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Protein yogurt and whey protein produce comparable muscle gains, but divergent microbiome shifts during strength training in older adults

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Sarcopenia, the age-related decline in muscle mass and strength, affects the functional capacity of older adults. Strength training (ST) combined with adequate protein intake is a key element in reversing and improving functional capacity. Protein, especially Whey Protein isolates (WPI), is widely used to improve muscle mass. In contrast, high-protein products, such as protein yogurt (PY), may offer similar benefits for muscle health and drive additional effects on gut health, which is altered in older adults. For this, we aim to compare WP and PY supplementation during ST on body composition, strength, and gut microbiome in untrained older adults. Seventeen untrained adults (60–70 years) were randomized to either consume WP (25 g) or PY (24.5 g) along with an 8-week supervised ST program (3 sessions/week). Initial and final assessments included body composition (BIA), strength (10RM, isokinetic torque, handgrip), gait speed, resting metabolic rate, and gut microbiome (16 S rRNA sequencing). Data were analyzed using repeated-measures ANOVA and diversity metrics. Both groups increased skeletal muscle mass (WP: +0.47 kg; PY: +0.50 kg) and improved strength and gait speed ($p < 0.01$), with no between-group differences. Fat mass decreased only in WP ($p = 0.02$), while resting metabolic rate increased in PY ($p = 0.03$). Microbiome analysis revealed distinct shifts: WP increased the Firmicutes/Bacteroidota ratio and enriched Subdoligranulum, whereas PY enhanced alpha diversity and increased the abundance of Coprococcus. Functional pathway predictions indicated differential enrichment in metabolic and signaling processes. High-protein yogurt and whey protein similarly improve muscle mass, strength, and functional capacity during ST, while exerting distinct effects on gut microbiome composition. Yogurt represents a cost-effective alternative to whey protein and may confer additional gut health benefits.

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Loss of muscle mass, strength, and functionality has been termed sarcopenia, a geriatric syndrome with a multifactorial etiology that increases with age[1]. The determinants of sarcopenia are diverse, encompassing a range of biological, nutritional, and environmental factors, including decreased anabolic processes[2], increased chronic low-grade inflammation[3], gut microbiota dysbiosis[4], protein-deficient diets[5], and cessation of physical exercise[6]. Exercise, particularly strength training (ST), is a potent stimulus for promoting skeletal muscle anabolism, resulting in metabolic and morphological improvements in this tissue, which can adapt to different frequencies, intensities, and exercise volumes at any age[7,8]. Therefore, ST is considered a primary strategy for delaying and treating sarcopenia[9].

In older adults, ST generates significant neuromuscular adaptations, including increases in power and strength-related abilities of daily living, such as rising from a chair, climbing and descending stairs, and active walking[10]. A recent systematic review found increases of 4.7–58.1% in functionality and 3.4–7.5% in muscle mass in 16 studies using ST as a therapeutic measure[11]. On the other hand, high-protein diet interventions may also potentiate ST-induced muscle mass gain. Vikberg et al. reported that a 10-week supervised ST resulted in a mean increase of ~ 1.4 kg lean mass and improved power and walking speed in functional tests in individuals under 70 years. Notably, these individuals consumed a milk-based high-protein supplement, although it was not mandatory as part of the intervention[12]. This result suggests an additive effect of high-protein intake on the anabolic effects of ST[13].

Protein is an essential nutrient primarily obtained from animal sources such as meat and dairy products (i.e., milk, yogurts, cheese) and, to a lesser extent, from plants (i.e., Soy, Peas, rice)[14]. Differences in protein quality are based on biological value, net utilization, and the Protein Digestibility-Corrected Amino Acid Score (PDCAAS)[15]. These factors make the selection of protein sources crucial for absorption and tolerance when ingested[16]. This is particularly relevant for dairy products, as these foods possess one of the highest biological values and PDCAAS[17]. For example, milk-derived proteins are composed of casein and whey, which the sports supplement industry utilizes in various forms (i.e., isolated, hydrolyzed, concentrated casein). These forms differ in their absorption speed and digestibility, which can impact protein synthesis rates[18]. Whey protein has rapid absorption; in contrast, casein, which constitutes about 80% of the protein content in milk, is absorbed more slowly, resulting in a different plasma amino acid appearance[19,20]. Although protein supplements are a popular option for increasing protein intake, recent evidence from our group[21] and others[22] suggests that different protein supplement brands do not consistently provide the quality and quantity of proteins they declare on their nutritional labels. This information should encourage the promotion of other high-quality food protein sources, especially for older adults, to support muscle and overall health[23]. In healthy, non-trained males, 12 weeks of Greek yogurt intake improved strength, muscle thickness, and fat-free mass (FFM) compared to a pudding (high in carbohydrates) placebo group[24]. More recently, Bagheri et al. have shown that the intake of 18 g of protein from Icelandic yogurt in older male adults (68 years) and ST improves lean body mass and strength compared to a placebo[25]. These results suggest that yogurts, which are exceptionally high in protein, can be a low-cost alternative to support muscle mass at various ages.

Yogurts not only possess almost the exact proportions of whey and casein that milk makes a good protein source[26] but are also recognized as functional foods by their impact on intestinal and metabolic health due to their digestibility[27,28]. In animal models, diets consisting of casein and whey refeed after a food restriction period differentially alter the bacterial population[29], providing evidence of the relevance of the protein source on the gut microbiome, which remains scarce in humans. Depending on the yogurt type (i.e., Stirred, extra protein, or Greek-style), fat, protein, physical, chemical, and probiotic characteristics can vary, as well as their functional effects on health[30]. We recently showed that high-protein yogurts in Chile have a good relationship with the content declared on the label[31]. Moreover, the amino acid profile found in most of the samples makes them similar to whey proteins. However, no study has directly compared protein intake from high-protein yogurts and whey protein supplements during a strength training program in older adults. Thus, we aimed to determine the effects of high-protein yogurt and whey protein intake on muscle strength, body composition, and microbiome diversity in older adults undergoing a guided strength training regimen.

Subjects and methods

Study population

Eligible participants were inactive men and women (60–70 years old) with <1 h of exercise per week and controlled blood pressure who had no impediments to being under an ST regime. Exclusion criteria were smoking, diabetes (type 1 or 2), renal and cardiac diseases (i.e., Heart Failure), alcohol consumption (Less than 0.1 fl. oz per day), lactose intolerance, regular intake of non-steroidal anti-inflammatory drugs or diuretics, and use of sports supplements that alter muscle mass (i.e., Whey protein, Creatine, HMB) in the last 6 months.

Participant recruitment

Recruitment was advertised locally in the community, the Motion Health and Performance Centre website, and social media (i.e., Instagram. All potential participants were contacted, and the project's general aims, objectives, and procedures were thoroughly explained to them. Potential candidates were identified based on their medical history, healthy habits, resting electrocardiogram (ECG), baseline fasting plasma glucose levels, and blood pressure profiles. This study enrolled participants from May 2023 to January 2024 and was conducted in accordance with the guidelines of the Declaration of Helsinki, with all procedures approved by the Ethics Committee of the Universidad de O´Higgins (N°027–2023, May 2023). Written informed consent was obtained from all participants. This study was registered at *ClinicalTrials.gov* (06/05/2024, *NCT06412302*,https://clinicaltrials.gov/study/NCT06412302).

The sample size was calculated using G-Power*, accepting a significance level for α of 0.05, a power of 0.80, and an effect size of 0.5; 8 subjects per group were needed.

Study design and intervention

The present study is a randomized trial in which participants were randomly assigned to receive 25 g of Whey protein (WP) (Vanilla, ISO100, Dymatize, USA) or 24.5 g of protein from high-protein yogurt (PY) (Vanilla, Loncoleche, Chile) immediately after training sessions in an 8-week strength training regimen (Fig. 1) (*randomizer.org*, MMA). As PY has a lower protein content per serving, two yogurts were prepared by the trainers (SB and FS) and mixed with filtered water to a total volume of 240mL. At the same time, one scoop of WP was diluted with 240 mL of water to ensure a similar volume and intake among groups and given by trainers to participants (Supplementary Fig. 1). The protein timing (immediately after) and frequency (training days only) of the supplementation were based on observations of Nabuco et al.[32]. Here, the appendicular muscle mass increased in older adults with strength training, regardless of the timing of intake.

Strength training (ST) sessions consisted of 3 × 8–12 reps with 1–2 repetitions in reserve (RIR). The training intensity ranged from 70% to 85% of 10 repetition maximum (RM), with 5% increases every 2 weeks. All sessions were supervised by a certified trainer at the Motion Health and Performance Center and were conducted in groups of 3 to ensure individualized prescriptions and participation. The training frequency was set at three days a week, with a (Monday, Wednesday, and Friday) lower, upper, and full body session schedule as recommended by the NSCA statements[33]. The exercise selection for lower-body sessions was as follows: leg press, one-leg step-up, hip thrust, leg curl, leg extension, and calf raise. Upper sessions: Pull-down, One-arm dumbbell row, Smith bench press, Smith shoulder press, Biceps curl and triceps extension, and a full body session included: Hex-bar deadlifts, Goblet squats, Leg curl, Leg extension, Lat pull-down wide, and Smith incline bench press. All exercises were performed using Ilus equipment (Ilus Fitness Company*, Santiago, Chile).

Measurements

Biochemical analysis

Before and after the 8-week intervention and after an overnight fast (8–12 h), approximately 5 mL of blood samples were drawn from the antecubital vein and immediately centrifuged. The serum was separated and frozen at −80 °C until analysis. Serum total cholesterol, Glucose, Triglycerides, HDL, Albumin, BUN, Uric acid, Creatinine, GOT, GPT, and GGT concentrations were determined in an automated dry-chemistry multi-analyzer (Spotchem EZ SP-4430; Menarini Diagnostics, UK) using specific soft reagent strips (ARKRAY, Shiga, Japan).

Body composition

Body composition analysis was performed using a multifrequency (5, 50, 250, 500, 1,000 and 3,000 kHz) bioelectric impedance analyzer with grab lead (InBody 970, InBody Co, South Korea). All participants were evaluated after an overnight fast (8–12 h) instructed to remove all metallic objects, refrain from consuming any drinks for 3–4 h and from consuming alcohol and engaging exercise for the 24 h before testing. Weight (kg), body fat mass (kg), total body water (L), skeletal muscle mass (kg), percent of body fat (%), visceral fat (kg),

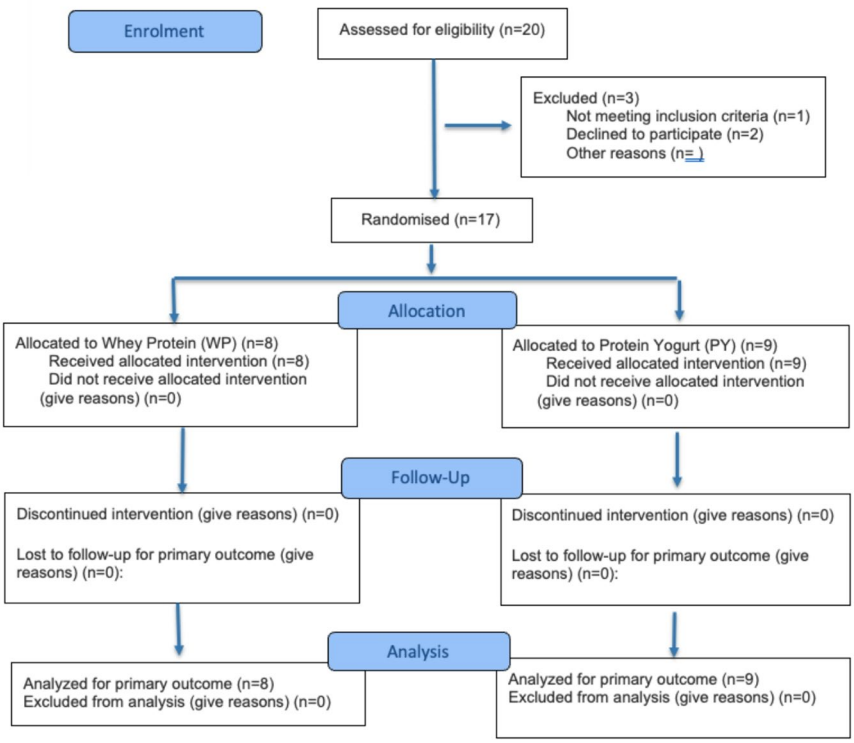


Fig. 1. CONSORT 2025 flow diagram.

trunk lean mass (kg), leg lean mass (kg), and whole-body phase angle (PhA°, 50 kHz) were determined before and after the 8-week intervention.

Cardiorespiratory fitness

Aerobic capacity was determined by indirect calorimetry using a calibrated breath-by-breath gas analyzer (Metalyzer, Cortex, Germany). Participants were subjected to an incremental-maximal protocol on a cycloergometer until exhaustion, as previously described[34]. Oxygen consumption (VO2) and carbon dioxide production (VCO2) were measured and compared at baseline and the end of the intervention.

Resting metabolic rate (RMR)

The resting metabolic rate was determined using an indirect calorimeter (QNRG, Cosmed, Italy) with a flow-dilution canopy hood. After an overnight fast (8–12 h), participants were instructed to remain in a resting condition for 15–20 min with a regular breathing pattern in a supine position, until a steady state (< 10% coefficient variation of one or more variables: VO_2, VCO_2, or RQ) was reached, following standardized recommendations[35].

24-hour food consumption recall

The nutritional information was obtained before and after the intervention through a 24-hour recall at the first and last laboratory visits, as previously described by our group and others[36,37]. The 24-hour recall has been shown to exhibit lower variation than other energy intake records in adults[38]. The total calories and macronutrient intake were calculated using the Photographic Atlas of Typical Chilean Food and Preparations by the Institute of Nutrition and Food Technology (INTA). Universidad de Chile[39] and performed by a registered dietitian of our team (RD).

Isokinetic strength

An isokinetic test was performed at the beginning and after 8 weeks following ST intervention. The test was performed bilaterally on an isokinetic machine (Genu Plus; Easytech Italy, Prato, Italy), which was calibrated by a physical therapist from our team before the test session. In brief, participants spent a short period gaining confidence in the isokinetic method. Then they warmed up with 6 repetitions at 120°/sec, followed by 1 repetition at 60°/sec at 50% of the maximum. After the warm-up, all participants rest for 2 min to perform 4 repetitions at 60°/s to determine the maximal knee extension and flexion peak torque (Nm)[40].

Maximal muscle strength

Maximal strength (1RM) was determined indirectly by the 10-repetition maximum (10RM) test as previously described[41,42]. In brief, 10RM was performed on the same day in the following order: Bench press (BP), Lat pull-down (LPD), and leg press (LP). Before testing, standard instructions for the exercise technique, performance, and guidance from a certified personal trainer are provided. During the 10RM test, participants performed incremental loads until they could no longer complete 11 repetitions, with a maximum of 5 attempts and 3–5-minute intervals between trials.

Hand grip strength

Hand grip strength (HGS) was measured in the dominant and non-dominant hands using a Jamar Plus dynamometer (Jamar, Jackson, MI, USA), as described previously, and reported as the maximal value of the three attempts[43]. Additionally, the HGS index was calculated by dividing HGS by height and expressed as (Kg/m^2) to account for body dimensions[44].

Time up and go (TUG)

Gait speed was determined using the Time Up and Go test (TUG) as previously described[45]. Briefly, all participants refrained from standing from a chair, walked through a 3 m away cone, turned around, and sat back on the chair, walking at the fastest and safest comfortable speed. All subjects were familiarized with the test by performing it before the trials, during which the best time was recorded, and gait speed was calculated in meters per second (m/s).

Microbiome

Microbiome diversity, abundance, and specific bacterial groups were determined using stool samples collected before and after the intervention, as described previously[46]. In brief, after consent, participants received a sample collection kit that included a fecal collection tube (DNA/RNA Shield™. Cat#R1101, Zymo Research), a disposable aluminum box to be used as a feces catcher, gloves, and an instruction manual. Each step of the procedure was thoroughly explained in detail to all subjects at the time they received the kit. Stool samples were collected 24 h before (Pre) and 24 h after (Post) the initiation of intervention and the last session, respectively. This sampling timing was selected to determine the long-term effects of both interventions. After collection, the samples were kept at room temperature for approximately two weeks and then stored at −80 °C until processing. DNA extraction from the fecal samples was performed using the Quick-DNA Fecal/Soil Microbe Miniprep Kit (Cat#D6010, Zymo Research), following the manufacturer's instructions. The quality of DNA extraction was verified using 1% agarose gel electrophoresis. The 16 S rRNA gene sequencing was conducted using the primers 341 F: CCTAYGGGRBGCASCAG and 806R: GGACTACHVGGGTWTCTAAT on the Illumina platform at Novogene (Beijing, China).

Statistical analysis

Sample size was determined based on a previous study on strength and muscular measurements after yogurt intake and strength training[25], it was calculated using G-Power that eight subjects per group (n = 16) were required for a significance level of α = 0.05, power of 0.80, and an effect size of 0.5. An unpaired t-test was used to determine baseline differences between groups, and a two-way ANOVA with repeated measures was used to examine both between-group and within-group differences between WP and YP, using GraphPad software (Prism, Version 10.4.2). Only participants who achieved > 85% compliance to exercise training were considered for statistical analyses.

For microbiome data analysis, the DADA2 and Phyloseq packages were used to process the 16 S rRNA sequences and identify and quantify the microbial diversity of the samples. The sequence reads were processed to remove the first 28 bases and discard low-quality reads, using standard filtering and trimming parameters, except for maxEE and truncQ, which were set to 2. The SILVA V138 database was used as a reference to assign amplicon sequence variants (ASVs) for taxonomic determination, ranging from kingdom to genus. The data analysis included the Centered Log Ratio (CLR) transformation of the ASV matrix. The iNext package was used to calculate diversity using the Shannon index. Using Euclidean distances, Principal Component Analysis (PCA) was performed with the CLR-transformed data.

Functional gene inference was performed using the PICRUSt2 pipeline, which predicts functional pathways from 16 S rRNA gene sequences. LEfSe (Linear Discriminant Analysis Effect Size) analysis was employed to identify significant differences in metabolism between groups (log LDA score > 2 and $p < 0.05$). Additionally, LEfSe was used to identify differentially represented genera among the different groups. A Spearman correlation analysis was conducted to explore the relationships between seven differential genera and the 30 metabolic pathways with the highest correlation across groups. Wilcoxon tests were used for statistical analyses of phylum and genus comparisons, with significance defined as $^*p < 0.05$, and non-significant results denoted as N.S. In the Spearman correlation analysis, the Benjamini-Hochberg correction was used to adjust p-values and control the false discovery rate (FDR), with results expressed as q-values ($q < 0.1$). This approach enabled the identification of key interactions and associations relevant to the study objectives. All microbiome analyses were performed using R (version 4.2.0) and RStudio (version 2022.7.1.554).

Results

Baseline and nutritional modifications induced by yogurt and whey protein intake

The flow diagram is illustrated in Fig. 1. The WP and PY groups were well-balanced in terms of sex and showed no differences in baseline hemodynamic, metabolic, and aerobic capacities, as shown in Table 1. After an 8-week intervention, calorie (kcal/d) and fat (g/d) intake did not differ within and between groups. In contrast, carbohydrate intake (g/d) decreased in the WP group after intervention (−36.9 ± 52.9 g/d, $p = 0.034$), with no change in the PY group (−2.4 g±37 g/d, $p = 0.87$) (Table 1). As expected, WP and PY increase their total and weight-adjusted (g/kg) protein intake compared with baseline (WP; 22.2 ± 19 g/d, 0.38 ± 0.0 g/kg and YP; 31.7 ± 12.6 g/d, 0.5 ± 0.0 g/kg), respectively, with no differences between groups (Table 2). Regarding serum lipid, renal, and hepatic markers, no significant changes were observed following the 8-week intervention with either WP or PY. (Supplementary Fig. 2).

Body composition, metabolic and functional changes after intervention

8-week intervention with ST consuming WP or PY did not affect body weight ($p = 0.45$) (Fig. 2A). However, skeletal muscle mass was similarly increased in both groups after intervention (WP 0.47 ± 0.74 kg, $p = 0.03$, PY 0.50 ± 0.40 kg, $p = 0.02$) (Fig. 2B), and phase angle (PhA) (Fig. 2C). In contrast, total fat mass (kg) significantly decreased only in WP group ($p = 0.02$) (Fig. 2D). Importantly, neither skeletal muscle mass, PhA or fat mass was different between groups at the end of the intervention. Regarding metabolic parameters, the resting metabolic rate increased significantly after intervention in the PY group (150.1 ± 132 kcal, $p = 0.03$) compared to WP (131.8 ± 253.7 kcal, $p = 0.08$) (Fig. 2E). In contrast, ST with neither nutritional intervention influenced $VO2_{max}$ (Fig. 2F). However, a significant increase in gait speed was found in both groups compared with baseline (WP 0.17 ± 0.16 m/s, $p = 0.007$, PY 0.16 ± 0.15 m/s, $p = 0.009$) with no group differences (Fig. 2F).

Variable	WP (n = 8, 5 F/3 M)	PY (n = 9, 6 F/3 M)	p-value
Age (yr)	66.2 ± 4.4	65.6 ± 4.1	0.79
Weight (kg)	72.3 ± 20.2	65.1 ± 13.8	0.48
Body Mass Index (kg/m^2)	25.9 ± 4.4	24.7 ± 2.9	0.65
Systolic Blood pressure (mmHg)	140.3 ± 12.9	131.4 ± 12.0	0.24
Diastolic Blood pressure (mmHg)	80.2 ± 6.7	80.3 ± 8.5	0.94
Fasting glucose (mg/dL)	114.5 ± 12.8	113.8 ± 10.0	0.81
Resting Heart Rate (bpm)	63 ± 8.5	68.4 ± 8.7	0.20
Resting Metabolic Rate (kcal/d)	1698 ± 440	1611 ± 205	0.60
$VO2_{max}$ (ml•kg•min^{-1})	21.3 ± 2.9	22.2 ± 3.2	0.58

Table 1. Baseline body composition, metabolic, and cardiovascular fitness differences. $VO2_{max}$: Maximal oxygen consumption. Data are presented as the mean ± standard deviation (SD). Unpaired t-test, $^*p < 0.05$.

Variable	WP (n = 8, 5 F/3 M)			PY (n = 9, 6 F/3 M)			ANOVA
	Pre	Post	p-value*	Pre	Post	p-value*	p-value #
Calories (kcal/d)	1953 ± 615.1	1835 ± 460.9	0.283	1816.7 ± 465.3	1940.9 ± 443.5	0.232	0.66
Protein (g/d)	82.9 ± 30.6	105.1 ± 20.5	0.001*	79.9 ± 20.4	111.6 ± 17.3	0.000*	0.55
Protein (g/kg/)	1.2 ± 0.4	1.5 ± 0.4	0.002*	1.2 ± 0.3	1.7 ± 0.3	< 0.001*	0.21
Fat (g/d)	68.7 ± 32.7	63.1 ± 39.9	0.503	63.2 ± 24.6	60.8 ± 18.2	0.762	0.87
Carbohydrates (g/d)	242.0 ± 84.5	205.1 ± 50.4	0.034*	242.0 ± 62.1	239.6 ± 71.9	0.874	0.30
Carbohydrates (g/kg)	3.4 ± 1.3	3.0 ± 0.8	0.061	3.7 ± 0.7	3.7 ± 0.1	0.852	0.14

Table 2. Calorie and macronutrient intake assessed by 24-hour recall. Data are presented as the mean ± standard deviation (SD). * Denotes significant intragroup differences between pre-and post-intervention, # Denotes significant differences post-intervention between WP and YP groups. Two-way ANOVA ($p < 0.05$).

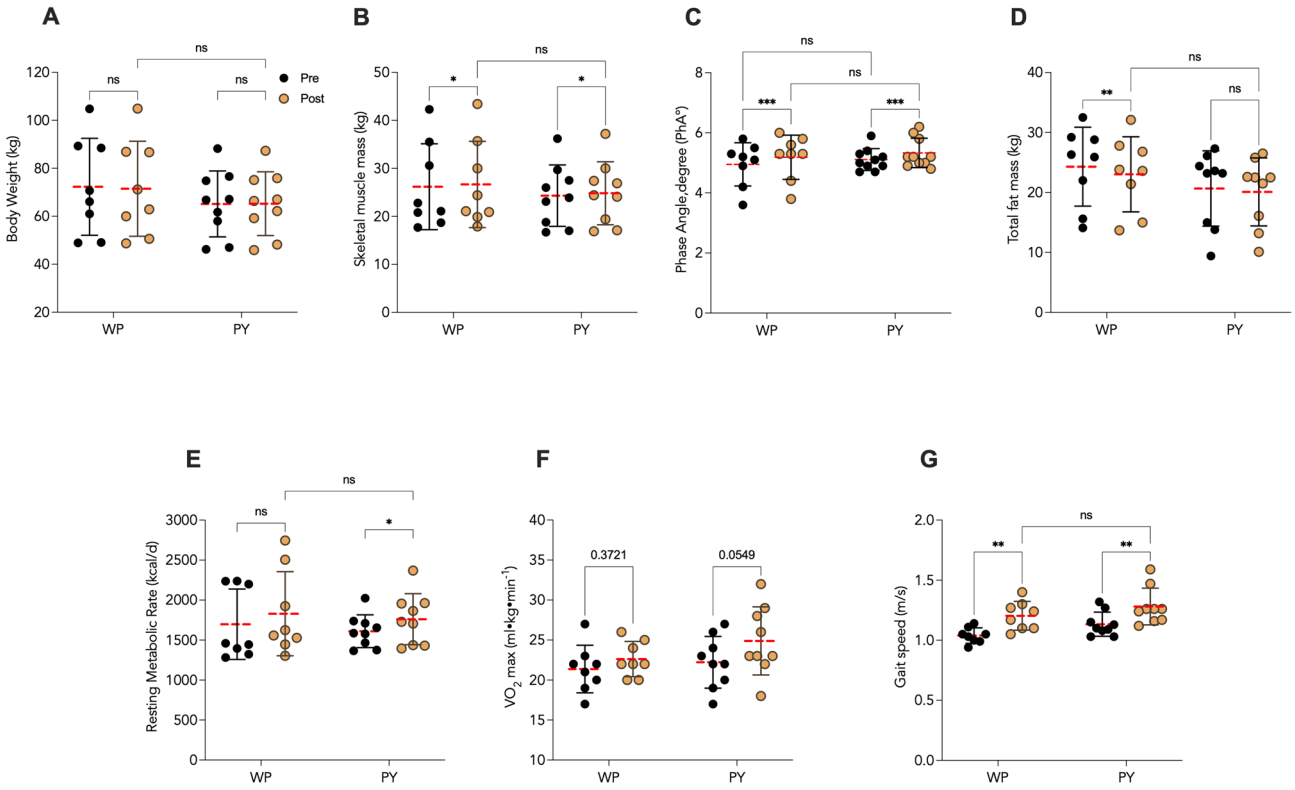


Fig. 2. Body composition and functional physical tests. (**A**) Body weight (kg), (**B**) Skeletal muscle Mass (SMM, kg), (**C**) Phase Angle (PhA, 50 kHz), (**D**) Total fat mass (kg), (**E**) REE (Kcal/day), (**F**) Maximal Oxygen Consumption (VO_{2max}, ml*kg*min^{-1}), (**G**) Gait speed in TUG test (m/s). Data are presented as the mean ± standard deviation (SD). Two-way ANOVA, * denotes significant intragroup differences between pre- and post-intervention (*$p < 0.05$, **$p < 0,001$, ns = no significant).

Strength improvements are similar between protein yogurt and whey protein

Bench press (BP) (Fig. 3A) and leg press (LP) (Fig. 3B) one-repetition maximum (1RM) increase significantly in both intervention groups compared to baseline (WP; BP: Δ10.9 ± 10.3 kg, $p = 0.002$, LP: Δ38.5 ± 24,3 kg, $p = 0.003$ and YP; BP: Δ12.4 ± 7.2 kg, p = < 0.0001, LP: Δ57.1 ± 19,7 kg, $p = 0.007$), while hand grip strength (HGS) normalized by height^2 improved only after PY intervention ($p = 0.02$) (Fig. 3C). Regarding isokinetic strength, WP and PY improve right (WP Δ19.9 ± 8.20 Nm, $p = 0.0004$, PY Δ16.0 ± 15.2 Nm, $p = 0.001$) and left quadriceps torque (WP Δ15.9 ± 11.46 Nm, $p = 0.003$, PY Δ12.4 ± 13.7 Nm, $p = 0.01$) with no differences between groups at the end of the intervention ($p = 0.18$) (Fig. 3D and F). Although neither left nor right hamstrings showed significant differences between groups, PY had a positive trend (Δ7,78 ± 10,33 Nm, $p = 0.0504$) after the intervention in the right hamstring compared to WP (Δ−1,1 ± 11,66 Nm, $p = 0.775$).

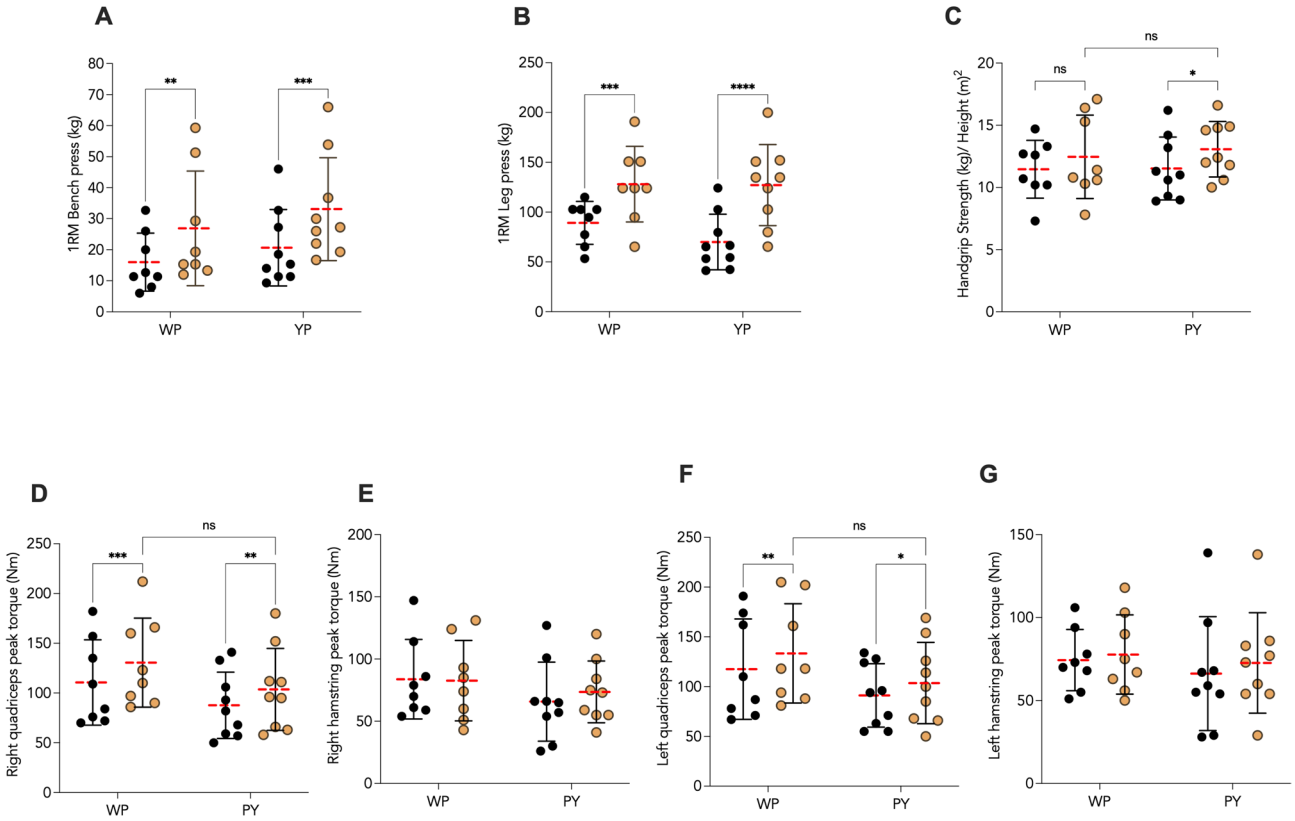


Fig. 3. Maximal and Isokinetic strength. (**A**) 1 repetition maximum Bench press (kg), (**B**) 1 repetition maximum Leg press (kg), (**C**) Dominant handgrip strength (Kg/Height^2), (**D**) Right quadriceps peak torque (Newton/m), (**E**) Right hamstring peak torque (Newton/m), (**F**) Left quadriceps peak torque (Newton/m), (**G**) Left hamstring peak torque (Newton/m). Data are presented as the mean ± standard deviation (SD). Two-way ANOVA, * and ** denote significant intragroup differences between pre-and post-intervention (*$p < 0.05$, **$p < 0.001$, and ns = no significant).

Protein intake from yogurt or whey protein alters the bacterial phyla, genus, and correlates with predicted functional pathways

Relative abundance of the six main phyla in the gut microbiome showed changes after ST in both WP and PY groups (Fig. 4A). From these phyla specifically, Firmicutes and Bacteroidota significantly increased and decreased, respectively, only in the WP group after ST ($p = 0.01$ and $p = 0.03$). In contrast, the PY group showed no change after the intervention (Fig. 4B and C). As expected, the individual changes in the most abundant microbiome phyla alter their Firmicutes/Bacteroidota ratio (F/B), where the F/B in the WP group increased significantly ($p = 0.01$) compared to the PY group ($p = 0.29$) after the intervention.

Regarding the genus, general changes were observed after intervention in both groups (Fig. 5A). Additionally, the genera *Copprococcus*, *Subdoligranulum*, *Prevotella*, and *DTU089* showed changes in their differential abundance. On the other hand, the genera *Klebsiella*, *Eggerthellaceae (DNF00809)*, *Butyricicoccaceae*, *and UCG-008* were identified in the WP group through LEfSe (see Methods for LEfSe, *data not shown*). *Copprococcus* and *Subdoligranulum* increased significantly ($p = 0.03$ and $p = 0.02$; Fig. 4B, PRE, and POST). At the same time, *Prevotella* decreased ($p = 0.038$) after the exercise intervention, regardless of supplementation (Fig. 4B, PRE, and POST). WP and PY interventions induced differential changes in the specific gut genus, where *Coprococcus* increased only on PY (Fig. 4B, Protein Yogurt) ($p = 0.012$), whereas *DTU089* increased ($p = 0.014$) and *Prevotella* decreased significantly in WP groups ($p = 0.022$) (Fig. 5B, Whey Protein). Although *Klebsiella*, *Subdoligranulum*, *DNF00809*, *and UCG-008* genus did not change significantly, there was a shift in gut populations confirmed with the changes in microbiome diversity in both groups expressed in Shannon entropy ($p = 0.010$), and where this modification where mainly on PY group ($p = 0.04$) compared to WP ($p = 0.14$) (Supplementary Fig. 3). Also, using PICRUSt2 and comparing it with the database (Permission N°254225), we conducted a Spearman analysis on the seven major abundant *genera* against the 50 most differential predicted functional pathways. Here, we observed similarities between intra- and intergroup bacteria enriched after WP and PY interventions (Fig. 6). These changes were associated with a marked correlation in different signaling and cellular processes, including metabolism, transporters, and genetic information processing.

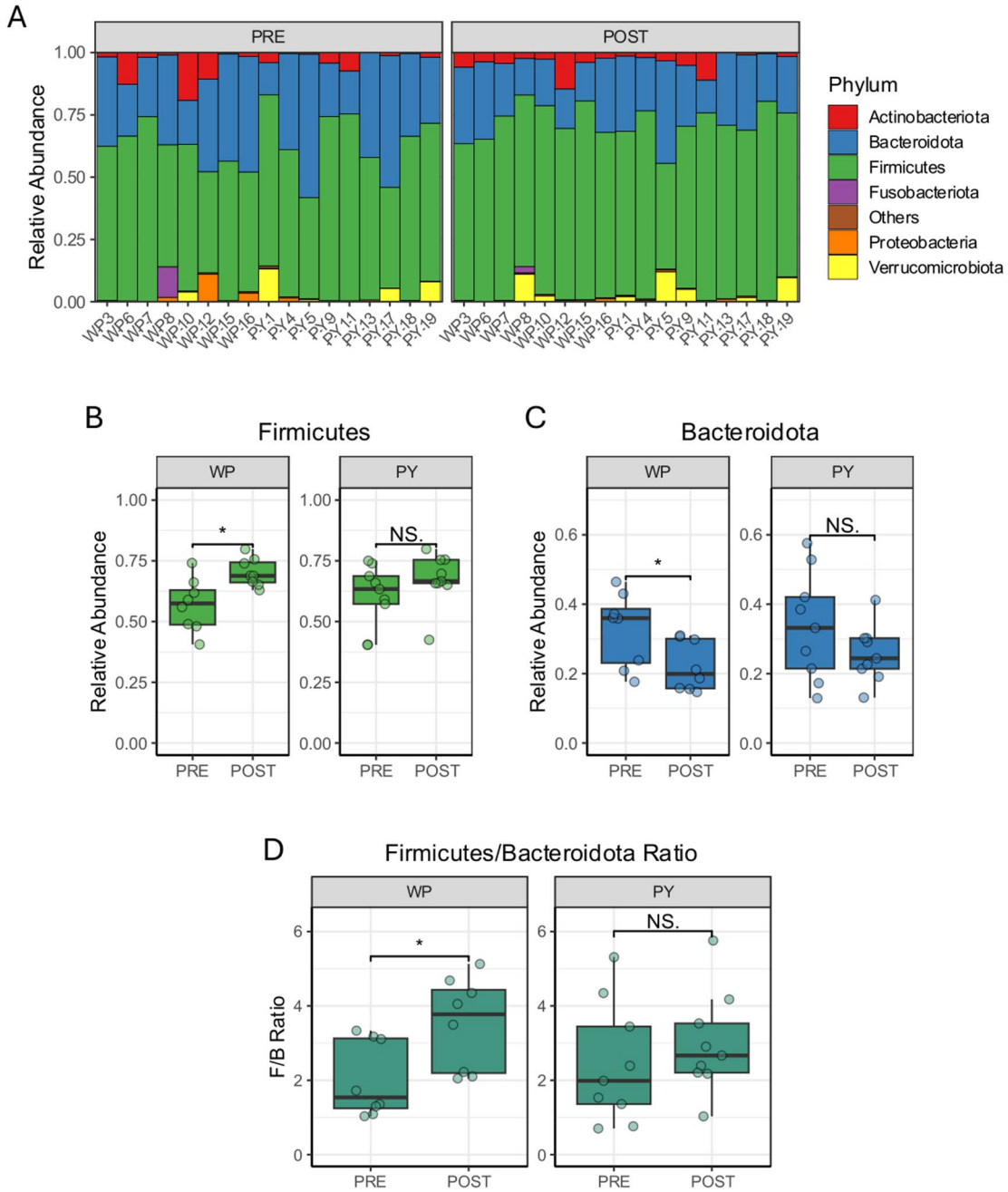


Fig. 4. Relative abundance of bacterial phyla in the gut microbiome. (**A**) Relative abundance of all samples before (PRE) and after (POST) the intervention of physical activity and protein supplementation. The six main phyla shown are *Actinobacteriota*, *Bacteroidota*, *Firmicutes*, *Fusobacteriota*, *Proteobacteria*, *and Verrucomicrobiota*; the rest were grouped as "Others." (**B** and **C**) Specific comparisons of the relative abundance of the two most abundant phyla. Firmicutes (green) and Bacteroidota (blue). (**D**) *Firmicutes/Bacteroidota* Ratio Comparison. The ratio between the phyla *Firmicutes and Bacteroidota* was evaluated before (PRE) and after (POST) the intervention involving physical activity and protein supplementation. Each point represents the ratio calculated for an individual sample. The black line in the bar represents the average, and the vertical line indicates the standard deviation of the group, as calculated using the applied supplement. Each circle represents the relative abundance of each sample. A Wilcoxon test was performed on the PRE and POST pairs of each protein supplement for statistical analysis. * $p < 0.05$. NS indicates not significant.

Discussion

This is the first study to compare the effects of consuming the same amount of protein from a high-protein yogurt or whey protein on body composition, physical function, and microbiome alterations during a strength training program in older adults. The key findings of the present study are that: (a) 8-week strength training (ST) induces important changes in muscle mass (SMM, PhA°), strength (1RM, Quadriceps peak torque (Nm)) and

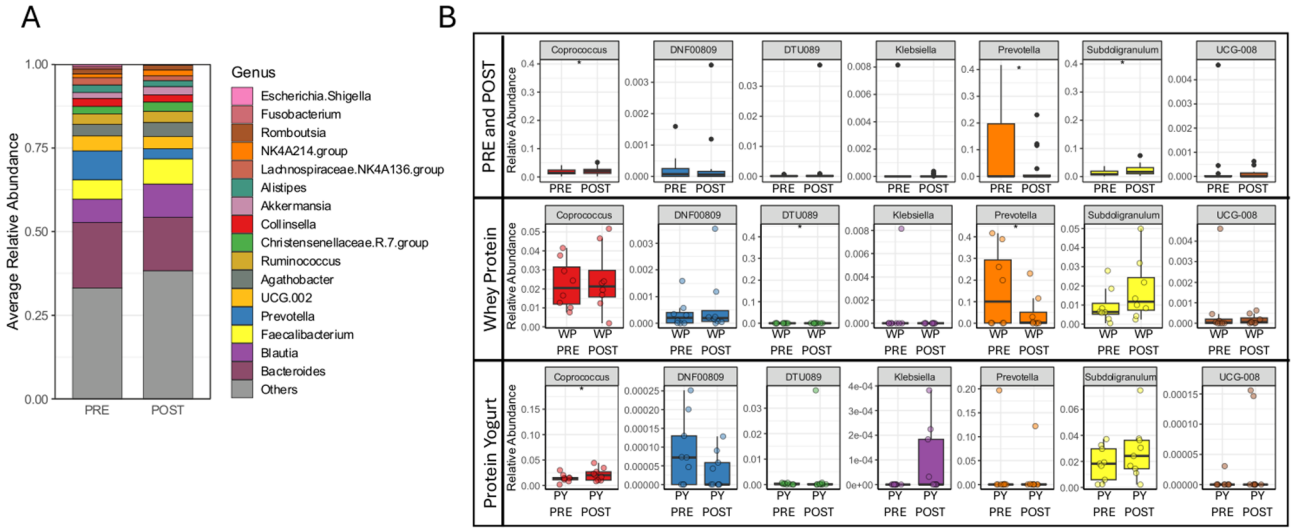


Fig. 5. Relative abundance of bacterial genera in the gut microbiota. (**A**) Distribution of the 16 most abundant genera before (PRE) and after (POST) the physical activity intervention. The remaining genera were grouped under the category "Others," denoted in gray. (**B**) Relative abundance of seven genera: *Coprococcus*, *Eggerthellanceae DNF00809*, *Ruminococcaceae DTU089*, *Klebsiella*, *Prevotella*, *Subdoligranulum*, and *Butyricicoccaceae UCG-008*. A Wilcoxon test was performed between PRE and POST pairs for statistical analysis of each protein supplement. * $p < 0.05$.

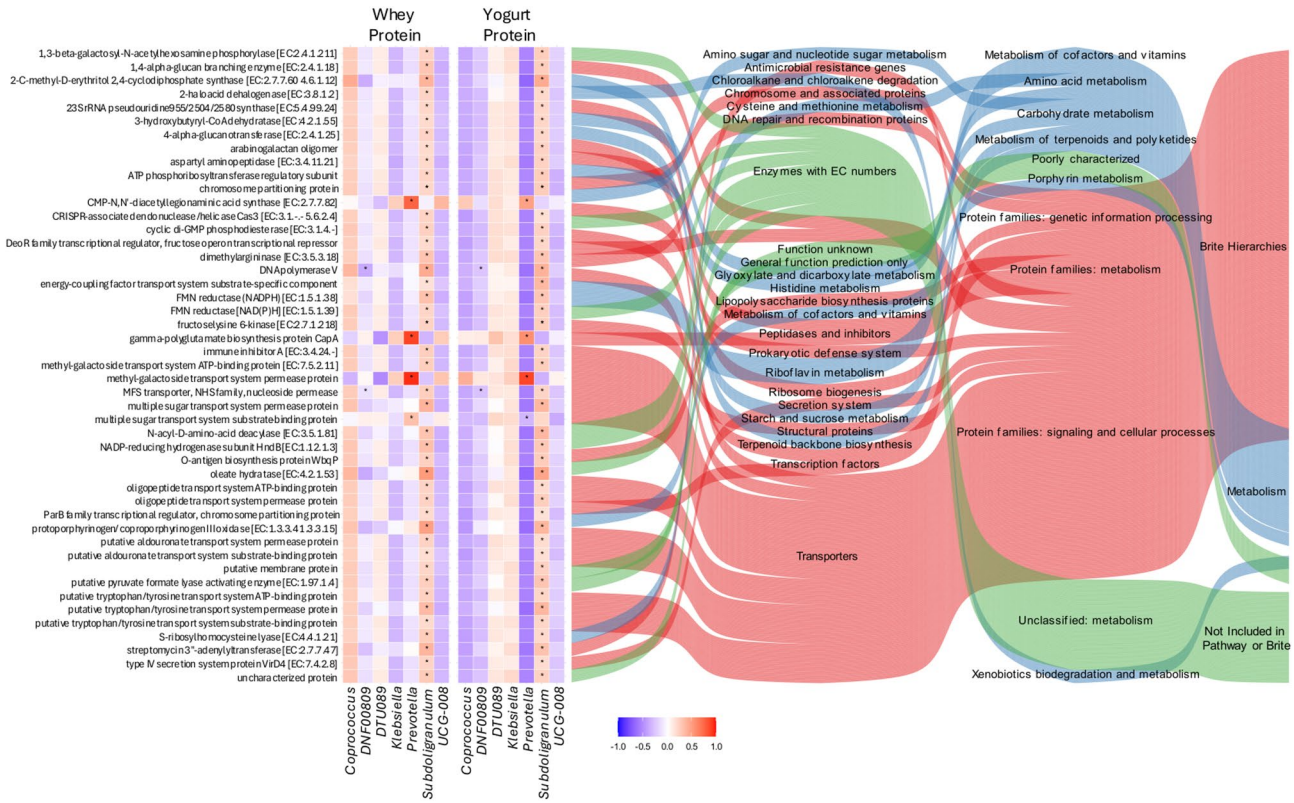


Fig. 6. Spearman correlation analysis between seven differential genera and the top 50 differential KEGG. The heat map displays the correlation coefficients, represented by color gradients ranging from red (positive correlation) to blue (negative correlation). The analysis includes post-intervention groups, one supplemented with WP and the other with PY. The Benjamini-Hochberg (p-value adjusted) correction was used to control for false discoveries and adjusted as q-values, with *q-value ($q < 0.1$). Pathway annotations were derived from the KEGG database[47].

functional (gait speed) that are independent of the source of protein intake; (b) ST with PY or WP differentially affect fat%, RMR, HGS/ht(m)^2 and gut microbiome phyla and genera and; (c) Microbiome changes induced by ST + PY or ST + WP are associated with the enrichment of different predicted metabolic pathways and cellular processes of potential relevance to host metabolism and physiological regulation, which should be interpreted cautiously and require confirmation with direct functional measurements.

Strength training (ST) is the most effective strategy for preventing and managing the majority of primary and secondary sarcopenia consequences in older adults[48,49]. Importantly, training-induced changes in muscle mass and body composition are relevant and can be produced at any age[8,50]. Here, the 8-week ST program decreased fat mass in the WP group and increased skeletal muscle mass and PhA° in both groups. PhA° is commonly used as a marker of cell membrane integrity and function and has been proposed as a marker of muscle function and quality[51]. Importantly, muscle quality is defined as the ability to generate force per unit of muscle mass or size, and it has also been correlated with muscle composition as a measure of muscle echogenicity[52,53]. Although in the present study, muscle quality was not determined, gait speed (m/s), 1RM on leg and chest press (kg), and quadriceps peak torque (Nm) increased significantly upon intervention, independent of the supplementation regime, thus supporting that ST, accompanied with adequate protein intake and quality, significantly improve muscle strength and function in older adults.

In order to promote an adequate protein intake during ST in the PY group, we provided individuals with two portions (240 g) of a commercially available Chilean high-protein yogurt (Extra-Proteina, Loncoleche, Chile) and the WP group with a whey protein isolated (Iso100, Dymatize)[54,55] to ensure a protein intake of 24.5 g and 25 g on training days respectively. Our research team has previously analyzed this particular yogurt and protein brand using proximate analysis for macronutrient composition and high-performance liquid chromatography (HPLC) for amino acid profile determination[56], demonstrating its nutritional value. Recently, Bagheri et al.. reported the efficacy of ST, along with the consumption of Icelandic yogurt (IR, formula verified) on training days (18 g protein) in untrained older men (68 years) compared to a placebo group[25]. Like our results, lean mass, bench press, and leg press increased significantly in both groups after the intervention ($p < 0.001$). Nevertheless, delta changes were more significant in the group consuming the Icelandic yogurt: lean mass (IR: Δ1.3 kg, PL: Δ0.6 kg), bench (IR: Δ4.3 kg, PL: Δ2.3 kg), and leg press (IR: Δ4.2 kg, PL: Δ2.5 kg) compared to the placebo. Also, the IR group had a ~ 20 g/d increase in their daily protein (pre 1.3 ± 0.9 g/kg per d; post 1.6 ± 0.1 g/kg per d) compared to placebo (pre 1.2 ± 0.1 g/kg per d, post 1.3 ± 0.1 g/kg per d) during the intervention[25] showing that yogurt is a cost-effective strategy to support ST in the older population. However, like Bagheri, the older males and females in the present study had adequate protein intake (~ 1.2 g/kg per d) at the beginning of the intervention and, as expected, a significant increase in the final intake (~ 1.5–1.7 g/kg per d), which is in line with the skeletal muscle mass and strength improvements shown by different protein sources when ST is performed on this particular population[19,57,58]. Yogurt has also been shown to be a good source of protein for muscle adaptations in younger participants. Bridge et al. report that untrained university males increase lean mass after 12 weeks of strength training by consuming Greek yogurt (GY) compared to a placebo pudding (0 g protein)[24]. Particularly in this study, the GY group received an additional 60 g of protein during training and 40 g on non-training days compared to a placebo group, which supports that the difference in body composition obtained in the GY group was possible by the higher protein intake per se and not for the nutrient characteristics of the yogurt used.

As mentioned, yogurts provide a high-quality protein source, particularly in terms of whey and casein content[59,60]. They also contain bioactive compounds, such as bacteriocins, amino acids, peptides, and short-chain fatty acids (SCFA), that can improve overall[61] and gut health[27]. It has been reported that aging induces gut microbiome dysbiosis, which could affect skeletal muscle mass and function[4,62]. Compared with young adults, older adults have higher levels of Bacteroidetes and a lower proportion of Firmicutes, reflected in a reduced Firmicutes/Bacteroidetes (F/B) ratio using 16 S rRNA sequencing[63]. We show that ST and WP significantly increase the F/B, while PY also tends to increase. Here, we reported the F/B ratio as a descriptive, phylum-level summary and not as a biomarker of gut health, dysbiosis, or inflammation. Several taxa within the Firmicutes phylum (particularly members of Clostridiales, such as Lachnospiraceae and Ruminococcaceae) are known butyrate producers, and SCFAs, including butyrate, have been implicated in muscle metabolism in pre-clinical aging models[64]. Although the F/B ratio has been observed to increase in some studies in adults with obesity[65,66], this ratio has also been positively correlated with cardiorespiratory fitness in young adults[67], suggesting a lack of consensus regarding this ratio when exercise is present.

Regarding the bacterial genera, we reported an increase in *Coprococcus* (a butyrate-producing genus) in PY, *a Subdoligranulum* enrichment in WP, and a decrease in *Prevotella*after training, with significant changes observed only in the WP group. Similarly, a recent study led by Duan et al[68]. reports that ultra-marathon runners had an increase in the F/B ratio and enrichment of *Coprococcus* compared to sedentary controls[68]. However, the mechanisms of this modulation are unknown. The results suggest that exercise intensity plays a vital role in altering the microbiome, especially in butyrate-producing genera[69]. This potential effect of exercise intensity could be explored by analyzing fecal SCFAs. This area requires further study, especially among older populations with varying fitness levels, and to determine whether yogurt could enhance these benefits.

Protein sources have also been considered relevant modulators of microbiome diversity[70]. As mentioned, whey and casein contents in yogurt make it a good option for sustaining aminoacidemia[71] and supporting muscle health. Several authors have described an inverse correlation between frailty and microbiome biodiversity[72,73]. In our study, the overall microbiome diversity of older adults increased in both groups. At the same time, PY intake resulted in the most significant increase in alpha diversity, as determined by Shannon entropy, compared to the WP group (Supplementary data). Recently, microbiome diversity was associated with physical activity levels in 101 older adults from 65 to 85 years[74], with a primary abundance of *Lactobacillus*, *F Faecalibacterium prausnitzii*, and *Roseburia intestinalis*, and a lower abundance of disease-associated bacteria such as *Desulfovibrio*

piger or potentially pathogenic Enterobacterales members. This result suggests that physical activity improves healthy microbiota in older adults. Although we did not assess spontaneous physical activity levels, training frequency may also be a relevant factor warranting further study in this population. Future research should focus on assessing the specific effects of exercise types, frequencies, dietary sources, and food-matrix differences. This last is especially relevant as yogurt, for example, can vary in carbohydrate and calcium content, which could affect the microbiome profiles and gut health in older adults despite similar protein content.

Conclusion

In conclusion, we demonstrate that older adults consuming either a classic whey protein supplement or a commercially available high-protein yogurt exhibit similar adaptations and improvements in muscle strength and functional capacity. At the same time, due to its digestibility, the protein matrix may have differential effects on the gut microbiome of older adults, especially on phyla and genera associated with metabolic predicted pathways related to overall health. These findings highlight two key aspects: the effect of strength training on improving functional capacity and muscle mass, which are lost due to aging-induced sarcopenia, and the relevance of different food matrices´ compositions in supporting muscle adaptations. Although this work focused on protein intake, future research should focus on varying the content of other macronutrients, minerals, and small peptides in whey and casein and examine their potential effects on gut health.

Limitations

Although the present results are relevant and highlight the role of strength training and different protein sources, such as whey supplements and yogurts, on the gut microbiome and overall health in older adults, they also have several limitations. First, our small sample size is limited to relatively healthy older adults with no diabetes, smoking, or other medical complications that have been shown to alter the gut microbiome (i.e., dysbiosis), and in whom specific changes in *phyla* or *genera* could be pronounced. Also, this small sample size does not allow for the detection of sex differences induced by the interventions. Second, the trial was designed to compare two protein sources under a standardized, supervised strength-training stimulus; therefore, we did not include a strength training–only arm, as estimating the independent effect of training per se was outside the scope of the present research question. Future studies could incorporate a training-only comparator to quantify the additive contribution of protein supplementation and to further examine dietary matrix features on gut-related outcomes in older adults. Third, functional profiles were predicted from 16 S rRNA gene data using PICRUSt2 rather than measured directly; therefore, these results should be considered hypothesis-generating and require confirmation with direct functional assessments. Lastly, although the BIA equipment used in the present study for body composition analysis (Inbody 970) is reliable and comparable with Dual energy X-ray absorptiometry (DXA), especially with upper body muscle mass[75], some of the differences observed in body composition between groups could not be detected by the small number of subjects.

Data availability

The datasets generated and/or analyzed during the current study are available in EMBL-EBI, specifically in the European Nucleotide Archive (ENA) repository under the accession code PRJEB104255, and may also be obtained upon request from M.MA or DV.I.

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Declarations

Competing interests

The authors declare no competing interests.

Additional information

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