Aging Research

ISSN 2957-8701 (print); 2957-9295 (online)
https://doi.org/10.26599/AGR.2024.9340028

Article

Effect of probiotics or synbiotics on skeletal muscle in older adults: a systematic review and meta-analysis of randomized controlled trials

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Received: 24 May 2024 | **Revised:** 4 June 2024 | **Accepted:** 9 June 2024

ABSTRACT

Probiotics and synbiotics have been widely used to modulate gut microbiome, which is associated with skeletal muscle aging and overall health in older adults. The aim of this systematic review and meta-analysis was to verify the effect of probiotics and synbiotics on muscle mass, muscle strength, physical performance, and quality of life (QOL) in older adults. PubMed, Embase, and Web of Science were searched from inception to Nov. 2023 for randomized controlled trials (RCTs). RCTs were identified that investigated the effects of supplementations containing probiotics or synbiotics on parameters of muscle mass, muscle strength, physical performance, and QOL in elderly adults. Standardized mean difference (SMD) with their 95%CI was used for the synthesis of the results. In total, 15 studies were included in this review. The administration of probiotics and synbiotics moderately improved muscle strength ($k = 7$, SMD = 0.667, 95%confidence interval (CI): 0.317–1.017, $I^2 = 72.5\%$, $p = 0.001$) and physical performance ($k = 6$, SMD = 0.597, 95%CI: 0.118–1.075, $I^2 = 80.6\%$, $p < 0.001$), whereas no significant effect was found on muscle mass ($k = 8$, SMD = 0.067, 95%CI: –0.090–0.225, $I^2 = 26.0\%$, $p = 0.221$) and QOL ($k = 7$, SMD = 0.421, 95%CI: –0.054–0.895, $I^2 = 86.2\%$, $p < 0.001$) in older adults. The subgroup analysis showed better performance in studies conducted in Asia, providing extra nutritional supplementation and exercise programs, and using long-term treatment, with more positive effects or lower heterogeneity. Furthermore, some evidence about the effects on gut microbiome, intestinal permeability, and immune and nutritional status was reported. This systematic review and meta-analysis demonstrate the safety and effectiveness of probiotics and synbiotics in improving muscle function among older people, possibly through alleviating intestinal permeability, immunosenescence, and nutritional status. Systematic review registration: https://www.crd.york.ac.uk/prospero/, Identifier: CRD 42023428400.

KEYWORDS

probiotics, synbiotics, muscle, quality of life, aging.

1 Introduction

With impressive advancements in medical science, the global population is aging and life expectancy continues to rise. However, healthy life expectancy is not keeping pace due to current therapeutic strategies focusing more on decreasing mortality but less on the elevation of quality of life (QOL) and overall health. Hence, there is a growing need to prioritize the framework of health aging to alleviate the medical, economic, and social burden associated with an aging population [1].

The skeletal muscle system, attracting more attention in older people, not only plays a vital role in maintaining posture and mobility but also serves as a mediator of systemic metabolism and inflammation. As individuals age beyond 40 years, there is a gradual decline in muscle mass and function, leading to multiple adverse outcomes (e.g., falls) and non-communicable diseases (NCDs, e.g., diabetes). The aging skeletal muscle is characterized by reduced regenerative capacity due to muscle stem cells (MuSCs) senescence, decreased anabolism, and increased catabolism of proteins causing myofiber atrophy, infiltration of ectopic adipose with fibrotic signatures, and loss and sclerosis of microvasculature capillaries. Both secretions and metabolic function from aged myofiber and the niche contribute to metabolic abnormalities and chronic low-grade systemic inflammation, thereby accelerating the aging process of skeletal muscle [2–4]. Elderly individuals with aging skeletal muscle who meet the criterion of declining muscle mass and function (muscle strength and physical performance) may be diagnosed with sarcopenia [5, 6].

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Cite this article as Zeng, Q., et al. *Aging Research*, **2024**, 2: 9340028.

Similar to the aging of skeletal muscle in humans, the gut microbiome undergoes dynamic changes. These changes involve continued drift with age in healthy people, while lacking consecutiveness in less healthy individuals [7]. Particularly in older adults with frailty and sarcopenia, decreasing gut microbiota diversity along with specific taxonomic alternation, such as increasing pathogenic bacteria and decreasing short-chain fatty acids (SCFAs)-producers, could be observed, implicating a potential link between gut microbiota and skeletal muscle aging [8, 9]. Furthermore, a Mendelian randomization study identified Alcaligenaceae, Family XIII, and Paraprevotella were positively associated with the risk of low hand-grip strength, while Streptococcaceae were negatively associated; moreover, eight bacterial taxa were associated with appendicular lean mass (ALM) [10]. The aging-related microbial communities may not only affect nutritional absorption and the integrity of the intestinal barrier but also trigger low-grade systemic inflammation and metabolic dysfunction [11–13], consequently leading to skeletal muscle aging.

Emerging evidence has further demonstrated that the alternation of gut microbiome had impacts on skeletal muscle, implicating the feasibility of modulating the gut microbiome to retard or even reverse the aging process of muscle [14, 15]. Probiotics and synbiotics, which are frequently used as gut microbiota regulators, have advantages in generating beneficial metabolites like SCFAs, promoting a friendly microbial community, strengthening the gut mucosal barrier, and ameliorating systemic inflammation and metabolic dysfunction [16–18], indicating potential benefits for muscle mass and function. Therefore, in order to prudently verify the effect of probiotics and synbiotics supplementation on muscle mass, muscle strength, physical performance, and QOL in older adults and explore other potential contributing factors, we performed a meta-analysis and subgroup analysis.

2 Results

2.1 Selection process

Through a comprehensive search of different databases according to our search strategy, a total of 3147 records were identified. After removing the duplicates, we screened 2471 papers based on their titles and abstracts, subsequently leaving us with 167 articles. Ultimately, following a meticulous review of the full texts, 15 studies were included in our review. Figure 1 illustrates the detailed flow diagram of the study selection process.

2.2 Characteristics of selected studies

The characteristics of the included studies are outlined in Table 1. Thirteen trials were double-blind RCTs [19–31], and two were open-label [32, 33]. These studies were conducted across various regions, including three in Sweden [21–23],

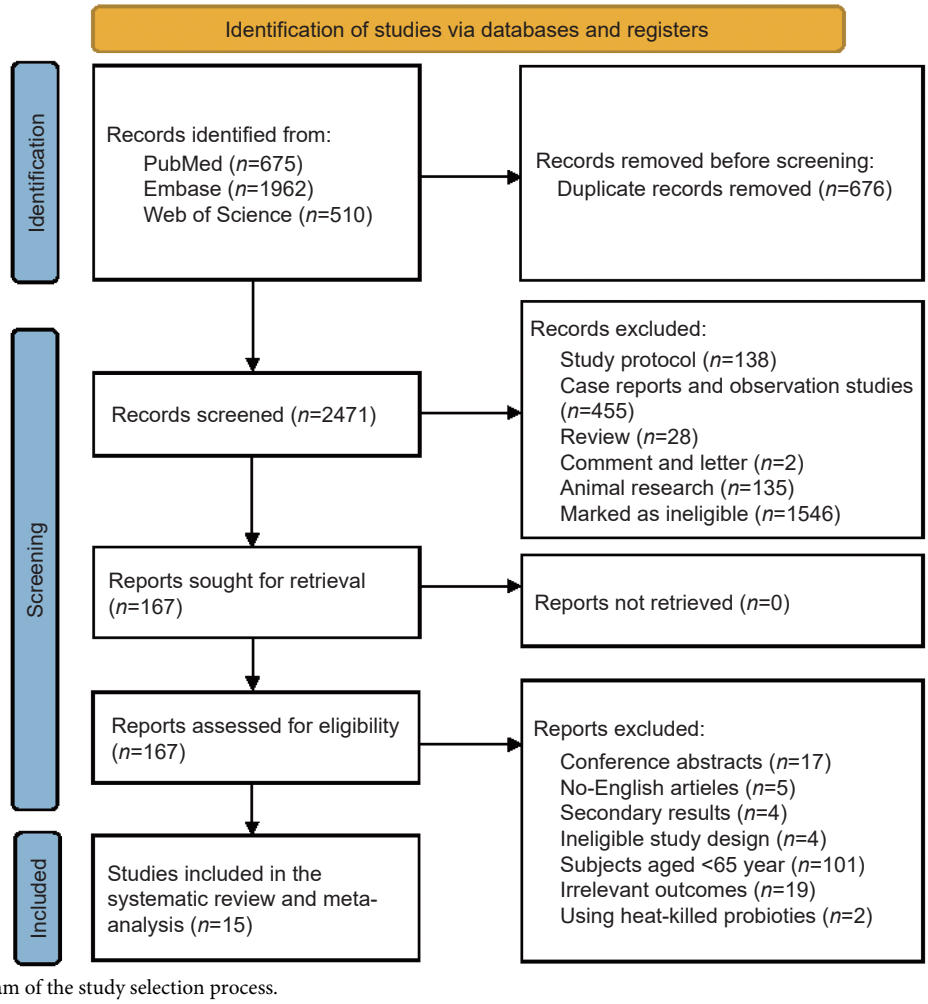

Figure 1 Flow diagram of the study selection process.

Table 1 The characteristics of eligible studies

Ref.	Region	Design	Participants			Intervention			Extracted outcomes
			Size (*N*)	Age	Health status	All subjects	Experimental treatment	Control	Period	
[33]	Japan	RCT	E:43 C:42	67.7 (59, 84)[a]	In good health	—	90 g of yogurt once per day containing *Lactobacillus bulgaricus* OLL1073R-1 (2.0×10^8–3.5×10^8 CFU/g) and *Streptococcus thermophilus* OLS3059 (6.3×10^8–8.8×10^8 CFU/g) (1073R-1 yoghurt)	100 g of milk once per day	12 weeks	QOL score
[26]	Brazil	Double-blind RCT	E:9 C:8	67.9 ± 4.5	Frailty	—	Synbiotics supplement: 6 g of Frutooligossacarides, 10^8–10^9 CFU *L. paracasei*, 10^8–10^9 CFU *L. rhamnosus*, 10^8–10^9 CFU *L. acidophilus* and 10^8–10^9 CFU *Bifidobacterium lactis*	Placebo: maltodex-trin	3 months	Fat free mass (BIA); Handgrip strength
[19]	China	Double-blind RCT	E:189 C:192	E:64.3 ± 4.1 C:65.1 ± 3.7	Non-displaced distal radius fracture	—	Skimmed milk containing a minimum of 6×10^9 CFU *L.casei* Shirota	Skimmed milk without probiotics	6 months	Handgrip strength
[21]	Sweden	Double-blind RCT	E:125 C:124	73.1 (65, 98)[a]	Free-living older adults	—	A stick-pack containing freeze-dried *L. reuteri* DSM17938 1×10^8 CFU, rhamnose, galactooligosaccharide, and maltodextrin to a total weight of 1 g	Placebo contained maltodex-trin	12 weeks	ED-5D-5L (EQ-5D index, EQ-5D VAS)
[28]	Japan	Double-blind RCT	E:20 C:19	E:69.9 ± 3.0 C:70.9 ± 3.2	Undergone stretch training for 1 year	Moderate resistance training	A sachet containing lyophilised power of *B. longum* BB536, *B. infantis* M-63, *B. breve* M-16V and *B. breve* B-3 (approximately 1.25 $\times 10^{10}$ CFU each) with the carrier dextrin	Placebo	12 weeks	Lean body mass (BIA)
[23]	Sweden	Double-blind RCT	E:45 C:45	E:76.4 ± 1.0 C:76.3 ± 1.1	Older women with low bone mineral density	—	Stick packs containing freeze-dried *L. reuteri* ATCC PTA 6475 in doses of 5×10^9 CFU and maltodextrin powder (twice daily)	Placebo product contained maltodex-trin powder	12 months	Total lean mass (DXA)
[29]	Spain	Double-blind RCT	E:18 C:18	E:65.8 ± 3.1 C:64.0 ± 2.6	Cirrhosis	—	A mixture of eight strains including *Streptococcus thermophilus* DSM 24731, *B. breve* DSM 24732, *B. longum* DSM 24736, *B. infantis* DSM 24737, *L. paracasei* DSM 24733, *L. acidophilus* DSM24735, *L. delbrueckii subsp bulgaricus* DSM 24734, *L. plantarum* DSM 24730 at total does of 4.5×10^{11} CFU bacteria per 4.4 g of sachet with maltose and silicon dioxide as excipients (twice daily)	Placebo contained maltose and silicon dioxide	12 weeks	Handgrip strength; Gait speed; HROQOL
[24]	Taiwan, China	Double-blind RCT	E(L):127.2 E(H):13 C:17	E(L):77.8 ± E(H):80.5 ± 9.4 C: 75.2 ± 7.2	Frailty	—	E(L): consuming one capsule including 1×10^{10} CFU *L. plantarum* TWK10 twice a day E(H): consuming one capsule including 3×10^{10} CFU *L. plantarum* TWK10 twice a day	Placebo	18 weeks	Muscle mass (iDXA); Handgrip strength; The 10 m walk test
[25]	Italy	Double-blind RCT	E:30 C:30	E:72 ± 3 C:71 ± 3	Metabolic syndrome based on the IDF criteria	Mediterra-nean diet; Increasing physical activity by walking or cycling	*L. plantarum* PBS067-DSM 24937 2×10^9 CFU per does, *L. acidophilus* PBS066-DSM 24936 2×10^9 CFU per dose, *L. reuteri* PBS072-DSM 25175 2×10^9 CFU per dose (total CFU/dose = 6×10^9 CFU), inulin, fructooligosaccharides (FOS), vegetable magnesium stearate, maltose	Placebo containing vegetable magnesiu-m stearate, maltose	60 days	EQ-5D index, EQ-5D VAS
[20]	Republic of Korea	Double-blind RCT	E:27 C:26	E:71.11 C:72.00	—	—	Two capsules after the meal in the morning and evening (a total of 1×10^9 CFU of *B. bifidum* BGN4 and *B. longum* BORI in soybean oil)	Placebo capsule contained 500 mg of soybean oil	12 weeks	QOL measured with the SWLS
[31]	Pakistan	Double-blind RCT	E:47 C:53	E:66.9 ± 3.4 C:68.3 ± 4.2	Stable COPD	—	One capsule (Vivomixx, 11.2×10^{10} CFU live bacteria including *Streptococcus thermophilus* DSM 24731, *B. longum* DSM 24736, *B. breve* DSM24732, DSM 24737, *L.* DSM 24735, DSM 24730, DSM24722, *L. delbrueckii* subsp. *bulgaricus* DSM 24734 along with maltose and silicon dioxide)	Placebo	16 weeks	ASM, ASMI (BIA); Handgrip strength; SPPB (4-meter walking speed)

(To be continued on the next page)

Table 1 (*Continued*)

Ref.	Region	Design	Participants			Intervention				Extracted outcomes
			Size (*N*)	Age	Health status	All subjects	Experimental treatment	Control	Period	
[30]	Pakistan	Double-blind RCT	E:44 C:48	E:67.6 ± 4.9 C:65.2 ± 5.6	Mild and moderate CHF	—	One capsule (Vivomixx, 11.2 ×10^{10} CFU live bacteria including *Streptococcus thermophilus* DSM 24731, *B. longum* DSM 24736, *B. breve* DSM24732, DSM 24737, *L.* DSM 24735, DSM 24730, DSM24722, *L. delbrueckii* subsp. *Bulgaricus* DSM 24734 along with maltose and silicon dioxide)	Placebo	12 weeks	ASM, ASMI (BIA); Handgrip strength; SPPB (4-meter walking speed)
[27]	Italy	Double-blind RCT	E:22 C:28	79.71 ± 4.84 E:78.84 ± 5.80 C:80.50 ± 3.74	Sarcopen-ia	Personal dietary program; Endurance and aerobic physical activity training	The water-dispersible powder contained omega-3 fatty acid (500 mg, consisting of EPA (64.71%), DHA (29.41%), and omega-3 (5.88%) in general), leucine (2.5 g), probiotic LPPS23 (3 × 10^{10} CFU, freeze-dried) (OLEP)	Placebo	2 months	ALM, ALM/h^2 (DXA); Handgrip strength; SPPB; Physical SF-12
[32]	Romania	RCT	E:93 C:107	E:68.0 ± 8.09 C:66.9 ± 7.81	Recent history of COVID-19 infection and SPPB score < 9	VitaminD (2000 IU) Specific physical training program	Additional high-protein diet therapy (recommended by a nutrition specialist) and probiotic therapy (containing *Enterococcus faecium*, *L. acidophilus, L. brevis*, *Lactococcus lactis*, *B. bifidum*, and *B.lactis*, at least 1 × 10^{10} CFU per portion = 4 g)	Without diet therapy and probiotics	2 months	ASM, ASM/h^2 (BIA)
[22]	Sweden	Double-line RCT	E:37 C:39	E: 71 (65,80) C: 70 (65, 81)[a]	Low-grade inflamma-tion (hs-CRP :1.5-6 mg/L)	—	Omega-3 with vitamin D (2 capsules of 1100 mg of fish oils and 200 IU Vitamin D) and four-strain probiotic, 2 capsules per day, each capsule containing *B. lactis* Bi-07, *B. lactis* Bl-04, *L. acidophilus* NCFM and *L paracasei* Lpc-37 (1.25 × 10^9 CFU each)	Maltodextrin capsules with sunflower oil	8 weeks	Sit-to-stand test; WOMAC

Notes: ASM: appendicular skeletal muscle mass; ASMI: appendicular skeletal muscle index; HROQOL: health-related quality of life; SF-12: the 12-item Short Form Survey; Values are given as mean ± standard deviation (SD). [a]Values are given as medians (Range). [b]Values are given as medians (IQR).

two in Italy [25, 27], one in Spain [29], one in Romania [32], one in Brazil [26], two in Pakistan [30, 31], two in Japan [28, 33], one in the Republic of Korea [20], one in Taiwan, China [24], and one in the Chinese mainland [19]. Four studies were conducted on elderly participants in good health [20, 21, 28, 33]; whereas the remaining studies included the elderly with various disease conditions, such as low bone mineral density [23], recent history of COVID-19 infection accompanying with short physical performance battery (SPPB) score < 9 [32], stable chronic obstructive pulmonary disease (COPD) [31], mild and moderate chronic heart failure (CHF) [30], cirrhosis [29], sarcopenia [27], frailty [24, 26], metabolic syndrome [25], low-grade-inflammation [22], and non-displaced distal radius fracture [19].

Furthermore, all participants received additional nutrition supplementation and physical training programs in three studies [25, 27, 32]; while in one study, moderate resistance training was required [28]. In our eligible studies, 11 of them conducted short-term treatment (≤ 3 months) [20–22, 25–30, 32, 33] while four of them had a duration of more than 3 months [19, 23, 24, 31], of which the minimum and maximum duration were 8 weeks and 12 months, respectively. Six studies utilized products containing one strain only [19, 21, 23, 24, 27, 33], and 9 trials used multi-strain probiotics [20, 22, 25, 26, 28–32], 3 of which were supplied with synbiotics in the experimental groups [21, 25, 26].

Regarding the various outcome measurements, eight studies assessed muscle mass via bioelectrical impedance analysis (BIA) in five studies [26, 28, 30–32] and dual-energy X-ray absorptiometry (DXA) in three studies [23, 24, 27]. Seven of them reported the values of muscle mass (e.g., ALM) [23, 24, 26–28, 30, 31], while four of them reported muscle mass indexes divided by height squared (e.g., skeletal muscle index (SMI)) [27, 30–32]. In addition, seven studies assessed handgrip strength [19, 24, 26, 27, 29–31], and six studies evaluated physical performance using gait speed in three [29–31], the 10-meter walk test in one [24], the sit-to-stand test in one [22], and the SPPB score in one [27]. In terms of QOL, one trial performed a QOL survey associated with lifestyle and health status [33], one study measured the Satisfaction With Life Scale (SWLS) [20], two trials used the EuroQol-5 Dimension questionnaire (EQ-5D index and EQ-5D VAS) [21, 25], one study used physical SF-12 [27], one trial assessed activity levels and QOL using the Western Ontario and McMaster Osteoarthritis Index (WOMAC) questionnaire [22], and one trial evaluated health-related quality of life (HRQOL) using the Nottingham Health Profile (NHP) [29].

2.3 Risk of bias of eligible studies

As displayed in Fig. S1 in the Electronic Supplementary Material (ESM), 8 studies were categorized as having "low risk" according to RoB2 [19, 21, 23, 25, 26, 28, 29, 31], four were classified as "some concerns" [20, 22, 30, 32], and three were labeled as "high risk" [24, 27, 33]. Specifically, 4 studies were thought to have some concerns in the randomization process

owing to a lack of information about allocation generation and concealment [24, 30, 32, 33]. Four trials had some concerns about missing outcome data due to the high participant dropout rates with balance distribution [20, 22, 30, 33], while two studies had high risks of deviations from intended intervention and missing outcome data because of the high and unbalanced dropout rates [24, 27]. Furthermore, one study had a high risk of the measurement of the outcomes because participants were aware of the intervention received, which might affect the results of the questionnaires about QOL [33].

2.4 The effect of probiotics or synbiotics supplementation on muscle mass

The results indicated that probiotics and synbiotics did not improve muscle mass significantly, compared to placebo, and displayed a low degree of heterogeneity among the eligible trials when using a fixed effect model (k = 8, standardized mean difference (SMD) = 0.067, 95%confidence interval (CI): −0.090–0.225, I^2 = 26.0%, p = 0.221, Fig. 2). Based on an array of subgroup analyses shown in Table S2 in the ESM, regrettably, we did not observe any significant impact of probiotics and synbiotics on muscle mass in different subgroups, but lower heterogeneity was observed in groups with subjects living in Asia, during long-term duration (> 3 months), without extra nutrition and exercise program, and using mono-strain probiotics. Furthermore, as shown in Figs. S3 and S4 in the ESM, there was no significant effect on either the values of muscle mass or their indexes divided by height squared. However, there was higher heterogeneity among eligible studies in the group that reported muscle mass indexes (k = 4, SMD: 0.187, 95%CI: −0.142–0.516, I^2 = 63.3%, p = 0.043).

2.5 The effect of probiotics and synbiotics supplementation on muscle strength

As the forest plot shown in Fig. 3, the supplementation of probiotics and synbiotics led to a moderate improvement in muscle strength, compared to placebo, with substantial heterogeneity among trials (k = 7, SMD = 0.667, 95%CI: 0.317–1.017, I^2 = 72.5%, p = 0.001). According to further subgroup analysis shown in Table S2 in the ESM, the studies conducted in Asia showed lower heterogeneity and significant benefits on muscle strength improvement, while those conducted in non-Asia regions displayed insignificant effects. Additionally, with higher heterogeneity, we did not observe a significant change in muscle strength following short-term supplementation. In contrast, long-term treatment elicited a moderate promotion with low heterogeneity. Furthermore, compared with the large improvement in one study with extra intervention in all individuals, less benefit was shown on muscle strength in the remaining trials. Moreover, mono-strain probiotics supplementation offered a moderate positive effect with low heterogeneity, whereas larger positive effect with substantial heterogeneity was observed following intervention with a combination of multi-strain probiotics and synbiotics.

2.6 The effect of probiotics and synbiotics supplementation on physical performance

Compared to the placebo, a moderate increase in physical performance was observed following probiotics and synbiotics supplementation with substantial heterogeneity among included trials (k = 6, SMD = 0.597, 95%CI: 0.118–1.075, I^2 = 80.6%, p < 0.001, Fig. 4). Furthermore, Table S2 in the ESM showed a moderate strengthening in physical performance in individuals from Asia, following probiotics and synbiotics supplementation, but no significant change in non-Asian older adults. As expected, long-term probiotics and synbiotics supplementation improved physical performance significantly with low heterogeneity. Consisted with the results of muscle strength, after removing one study under the background of extra intervention, less impact of probiotics and synbiotics supplementation on physical performance was displayed in

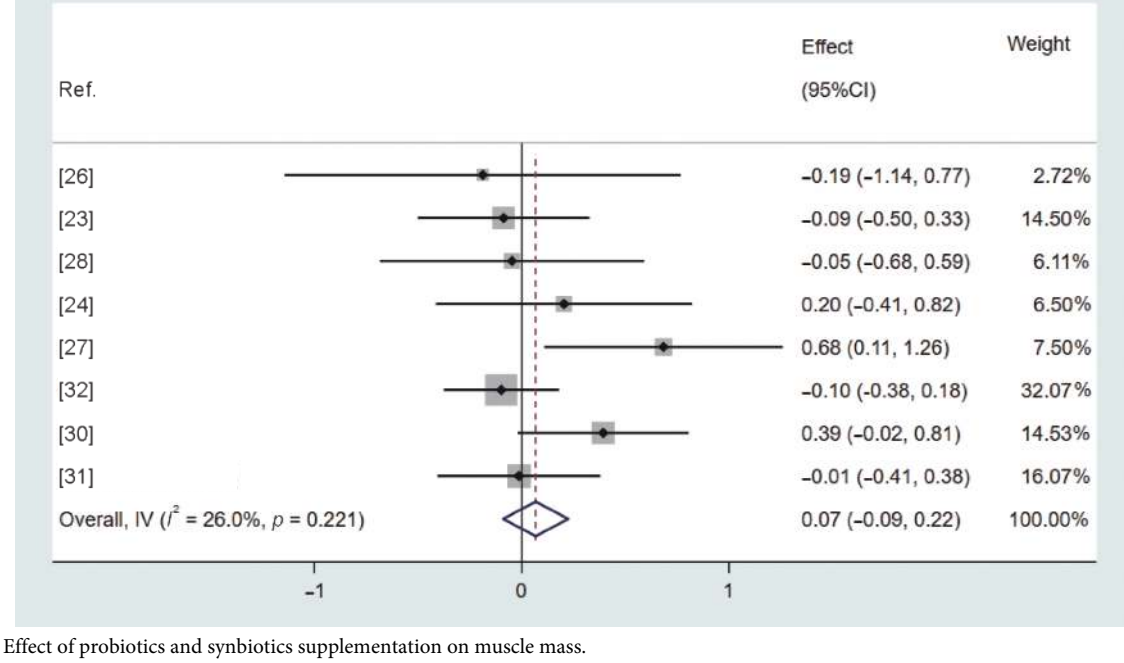

Figure 2 Effect of probiotics and synbiotics supplementation on muscle mass.

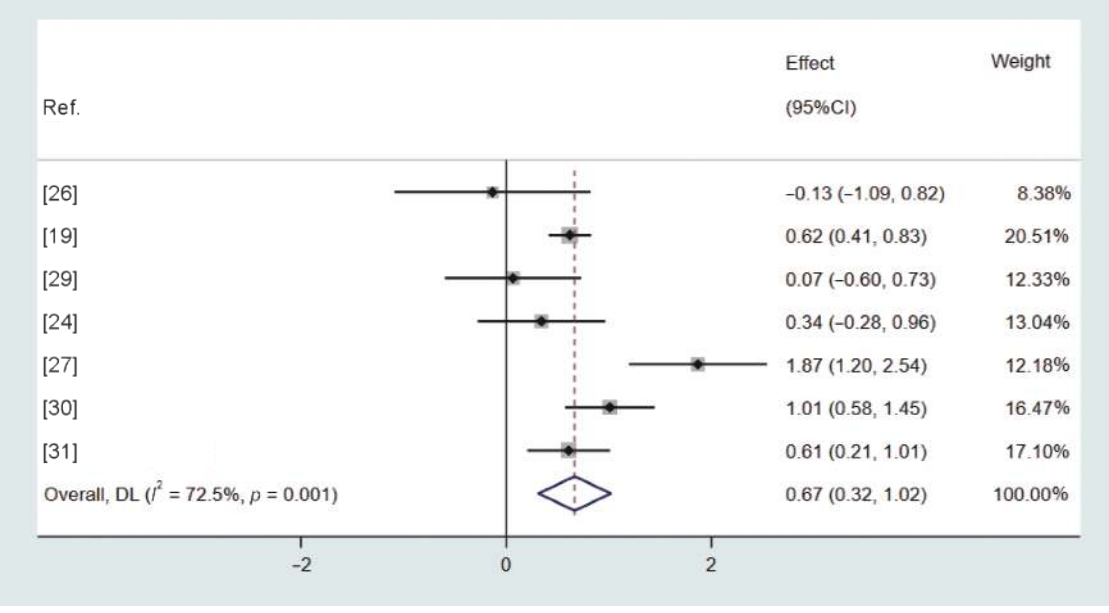

Figure 3 Effect of probiotics and synbiotics supplementation on muscle strength.

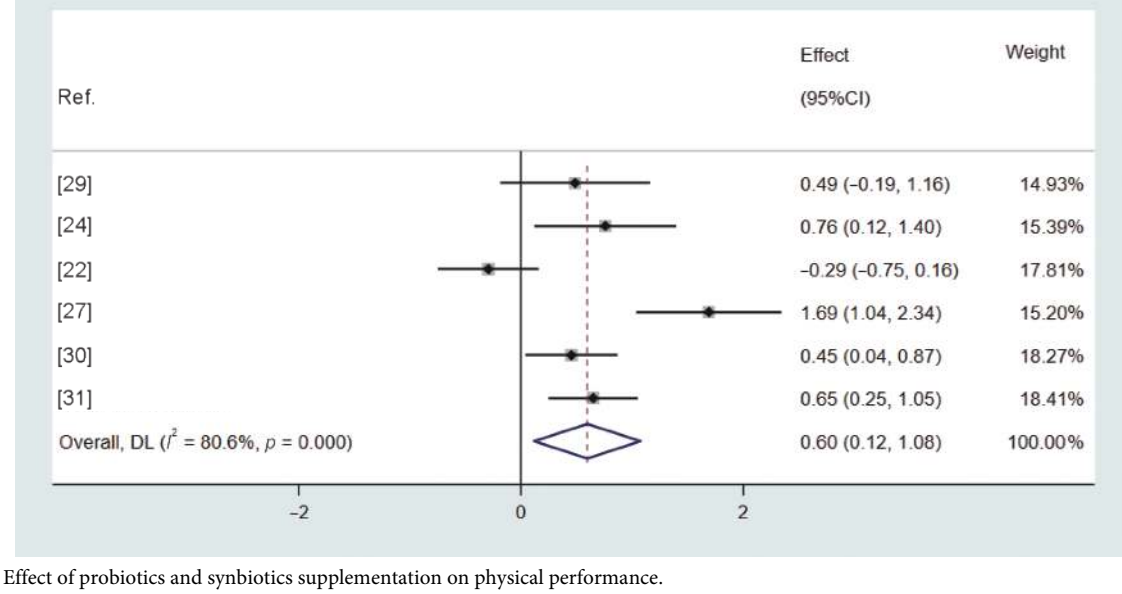

Figure 4 Effect of probiotics and synbiotics supplementation on physical performance.

the remaining studies. In addition, significant promotions of physical performance were elicited in both studies using mono-strain probiotics and the trials using multi-strain probiotics and synbiotics, while the latter showed substantial heterogeneity.

2.7 The effect of probiotics and synbiotics supplementation on QOL

A meta-analysis revealed that probiotics and synbiotics did not elicit significant improvement in QOL with substantial heterogeneity when compared to placebo (k = 7, SMD = 0.421, 95%CI: −0.054–0.895, I^2 = 86.2%, p < 0.001, Fig. 5). Additionally, no significant change in QOL after probiotics and synbiotics treatments was identified in different subgroups, although lower heterogeneity was identified in the subgroup performed in Asia, with individuals in good health, and without providing additional nutrition and exercise projects in all participants.

2.8 The effect of probiotics and synbiotics supplementation on gut microbiome and nutritional and immune status

To assess the alternation of the gut microbiota and metabolites, two studies performed fecal microbiome analysis [20, 29], and one conducted fecal metabolites (SCFAs) analysis [22]. Both fecal microbiome analyses showed no significant change in either diversity or gut microbial composition at the phylum level among different groups during the treatment period. However, one trial observed a significant change at the genus level in the experimental group rather than the placebo group, with significant decreases in the relative abundances of *Eubacterium*, *Allisonella*, *Clostridiales*, and Prevotellaccae [20]. In another study performing analysis targeting SCFAs, active treatment resulted in increased valeric acid and iso-butyric acid in feces [22]. Furthermore, four studies demonstrated that probiotics

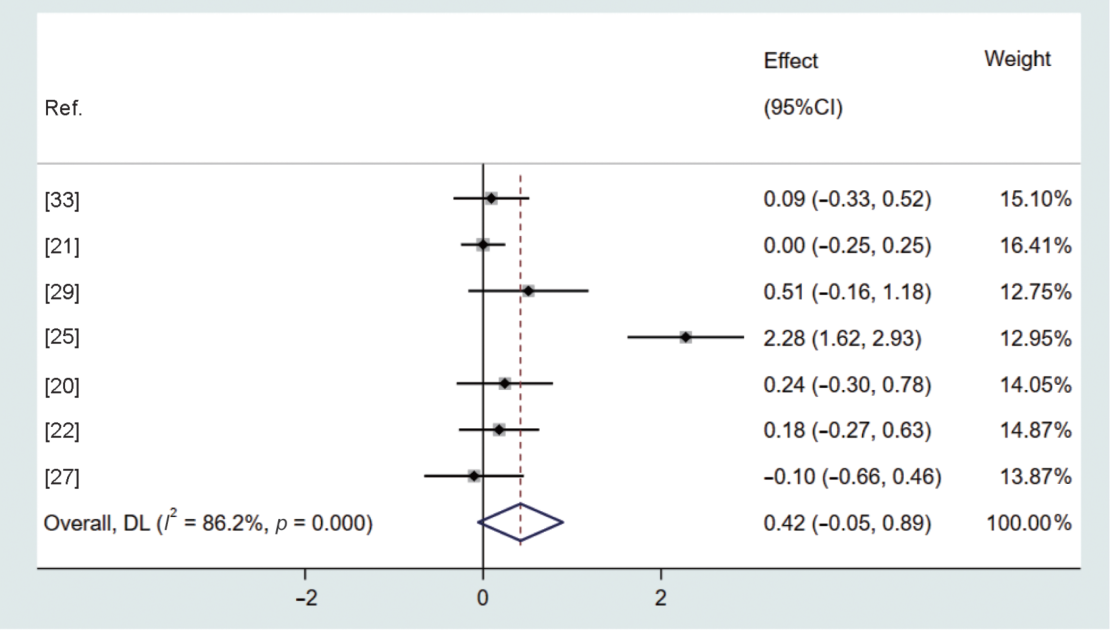

Figure 5 Effect of probiotics and synbiotics supplementation on quality of life.

and synbiotics elicit various effects on the change of gastrointestinal permeability. Tingö, et al. did not observe any difference in intestinal permeability due to active treatment using either the muti-sugar urinary recovery test or measurement of the plasma intestinal fatty acid-binding protein (I-FABP) [22], while the significant decline of the plasma FABP-6 and urinary claudin-3 but no FABP-2, zonulin, lipopolysaccharide-binding protein (LBP) level was detected in experimental group of another study [29]. Karim, et al. also found the probiotics supplementation significantly reduced the plasma zonulin level in sarcopenia patients with CHF or COPD [30, 31].

To dig deeper into the mechanism of probiotics and synbiotics, eight trials also assessed the inflammation status and immune function of participants [22, 25–27, 29–31, 33]. Besides one study with a small sample size did not yield significant changes between groups or over time [26], six trials demonstrated the effect of probiotics and synbiotics on the alleviation of inflammation, particularly in lowering levels of C-reaction protein (CRP) and hypersensitive CRP (hs-CRP) [22, 25, 27, 29–31]. The levels of the tumor necrosis factor alpha (TNF-α) and 8-isoprostanes were significantly decreased [25, 29–31] while the level of interleukin 10 (IL-10) was elevated following probiotics treatment compared to placebo [22]. Furthermore, while assessing the effect of fermented yogurt on the risk of infection and immune defense, Makino, et al. found a lower risk of infection and higher activity of natural killer cells in the yogurt group rather than the milk group [33].

Moreover, regarding nutritional status, Makino, et al. reported an increase in vitamin D, eicosapentaenoic acid (EPA), docosahexaenoic acid (DHA), and omega-3 polyunsaturated fatty acids (n-3 PUFA), as well as a tremendous decrease in the n-6: n-3 ratio after active treatment rather than placebo [33]. Additionally, a significant elevation of serum hemoglobin level was observed after the hyper-protein diet and probiotics in one study [32], and significant increases in the serum leucine, isoleucine, valine, and total amino acids levels were identified in experimental group compared to placebo group in another study [27]. There was also one study found that probiotics could increase dickkopf-1 (Dkk-1) and decrease dickkopf-3 (Dkk-3) and sterol regulatory element-binding protein-1 (SREBP1) in the sarcopenia patients with CHF, indicating the effect of probiotics on the Wnt signaling pathway [30].

2.9 Adverse events

There were 10 studies reported the adverse events, while the remaining studies didn't [24, 26, 30–32]. In half of the studies reported adverse events, no clinically adverse or serious adverse events were noted related to the administration of probiotics and synbiotics [19, 20, 27, 28, 33]. Moreover, five studies reported some adverse events (e.g., gastrointestinal symptoms, joint and muscle pain, and headache), which displayed an even distribution between the experimental groups and the comparator groups [21–23, 25, 29]. In conclusion, the administration of probiotics and synbiotics was well-tolerated and safe.

2.10 Sensitive analysis and publication bias

As illustrated in Fig. S8 in the ESM, the sensitivity analyses revealed no considerable changes in the effect sizes across all parameters. The mean changes in muscle mass and QOL for treatment versus placebo ranged from 0.012 to 0.146, and from 0.085 to 0.511, and both of their 95%CI encompassing 0, suggesting robust evidence to support that probiotics and synbiotics have a slight effect on muscle mass and QOL. Additionally, the mean change in muscle strength when comparing treatment to placebo ranged from 0.553 to 0.752, indicating probiotics and synbiotics have a generally positive effect on muscle strength. However, sensitivity analyses for physical performance showed that the changes for treatment versus placebo ranged from 0.393 to 0.773, but removing Karim 2022 (COPD) [31] caused the 95%CI containing 0, which might cause estimating a less significant effect.

Funnel plots for probiotics and synbiotics supplementation

on four measure outcomes were performed and shown in Fig. S9 in the ESM, all of which illustrated comparatively uniform distributions, and Egger's test (Fig. S10 in the ESM) revealed no evidence for publication bias either.

3 Discussion

Our meta-analysis revealed a noteworthy moderate effect of probiotics and synbiotics on the improvement of muscle strength and physical performance, but no significant difference was identified in the muscle mass and QOL, suggesting a unique effect of probiotics and synbiotics on enhancing muscle function in older adults. Additionally, compared to the placebo, the application of probiotics and synbiotics did not increase adverse events in older adults, even with either high-dose or long-term intervention, displaying good toleration and safety.

3.1 Findings from the subgroup analysis

It is important to note that discussing the effectiveness of probiotics should presuppose the fact that they successfully colonized gut mucosal, which is influenced by host features and interventions taken [34]. Therefore, subgroup analysis was conducted to investigate the impact of host unique characteristics on the effectiveness of probiotics and synbiotics based on their health status or region. Some studies have indicated that individuals with different health statuses respond differently to probiotic supplementation [35, 36]. Consistently, the effects of probiotics and synbiotics showed higher heterogeneity in studies involving individuals with various diseases than in good health. Additionally, a moderate enhancement of muscle function with lower heterogeneity was identified in the groups conducted in Asia rather than in non-Asia area. These results suggest that host genetic factors like race, may affect the colonization and efficacy of probiotics and synbiotics. On the other hand, lifestyle factors, particularly dietary patterns which are associated with the compositions and functions of the gut microbiome (e.g., diversity and enterotype) [37], should be taken into account. These could be roughly divided into the Western diet high in saturated fat and calories but low in fiber, and the Oriental diet, which is low in fat but high in fiber [38]. It has been demonstrated that a fiber-rich diet may promote probiotic colonization and efficacy by providing sufficient and preferential fuel sources [39]. The coincidence of higher SMDs showed in some studies providing additional interventions (e.g., limiting consumption of red meats and Mediterranean diet) than those without extra nutritional interventions in non-Asia groups may also prompt the potential effect of dietary patterns.

Further, we delved into the relationship between intervention methods and their effectiveness, as well as analyzed the heterogeneity caused by intervention methods. Besides dietary patterns, additional nutrition supplementation and exercise programs also affect the gut microbiome. Exercise training and nutrition supplementation, which are recommended in guidelines for sarcopenia and frailty [40–42], could potentially affect gut microbiome [43, 44], so there is an interest in exploring the interaction effect between nutritional supplementation and exercise training and probiotics and synbiotics on skeletal muscle and overall health. While higher heterogeneity was shown in the subgroups accepting extra diverse interventions, it is worth noting that the highest SMD of each aspect appeared in these subgroups, probably suggesting a mutual promotion between probiotics or synbiotics and extra nutrition and exercise programs. Nevertheless, to figure out the interaction effect among various interventions, more intervention studies and network meta-analysis should be conducted in the future.

Furthermore, in terms of the types of probiotics and synbiotics utilized, multi-strain probiotics and synbiotics may offer more stable and effective colonization and functionality than mono-strain probiotics [45, 46]. However, while studies have shown a higher enhancement of muscle strength with the use of multi-strain probiotics and synbiotics, no prominent advantage over mono-strain probiotics has been demonstrated. Besides the small size of the studies and other factors causing heterogeneous, it should be under consideration that not all combinations of muti-strain probiotics and prebiotics would bring synergy advantages with a composite of quorum sensing system, providing sources between bacteria, anaerobic conditions, etc. [47], suggesting that an appropriate combination should be considered in design. Additionally, long-term intervention tends to bring stable gut mucosal colonization and uniform promotion of muscle function, highlighting the importance of adequate interventional duration. However, unlike probiotics increased muscle mass and weight in mice [48, 49], no significant elevation of muscle mass was identified in the studies with long-term intervention in humans, presumably because the duration was still too short to observe considerable improvement in muscle mass.

3.2 Related mechanisms

Since the results of fecal microbiota analysis are just the tip of the iceberg [50], slight changes in fecal microbiota and metabolites do not mean that probiotics and synbiotics have little effect. In fact, research has shown that the modulation of microbiota from probiotics and synbiotics can pronouncedly alleviate intestinal permeability and pro-inflammatory status, as well as facilitate anti-inflammatory and anti-infective responses in the battle against immunosenescence. Probiotics and synbiotics contribute to competing with pathogenic bacteria, maintaining microbiota homeostasis, generating SCFAs or other metabolites, etc. [17, 18, 51], subsequently alleviating TNF-α-induced intestinal damage, promoting regulatory T cell-derived IL-10 production [52], facilitating the production of sIgA [53], and thereupon fortifying the gut mucosal barriers and modulating the innate immune response. Furthermore, they further regulate the balance of anti- and pro-inflammatory status in the circulatory system and muscle tissue by reducing harmful factors from sneaking out of the "leaky gut" into the circulatory system, increasing beneficial bacteria and metabolites, modulating inflammatory cytokines, etc. [54–59].

In addition to alleviating chronic low-grade inflammation, the supplementation of probiotics and synbiotics enhances nutrition absorption, either from the gut lumen into the bloodstream, or from the bloodstream into muscle tissues. The increased levels of amino acids and SCFAs in the blood after probiotics and synbiotics supplementation demonstrate a positive impact on nutrition absorption. Finally,

incorporating lower pro-inflammatory status and adequate energy and nutrient supply, they could significantly suppress NF-κB activation, decrease expression levels of ubiquitin-proteasome pathways [18, 60], activate Wnt, AKT, and AMPK signal pathway [61, 62], enhance the antioxidative capacity and mitochondrial function [55–57], etc. in muscle tissue. These facilitate muscle protein synthesis and alleviate muscle protein breakdown, while also promoting a favorable microenvironment for restoration of muscle function and maintenance of muscle mass.

However, it is important to note that muscle mass and quality are primarily influenced by muscle protein homeostasis, which involves a balance of protein synthesis and degradation [63]. There is growing evidence suggesting that probiotics can help alleviate anabolic resistance, a common occurrence in older individuals who often exhibit lower rates of muscle protein synthesis in response to nutritional supplementation and exercise [64, 65]. For example, the RCT conducted by Rondanelli, et al. demonstrated that when probiotics were combined with appropriate nutritional supplementation and an exercise program, both muscle mass and function significantly improved [27]. However, it should be acknowledged that muscle mass and quality are also affected by long-term and complex mechanisms such as loss of motor innervation, reduced vascular perfusion, adipose infiltration, and stem cell exhaustion. These factors may present challenges in fully reversing age-related loss of muscle mass within a short timeframe, potentially contributing to the uncertain effects of probiotics and synbiotics on muscle mass in older individuals.

3.3 The effect of probiotics and synbiotics on QOL

We further explore the potential benefits for QOL, which is associated with physical function and mental health. Given high subjectivity, QOL may be significantly elevated in cases of surprisingly large improvements in muscle mass and function, as well as other inspiring health improvements. In our eligible studies, significant improvement in QOL was observed in elderly patients with metabolic syndrome following treatment, because of not only alleviation of metabolic and inflammatory issues and improvement of physical function but also mentally positive feedback from visualized pleasing inspection results.

3.4 Limitations

Since no specific probiotic has been reported to have a unique effect on treating muscle dysfunction and muscle mass loss, we aimed to investigate the overall effects of probiotics and synbiotics on aging muscle. Although we included 15 RCTs in total, the number of studies in each aspect was relatively small, with a maximum of eight studies per meta-analysis, suggesting the challenge of dealing with small sample sizes. While exploring the heterogeneity from various intervention methods, we just explored the impact of extra interventions, treatment durations and types of probiotics and synbiotics in subgroup analysis, but failed to take the dosage and strains of probiotics into account. Additionally, language bias cannot be ruled out as our search strategy was exclusively based on English-language-dominated databases.

4 Conclusions

In conclusion, the eligible studies provide sufficient evidence that probiotics and synbiotics have significantly moderate effects on improving muscle strength and physical performance, but indeterminate impact on the elevation of muscle mass and QOL. Host health status and the intervention methods will affect the impact of probiotics and synbiotics on skeletal muscle. These supplements may reshape the gut microbiota community, and modulate mucosal barriers and innate immune system, subsequently restoring chronic low-grade systemic inflammation and immunosenescence. Moreover, they may improve the absorption and utilization of nutrition and energy sources in enterocytes and muscle cells. Considering their effects on skeletal muscle in elderly people, they may be potentially effective microbiota modulators to improve muscle function. Therefore, an individual health plan could be proposed for promoting muscle and overall health in older adults. The plan would combine the appropriate use of probiotics or synbiotics considering host features and the intervention itself with personalized nutrition and exercise programs (e.g., Mediterranean diet, high-protein diet, moderate resistance training), particularly for those with poor nutritional status or chronic low-grade inflammation.

5 Materials and methods

This systematic review and meta-analysis was conducted following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. The protocol was registered in the International Prospective Register of Systematic Reviews (PROSPERO) (CRD42023428400).

5.1 Search strategy

Following the PRISMA guidelines, two independent reviewers (Q.Z. and Y.L.) systematically searched PubMed (MEDLINE), EMBASE, and Web of Science databases using a combination of relevant Medical Subject Headings (MeSH) and free-text terms from database inception to November 2023. The full details of the search strategy are presented in Table S1 in the ESM.

5.2 Inclusion and exclusion criteria

Based on PICOS (Population, Intervention, Comparison, Outcome, Study design) principle, the inclusion criteria were as follows: (1) randomized controlled trial (RCT) study design; (2) participants aged ≥ 65 years on average; (3) intervention involving probiotics or synbiotics supplementation; (4) outcomes including indicators of muscle mass, muscle strength, physical performance, or QOL. Outcomes had to be presented as medians or means at baseline and endpoint or change from baseline for both the experimental and comparator groups.

The exclusion criteria were: (1) animal studies, *in vitro* studies, protocol papers, observational studies, case reports, reviews, letters, conference abstracts, editorials, or other undistinctive forms; (2) studies reporting insufficient data or results; (3) studies with duplicate datasets; (4) intervention included enteral nutrition or other pharmacological agents

may affect the composition of gut microbiota (e.g., use of antibiotics); (5) non-English article.

5.3 Study selection

After removing the duplicate studies, two reviewers (Q.Z. and Y.L.) screened the records based on the titles and abstracts according to the inclusion and exclusion criteria. The potential records then underwent a full-text review for eligibility. Any disagreements were resolved through discussion and consensus with an independent reviewer (WH). The results were displayed following the PRISMA statement [66].

5.4 Data extraction

Based on the RCT template provided by the Cochrane Collaboration, two authors (Q.Z. and Y.L.) extracted the following information from eligible studies independently: authors, region, participant characteristics (e.g., age), sample size, description of intervention methods (e.g., dose and duration), blinding, and tools and results of outcome measurement. In the case of discrepancies in the data extraction, a third investigator (W.H.) was involved.

5.5 Assessment of risk of bias

The risk of bias and methodological quality of the included studies was assessed using Cochrane's Risk-of-Bias 2 (RoB2) tool by two independent authors (Q.Z. and Y.L.). Any discrepancies were resolved through discussion with a third author (W.H.). The RoB2, recommended by the Cochrane Handbook for Systematic Reviews of Interventions, is used to assess bias in RCTs across five domains: randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome, and selection of the reported result [67, 68]. According to the scoring system, the risk of bias in each domain and the overall bias was defined as "high", "some concerns", or "low".

5.6 Data synthesis and statistical analysis

The changes in outcome indicators from baseline to post-intervention were extracted from each eligible trial in both control and experimental groups, presented as mean ± standard deviation (SD), and *p*-value, if applicable. Graphic values were calculated using GetData Graph Digitizer Version 2.26 software if the numerical data were not reported. While just standard error of mean (SEM), 95%CI, or median (interquartile range or range) were reported, they were transformed into SD using the method recommended in the Cochrane Handbook and other relevant methods [69–71]. According to Section 6.5.2.10 of the Cochrane Handbook guideline, when comparing three or more groups, interventional groups with similar intervention categories were combined into a single group for comparison with the control group (e.g., the high-dose probiotics group and the low-dose probiotics group). Furthermore, when change-from-baseline mean and SD were not reported, the mean was estimated by subtracting initial data from the final data, and the SD was estimated using the equation from Follmann, et al.: $SD_{E,change}=\sqrt{SD^2_{E,baseline}+SD^2_{E,final}-(2\times Corr\times SD_{E,baseline}\times SD_{E,final})}$, which is commended in the Cochrane Handbook Section 6.5.2.8. Given no appropriate study to refer to, we assumed a Corr of 0.50 in the values of muscle mass, muscle strength, physical performance, and QOL between baseline and post-intervention, and further sensitivity analyses testing different values of Corr (0.2 and 0.8) yielded similar results.

Statistical analyses were conducted using Stata version 17.0. To assess statistical heterogeneity between studies, the overlap of their 95%CI was measured and Cochran's Q (Chi-square test) and I^2 were performed. If the I^2 values were ≤ 50%, the pooled data were considered low heterogeneity and analyzed using a fixed-effects model. While I^2 values were > 50%, we considered the pooled data had substantial heterogeneity and the random-effects model was adopted. According to region, health status, treatment duration, additional intervention methods (whether provided exercise training and nutrition supplementation in all participants), and types of probiotics and synbiotics (mono-strain probiotics or multi-strain probiotics and synbiotics), a subgroup analysis was separately performed to analyze the heterogeneity and effects of probiotics and synbiotics on muscle mass, muscle strength, physical performance, or QOL. Given that studies exhibited data using different tools, the resultant data were presented as SMD and 95%CI, and the statistical significance was set at *p*-value < 0.05. Those SMDs of 0.2, 0.5, and 0.8 represented small, moderate, and large effect sizes, respectively. All meta-analysis results were visualized as forest plots.

Furthermore, a funnel plot, Egger's test, and sensitivity analysis were performed. The funnel plot and Egger's test were used to evaluate the publication bias of individual studies based on the pooled estimates. Sensitivity analysis was performed to assess the robustness of results by removing them one by one, and *p*-values less than 0.05 were considered statistically significant.

Author contributions

W.H. came with the idea of this systematic review and designed the study. All authors contributed to the development of the selection criteria, the risk of bias assessment strategy, and the data extraction criteria. Q.Z. and Y.L. contributed to article selection, quality assessment of the eligible studies, data extraction, analyzed data and performed statistical analysis, and writing manuscript.

Acknowledgments

This work was supported by the Science and Technology Planning Project of Guangzhou (No. 202002020011) and the National Natural Science Foundation of China (No. 62175263).

Conflict of interests

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interests.

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