiveness of resistance training compared with aerobic training in enhancing gut microbiome health. Overall, these indices highlight the positive impact of regular exercise on restoring beneficial gut populations during aging.

To examine the relationship between plasma irisin levels and key gut microbiome indices, correlation analysis was performed. The analyzed indices included the beneficial bacterium *Faecalibacterium prausnitzii*, the composite ratio F/BAD (*F. prausnitzii* / (*C. difficile* + *E. faecalis*)), and the ratio F/Cd. Figure 2 illustrates these associations using scatterplots accompanied by linear regression lines for each index.

Figure 2

Correlation Between Irisin Levels and Microbial Indices



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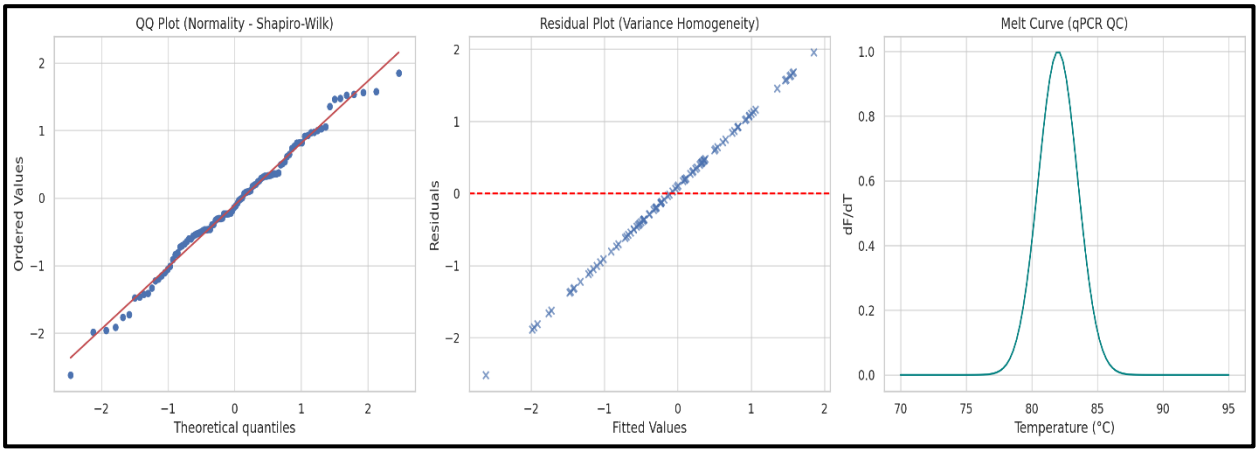
The scatterplots show a notable positive correlation between irisin levels and *Faecalibacterium prausnitzii* (purple), the F/BAD ratio (green), and the F/Cd ratio (orange). These findings confirm that increased irisin levels are associated with improved gut microbial ratios linked to intestinal health. Furthermore, the slope of the regression lines in all three indices reflects this reinforcing association and demonstrates an overall pattern of positive interaction along the "irisin–microbiome axis" in response to physical training in aged mice.

To ensure the validity of statistical analyses, assumption tests for normality, homogeneity of variance, absence of outliers, and biological data quality were conducted. Figure 3 presents the diagnostic plots related to these evaluations.

Figure 3

Diagnostic Plots for Assumption Testing and Data Quality Control



The results of these plots indicated that the data were normally distributed (QQ-plot pattern close to the diagonal line), the spread of residuals remained constant around zero (indicating homogeneity of variance), and the qPCR melt curve showed a single distinct peak with no contamination. Therefore, the assumptions required for parametric analyses were satisfied, and the overall data quality was at an optimal level.

4. Discussion

The present study investigated the differential effects of aerobic and resistance training on plasma irisin levels and gut microbiome composition in aged mice, with a particular focus on *Faecalibacterium prausnitzii*, *Clostridium difficile*, and *Enterococcus faecalis*. The findings revealed that both aerobic and resistance training significantly increased circulating irisin levels compared with the aged sedentary group, with resistance training inducing the most pronounced rise. This is consistent with evidence that aging reduces FNDC5/irisin expression in skeletal muscle, contributing to sarcopenia and diminished metabolic resilience (24). The observed increase in irisin following training suggests that muscular contraction–driven myokine release remains responsive even in late life and can be enhanced through structured exercise. This aligns with research demonstrating that exercise-induced myokine signaling regulates metabolic, inflammatory, and neural pathways relevant to aging (12, 16).

The elevation of irisin in the resistance training group is supported by previous findings that mechanical overload and load-bearing contractions produce more robust activation of FNDC5 transcription than endurance-type activity (28). Moreover, recent work in aged mice indicates that resistance training induces stronger irisin-related gene expression and exerts more potent regulatory effects on inflammatory mediators including TGF-β, NF-κB, and SIRT1 in intestinal lymphocytes (29). These molecular interactions may explain the substantial improvements observed in the present study, suggesting that resistance training is particularly effective in overcoming age-related declines in irisin. Given that irisin deficiency itself can induce gut dysbiosis and depressive-like behaviors through the gut–brain axis (25), restoring irisin levels through exercise may serve as a critical mechanism linking training to systemic health benefits in aging.

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The observed remodeling of gut microbiota in response to exercise further reinforces the role of physical activity in alleviating age-associated dysbiosis. The increased abundance of *F. prausnitzii* in both training groups, particularly resistance training, aligns with research showing that physically active older adults display enriched representation of this beneficial species (23). This bacterium is a major butyrate producer with anti-inflammatory activity, playing an essential role in maintaining epithelial barrier integrity, immunomodulation, and metabolic homeostasis (8, 9). Its decline in aging contributes to chronic inflammation, reduced SCFA availability, and metabolic vulnerability (3, 6). Therefore, the training-induced elevation of *F. prausnitzii* observed in this study suggests that both aerobic and resistance training can partially restore SCFA-related microbial function in older organisms.

Similarly, the significant reduction in *C. difficile* and *E. faecalis* following training has meaningful implications for aging physiology. Both species increase with age and contribute to intestinal inflammation, barrier disruption, and systemic immune activation (10, 11). Endurance exercise has been reported to reduce *C. difficile* abundance and improve gut health in older adults (10), which mirrors the improvements observed here. The more pronounced decrease in the resistance training group may be related to stronger irisin induction, as irisin modulates intestinal integrity and microbial composition directly (26). This suggests that myokine-mediated mechanisms, not only biomechanical effects of training, contribute to the suppression of harmful gut taxa in aging.

Composite microbial health indices (F/BAD, F/Cd, F/Ef) provided an integrated view of microbial balance, revealing that resistance training resulted in the most favorable profiles. These indices, which express beneficial-to-harmful bacterial ratios, are important because shifts in single taxa may not accurately reflect systemic gut health. The present results are consistent with studies showing that well-trained older adults exhibit higher SCFA-producer dominance relative to opportunistic pathogens (5, 22). The improvement in microbial balance in this study may reflect enhanced intestinal barrier function, reduced inflammation, increased SCFA availability, and a healthier metabolic milieu—all hallmarks of adaptive responses to sustained physical activity (1, 12).

An important aspect of the findings is the strong positive correlation between irisin levels and beneficial microbial indices (F/BAD, F/Cd, and *F. prausnitzii* abundance). This supports the emerging view of a bidirectional irisin–microbiome axis, in which muscle-derived myokines and gut microorganisms mutually influence each other's activity. Irisin has been shown to protect intestinal barrier function, reduce permeability, and shift gut microbial composition toward SCFA-producing taxa (26). Conversely, gut-derived metabolites such as butyrate promote mitochondrial biogenesis and muscle protein synthesis, potentially influencing irisin secretion (2, 4). This synergistic interaction provides a physiological explanation for the co-modulation observed in the present study, where both irisin and healthy microbiota improved most prominently in the resistance training group.

The divergence in the effects of aerobic versus resistance training is an important finding. Although both interventions improved microbiota and irisin to some extent, resistance training produced more substantial adaptations. This may be attributed to greater mechanical tension, higher recruitment of type II muscle fibers, and stronger induction of systemic anabolic signals—all of which are more severely compromised in aging (24). Additionally, resistance training can reduce oxidative stress and inflammatory signaling more effectively than moderate aerobic exercise (28). Taken together, these findings suggest that resistance training may be an especially potent modality for restoring both microbiome integrity and myokine function in older organisms.

The present study aligns with a growing body of evidence demonstrating that exercise interventions can reshape the aging microbiome independently of diet. Systematic reviews have shown consistent associations between physical activity and increased microbial diversity, enrichment of butyrate producers, and improvement of metabolic pathways (16-18). However, the precise microbial changes observed may depend on training intensity, duration, and modality. High-intensity aerobic exercise, for instance, has been shown to alter specific genera such as *Lactobacillus*, *Bifidobacterium*, and *E. coli* in older mice (30). Similarly, systematic reviews report that resistance training may generate stronger anti-inflammatory microbial shifts compared with aerobic exercise (27). The present findings

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support this pattern, demonstrating greater microbiome normalization under resistance training.

Moreover, endurance exercise is traditionally associated with systemic metabolic improvements, whereas resistance training has been less widely studied in relation to the gut microbiota. However, the present study adds to a small but growing literature highlighting the potential of resistance training to improve microbial ecosystems in aging. Given the complementary benefits of aerobic exercise in modulating metabolic efficiency and cardiorespiratory fitness (14, 15), both modalities likely contribute to different aspects of microbiome and irisin regulation. Nonetheless, resistance training appears particularly well suited for reversing age-related declines in muscle-derived signaling molecules and restoring beneficial microbial populations.

5. Conclusion

Taken together, the results of this study highlight the potential of structured exercise—particularly resistance training—to restore key biological axes disrupted during aging, including the gut microbiota, irisin signaling pathways, and the beneficial–harmful microbial balance. By demonstrating concurrent improvements in both microbial composition and myokine levels, this research underscores the mechanistic interplay between physical activity, intestinal health, and musculoskeletal resilience in aging. These findings further support the concept of a unified gut–muscle axis as a key target for geroscience interventions (2-4).

6. Limitations and Suggestions

This study, although methodologically rigorous, has several limitations. First, it was conducted on an animal model, which limits the direct generalizability of findings to humans, especially given interspecies differences in microbiome composition and myokine kinetics. Second, the study duration was limited to eight weeks; longer-term interventions may reveal more pronounced or different microbial adaptations. Third, while three key bacterial species were analyzed, the gut microbiome is highly complex, and interactions among hundreds of taxa were not captured. Fourth, dietary intake was controlled but not manipulated, preventing assessment of exercise–diet interactions. Finally, the study did not measure SCFA concentrations or intestinal permeability markers, which could have provided mechanistic insight into the functional consequences of microbial changes.

Future investigations should explore longer-duration interventions to evaluate the stability and long-term effects of exercise-induced microbial changes. Studies that combine exercise with dietary manipulations, such as high-fiber or probiotic supplementation, could help identify synergistic strategies for restoring gut health in aging. Multi-omics approaches—including metabolomics, transcriptomics, and proteomics—should be incorporated to assess functional pathways linking microbiota, irisin, inflammation, and musculoskeletal outcomes. Furthermore, a broader set of microbial species should be analyzed to capture the full ecological restructuring of the gut in response to different training modalities. Finally, clinical studies in older adults are needed to confirm the translational relevance of findings from animal models.

Exercise programs designed for older adults should prioritize resistance training as a primary modality to improve muscle health, irisin levels, and gut microbial balance. Incorporating moderate-intensity aerobic exercise may provide complementary benefits for cardiovascular and metabolic health. Practitioners should structure training routines that are progressive, feasible, and tailored to individual fitness levels to ensure safety and maximize biological responsiveness. Additionally, evaluating gut health indicators in aging individuals may help personalize exercise interventions and better monitor improvements over time.

Authors' Contributions

M. E. S. coordinated the study design, supervised animal handling, and ensured methodological accuracy throughout the intervention period. J. B. B. led the laboratory analyses, including ELISA and qPCR procedures, and contributed to statistical modeling and interpretation of microbiome–irisin relationships. H. M. assisted in implementing aerobic and resistance training protocols, monitoring physiological responses, and organizing data collection. L. S. contributed to data analysis, synthesis of findings, and preparation and critical revision of the manuscript. All authors reviewed and approved the final version of the article.

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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

This study was conducted in accordance with institutional and national guidelines for the care and use of laboratory animals. All experimental procedures were reviewed and approved by the Ethics Committee of Islamic Azad University, Najafabad Branch (Approval Code: IR.IAU.NAJAFABAD.REC.1404.098). Efforts were made to minimize animal discomfort and reduce the number of animals used.

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