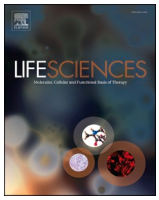
Life Sciences 404 (2026) 124646

Contents lists available at ScienceDirect

Life Sciences

journal homepage: www.elsevier.com/locate/lifescie



High-protein intake shapes physiological adaptation and gut microbiota in response to resistance training

ARTICLE INFO

Keywords:
High-protein diet
Progressive resistance training
Physical performance
Gut microbiota
Metabolic adaptation

ABSTRACT

Aims: High-protein diets stimulate muscle anabolism and influence gut microbiota composition, while resistance training improves neuromuscular function and metabolic health. We investigated whether dietary protein acts as the primary driver of physiological and microbial adaptations, and whether resistance training provides additional benefits.

Materials and methods: Twenty-four male C57BL/6 mice were randomly assigned to four groups ($n = 6$ per group): normal control (NC), high-protein diet (HPD), normal diet with exercise (NC + EX), and high-protein diet with exercise (HPD + EX). The HPD provided 60% of total energy from protein. Resistance training consisted of progressive ladder-climbing performed three times per week for eight weeks. Physical performance was evaluated by grip strength, weight-holding capacity, rotarod performance, and functional strength performance. Gut microbiota was analyzed using 16S rRNA sequencing, and blood and tissue samples were collected for biochemical and histological analyses.

Key findings: Compared with NC, the HPD + EX group exhibited significantly enhanced physical performance, and reduced mesenteric fat mass and adipocyte size ($p < 0.05$). HPD alone decreased gut microbial richness, whereas the HPD + EX group did not differ significantly from the HPD group in microbial richness or overall microbiota composition. LEfSe analysis identified higher relative abundances of selected genera, including *Lactobacillus* and *Faecalibaculum*, in the HPD + EX group than in the HPD group.

Significance: These findings identify dietary protein as a key determinant of physiological and gut microbial adaptations, while resistance training primarily improves physical performance and may influence selected microbial taxa without restoring or stabilizing the overall microbial community. Together, these findings suggest that dietary protein and resistance training may play distinct and complementary roles in host metabolic regulation and physical function.

1. Introduction

With the growing global awareness of health, fitness, and body composition management, dietary strategies aimed at supporting training adaptation and metabolic health have received increasing attention. Diet and exercise are recognized as two interrelated lifestyle factors that play complementary roles in supporting overall physiological health and functional capacity [1,2]. Among various exercise modalities, resistance training is widely acknowledged for its efficacy in improving musculoskeletal strength, functional capacity, and metabolic resilience [3]. High-intensity exercise, particularly resistance training, induces skeletal muscle damage and increases protein turnover, thereby elevating the demand for effective nutritional support to facilitate recovery and physiological adaptation [4,5]. Protein plays a central role in repairing exercise-induced muscle damage, promoting hypertrophy, and regulating metabolic signaling [6,7]. High-protein diets have been consistently shown to enhance muscle protein synthesis when combined with resistance training, while also preserving muscle mass during caloric restriction and mitigating age-related declines in muscle function [8]. As such, strategic protein supplementation is widely adopted to support post-exercise recovery and optimize training adaptations.

In recent years, the gut microbiota has been increasingly recognized as a critical mediator of host metabolic health, with mechanistic insights underscoring its involvement in energy regulation, immune homeostasis, and nutrient metabolism [9,10]. While the beneficial effects of high-protein diets on muscle protein synthesis and physical adaptation are well established, recent studies have begun to explore their broader systemic impacts through microbiota-mediated mechanisms [11]. Dietary protein not only supports anabolic processes in host tissues but also influences the composition and functional activity of the gut microbiota. This complex microbial ecosystem is highly diverse and metabolically active, and it plays a central role in maintaining physiological homeostasis [12]. These findings suggest that gut microbes may act as intermediaries in mediating the systemic effects of dietary patterns. Importantly, both dietary patterns and physical activity are recognized as major modulators of gut microbial ecology, and their interaction may contribute to physiological adaptation and performance outcomes in physically active individuals [13,14]. Understanding these relationships is essential for optimizing dietary strategies aimed at enhancing performance while preserving systemic health. Resistance training imposes physiological demands distinct from those of aerobic exercise, including greater mechanical loading, increased protein turnover, and unique recovery processes [15]. These training-specific characteristics may influence the gut environment and host adaptations in

https://doi.org/10.1016/j.lfs.2026.124646
Received 24 March 2026; Received in revised form 18 August 2026; Accepted 19 August 2026
Available online 22 August 2026

ways not observed with endurance-based exercise. Investigating how protein intake shapes microbial composition and host physiology under resistance training conditions may provide new insights into how combined nutrition and training approaches influence health and performance outcomes.

Therefore, the present study investigated the effects of a high-protein diet combined with progressive resistance training on muscular performance, adiposity, and gut microbiota composition. Muscular performance and adiposity were selected as key physiological outcomes because they represent functional adaptations and metabolic status in response to dietary protein intake and resistance training. The resistance training protocol was designed to mimic progressive overload resistance exercise commonly performed in human strength training. This integrative approach aimed to clarify how high-protein intake and progressive resistance training influence physiological adaptations and gut microbiota composition, providing further insight into the interactions between nutrition, exercise, and metabolic health.

2. Materials and methods

2.1. Experimental design

Eight-week-old male C57BL/6 mice were obtained from BioLASCO (Taipei, Taiwan). All animals were housed in the animal facility of the Graduate Institute of Sport Science at National Taiwan Sport University under controlled conditions: a temperature of 22 ± 2 °C, relative humidity of 60 ± 10%, and a 12-h light/dark cycle. During the experiment, mice were fed either standard laboratory chow (LabDiet 5001; LabDiet, St. Louis, MO, USA; 3.02 kcal/g; 28.5 kcal% protein, 58 kcal% carbohydrate, and 13.5 kcal% fat) or a high-protein diet (D13110701; Research Diets, Inc., New Brunswick, NJ, USA; 3.81 kcal/g; 60 kcal% protein, 25 kcal% carbohydrate, and 15 kcal% fat). Water was provided ad libitum. Food intake and body weight were monitored weekly throughout the intervention. All animal procedures were approved by the Institutional Animal Care and Use Committee (IACUC) of National Taiwan Sport University, Taoyuan City, Taiwan (approval number: IACUC No. 10820).

After a one-week acclimation period, 24 mice were randomly assigned to one of four groups based on body weight: (1) normal control group (NC), (2) high protein diet group (HPD), (3) normal control + exercise training group (NC + EX), and (4) high protein diet + exercise training group (HPD + EX). The intervention lasted for 8 weeks. At the end of the study period, the mice were subjected to a battery of physical performance tests, including grip strength assessment, body weight recording, rotarod balance, and grip force retention evaluation. Following these assessments, the mice were sacrificed using carbon dioxide.

2.2. Progressive resistance training

A progressive resistance training protocol using a weighted sled-pulling system was implemented as previously described [16]. The resistance training protocol used in this study was designed to mimic progressive overload resistance exercise commonly performed in human strength training and to allow investigation of physiological adaptations to resistance exercise under controlled dietary conditions.

Prior to the training intervention, mice were familiarized with the sled-pulling apparatus to reduce stress and ensure proper task performance. The maximal pulling strength of each mouse was then determined and defined as the one-repetition maximum (1RM). For the 1RM test, the initial load was set at 300 g and increased by 50 g after each successful pull until the mouse could no longer complete the full pulling distance without assistance. The maximum load that could be pulled through the full track without assistance was recorded as the 1RM. During the 8-week intervention, mice performed resistance training three times per week. Training loads were prescribed relative to each mouse's individual 1RM (%1RM), and 1RM was reassessed weekly to adjust training loads and maintain relative intensity across animals. Each session consisted of 8–12 weighted sled-pulling bouts with a 2-min rest interval between bouts. Within each training session, the load was progressively increased from approximately 50% to 100% of each mouse's 1RM, with the final bouts performed at maximal load. If a mouse failed to initiate movement after three gentle tactile prompts, a single 1 mA electrical stimulus was applied to encourage task completion.

2.3. Forelimb grip strength

Forelimb grip strength was measured using a grip strength meter (Model RX-5, Aikoh Engineering, Nagoya, Japan) following previously described procedures [17]. Mice grasped a metal grid with their forepaws and were gently pulled backward by the tail until release. Each mouse performed 10 trials, and the maximum peak force was used for analysis.

2.4. Weights test

The weights test was performed to evaluate forelimb strength and endurance following a modified protocol described previously [18]. A 15 g steel wool ball served as the base weight, and additional 15 g chain links were added to progressively increase the load. Each stage required three successful lifts within 3 s, with a 10-s rest between stages. The test ended when the mouse failed to lift the weight across three attempts. Final scores were calculated based on completed stages as previously described [18].

2.5. Rotarod balance test

Rotarod performance was evaluated using a motorized rotarod apparatus (ROTAROD LE82050, Panlab, Barcelona, Spain), following previously established protocols [19]. Mice were placed individually on the rotating rod, which was set to an initial speed of 4 rpm and progressively accelerated to 40 rpm over a 5-min period. The latency to fall (in seconds) and the corresponding rotational speed (rpm) at the time of falling were recorded for each mouse.

2.6. Functional strength test

Functional strength was assessed to evaluate isometric grip endurance, following a previously established protocol [20]. Each mouse was equipped with a lead weight equivalent to 10% of its body weight, which was affixed to the dorsal surface. Mice were then allowed to grasp a vertical wire mesh with their forepaws. Timing began immediately after the load was applied, and the maximum duration the mouse could maintain its grip before releasing both hind limbs was recorded.

2.7. Bacterial DNA extraction and 16S rRNA sequencing

Cecal samples were collected at the time of sacrifice for gut microbiota analysis and immediately stored at −80 °C. Bacterial DNA was isolated using the cetyltrimethylammonium bromide/sodium dodecyl sulfate (CTAB/SDS) method and assessed for concentration and purity via 1% agarose gel electrophoresis. Extracted DNA was kept at −80 °C until further analysis. The hypervariable V3–V4 region of the bacterial 16S rRNA gene was amplified by polymerase chain reaction (PCR) using barcoded universal primers 341F (5'-CCTAYGGGRBGCASCAG-3′) and 806R (5'-GGACTACNNGGGTATCTAAT-3′). Amplicon libraries were prepared using the TruSeq DNA PCR-Free Sample Preparation Kit (Illumina, San Diego, CA, USA) with an average insert size of 450–470 bp. Library preparation and paired-end high-throughput sequencing were performed by BIOTOOLS Co., Ltd. (New Taipei City, Taiwan) on the Illumina HiSeq2500 platform.

2.8. Haematological assessment

At the end of the experimental period, mice were euthanized using carbon dioxide, and blood samples were collected via cardiac puncture. The collected blood was centrifuged at 1500 ×*g* for 15 min at 4 °C to separate the serum. Serum levels of glucose (GLU), blood urea nitrogen (BUN), creatinine (CREA), uric acid (UA), glutamic oxaloacetic transaminase (GOT), glutamic pyruvic transaminase (GPT), total protein (TP), albumin (ALB), and creatine kinase (CK) were subsequently analyzed.

2.9. Histological staining of tissue

Following sacrifice, major organs including the liver, kidney, skeletal muscle, brown adipose tissue (BAT), and epididymal fat pad (EFP) were carefully excised and weighed. Tissues were fixed in 10% neutral-buffered formalin, dehydrated, and embedded in paraffin. Sections were cut at a thickness of 4 μm and stained with hematoxylin and eosin (H&E) for histopathological evaluation. Stained slides were examined under a light microscope equipped with a charge-coupled device (CCD) camera (BX-51; Olympus, Tokyo, Japan) by a clinical pathologist. Adipocyte size in the EFP was analyzed using H&*E*-stained sections, where intracellular lipid vacuoles were manually traced, and cross-sectional areas were quantified using ImageJ software (National Institutes of Health, Bethesda, MD, USA). For skeletal muscle analysis, H&*E*-stained gastrocnemius and quadriceps sections were photographed under light microscopy at 200× magnification. The average fiber cross-sectional area (CSA, μm^2) was quantified with reference to the H & E staining results for each group observed using ImageJ software (NIH, USA).

2.10. Glycogen measurement

After sacrifice, liver and skeletal muscle tissues were collected. Approximately 45–50 mg of each tissue was weighed and immediately stored at −80 °C for subsequent glycogen analysis. Tissues were placed in microcentrifuge tubes and homogenized in five volumes of homogenization buffer using a tissue homogenizer (Bullet Blender, Next Advance, Cambridge, MA, USA). The homogenates were then centrifuged at 15,000 ×*g* for 15 min at 4 °C, and the supernatants were collected. Glycogen content was determined by transferring the supernatants and glycogen standards into a 96-well microplate, followed by the addition of reaction reagents. Absorbance was measured at 460 nm using a microplate spectrophotometer, and glycogen levels were quantified based on a standard curve.

2.11. Statistical analysis

All data are expressed as mean ± standard deviation (SD), with $n = 6$ mice per group unless otherwise specified. For gut microbiota analysis, cecal samples from four mice per group ($n = 4$) were randomly selected for 16S rRNA sequencing analysis. Body weight changes over time were analyzed using two-way mixed ANOVA, with time as the within-subject factor and group as the between-subject factor.

Statistical analyses of physiological and biochemical parameters were performed using one-way analysis of variance (ANOVA) followed by Duncan's multiple range test to determine differences among the four treatment groups. In addition, a two-way ANOVA was performed to evaluate the main effects of diet (D), exercise (E), and their interaction (D × E). When a significant diet × exercise interaction was observed, the interaction effect was interpreted and reported. When no significant interaction was observed, the main effects of diet and exercise were reported where appropriate. Analyses were conducted using SPSS Statistics for Windows, Version 20.0 (IBM Corp., Armonk, NY, USA), and statistical significance was defined as $p < 0.05$.

For gut microbiota analysis, both alpha and beta diversity were evaluated. Alpha diversity metrics, including the observed number of operational taxonomic units (OTUs) and the Shannon diversity index, were compared using the Kruskal–Wallis test, followed by Dunn's post hoc test. Beta diversity was assessed using non-metric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarity matrices and visualized via QIIME software. Differences in beta diversity among groups were tested using permutational multivariate analysis of variance (PERMANOVA). Bray–Curtis distances and the relative abundance of selected bacterial taxa were compared using the Kruskal–Wallis test followed by Dunn's post hoc test. These non-parametric tests were performed using GraphPad Prism (version 9.3.1; La Jolla, CA, USA), and a p-value <0.05 was considered statistically significant. Additionally, the linear discriminant analysis effect size (LEfSe) algorithm was applied to identify differentially abundant bacterial taxa across groups. Taxa with a logarithmic LDA score ≥ 4.0 and Kruskal–Wallis $p < 0.05$ were considered significantly discriminative.

3. Results

3.1. Effects of high-protein intake and progressive resistance training on body weight and food consumption

Weekly changes in body weight throughout the experimental period are presented in Fig. 1. Before the intervention, the body weights of the NC, HPD, NC + EX, and HPD + EX groups were 23.1 ± 1.2, 23.3 ± 1.1, 22.9 ± 1.0, and 23.0 ± 0.6 g, respectively. Over the 8-week intervention, body weight increased steadily in all groups. Two-way mixed ANOVA revealed a significant main effect of time ($p < 0.001$, $\varepsilon = 0.274$), indicating progressive body weight gain throughout the study period. However, the main effect of group was not significant ($p = 0.770$), and no significant time × group interaction was observed ($p = 0.364$, $\varepsilon = 0.274$).

Throughout the study, mice were provided ad libitum access to food and water. Average daily food and water intake per mouse are summarized in Table 1. The average food intake for the NC, HPD, NC + EX, and HPD + EX groups was 5.7 ± 0.6, 3.4 ± 0.3, 5.4 ± 0.4, and 3.1 ± 0.3 g/mouse/day, respectively. One-way ANOVA showed significant differences among all groups ($p < 0.001$), with the lowest intake observed in the HPD + EX group. Two-way ANOVA further revealed significant main effects of diet and exercise (both $p < 0.001$) on food intake, whereas the diet × exercise interaction ($p = 0.426$) was not significant.

Similarly, daily caloric intake was 19.2 ± 2.1, 12.8 ± 1.1, 18.0 ± 1.2, and 11.8 ± 1.0 kcal/mouse/day in the NC, HPD, NC + EX, and HPD + EX groups, respectively. Caloric intake differed significantly among all groups ($p < 0.001$), and two-way ANOVA showed significant main effects of diet and exercise (both $p < 0.001$), with no significant diet × exercise interaction ($p = 0.663$).

In contrast, the average daily water intake in the NC, HPD, NC + EX, and HPD + EX groups was 6.0 ± 0.8, 7.7 ± 0.8, 6.2 ± 0.7, and 6.6 ± 0.9 mL, respectively. Water intake was significantly higher in the HPD group than in the other groups ($p < 0.001$), while the HPD + EX group was higher than the NC and NC + EX groups but lower than the HPD group. Two-way ANOVA revealed a significant diet × exercise interaction ($p < 0.001$) for water intake.

3.2. Effects of high-protein intake and progressive resistance training on adiposity and organ weights

Organ and tissue weights are presented in Table 2. One-way ANOVA showed that absolute and relative mesenteric fat weights were

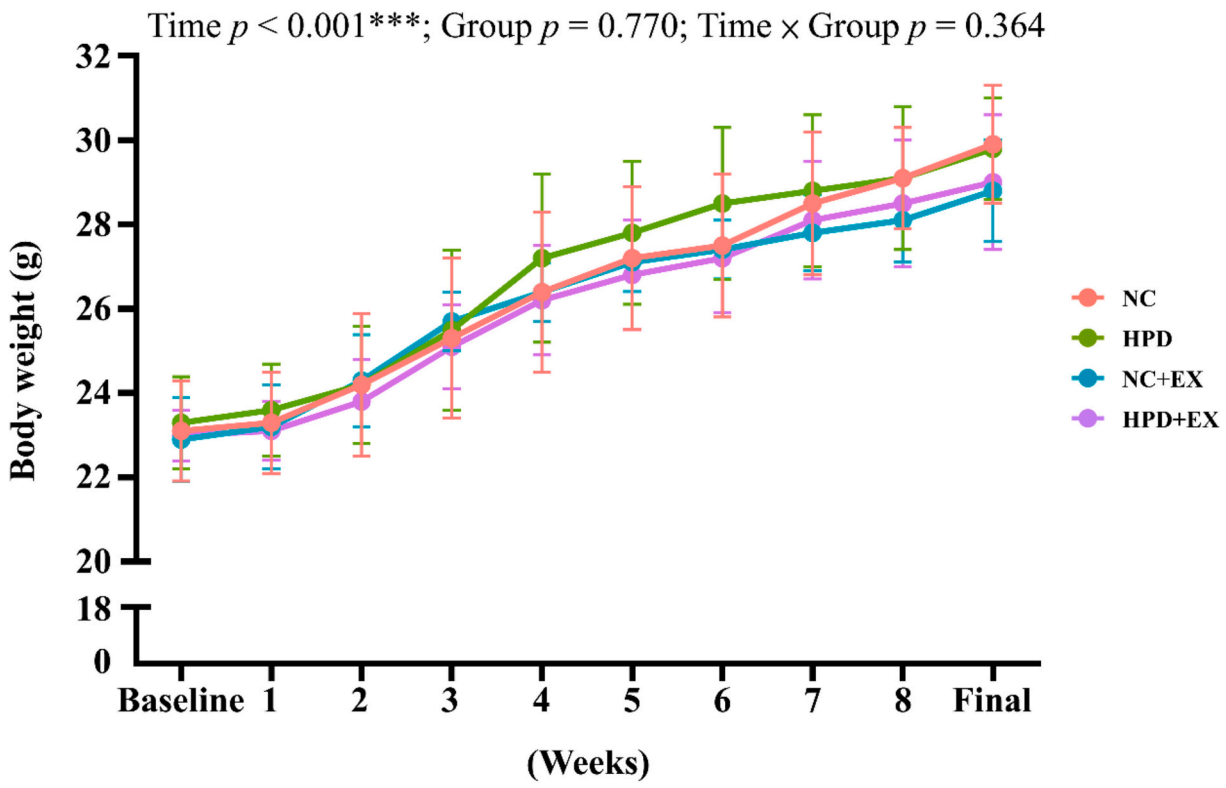


Fig. 1. Effects of High-Protein Intake and Progressive Resistance Training on body weight. Mice were divided into four groups: NC, HPD, NC + EX, and HPD + EX ($n = 6$ per group). Data are presented as mean ± SD. Body weight changes were analyzed using two-way mixed ANOVA, with time as the within-subject factor and group as the between-subject factor. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

Table 1
Effects of high-protein intake and progressive resistance training on dietary intake and water consumption.

Characteristic	NC	HPD	NC + EX	HPD + EX	*p* value		
					E	D	E × D
Diet intake (g/mouse/day)	5.7 ± 0.6^{d}	3.4 ± 0.3^{b}	5.4 ± 0.4^{c}	3.1 ± 0.3^{a}	<0.001***	<0.001***	0.426
Diet intake (kcal/mouse/day)	19.2 ± 2.1^{d}	12.8 ± 1.1^{b}	18.0 ± 1.2^{c}	11.8 ± 1.0^{a}	<0.001***	<0.001***	0.663
Water intake (mL/mouse/day)	6.0 ± 0.8^{a}	7.7 ± 0.8^{c}	6.2 ± 0.7^{a}	6.6 ± 0.9^{b}	<0.001***	<0.001***	<0.001***

Mice were divided into four groups: NC, HPD, NC + EX, and HPD + EX ($n = 6$ per group). Data are presented as mean ± SD. Different superscript letters (a, b, c, d) indicate significant differences among groups based on one-way ANOVA followed by Duncan's multiple range test ($p < 0.05$). The *p* values for exercise (E), diet (D), and their interaction (E × D) were obtained from two-way ANOVA and represent the main effects of exercise, diet, and their interaction, respectively. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

significantly lower in the HPD, NC + EX, and HPD + EX groups than in the NC group ($p = 0.003$ and $p = 0.004$). Two-way ANOVA revealed significant diet × exercise interactions for absolute and relative mesenteric fat weights ($p = 0.007$ and $p = 0.006$). For variables without significant interactions, main effects were reported, with a significant exercise effect observed for relative BAT weight ($p = 0.048$). No significant differences were found in other organ or tissue weights.

3.3. Effects of high-protein intake and progressive resistance training on physical performance

Following the 8-week intervention, multiple parameters of physical performance were evaluated (Fig. 2). Forelimb grip strength is shown in Fig. 2A. Relative grip strength, expressed as a percentage of body weight (g/g × 100), increased from 408.38 ± 27.96% in the NC group to 411.61 ± 37.65%, 438.05 ± 29.06%, and 442.86 ± 12.98% in the HPD, NC + EX, and HPD + EX groups, respectively. Although no significant differences were observed among the four groups by one-way ANOVA ($p = 0.103$), two-way ANOVA showed a significant main effect of exercise on relative grip strength ($p = 0.016$), with no significant diet effect ($p = 0.732$) or diet × exercise interaction ($p = 0.946$).

Weights test performance is shown in Fig. 2B. Mice in the NC, HPD, NC + EX, and HPD + EX groups achieved average hold durations of 6.83 ± 1.47, 7.17 ± 1.17, 10.67 ± 2.34, and 12.33 ± 1.51 s, respectively. The NC + EX and HPD + EX groups performed significantly better than the NC and HPD groups ($p < 0.001$), with no significant difference between the two exercise groups. Two-way ANOVA further revealed a significant main effect of exercise ($p < 0.001$), while diet ($p = 0.160$) and diet × exercise interaction ($p = 0.342$) were not significant.

The rotarod balance test results are shown in Fig. 2C. The average balance time for the NC, HPD, NC + EX, and HPD + EX groups was 57.50 ± 20.23, 58.17 ± 13.08, 72.94 ± 10.03, and 83.50 ± 9.50 s, respectively. The HPD + EX group exhibited significantly higher balance performance than the NC and HPD groups ($p = 0.010$), whereas the NC + EX group did not differ significantly from the other groups. Two-way ANOVA showed a significant main effect of exercise ($p = 0.002$), with no significant diet effect ($p = 0.334$) or diet × exercise interaction ($p = 0.393$).

Table 2
Effects of high-protein intake and progressive resistance training on organ weights.

Characteristic	NC	HPD	NC + EX	HPD + EX	*p* value		
					E	D	E × D
Weight(g)							
Liver (g)	1.59 ± 0.19[a]	1.63 ± 0.20[a]	1.45 ± 0.18[a]	1.57 ± 0.14[a]	0.175	0.283	0.560
BAT (g)	0.07 ± 0.02[a]	0.07 ± 0.02[a]	0.08 ± 0.01[a]	0.09 ± 0.01[a]	0.135	1.000	1.000
EFP (g)	0.35 ± 0.07[a]	0.33 ± 0.08[a]	0.32 ± 0.09[a]	0.33 ± 0.10[a]	0.619	0.906	0.687
Perirenal fat (g)	0.14 ± 0.03[a]	0.15 ± 0.04[a]	0.15 ± 0.05[a]	0.17 ± 0.05[a]	0.580	0.462	0.853
Mesenteric fat (g)	0.52 ± 0.14[b]	0.30 ± 0.05[a]	0.30 ± 0.14[a]	0.33 ± 0.05[a]	0.043*	0.034*	0.007**
Total fat (g)	1.01 ± 0.17[a]	0.78 ± 0.12[a]	0.77 ± 0.25[a]	0.83 ± 0.17[a]	0.208	0.265	0.070
Gas (g)	0.37 ± 0.05[a]	0.36 ± 0.02[a]	0.35 ± 0.02[a]	0.34 ± 0.03[a]	0.262	0.548	0.947
QUA (g)	0.47 ± 0.05[a]	0.47 ± 0.05[a]	0.44 ± 0.03[a]	0.46 ± 0.03[a]	0.247	0.558	0.844
Relative tissue weight (%)							
Liver (%)	5.35 ± 0.69[a]	5.46 ± 0.68[a]	5.02 ± 0.59[a]	5.39 ± 0.25[a]	0.423	0.321	0.587
BAT (%)	0.25 ± 0.04[a]	0.25 ± 0.05[a]	0.28 ± 0.02[a]	0.28 ± 0.04[a]	0.048*	0.985	0.959
EFP (%)	1.17 ± 0.23[a]	1.11 ± 0.27[a]	1.10 ± 0.28[a]	1.14 ± 0.35[a]	0.837	0.919	0.699
Perirenal fat (%)	0.48 ± 0.10[a]	0.51 ± 0.14[a]	0.52 ± 0.17[a]	0.58 ± 0.17[a]	0.420	0.466	0.844
Mesenteric fat (%)	1.75 ± 0.45[b]	0.99 ± 0.15[a]	1.04 ± 0.45[a]	1.16 ± 0.23[a]	0.072	0.036*	0.006**
Total fat (g)	3.40 ± 0.54[a]	2.62 ± 0.39[a]	2.66 ± 0.83[a]	2.87 ± 0.64[a]	0.345	0.272	0.064
Gas (%)	1.22 ± 0.12[a]	1.20 ± 0.03[a]	1.22 ± 0.06[a]	1.19 ± 0.12[a]	0.834	0.464	0.833
QUA (%)	1.56 ± 0.10[a]	1.59 ± 0.13[a]	1.54 ± 0.09[a]	1.58 ± 0.10[a]	0.701	0.501	0.852

Mice were divided into four groups: NC, HPD, NC + EX, and HPD + EX ($n = 6$ per group). Data are presented as mean ± SD. Different superscript letters (a, b) indicate statistically significant differences among groups based on one-way ANOVA followed by Duncan's multiple range test ($p < 0.05$). The p values for exercise (E), diet (D), and their interaction (E × D) were obtained from two-way ANOVA and represent the main effects of exercise, diet, and their interaction, respectively. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. Relative tissue weight (%) = tissue weight / body weight (g) × 100%. Tissues analyzed include liver, brown adipose tissue (BAT), epididymal fat pad (EFP), gastrocnemius muscle (Gas), and quadriceps muscle (QUA).

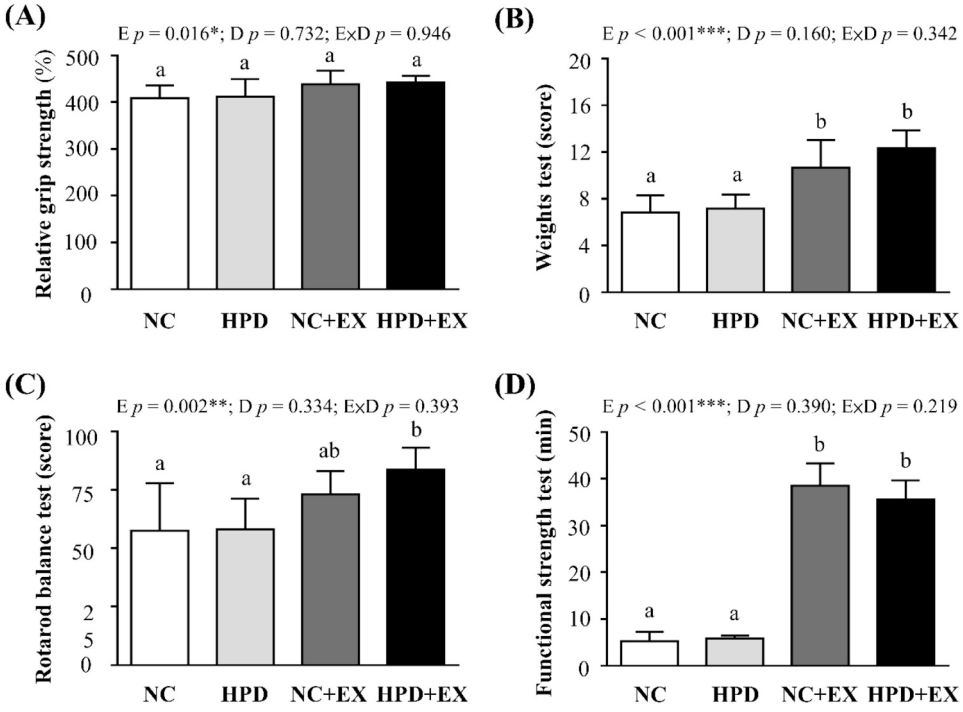


Fig. 2. Effects of High-Protein Intake and Progressive Resistance Training on (A) Relative grip strength. (B) weights test. (C) rotarod balance performance. (D) functional strength test. Mice were divided into four groups: NC, HPD, NC + EX, and HPD + EX ($n = 6$ per group). Data are presented as mean ± SD. Different superscript letters (a, b) indicate statistically significant differences among groups based on one-way ANOVA followed by Duncan's multiple range test ($p < 0.05$). The p values for exercise (E), diet (D), and their interaction (E × D) were obtained from two-way ANOVA and represent the main effects of exercise, diet, and their interaction, respectively. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

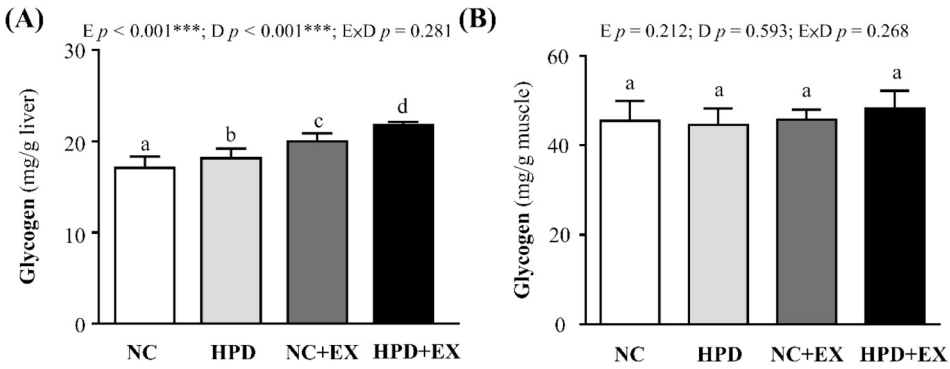


Fig. 3. Effects of High-Protein Intake and Progressive Resistance Training on glycogen storage. (A) Hepatic glycogen content. (B) Muscle glycogen content. Mice were divided into four groups: NC, HPD, NC + EX, and HPD + EX ($n = 6$ per group). Data are presented as mean ± SD. Different superscript letters (a, b, c, d) indicate statistically significant differences among groups based on one-way ANOVA followed by Duncan's multiple range test ($p < 0.05$). The p values for exercise (E), diet (D), and their interaction (E × D) were obtained from two-way ANOVA and represent the main effects of exercise, diet, and their interaction, respectively. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

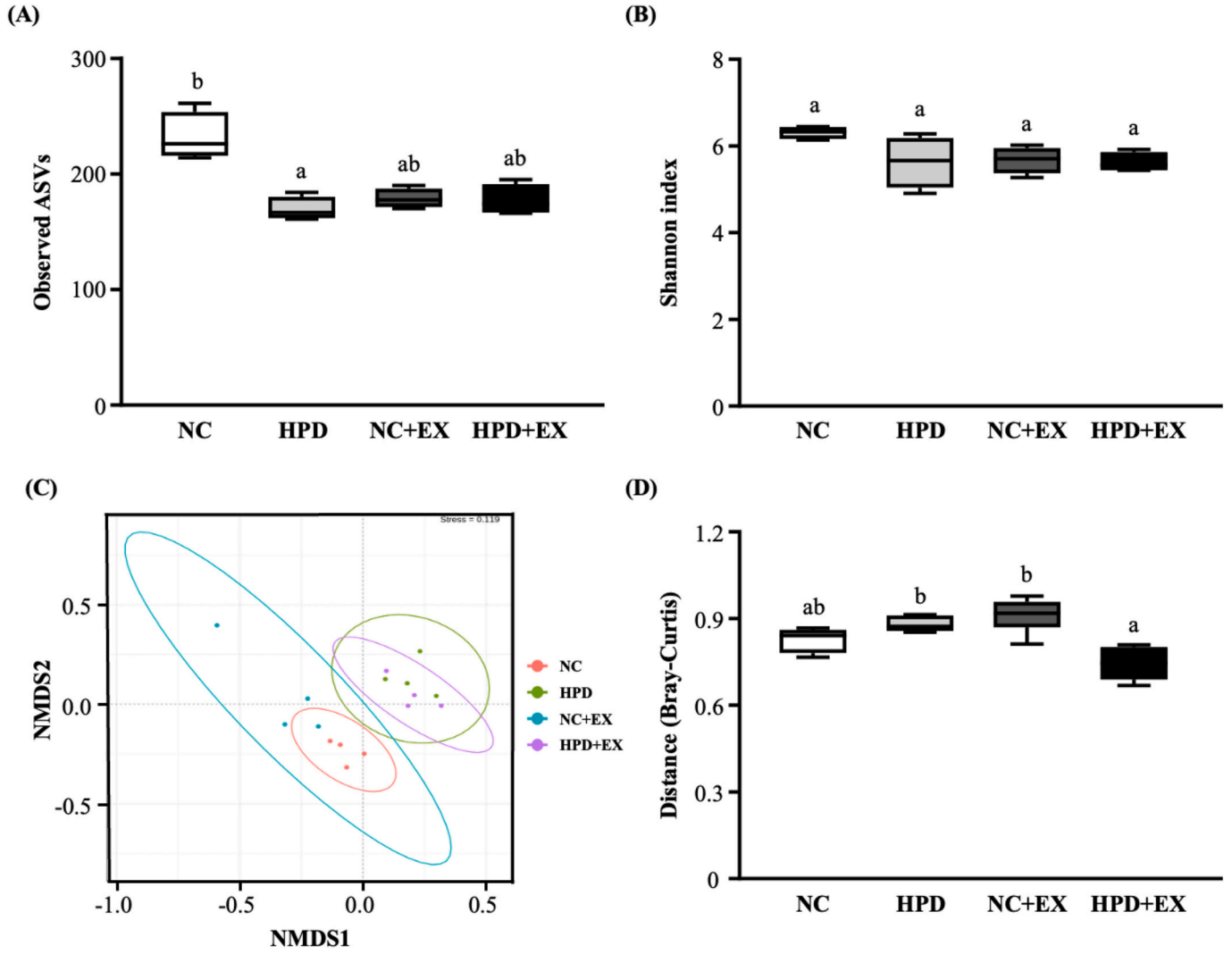


Fig. 4. Effects of high-protein intake and progressive resistance training on gut microbial diversity. (A) Observed amplicon sequence variants (ASVs). (B) Shannon diversity index. (C) Non-metric multidimensional scaling (NMDS) based on Bray–Curtis dissimilarities. Differences in overall microbial composition were evaluated using PERMANOVA with adjustment for multiple pairwise comparisons. (D) Inter-individual Bray–Curtis distances among groups. Mice were divided into four groups: NC, HPD, NC + EX, and HPD + EX ($n = 4$ per group). Data are presented as box-and-whisker plots. Different superscript letters (a, b) indicate statistically significant differences among groups based on the Kruskal–Wallis test followed by Dunn's post hoc test ($p < 0.05$).

The Functional Strength Test results are presented in Fig. 2D. The average endurance performance for the NC, HPD, NC + EX, and HPD + EX groups was 5.28 ± 1.95, 5.80 ± 0.63, 38.51 ± 4.81, and 35.62 ± 4.04 m, respectively. The NC + EX and HPD + EX groups showed significantly greater endurance than the NC and HPD groups ($p < 0.001$), while no significant difference was observed between the two exercise groups. Two-way ANOVA further indicated a significant main effect of exercise ($p < 0.001$), whereas diet ($p = 0.390$) and diet × exercise interaction ($p = 0.219$) were not significant.

3.4. Effects of high-protein intake and progressive resistance training on glycogen content

The glycogen concentrations in hepatic and muscle tissues following the intervention are presented in Fig. 3. Fig. 3A shows hepatic glycogen levels, which were 17.21 ± 1.11, 18.28 ± 0.93, 20.12 ± 0.74, and 21.93 ± 0.18 mg/g in the NC, HPD, NC + EX, and HPD + EX groups, respectively. One-way ANOVA showed that hepatic glycogen content was significantly increased in the HPD, NC + EX, and HPD + EX groups compared with the NC group ($p < 0.001$). In addition, the HPD + EX group showed the highest hepatic glycogen level among all groups. Two-way ANOVA further revealed significant main effects of exercise and diet on hepatic glycogen content (both $p < 0.001$), whereas the diet × exercise interaction was not significant ($p = 0.281$). Fig. 3B shows muscle glycogen levels, which were 45.47 ± 4.42, 44.58 ± 3.66, 45.70 ± 2.30, and 48.22 ± 3.96 mg/g in the NC, HPD, NC + EX, and HPD + EX groups, respectively. No statistically significant differences were observed among groups ($p = 0.379$). Consistently, two-way ANOVA showed no significant main effects of exercise ($p = 0.212$) or diet ($p = 0.593$) and no significant diet × exercise interaction ($p = 0.268$) on muscle glycogen content.

3.5. Effects of high-protein intake and progressive resistance training on gut microbiota composition

Observed amplicon sequence variants (ASVs) differed significantly among groups ($p = 0.0058$, Kruskal-Wallis test), with higher values in NC compared to HPD ($p = 0.0156$), while NC + EX and HPD + EX showed intermediate levels without significant differences (Fig. 4A). No significant differences were observed in the Shannon index (Fig. 4B). Non-metric multidimensional scaling (NMDS) based on Bray-Curtis dissimilarities revealed distinct clustering patterns across groups (Fig. 4C). Compositional differences were further supported by PERMANOVA, which showed significant differences between most group pairs (adjusted $p = 0.0396$), except between HPD and HPD + EX (adjusted $p = 0.073$).

Analysis of Bray-Curtis distances showed a significant overall group effect ($p = 0.0011$, Kruskal–Wallis test). Post hoc comparisons indicated that the HPD + EX group had significantly lower inter-individual distances than the HPD ($p = 0.021$) and NC + EX ($p = 0.0017$) groups, while no significant differences were found between the NC group and any other group (Fig. 4D).

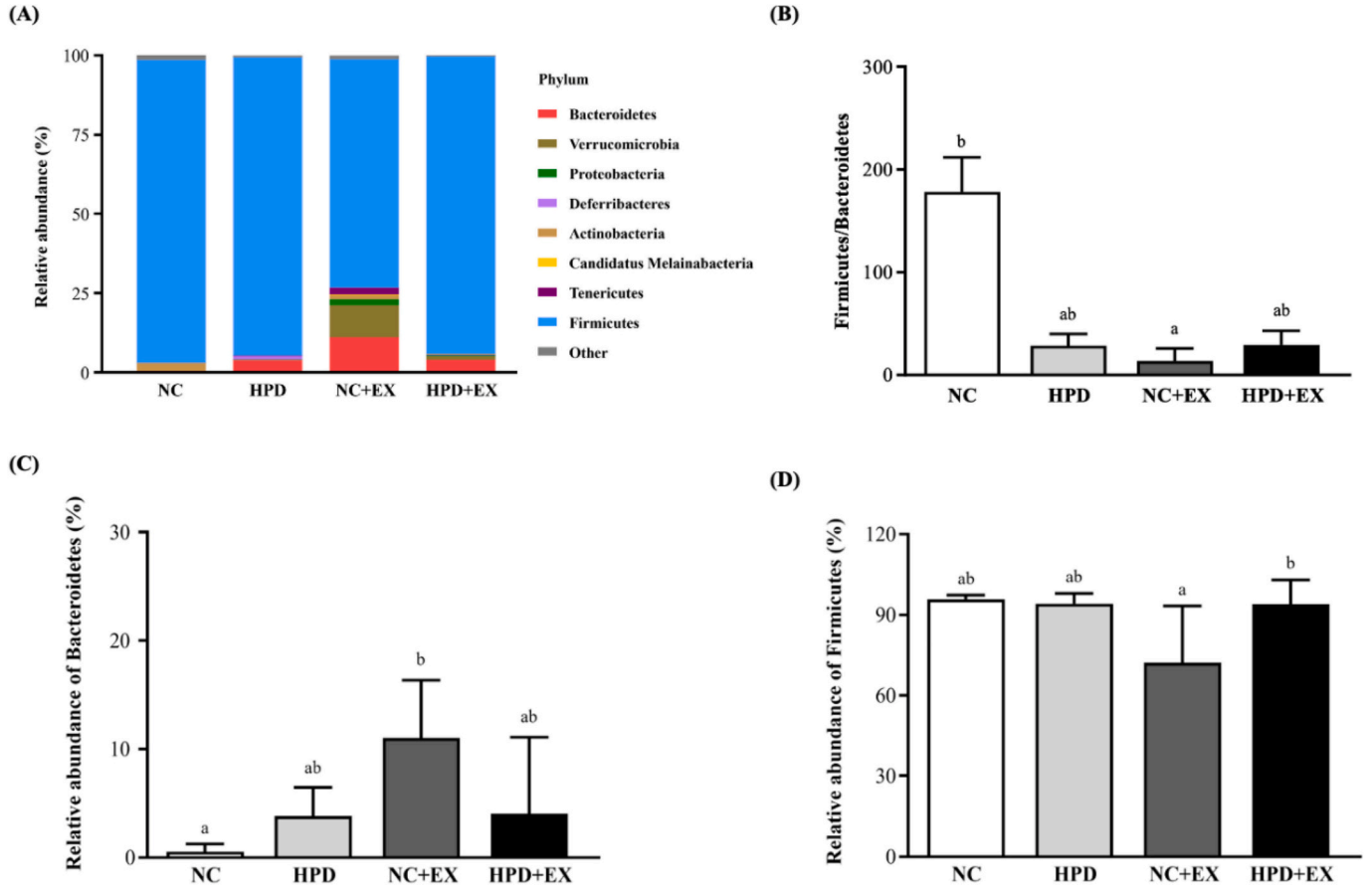


Fig. 5. Effects of High-Protein Intake and Progressive Resistance Training on gut microbiota composition at the phylum level. (A) Stacked bar plot showing the relative abundance of bacterial phyla in each group. (B) *Firmicutes/Bacteroidetes* (F/B) ratio. (C) Relative abundance of *Bacteroidetes*. (D) Relative abundance of *Firmicutes*. Mice were divided into four groups: NC, HPD, NC + EX, and HPD + EX ($n = 4$ per group). Data are presented as mean ± SD. Different superscript letters (a, b) indicate statistically significant differences among groups based on the Kruskal–Wallis test followed by Dunn's post hoc test ($p < 0.05$).

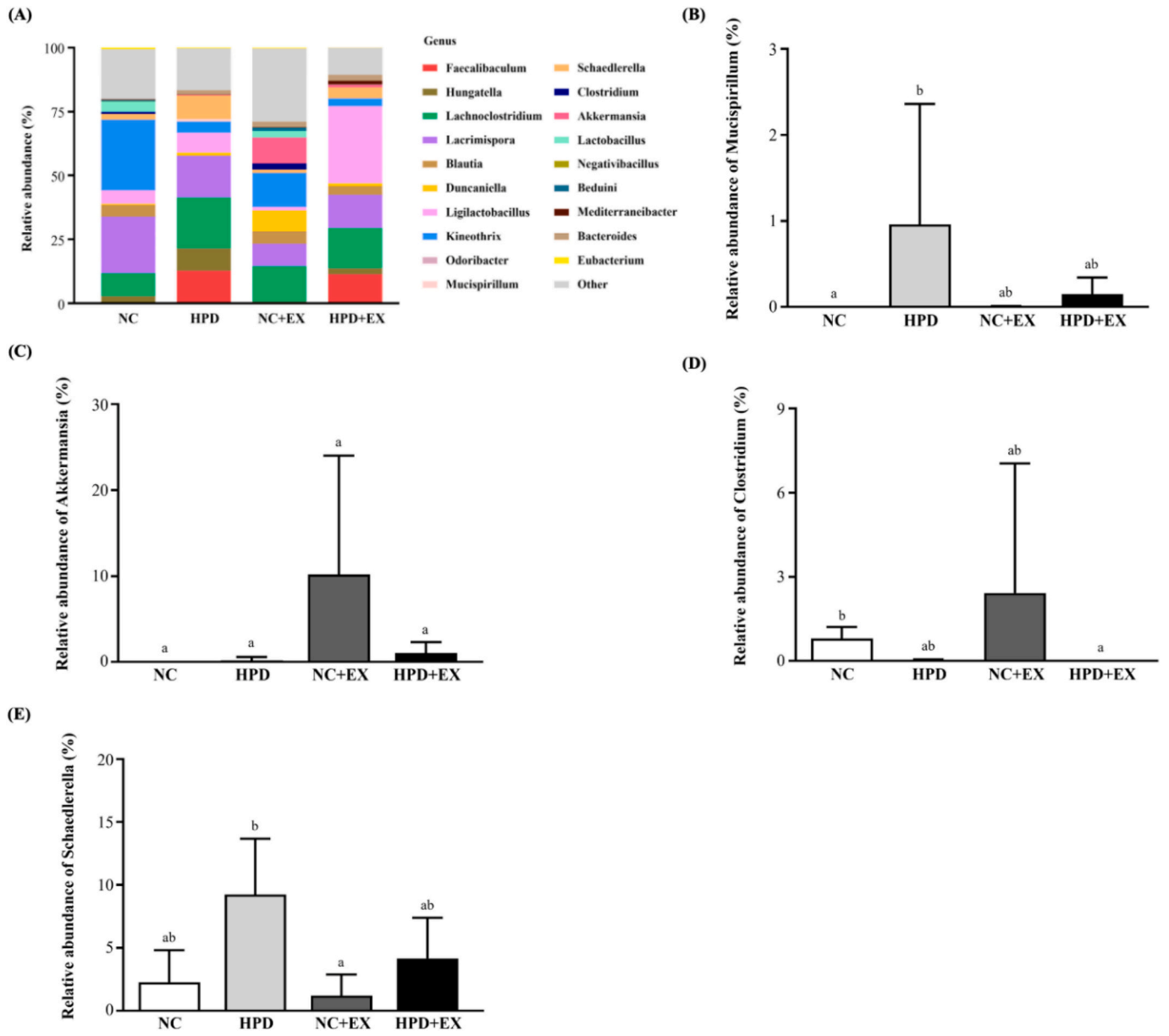


Fig. 6. Effects of High-Protein Intake and Progressive Resistance Training on gut microbiota composition at the genus level. (A) Stacked bar plot showing the relative abundance of bacterial genera in each group. (B) Relative abundance of *Mucispirillum*. (C) Relative abundance of *Akkermansia*. (D) Relative abundance of *Clostridium*. (E) Relative abundance of *Schaedlerella*. Mice were divided into four groups: NC, HPD, NC + EX, and HPD + EX ($n = 4$ per group). Data are presented as mean ± SD. Different superscript letters (a, b) indicate statistically significant differences among groups based on the Kruskal–Wallis test followed by Dunn's post hoc test ($p < 0.05$).

3.6. Effects of high-protein intake and progressive resistance training on gut microbiota composition at the phylum and genus levels

Analysis of gut microbiota composition using 16S rRNA gene sequencing revealed significant differences at both the phylum and genus levels (Figs. 5 and 6). At the phylum level, *Firmicutes* and *Bacteroidetes* were predominant across all groups, with notable compositional shifts following the interventions (Fig. 5A). The *Firmicutes/Bacteroidetes* (F/B) ratio differed significantly among groups ($p = 0.0016$, Kruskal-Wallis test), with a higher ratio observed in the NC group compared to NC + EX ($p = 0.0065$), while no differences were detected between NC and HPD or HPD + EX (Fig. 5B). *Bacteroidetes* abundance differed significantly among groups ($p = 0.0096$, Kruskal–Wallis test), with significantly higher levels in NC + EX compared to NC ($p = 0.0227$), while HPD and HPD + EX exhibited intermediate abundances without reaching statistical significance (Fig. 5C). *Firmicutes* abundance differed significantly among groups ($p = 0.0305$, Kruskal-Wallis test), with lower levels observed in NC + EX compared to HPD + EX ($p = 0.0451$), and no significant difference between NC and HPD (Fig. 5D).

At the genus level, several taxa exhibited significant differences in relative abundance among groups (Fig. 6A). *Mucispirillum* abundance differed significantly ($p = 0.0155$, Kruskal-Wallis test), with higher levels observed in the HPD group compared to NC ($p = 0.0324$), while only minimal levels were detected in NC + EX and HPD + EX (Fig. 6B). *Akkermansia* abundance showed a significant overall group effect based on the Kruskal–Wallis test ($p = 0.033$); however, Dunn's post hoc test did not identify significant pairwise differences among groups (Fig. 6C). *Clostridium* abundance differed significantly among groups ($p = 0.0167$, Kruskal-Wallis test), with significantly higher levels in the NC group

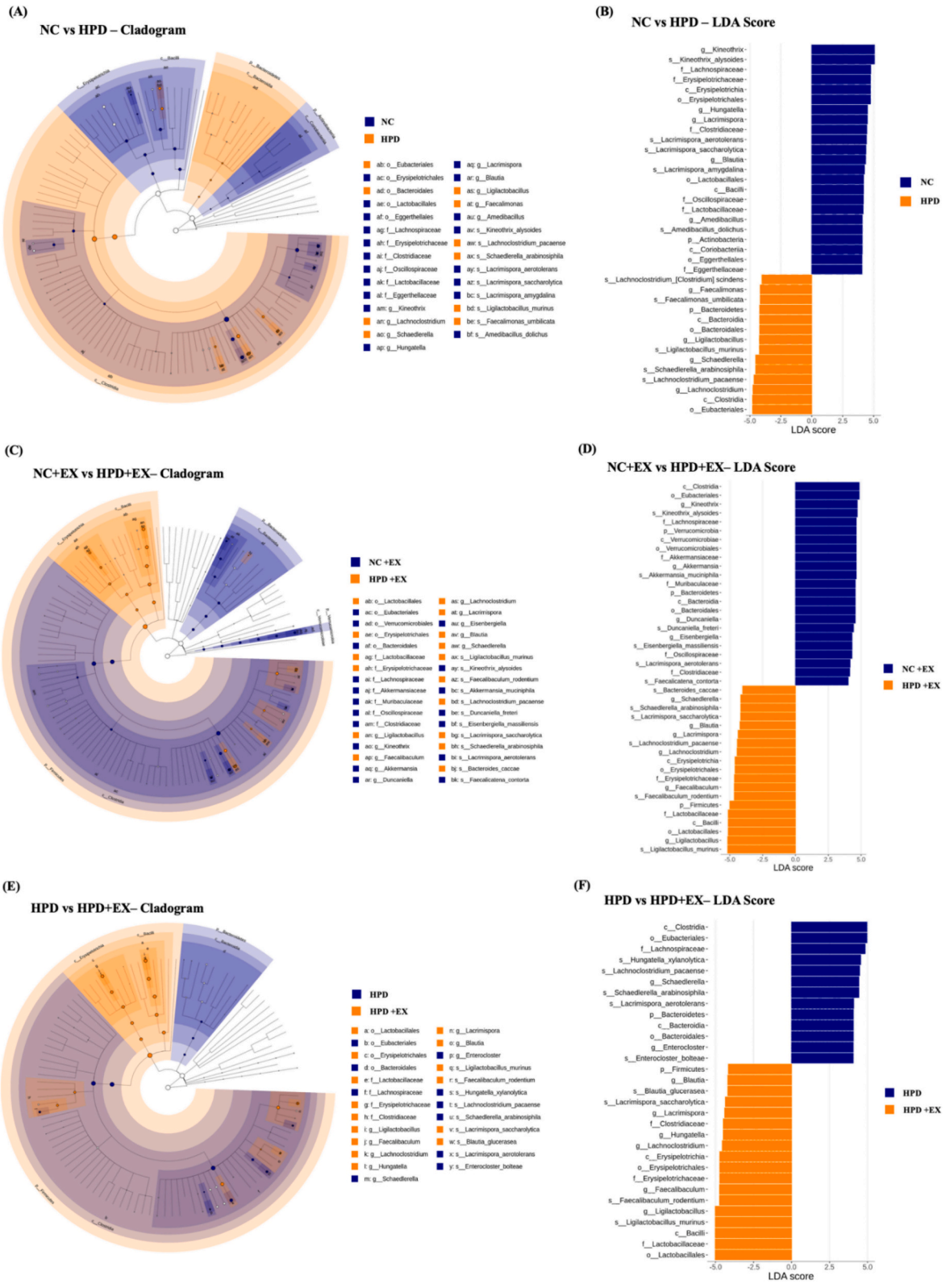


Fig. 7. Pairwise linear discriminant analysis effect size (LEfSe) analysis of differentially abundant gut microbial taxa among the experimental groups. (A) LEfSe taxonomic cladogram showing the phylogenetic distribution of differentially abundant taxa between the NC and HPD groups. (B) Corresponding LDA score plot for the NC and HPD groups. (C) LEfSe taxonomic cladogram showing the phylogenetic distribution of differentially abundant taxa between the NC + EX and HPD + EX groups. (D) Corresponding LDA score plot for the NC + EX and HPD + EX groups. (E) LEfSe taxonomic cladogram showing the phylogenetic distribution of differentially abundant taxa between the HPD and HPD + EX groups. (F) Corresponding LDA score plot for the HPD and HPD + EX groups. Mice were divided into four groups: NC, HPD, NC + EX, and HPD + EX ($n = 4$ per group). Taxa with an LDA score ≥ 4.0 and Kruskal–Wallis $p < 0.05$ were considered significantly discriminative.

compared to HPD + EX ($p = 0.0193$). No significant differences were found among the remaining groups (Fig. 6D). *Schaedlerella* abundance also differed significantly across groups ($p = 0.031$, Kruskal-Wallis test), with higher levels detected in the HPD group compared to NC + EX ($p = 0.037$), while NC and HPD + EX exhibited intermediate abundances without statistical significance (Fig. 6E).

3.7. Effects of high-protein intake and progressive resistance training on differentially abundant gut microbial taxa identified by LEfSe analysis

Pairwise linear discriminant analysis effect size (LEfSe) analysis was performed to identify differentially abundant gut microbial taxa among the experimental groups (Fig. 7). The NC and HPD groups were compared to identify diet-associated differences under sedentary conditions (Fig. 7A,B). The NC + EX and HPD + EX groups were compared to identify diet-associated differences under exercise conditions (Fig. 7C, D). The HPD and HPD + EX groups were compared to identify exercise-associated differences under high-protein feeding conditions (Fig. 7E,F).

The LEfSe taxonomic cladogram comparing the NC and HPD groups is shown in Fig. 7A, and the corresponding LDA scores are presented in Fig. 7B. At the genus level, *Kineothrix*, *Hungatella*, *Lacrimispora*, *Blautia*, and *Amedibacillus* were enriched in the NC group, whereas *Faecalimonas*, *Ligilactobacillus*, *Schaedlerella*, and *Lachnoclostridium* were enriched in the HPD group. These findings demonstrate differences in selected microbial taxa between the two dietary groups under sedentary conditions.

The LEfSe taxonomic cladogram comparing the NC + EX and HPD + EX groups is shown in Fig. 7C, and the corresponding LDA scores are presented in Fig. 7D. At the genus level, *Kineothrix*, *Akkermansia*, *Duncaniella*, and *Eisenbergiella* were enriched in the NC + EX group, whereas *Schaedlerella*, *Blautia*, *Lacrimispora*, *Lachnoclostridium*, *Faecalibaculum*, and *Ligilactobacillus* were enriched in the HPD + EX group. These findings demonstrate differences in selected microbial taxa between the two dietary groups under exercise conditions.

The LEfSe taxonomic cladogram comparing the HPD and HPD + EX groups is shown in Fig. 7E, and the corresponding LDA scores are presented in Fig. 7F. At the genus level, *Schaedlerella* and *Enterocloster* were enriched in the HPD group, whereas *Blautia*, *Faecalibaculum*, and *Ligilactobacillus* were enriched in the HPD + EX group. LEfSe therefore identified differences in selected microbial taxa between high-protein–fed mice with and without exercise. However, pairwise PERMANOVA did not detect a statistically significant difference in overall microbial composition between the HPD and HPD + EX groups after adjustment for multiple comparisons (adjusted $p = 0.073$).

3.8. Effects of high-protein intake and progressive resistance training on blood biochemical parameters

Blood biochemical parameters following the 8-week intervention are presented in Table 3. Serum glucose (GLU) was measured to assess glycemic status. Markers of renal function included blood urea nitrogen (BUN), creatinine (CREA), and uric acid (UA). Liver-related markers included aspartate aminotransferase (AST), alanine aminotransferase (ALT), albumin (ALB), and total protein (TP). Creatine kinase (CK) was measured as an indicator of muscle damage.

No significant differences were observed among groups in GLU, UA, ALT, TP, or ALB levels, indicating that neither high-protein intake nor resistance training significantly affected these parameters.

Renal and muscle-related biochemical markers are shown in Table 3. The BUN concentrations in the NC, HPD, NC + EX, and HPD + EX groups were 34.73 ± 1.45, 41.13 ± 6.67, 31.92 ± 2.02, and 41.83 ± 5.60, respectively. Compared with the NC and NC + EX groups, BUN levels were significantly higher in the HPD and HPD + EX groups ($p = 0.002$). Two-way ANOVA further showed a significant main effect of diet on BUN levels ($p < 0.001$), while no significant exercise effect ($p = 0.573$) or diet × exercise interaction ($p = 0.353$) was observed.

Serum creatinine (CREA) concentrations were 0.44 ± 0.01, 0.38 ± 0.02, 0.44 ± 0.01, and 0.38 ± 0.01 in the NC, HPD, NC + EX, and HPD + EX groups, respectively. CREA levels in both the HPD and HPD + EX groups were significantly lower than those in the NC and NC + EX groups ($p < 0.001$). Two-way ANOVA revealed a significant main effect of diet on CREA levels ($p < 0.001$), with no significant exercise effect ($p = 0.849$) or diet × exercise interaction ($p = 0.570$).

Aspartate aminotransferase (AST) concentrations were 106.67 ± 25.41, 146.83 ± 22.63, 154.83 ± 17.13, and 155.33 ± 41.01 in the NC, HPD, NC + EX, and HPD + EX groups, respectively. AST levels were significantly increased in the HPD, NC + EX, and HPD + EX groups compared with the NC group ($p = 0.020$). Two-way ANOVA showed a significant main effect of exercise on AST levels ($p = 0.022$), while the diet effect ($p = 0.090$) and diet × exercise interaction ($p = 0.098$) were not significant.

Creatine kinase (CK) levels were 256.17 ± 30.31, 357.67 ± 19.16, 385.17 ± 57.77, and 417.67 ± 69.55 in the NC, HPD, NC + EX, and HPD + EX groups, respectively. CK concentrations were significantly elevated in the HPD, NC + EX, and HPD + EX groups compared with the NC group ($p < 0.001$). Two-way ANOVA further indicated significant main effects of exercise ($p < 0.001$) and diet ($p = 0.003$) on CK levels, whereas the diet × exercise interaction ($p = 0.098$) was not significant.

3.9. Effects of high-protein intake and progressive resistance training on skeletal muscle morphology

Histological examination revealed increased myofiber cross-sectional area (CSA) in the quadriceps (QUA) muscle of the exercise-trained groups (Fig. 8). One-way ANOVA showed that QUA myofiber CSA was significantly increased in the NC + EX and HPD + EX groups compared with the NC and HPD groups ($p < 0.001$). Two-way ANOVA

Table 3
Effects of High-Protein Intake and Progressive Resistance Training on Blood Biochemical Parameters.

Characteristic	NC	HPD	NC + EX	HPD + EX	*p* value		
					E	D	E × D
GLU (mg/dL)	227.00 ± 9.82[a]	237.83 ± 21.35[a]	233.50 ± 28.19[a]	242.17 ± 35.61[a]	0.609	0.361	0.918
BUN (mg/dL)	34.73 ± 1.45[a]	41.13 ± 6.67[b]	31.92 ± 2.02[a]	41.83 ± 5.60[b]	0.573	<0.001***	0.353
CREA (mg/dL)	0.44 ± 0.01[b]	0.38 ± 0.02[a]	0.44 ± 0.01[b]	0.38 ± 0.01 [a]	0.849	<0.001***	0.570
UA (mg/dL)	1.87 ± 0.23[a]	2.17 ± 0.38 [a]	2.30 ± 0.27[a]	2.12 ± 0.44 [a]	0.183	0.679	0.097
AST (U/L)	106.67 ± 25.41[a]	146.83 ± 22.63[b]	154.83 ± 17.13[b]	155.33 ± 41.01[b]	0.022*	0.090	0.098
ALT (U/L)	38.17 ± 5.46[a]	36.33 ± 10.88[a]	38.33 ± 6.50[a]	33.83 ± 8.18[a]	0.725	0.345	0.688
ALB (g/dL)	3.34 ± 0.09[a]	3.29 ± 0.15[a]	3.33 ± 0.12[a]	3.30 ± 0.11[a]	0.973	0.376	0.837
TP (g/dL)	5.72 ± 0.15[a]	5.52 ± 0.25[a]	5.68 ± 0.19[a]	5.75 ± 0.23[a]	0.257	0.445	0.135
CK (U/L)	256.17 ± 30.31[a]	357.67 ± 19.16[b]	385.17 ± 57.77[b]	417.67 ± 69.55[b]	<0.001***	0.003**	0.098

Mice were divided into four groups: NC, HPD, NC + EX, and HPD + EX ($n = 6$ per group). Data are presented as mean ± SD. Different superscript letters (a, b) indicate statistically significant differences among groups based on one-way ANOVA followed by Duncan's multiple range test ($p < 0.05$). The *p* values for exercise (E), diet (D), and their interaction (E × D) were obtained from two-way ANOVA and represent the main effects of exercise, diet, and their interaction, respectively. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$.

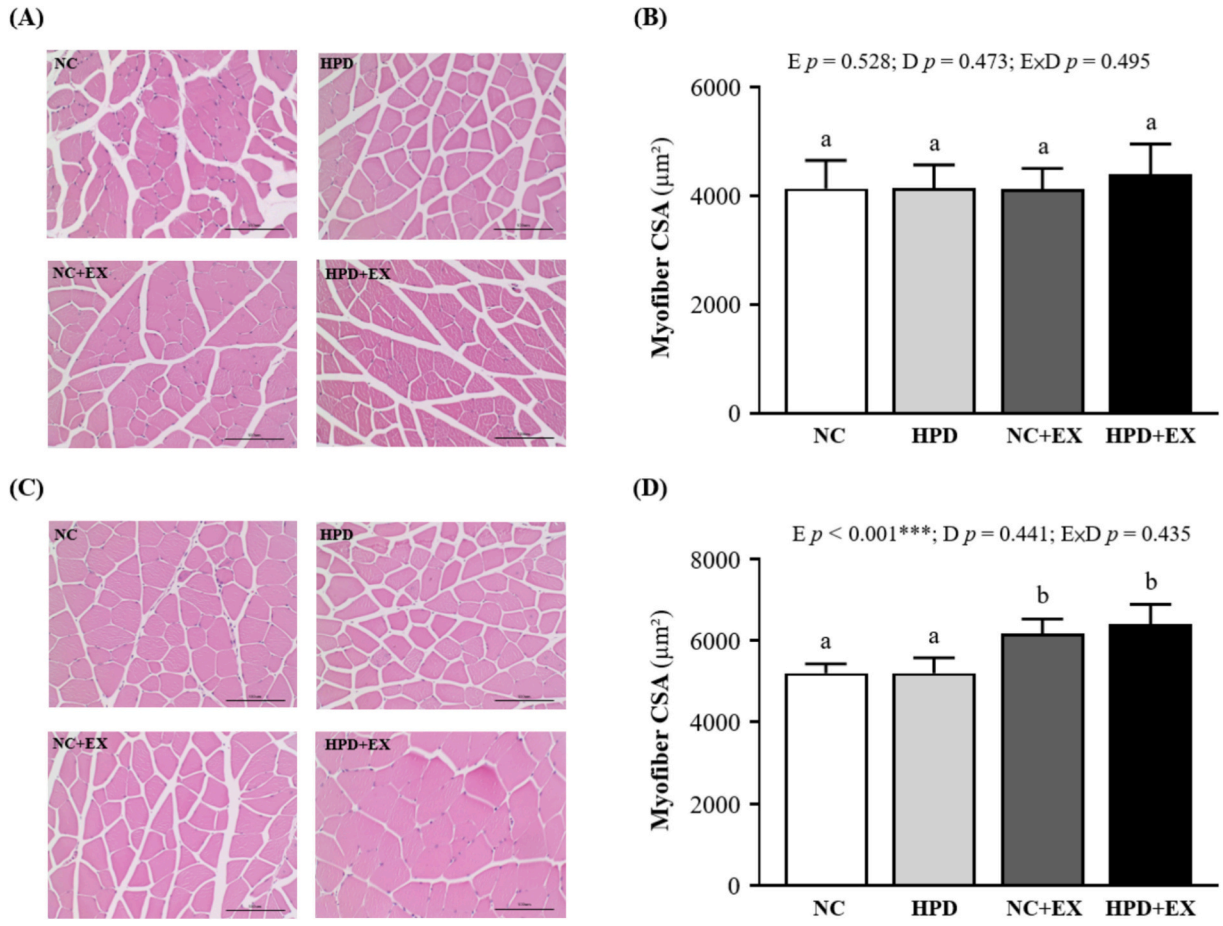


Fig. 8. Effects of High-Protein Intake and Progressive Resistance Training on (A) gastrocnemius muscle (GAS) morphology, (B) cross-sectional area (CSA) of GAS myofibers, (C) quadriceps muscle (QUA) morphology, and (D) CSA of QUA myofibers. Mice were divided into four groups: NC, HPD, NC + EX, and HPD + EX ($n = 6$ per group). Data are presented as mean ± SD. Different superscript letters (a, b) indicate significant differences among groups based on one-way ANOVA followed by Duncan's multiple range test ($p < 0.05$). The p values for exercise (E), diet (D), and their interaction (E × D) were obtained from two-way ANOVA and represent the main effects of exercise, diet, and their interaction, respectively. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. H&E staining; magnification 200×; scale bar = 100 μm.

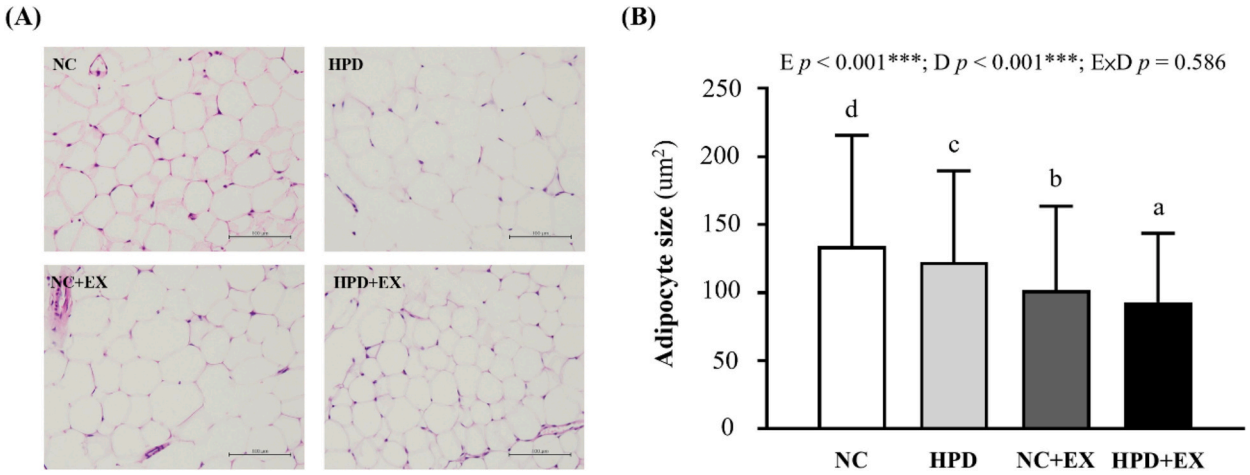


Fig. 9. Effects of High-Protein Intake and Progressive Resistance Training on (A) epididymal fat pad morphology. (B) adipocyte size. Mice were divided into four groups: NC, HPD, NC + EX, and HPD + EX ($n = 6$ per group). Data are presented as mean ± SD. Different superscript letters (a, b, c, d) indicate significant differences among groups based on one-way ANOVA followed by Duncan's multiple range test ($p < 0.05$). The p values for exercise (E), diet (D), and their interaction (E × D) were obtained from two-way ANOVA and represent the main effects of exercise, diet, and their interaction, respectively. *$p < 0.05$, **$p < 0.01$, ***$p < 0.001$. H&E staining; magnification 200×; scale bar = 100 μm.

further revealed a significant main effect of exercise on QUA myofiber CSA ($p < 0.001$), whereas the diet × exercise interaction was not significant ($p = 0.435$). These findings indicate that progressive resistance training promoted QUA myofiber hypertrophy irrespective of dietary protein intake.

3.10. Effects of high-protein intake and progressive resistance training on body fat

Histological examination of epididymal fat pads revealed a progressive reduction in adipocyte size among the intervention groups (Fig. 9). One-way ANOVA showed that adipocyte size was significantly decreased in the HPD, NC + EX, and HPD + EX groups compared with the NC group, with the lowest value observed in the HPD + EX group ($p < 0.001$). Two-way ANOVA further revealed significant main effects of exercise and diet on adipocyte size (both $p < 0.001$), whereas the diet × exercise interaction was not significant ($p = 0.586$). These findings indicate that both high-protein intake and progressive resistance training independently contributed to the attenuation of adipocyte hypertrophy.

4. Discussion

High-protein diets have been extensively studied for their physiological benefits, particularly in reducing adiposity and suppressing energy intake through enhanced satiety and thermogenic responses [21,22]. In the present study, high-protein feeding resulted in significant reductions in mesenteric fat weight and epididymal adipocyte size, accompanied by decreased food intake and increased water consumption. However, energy intake was substantially lower in both high-protein diet groups. Therefore, the reductions in adiposity and the accompanying physiological and microbial changes cannot be attributed solely to the higher protein content. The absence of a pair-fed control group prevented us from distinguishing the effects of increased dietary protein from those of reduced energy intake.

Although mesenteric fat showed the most pronounced reduction in tissue weight, epididymal fat was selected for histological analysis because it is a commonly used and anatomically well-defined adipose depot in rodent studies. This depot allows consistent evaluation of adipocyte morphology and comparison with previous studies. These findings are consistent with previous research indicating that protein-rich diets regulate protein-specific appetite and reduce spontaneous energy intake, thereby contributing to reduced fat accumulation and improved metabolic control [23]. The observed reduction in food intake may be attributed to the role of dietary protein in promoting satiety and maintaining nutritional homeostasis through protein-driven signaling mechanisms [24].

The dietary and exercise interventions were associated with distinct physiological and microbial outcomes. In particular, resistance training was primarily associated with improvements in physical performance, whereas the high-protein dietary intervention, together with the accompanying reduction in energy intake, was associated with reduced adiposity, increased hepatic glycogen storage, and alterations in gut microbiota composition. Resistance training did not significantly alter microbial richness or overall microbiota composition in mice fed a high-protein diet. Nevertheless, LEfSe identified differences in selected microbial taxa between the HPD and HPD + EX groups, suggesting limited taxon-specific differences associated with exercise under high-protein feeding conditions. Although BUN and AST levels were moderately elevated in some intervention groups, these biochemical changes should be interpreted cautiously and warrant further investigation.

From a performance perspective, resistance training significantly enhanced muscular strength, endurance, and motor coordination, as evidenced by improvements in relative forelimb grip strength, weights test holding time, rotarod test latency, and functional strength test performance. These findings are consistent with previous studies indicating that progressive resistance exercise promotes neuromuscular coordination, muscle strength development, and balance control [16,25]. Overall, the significant main effects of exercise across the performance variables indicate that progressive resistance training was the primary factor associated with improved physical performance. Although the HPD + EX group showed the highest mean values in several outcomes, no significant diet × exercise interaction was detected for these performance variables.

Although progressive resistance training significantly improved physical performance, no significant differences were observed in gastrocnemius or quadriceps muscle weight among groups. Nevertheless, histological analysis revealed a significant increase in quadriceps myofiber cross-sectional area in the NC + EX and HPD + EX groups, with a significant main effect of exercise. These findings suggest that resistance training induced quadriceps myofiber hypertrophy despite the absence of detectable changes in whole-muscle weight. The lack of significant diet and diet × exercise effects further indicates that high-protein intake did not enhance the exercise-associated increase in quadriceps myofiber size.

The absence of significant changes in whole-muscle weight differs from previous studies utilizing weight-pulling resistance exercise models that reported significant increases in muscle mass following progressive resistance training [16,26]. Several factors may explain this discrepancy, including differences in training protocols, training volume, intervention objectives, and animal responses to exercise. Importantly, improvements in muscle function do not necessarily require substantial increases in whole-muscle mass. Such improvements may reflect a combination of localized myofiber hypertrophy and adaptations in neuromuscular coordination, motor unit recruitment, and muscle quality [27,28]. These adaptations may collectively have contributed to the observed improvements in strength, endurance, and motor performance.

In terms of metabolic fuel storage, hepatic glycogen content was significantly elevated in the HPD, NC + EX, and HPD + EX groups, while no significant changes were observed in muscle glycogen. Two-way ANOVA further revealed significant main effects of both diet and exercise on hepatic glycogen content, without a significant diet × exercise interaction. Although muscle glycogen is typically emphasized in exercise studies, hepatic glycogen plays a critical role in maintaining glucose homeostasis and sustaining prolonged physical activity, particularly during fasting or the early stages of exercise [29,30]. The elevated hepatic glycogen content associated with the dietary and exercise interventions may reflect alterations in hepatic glycogen storage or metabolism; however, its direct contribution to exercise performance was not evaluated in the present study. These findings are consistent with previous studies suggesting that high-protein diets may help preserve hepatic energy stores, although the underlying mechanisms require further investigation [31].

This study showed that high-protein intake and progressive resistance training were associated with distinct physiological and microbial responses. High-protein feeding in the absence of exercise was associated with altered gut microbial composition and reduced microbial richness. LEfSe further identified enrichment of *Faecalimonas*, *Ligilactobacillus*, *Schaedlerella*, and *Lachnoclostridium* in the HPD group compared with the NC group. Although observed ASVs were significantly reduced in the HPD group, the Shannon index did not differ significantly among groups. This discrepancy may be explained by the different ecological information captured by these indices. Observed ASVs primarily reflect microbial richness, whereas the Shannon index incorporates both richness and evenness. Therefore, a reduction in the number of detected ASVs does not necessarily lead to a significant change in Shannon diversity if the relative abundance distribution of dominant taxa remains relatively stable. These findings suggest that the high-protein dietary intervention may be associated with reduced microbial richness without a detectable change in Shannon diversity. These changes align with earlier findings suggesting that increased dietary protein availability

and protein fermentation may favor bacteria capable of utilizing nitrogen-containing substrates and alter microbial community structure [32,33]. However, microbial functional activity was not directly assessed in the present study, and the functional implications of these taxonomic changes therefore remain uncertain.

Under exercise conditions, LEfSe identified *Akkermansia* and *Duncaniella* as taxa enriched in the NC + EX group relative to the HPD + EX group. Previous studies have associated these genera with metabolic regulation, mucosal barrier integrity, and inflammatory responses [34–36]. However, the present comparison reflects differences between dietary groups under exercise conditions and does not demonstrate that resistance training increased the abundance of these genera. Moreover, although an overall group effect was observed for *Akkermansia*, Dunn's post hoc test did not identify any significant pairwise differences. Therefore, its variation cannot be specifically attributed to resistance training.

Previous studies have suggested that exercise may influence microbial resilience and favorably modulate gut microbial composition [37]. In the present study, however, microbial richness and overall microbiota composition did not differ significantly between the HPD and HPD + EX groups. Although LEfSe identified differences in selected taxa and the HPD + EX group showed lower inter-individual Bray–Curtis distances than the HPD group, these findings alone do not demonstrate restoration or stabilization of the gut microbiota. Thus, the present results support taxon-specific differences associated with exercise under high-protein feeding conditions but do not establish a broader improvement in microbial community composition.

The microbiota analysis has several limitations. Functional prediction based on 16S rRNA gene sequencing is inferential, and the limited sample size ($n = 4$ per group) provided insufficient statistical power for robust physiological–microbial correlation and subgroup analyses. Future studies incorporating larger sample sizes, shotgun metagenomic sequencing, metabolomic profiling, and targeted correlation analyses are needed to clarify the functional relevance of the observed microbial changes.

Collectively, the high-protein dietary intervention, accompanied by reduced energy intake, was associated with reduced adiposity, increased hepatic glycogen storage, and alterations in gut microbial composition, whereas progressive resistance training improved physical performance and increased quadriceps myofiber cross-sectional area. LEfSe identified differences in selected microbial taxa between the HPD and HPD + EX groups, although microbial richness and overall microbiota composition did not differ significantly between these groups. These findings indicate distinct physiological and microbial outcomes associated with the dietary and exercise interventions, without consistent evidence that high-protein intake enhanced the effects of resistance training. The functional significance of the observed microbial changes remains to be established.

5. Conclusions

This study shows that the high-protein dietary intervention, accompanied by reduced energy intake, was associated with reduced adiposity, increased hepatic glycogen storage, and alterations in gut microbial composition. Progressive resistance training improved physical performance and increased quadriceps myofiber cross-sectional area. Although differences in selected microbial taxa were observed between the HPD and HPD + EX groups, resistance training did not significantly alter microbial richness or overall microbiota composition in high-protein-fed mice. Overall, the dietary and exercise interventions were associated with distinct physiological and microbial outcomes, without consistent evidence that high-protein intake enhanced the effects of resistance training. Further studies incorporating direct functional analyses are needed to determine the biological significance of the observed microbial changes.

CRediT authorship contribution statement

Yi-Ju Hsu: Writing – review & editing, Writing – original draft, Methodology, Formal analysis, Data curation, Conceptualization. **Chun-Yu Kuo:** Formal analysis, Data curation. **Yu-Ching Lo:** Data curation.

Declaration of competing interest

The authors have no conflict of interest to declare.

Acknowledgements

This work was supported by the National Science and Technology Council, Taiwan (grant number: MOST 109-2410-H-179-001-MY2). We would like to thank the animal care team at the National Taiwan Sport University for their assistance and support in animal husbandry and management throughout the study.

Data availability

No data was used for the research described in the article.

References

[1] M.C. Serra, K.R. Dondero, D. Larkins, A. Burns, O. Addison, Healthy lifestyle and cognition: interaction between diet and physical activity, Curr. Nutr. Rep. 9 (2020) 64–74.
[2] L. Zhang, Y. Liu, Y. Sun, X. Zhang, Combined physical exercise and diet: regulation of gut microbiota to prevent and treat of metabolic disease: a review, Nutrients 14 (2022) 4774.
[3] G. D'Onofrio, J. Kirschner, H. Prather, D. Goldman, A. Rozanski, Musculoskeletal exercise: its role in promoting health and longevity, Prog. Cardiovasc. Dis. 77 (2023) 25–36.
[4] W.J. Smiles, J.A. Hawley, D.M. Camera, Effects of skeletal muscle energy availability on protein turnover responses to exercise, J. Exp. Biol. 219 (2016) 214–225.
[5] A. Caballero-García, A. Córdova-Martínez, Muscle recovery and nutrition, Nutrients 14 (2022) 2416.
[6] A.G. Pearson, K. Hind, L.S. Macnaughton, The impact of dietary protein supplementation on recovery from resistance exercise-induced muscle damage: a systematic review with meta-analysis, Eur. J. Clin. Nutr. 77 (2023) 767–783.
[7] O.C. Witard, L. Bannock, K.D. Tipton, Making sense of muscle protein synthesis: a focus on muscle growth during resistance training, Int. J. Sport Nutr. Exerc. Metab. 32 (2022) 49–61.
[8] R.W. Morton, K.T. Murphy, S.R. McKellar, B.J. Schoenfeld, M. Henselmans, E. Helms, A.A. Aragon, M.C. Devries, L. Banfield, J.W. Krieger, S.M. Phillips, A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults, Br. J. Sports Med. 52 (2018) 376–384.
[9] Y. Fan, O. Pedersen, Gut microbiota in human metabolic health and disease, Nat. Rev. Microbiol. 19 (2021) 55–71.
[10] W.M. de Vos, H. Tilg, M. Van Hul, P.D. Cani, Gut microbiome and health: mechanistic insights, Gut 71 (2022) 1020–1032.
[11] A. Bartlett, M. Kleiner, Dietary protein and the intestinal microbiota: an understudied relationship, iScience 25 (2022) 105313.
[12] A. Nishida, Y. Ando, I. Kimura, J. Miyamoto, Involvement of gut microbial metabolites derived from diet on host energy homeostasis, Int. J. Mol. Sci. 23 (2022) 5562.
[13] A. Kårlund, C. Gómez-Gallego, A.M. Turpeinen, O.M. Palo-Oja, H. El-Nezami, M. Kolehmainen, Protein supplements and their relation with nutrition, microbiota composition and health: is more protein always better for sportspeople? Nutrients 11 (2019) 829.
[14] R.L. Hughes, H.D. Holscher, Fueling gut microbes: a review of the interaction between diet, exercise, and the gut microbiota in athletes, Adv. Nutr. 12 (2021) 2190–2215.
[15] C. Lim, E.A. Nunes, B.S. Currier, J.C. McLeod, A.C.Q. Thomas, S.M. Phillips, An evidence-based narrative review of mechanisms of resistance exercise-induced human skeletal muscle hypertrophy, Med. Sci. Sports Exerc. 54 (2022) 1546–1559.
[16] W.G. Zhu, J.E. Hibbert, K.H. Lin, N.D. Steinert, J.L. Lemens, K.W. Jorgenson, S. M. Newman, D.W. Lamming, T.A. Hornberger, Weight pulling: a novel mouse model of human progressive resistance exercise, Cells 10 (2021) 2459.
[17] Y.J. Hsu, W.C. Huang, C.C.C. hiu, Y.L. Liu, W.C. Chiu, C.H. Chiu, Y.S. Chiu, C. C. Huang, Capsaicin supplementation reduces physical fatigue and improves exercise performance in mice, Nutrients 8 (2016) 648.
[18] R.M. Deacon, Measuring the strength of mice, J. Vis. Exp. 76 (2013) 2610.
[19] T.G. Graber, L. Ferguson-Stegall, H. Liu, L.V. Thompson, Voluntary aerobic exercise reverses frailty in old mice, J. Gerontol. A Biol. Sci. Med. Sci. 70 (2015) 1045–1058.

[20] K. Krüger, D.K. Gessner, M. Seimetz, J. Banisch, R. Ringseis, K. Eder, N. Weissmann, F.C. Mooren, Functional and muscular adaptations in an experimental model for isometric strength training in mice, PLoS One 8 (2013) e79069.
[21] W.W. French, S. Dridi, S.A. Shouse, H. Wu, A. Hawley, S.O. Lee, X. Gu, J.I. Baum, A high-protein diet reduces weight gain, decreases food intake, decreases liver fat deposition, and improves markers of muscle metabolism in obese Zucker rats, Nutrients 9 (2017) 587.
[22] S. Jiang, S. Ji, X. Tang, T. Wang, H. Wang, X. Meng, A comparison study on the therapeutic effect of high protein diets based on pork protein versus soybean protein on obese mice, Foods 11 (2022) 1227.
[23] P.C. Even, J. Gehring, D. Tomé, What does self-selection of dietary proteins in rats tell us about protein requirements and body weight control? Obes. Rev. 22 (2021) e13194.
[24] D. Tomé, C. Chaumontet, P.C. Even, N. Darcel, S.N. Thornton, D. Azzout-Marniche, Protein status modulates an appetite for protein to maintain a balanced nutritional state-a perspective view, J. Agric. Food Chem. 68 (2020) 1830–1836.
[25] W.L. Westcott, Resistance training is medicine: effects of strength training on health, Curr. Sports Med. Rep. 11 (2012) 209–216.
[26] H. Al-Sarraf, A. Mouihate, Muscle hypertrophy in a newly developed resistance exercise model for rats, Front. Physiol. 13 (2022) 851789.
[27] T. Moritani, H.A. de Vries, Neural factors versus hypertrophy in the time course of muscle strength gain, Am. J. Phys. Med. 58 (1979) 115–130.
[28] A. Del Vecchio, A. Casolo, F. Negro, M. Scorcelletti, I. Bazzucchi, R.M. Enoka, F. Felici, D. Farina, The increase in muscle force after 4 weeks of strength training is mediated by adaptations in motor unit recruitment and rate coding, J. Physiol. 597 (2019) 1873–1887.
[29] A.F. Alghannam, J.T. Gonzalez, J.A. Betts, Restoration of muscle glycogen and functional capacity: role of post-exercise carbohydrate and protein co-ingestion, Nutrients 10 (2018) 253.
[30] J. Mandl, Glycogen-endoplasmic reticulum connection in the liver, Int. J. Mol. Sci. 24 (2023) 1074.
[31] S.M. Pasiakos, W.F. Martin, C.S. Sharma, M.M. Pikosky, P.C. Gaine, D.R. Bolster, B. T. Bennett, N.R. Rodriguez, Level of dietary protein intake affects glucose turnover in endurance-trained men, J. Int. Soc. Sports Nutr. 8 (2011) 20.
[32] M. Beaumont, K.J. Portune, N. Steuer, A. Lan, V. Cerrudo, M. Audebert, F. Dumont, G. Mancano, N. Khodorova, M. Andriamihaja, G. Airinei, D. Tomé, R. Benamouzig, A.M. Davila, S.P. Claus, Y. Sanz, F. Blachier, Quantity and source of dietary protein influence metabolite production by gut microbiota and rectal mucosa gene expression: a randomized, parallel, double-blind trial in overweight humans, Am. J. Clin. Nutr. 106 (2017) 1005–1019.
[33] S.H. Duncan, A. Iyer, W.R. Russell, Impact of protein on the composition and metabolism of the human gut microbiota and health, Proc. Nutr. Soc. 80 (2021) 173–185.
[34] C. Bressa, M. Bailén-Andrino, J. Pérez-Santiago, R. González-Soltero, M. Pérez, M. G. Montalvo-Lominchar, J.L. Maté-Muñoz, R. Domínguez, D. Moreno, M. Larrosa, Differences in gut microbiota profile between women with active lifestyle and sedentary women, PLoS One 12 (2017) e0171352.
[35] C.S. Chang, Y.C. Liao, C.T. Huang, C.M. Lin, C.H.Y. Cheung, J.W. Ruan, W.H. Yu, Y. T. Tsai, I.J. Lin, C.H. Huang, J.S. Liou, Y.H. Chou, H.J. Chien, H.L. Chuang, H. F. Juan, H.C. Huang, H.L. Chan, Y.C. Liao, S.C. Tang, Y.W. Su, C.Y. Kao, et al., Identification of a gut microbiota member that ameliorates DSS-induced colitis in intestinal barrier enhanced Dusp6-deficient mice, Cell Rep. 37 (2021) 110016.
[36] H. Jian, Y. Liu, X. Wang, X. Dong, X. Zou, *Akkermansia muciniphila* as a next-generation probiotic in modulating human metabolic homeostasis and disease progression: a role mediated by gut-liver-brain axes? Int. J. Mol. Sci. 24 (2023) 3900.
[37] J.H. Moore, K.S. Smith, D. Chen, D.A. Lamb, M.A. Smith, S.C. Osburn, B.A. Ruple, C.D. Morrow, K.W. Huggins, J.R. McDonald, M.D. Brown, K.C. Young, M. D. Roberts, A.D. Frugé, Exploring the effects of six weeks of resistance training on the fecal microbiome of older adult males: secondary analysis of a peanut protein supplemented randomized controlled trial, Sports (Basel, Switzerland) 10 (2022) 65.

Yi-Ju Hsu[a,*], Chun-Yu Kuo[a], Yu-Ching Lo[a]
[a] *Graduate Institute of Sports Science, National Taiwan Sport University, Taoyuan, 33301, Taiwan*

[*] Corresponding author at: School of Graduate Institute of Sports Science, National Taiwan Sport University, No. 250, Wenhua 1st Rd., Guishan Township, Taoyuan County, 33301, Taiwan.
E-mail address: ruby780202@ntsu.edu.tw (Y.-J. Hsu).