

Leucine metabolite β -hydroxy- β -methyl butyrate (HMB) supplementation on muscle mass during resistance training in older subjects: meta-analysis

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Abstract

Background: Aging, which is accompanied by loss of muscle mass, strength, and function, may contribute to the development of frailty and fractures in older people. Interventions such as β -hydroxy- β -methyl butyrate (HMB) treatment and resistance exercise training (RET) have been well established independently to attenuate muscle loss in previous researches. Nevertheless, no consensus exists on whether the combination of HMB intervention and RET could obtain an additional benefit to the older population. Our aim was to systematically quantify whether HMB supplementation combined with RET has a synergistic effect on improving muscle mass, strength, and function in older adults.

Methods: A systematic search was performed using the electronic databases Medline, Embase, Cochrane Library, and Web of Science from inception of the study until Oct 30, 2021. The articles included were all randomized controlled trials and met the inclusion. A fixed or randomized (if data were heterogeneous) effects meta-analysis was performed using Stata.

Results: A total of 256 articles were screened, with eight studies matching the eligibility criteria, which enrolled 333 subjects (≥ 65 years old). A meta-analysis was conducted, and the results showed no significant difference between the groups in lean mass, fat mass, or physical performance. In the subgroup analysis regarding the differences in muscle strength between appendicular muscles, HMB supplementation combined with RET contributed to significantly improving the muscle strength of the lower limbs ($n = 6$, SMD: 0.55, 95% confidence interval: 0.06 to 1.04).

Conclusion: A combination of HMB supplementation and RET in older people has an additional benefit for muscle strength, especially in the lower limbs, instead of muscle function and physical performance. Further studies are needed to demonstrate the mechanism.

Keywords: PHMB, muscle mass, resistance exercise training, elderly

Introduction

The number of elderly people is estimated to reach 2 billion by 2050. Age-related muscle loss accompanied by

a decrease in muscle strength and function, which leads to multiple adverse consequences, including disability, frailty, morbidity, and mortality is an important clinical problem in elderly people [1]. The prevalence of sarcopenia, which is characterized by muscle loss and dysfunction reported by the European Working Group on Sarcopenia in Older People (EWGSOP), has reached 9.9–40.4% in older adults [2]. Methods to relieve sarcopenia have been intensively researched, including resistance training, nutritional supplements, hormones, drug treatments, and so on. For the elderly, especially disabled elderly, because of the decline in physical function, the interventions mentioned above are greatly restricted in clinical conduction. Therefore, for high-risk groups for sarcopenia, and the elderly, providing intervention as soon as possible, to delay progression and to explore efficient and multi-target strategies is the key clinical priorities.

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Muscle atrophy is mainly due to a dynamic imbalance between muscle protein synthesis (MPS) and muscle protein breakdown. Resistance exercise and nutrition therapy are two promising ways to maintain protein balance [3, 4]. However, some elderly people experience some difficulty in implementing and persisting in resistance exercise, particularly during acute illness and disability. In addition, the elderly need professional guidance to carry out resistance training to reduce the risk of sports injuries and falls. Furthermore, nutritional supports, including protein and amino acid supplements, are considered safe and convenient. However, their efficiency depends on the appetite and digestive functions of the elderly. For this reason, novel nutrients those are more available and efficient should be investigated.

β -Hydroxy- β -methyl butyrate (HMB) is a metabolite of the amino acid leucine. Studies have confirmed its role in relieving and promoting muscle synthesis and relieving muscle atrophy [5, 6]. HMB stimulates protein synthesis through increasing growth hormone/insulin-like growth factor I activity (IGF-1) axis in skeletal muscle; downregulating eukaryotic initiation factor 2 α (eIF2 α) and upregulating the mammalian target of rapamycin (mTOR)/p70 ribosomal protein S6 kinase (S6K1) pathway; and activating mitogen-activated protein kinase/extracellular signal-regulated protein kinase (MAPK/ERK) and phosphatidylinositol-3-OH kinase (PI3K)/Akt pathways, facilitated by IGF-I. The possible mechanisms of HMB inhibition of protein degradation are as follows. First, HMB inhibits protein kinase C (PKC)-signaling, which diminishes the ubiquitin-proteasome protein hydrolysis

pathway. Second, HMB decreases mitochondria-associated cystathionase activity and subsequent reactive oxygen species (ROS) production. These effects lead to the inhibition of apoptosis in myonuclear cells. Third, HMB increases cell membrane integrity via cholesterol synthesis to reduce tissue injury-induced protein hydrolysis. Finally, HMB may act as a structural component within cell membranes, but its role remained unclear. The molecular mechanism is illustrated in Figure 1 [7]. HMB may also enhance the stability of the cell membrane by undergoing polymerization. In clinical studies, the role of HMB combined with resistance exercise training (RET) treatment has not yet reached consensus because of study-specific characteristics. Researchers have found both gains and no changes in strength with HMB supplementation. Whether HMB supplementation combined with RET is more effective than RET alone, which component focuses on muscle mass, strength, and function, is unclear in the elderly [8] [9]. Hence, we conducted a meta-analysis to assess the influence of HMB supplementation combined with RET on lean mass, body fat mass (FM), muscle strength, and muscle function in older people.

Methods

Study inclusion/exclusion criteria

Studies that met the following criteria were eligible for inclusion according to the participant, intervention, control, outcome measures, and study design strategy (Table 1).

Data sources and searches

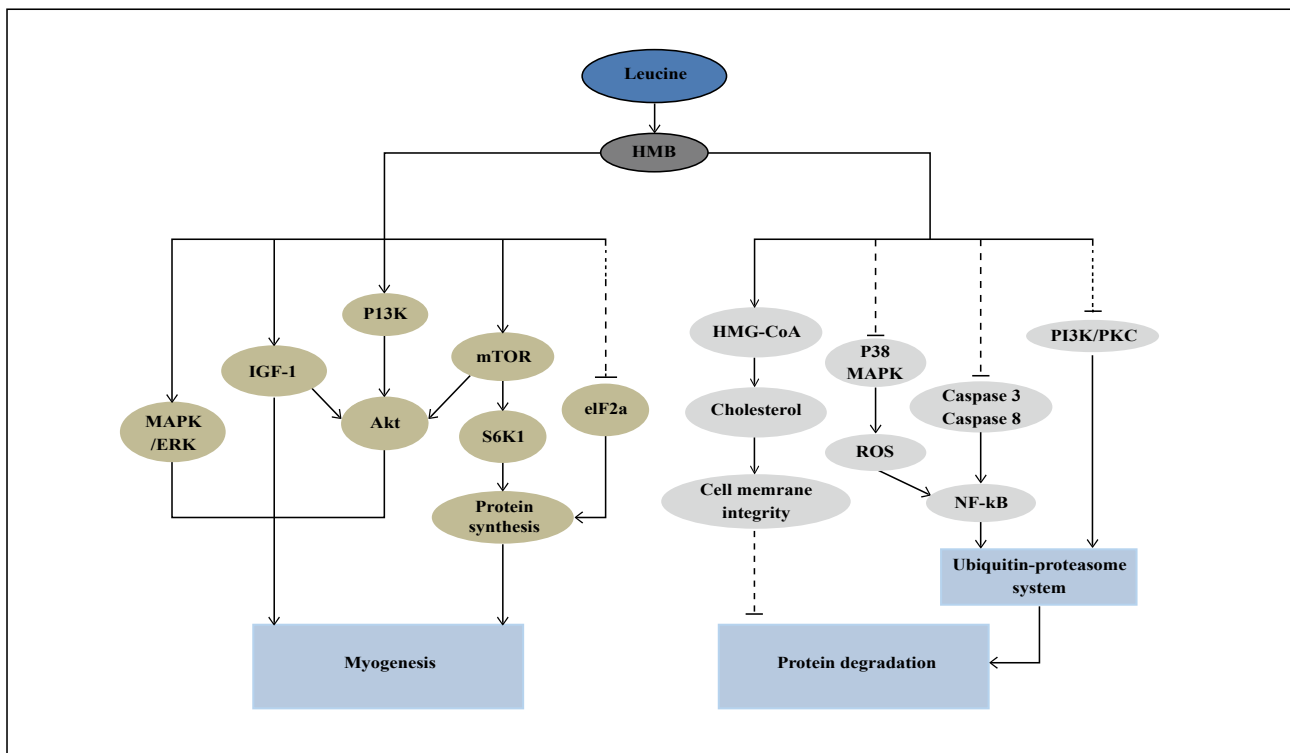


Figure 1. Possible mechanisms for HMB to stimulate myogenesis and inhibit protein degradation in skeletal muscle. S6K1: p70 ribosomal protein S6 kinase, MAPK: Mitogen-activated protein kinase, ERK: extracellular signal-regulated protein kinase, IGF-I: Insulin-like growth factor I, PI3K: Phosphoinositide-3-OH kinase, eIF2 α : Eukaryotic initiation factor 2 α , ROS: Reactive oxygen species, NF- κ B: Nuclear factor- κ B, mTOR: Mammalian target of rapamycin, PKC: Protein kinase C, HMG-CoA: β -hydroxy- β -methyl glutaryl-CoA. Dashed lines indicate suppression, solid lines indicate enhancement.

Table 1. Inclusion and exclusion criteria used to evaluate studies for the meta-analysis.

Inclusion criterion	Description
Participants	Aged 65 or older.
Intervention	HMB oral supplementation in addition to resistance training.
Control	Participants were not provided with HMB supplementation (controls or placebo).
Outcome	Body composition, muscle strength, muscle function.
Study design	Randomized controlled trial.

A literature search was conducted by searching relevant databases to investigate the effects of HMB combined with resistance exercise on body composition and muscle strength and function in older adults. Relevant articles from the earliest year to 2000 were searched. Search terms were used, including (HMB or beta-hydroxy-beta-methyl butyrate or β -hydroxy- β -methyl butyrate) and (exercise or training or “resistance exercise”) and (“older adults” or elderly or elder) and (“muscle mass” or “muscle strength” or “sarcopenia”). Search electronic libraries included PubMed, Web of Science, Cochrane Library, and Embase, with keywords used in various combinations, and maximum search results have been reached. The search covered original papers written in English and published before Oct 30, 2021, in these databases. Trials were conducted in humans.

Data extraction and outcome measures

The data in the papers were extracted by 2 independent and parallel investigators. First, all the papers were downloaded by the two researchers. Second, duplicates were removed, and titles and abstracts were screened to identify studies that met the eligibility criteria. Thereafter, the full texts were assessed. We manually searched the references meeting the inclusion criteria for further analysis. The following data were extracted for each study: authors, year of publication, sample size, gender, mean age, RET intervention, placebo/control information, body composition results, information on muscle strength and muscle function, and any other noteworthy information (e.g., source of bias/conflict of interest). The original data were obtained from the corresponding authors when the required data were missing in the articles. Otherwise, the data were imputed according to the methods given in the Cochrane Handbook. For studies in which no standard deviation (SD) was given, the post-intervention SD could be derived from the sample size (95% confidence interval [CI]).

Risk of bias analysis

The methodological quality of the included articles was assessed by the two investigators according to the Cochrane Collaboration risk-of-bias tool [10], including seven separate areas: (1) random sequence generation; (2) allocation concealment; (3) blinding of participants and personnel; (4) blinding of outcome assessments; (5)

incomplete outcome data; (6) selective reporting and (7) other sources of bias. When the two researchers disagreed on study eligibility, data extraction, and risk-of-bias assessment, a third investigator was available to arbitrate. Judgments for each entry involved assessing the risk of bias as “low risk,” “high risk,” and “unclear,” the latter indicating a lack of information or uncertainty about potential bias.

Data analysis

The outcomes of interest in this paper included the effect on body composition, strength, and physical function. If the dependent variable had multiple time points, only the pre-intervention and post-intervention values were selected. Meta-analysis was performed on the extracted data with Stata-SE. For the included studies, given the different ways used to measure muscle mass and strength, effect sizes were expressed as standardized mean differences (SMDs) with a 95% CI. SMD values of 0.2, 0.5, and 0.8 were defined as small, medium, and large effect sizes, respectively [11]. The heterogeneity of the included studies was determined by I^2 (< 50% was considered low, 50%–74.9% was considered moderate, and 75%–100% was considered high heterogeneity). The fixed-effects model was used when I^2 was less than 50%; otherwise, the random-effects model was used.

Results

Study selection

A total of 256 studies were identified from the search strategy and other searches. After eliminating duplicates, 215 records were available for title and abstract screening. A total of 37 articles were screened for full text, and after further screening based on our selection criteria, 10 of these articles were reviewed for inclusion. After further exclusion based on our selection criteria, eight randomized controlled trials met the inclusion criteria and underwent a final analysis. Figure 2 shows the PRISMA flow diagram.

Study characteristics

The eight eligible randomized controlled trials involved a total of 333 older adults: 159 were included in the experience group, and 174 were assigned to the control group. Because of unavailable data in two studies [12, 13], the authors were contacted to provide additional data by e-mail but no reply was received. The characteristics of these studies are shown in Table 2 and individual study outcomes included in the meta-analysis are shown in Table 3. The studies were conducted in healthy older adults with an average age of over 65 years. No studies mentioned the race of the subjects. The duration of the intervention varied widely, from 6 weeks to 12 months, and the frequency was two to three times per week. The HMB dose varied between 1.5 g/day ($n = 2$) and 3.0 g/day ($n = 6$).

Quality of included studies and risk of bias

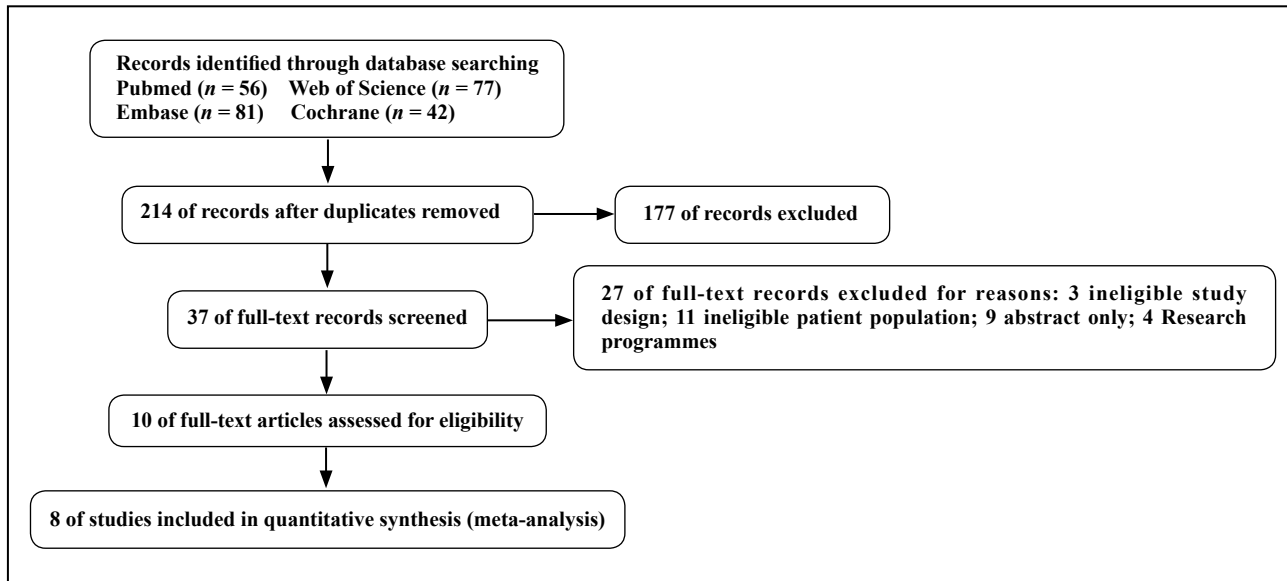


Figure 2. Flow through of articles through the search and review process.

No study was considered to have a low risk of bias in all categories. Scout showed a high risk of selective reporting because of a difference from the pre-registered trial [12]. The smallest biases were found in allocation concealment and blinding of outcome assessments. Figure 3 indicates the risk of bias assessment. The heterogeneity of the included studies was determined by I^2 (< 50% was considered low, 50–74.9% was considered moderate, and 75–100% was considered high heterogeneity). The fixed-effects model was used when I^2 was less than 50%. Otherwise, a random-effects model was used.

Main outcomes

All studies included reported measurements of body composition, including FM and fat-free mass, which is based on dual X-ray absorptiometry (DXA) or computed tomography, with a measurement cycle ranging from 6 weeks to 12 months. Five studies reported the effects of HMB on lower body strength, including knee flexion/extension by isokinetic, isometric, one maximum repetition, maximal voluntary contraction, peak torque isometric and isokinetic strength of the lower limbs, and hip adductor strength. Four studies reported the effects on upper-body strength muscle strength, including handgrip strength and handgrip endurance [13–16], and one study reported a percentage change in upper and lower body strength [17]. Four studies reported the effects on muscle function, including short physical performance battery (SPPB), get up and go, 4-meter walk time, 6-second walk distance, usual gait speed, and five-repetition sit-to-stand [13–16]. The SMD of variables at the end of the intervention period between the HMB groups and the placebo combined with resistance exercise was used for analysis.

All included studies were tested for heterogeneity: FM, fat-free mass, lower-body strength, upper-body strength, and muscle function, and the results revealed high heterogeneity. Therefore, random-effects models were applied to the meta-analysis. Eight studies were included in the me-

ta-analysis, revealing evidence that HMB or supplements containing HMB improved lower body strength compared with controls, but with a moderate to large effect size (SMD = 0.55; 95% CI: 0.06, 1.04; $P = 0.000$; $I^2 = 86.9\%$, Figure 4). HMB showed no significant effect on upper body strength (SMD = 0.27; 95% CI: -0.55, 1.09; $P = 0.000$; $I^2 = 92.0\%$, Figure 5). Almost no effect was found in FM (SMD = 0.25; 95% CI: -0.18, 0.75; $P = 0.001$; $I^2 = 82.1\%$, Figure 6), fat-free mass (SMD = 0.04; 95% CI: -0.26, 0.33; $P = 0.000$; $I^2 = 76.3\%$, Figure 7), and muscle function (SMD = 0.15; 95% CI: -0.21, 0.51; $P = 0.000$; $I^2 = 83.7\%$, Figure 8).

Sensitivity analyses and publication bias

Following the recommendations of the Cochrane Handbook for Systematic Reviews of Interventions, statistical tests to build funnel plot asymmetry were not undertaken owing to the use of SMDs in the meta-analysis.

Discussion

This study was conducted to compare the effects of interventions of HMB supplementation in addition to RET and RET alone on the muscle mass, strength, and function of healthy community-dwelling elder adults. HMB supplementation and RET are well-recognized effective treatments for sarcopenia, and the synergistic effects of the intervention methods mentioned above have been previously evaluated [18, 19]. However, no consensus was reached. Jakubowski concluded that HMB and RET treatment has a small effect on total body mass gain, but this effect was not seen in free FM (FFM), muscle strength, or decreases in (FM) [20]. Rowlands and Thomson determined a synergistic effect in leg strength gains in previously untrained men by supplementing them with HMB during resistance training, but this effect in trained lifters was not significant [18]. Another recent study in untrained young adults showed that additional supplementation with

Table 2. Study characteristics of included trials.

Author, Year	Subjects	No. (C; T)	Age. (C; T)	Training Regimen				HMB Supplementation		
				Length	Frequency	Intensity (Reps)	Sets	Loading	Daily dose	Control
Stout, 2013	Man, woman; healthy	N _C = 20; N _T = 16	73.0 ± 1.0; 73.0 ± 1.0	21 (wk)	3/wk	8-12 at 80% 1-RM	66 (77.6%)	Mixture	3 g HMB/d	Placebo
Stout, 2015	Man; healthy	N _C = 12; N _T = 12	72.1 ± 5.7; 72.1 ± 5.7	12 (wk)	3/wk	N.R.	6 (54.54%)	Mixture	3 g HMB/d	Placebo
Din, 2019	Man; healthy	N _C = 8; N _T = 8	68.5 ± 1.0; 67.8 ± 1.15	6 (wk)	3/wk	6-8 at 75% 1-RM	7 (87.5%)	Mixture	3 g HMB/d	Placebo
Vukovich, 2000	Man, woman; healthy	N _C = 17; N _T = 14	70.0 ± 1.0; 70.0 ± 1.0	8 (wk)	2/wk	10-12 at 70% 1-RM	4 (80%)	Capsules	3 g HMB/d	Placebo
Berton L, 2016	Women; healthy	N _C = 33; N _T = 32	69.5 ± 5.3; 69.5 ± 5.3	8 (wk)	2/wk	Mild fitness	7 (87.5%)	Drink	1.5 g ca-HMB/d	Standard diet
Deutz, 2013	Men, women; healthy	N _C = 11; N _T = 8	67.1 ± 1.7; 67.4 ± 1.4	8 (wk)	3/wk	8-10 at 80% 1-RM	4 (80%)	Sachet	3 g HMB/d	Placebo
Rathmacher, 2020	Men, women; healthy	N _C = 34; N _T = 30	67.7 ± 0.7; 67.2 ± 0.7	12 (mon)	3/wk	60 minutes of supervised resistance	4.8+6.5, [0.04-30]	Capsules	3 g ca-HMB/ dplus vitamin D3 (2,000 IU/ day)	Placebo
Osuka, 2021	Woman	N _C = 39; N _T = 39	71.8 ± 4.1; 73.5 ± 4.2	12 (wk)	2/wk	50 min resistance training	N.R.	Powder	1.5 g ca-HMB/d	Placebo

C: control; T: treatment; No: number; NC: number of control; NT: number of treatment; PL: Placebo; ca-HMB: calcium beta-hydroxy-beta-methylbutyrate; N.R.: not reported; 1-RM: one repetition maximum.

Table 3. Individual study outcome included in the meta-analysis.

Author, Year	Subjects	No. (C; T)	Age. (C; T)	Training Regimen
Stout, 2013	DXA	Total lean mass, kg Leg lean mass, kg Total fat mass, kg	Extensor 180°·s ⁻¹ Flexor 180°·s ⁻¹ Extensor 60°·s ⁻¹ Flexor 60°·s ⁻¹ Hand grip strength, kg	Get up and go, s
Stout, 2015	DXA	Abdominal fat mass, kg		
Din, 2019	DXA	Thigh lean muscle mass, g Thigh fat free mass, g	MVC, Nm 1-RM: Nm	
Vukovich, 2000	DXA	Body fat (%) Fat-free mass, kg	Upper body strength (%) Lower body strength (%)	
Berton L, 2016	DXA pQCT Radial pQCT Tibial pQCT	ASMMI Abdominal fat mass, kg Fat-free mass, kg Muscle area, mm ² Fat area, mm ² Muscle area, mm ² Fat area, mm ²	PT isokinetic Nm: PT isokinetic ext, Nm: PT isokinetic flex, Nm: Handgrip strength, kg Handgrip endurance, s	Chair stand times, s 6 MWT, m Walking time, s Balance test, score SPPB
Deutz, 2013	DXA	Leg Lean, kg Total body fat mass, kg Total lean Mass, kg	Knee extensor (60°) strength, Nm Knee extensor (180°) strength, Nm	
Rathmacher, 2020	DXA	Lean Mass, kg Body fat (%)	Hand grip strength, kg	Get up and go, s Get up (reps)
Osuka, 2021	DXA	Appendicular lean mass, kg Fat-free mass, kg Skeletal muscle index, kg/m ² Fat mass, kg Upper-extremity lean mass, kg Lower-extremity lean mass, kg	Knee extensor strength, N Hip adductor strength, N Handgrip strength, kg	Usual gait speed, m/s Maximal gait speed, m/s Timed up-and-go, s Five-repetition sit-to-stand, s

DXA: dual X-ray absorptiometry; SPPB: short physical performance battery; MVC: maximal voluntary contraction; 1-RM: one repetition maximum.

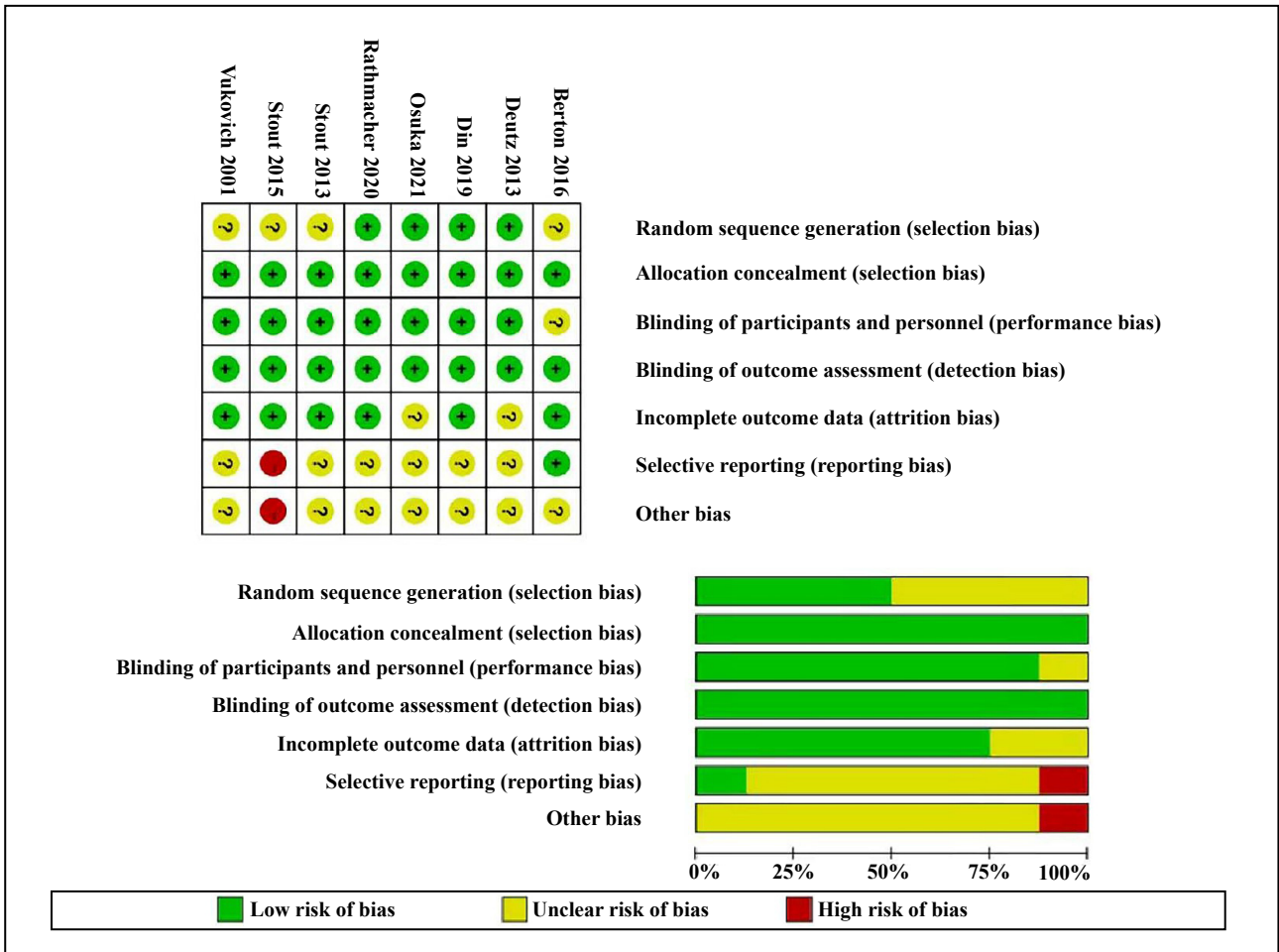


Figure 3. Risk-of-bias summary for all studies and outcomes, drawing by Review Manager 5.3.

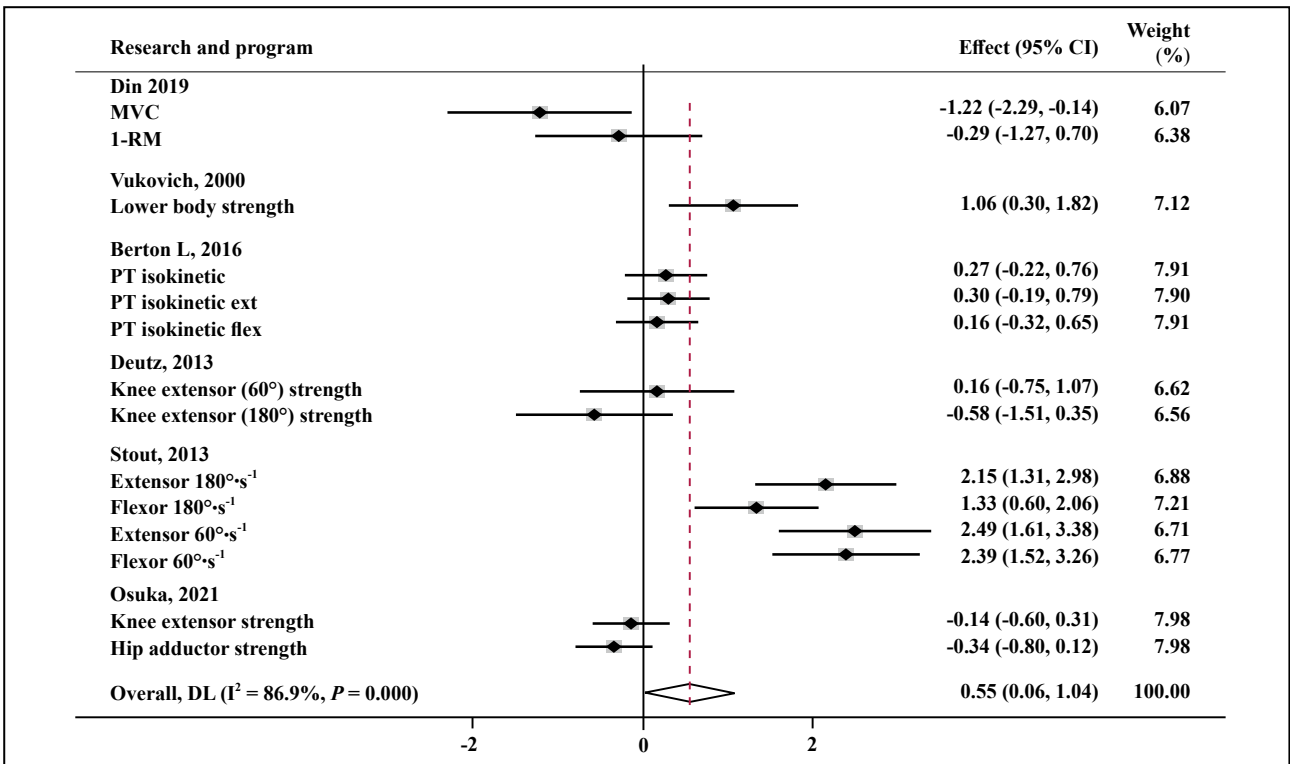


Figure 4. Forest plot of the results of a random-effects meta-analysis of HMB shown as pooled standard mean differences with 95% CIs on lower-body strength. Data were analyzed by stata 15. Vertical lines and diamonds indicate the overall measure of effects and confidence intervals.

Note: Weights are from random-effects model.

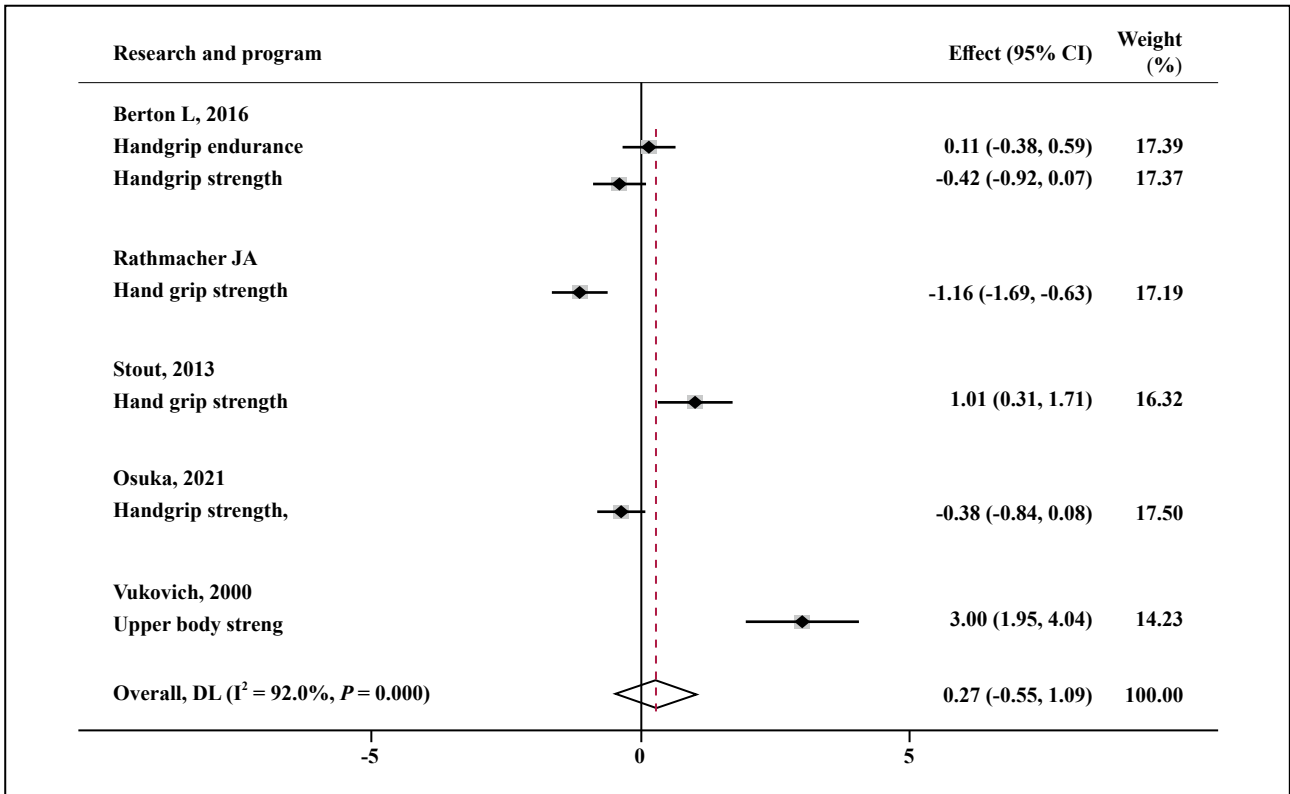


Figure 5. Forest plot of the results of a random-effects meta-analysis of HMB shown as pooled standard mean differences with 95% CIs on upper-body strength. Data were analyzed by stata 15. Vertical lines and diamonds indicate the overall measure of effects and confidence intervals. **Note:** Weights are from random-effects model.

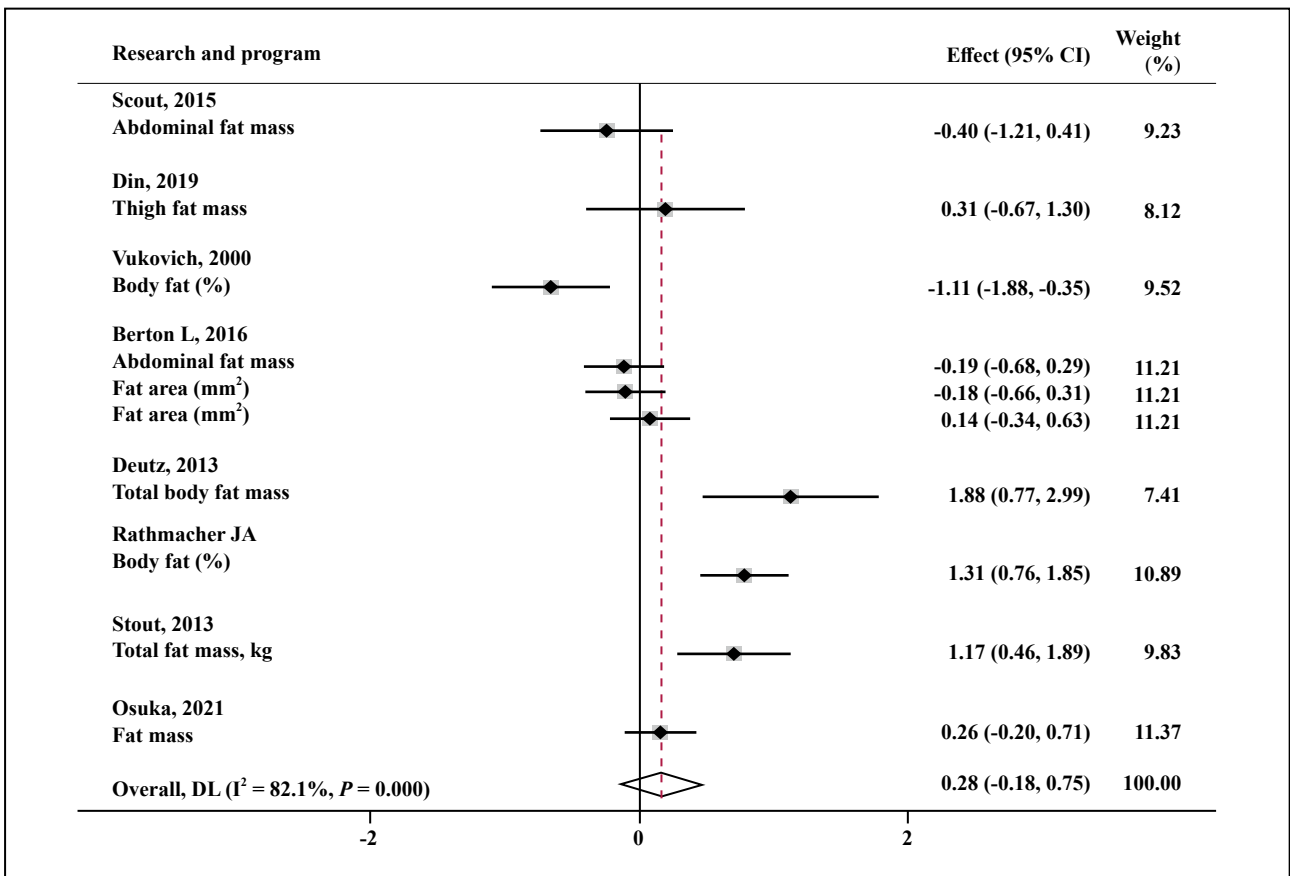


Figure 6. Forest plot of the results of a random-effects meta-analysis of HMB shown as pooled standard mean differences with 95% CIs on fat mass. Data were analyzed by stata 15. Squares are effect sizes. Vertical lines and diamonds indicate the overall measure of effects and confidence intervals. **Note:** Weights are from random-effects model.

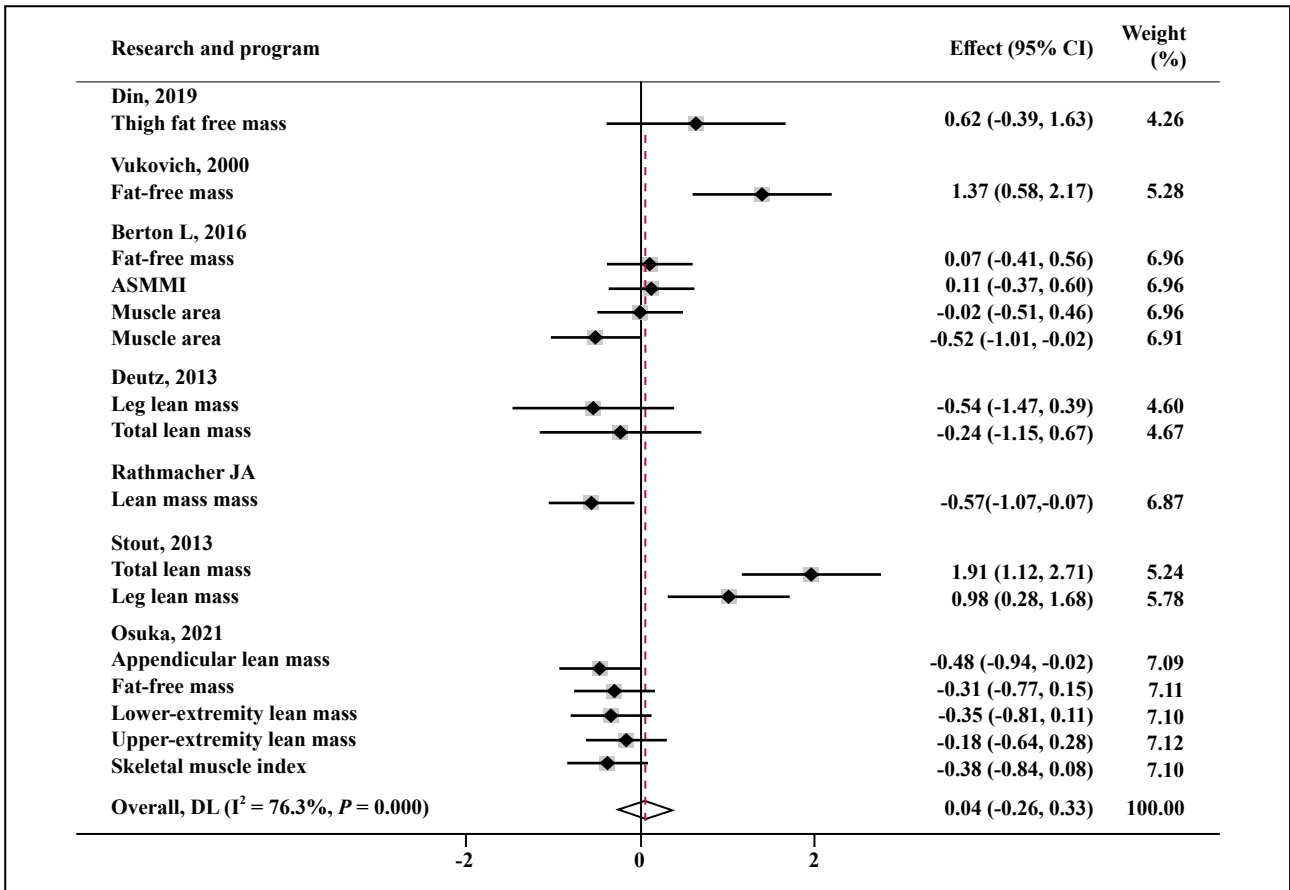


Figure 7. Forest plot of the results of a random-effects meta-analysis of HMB shown as pooled standard mean differences with 95% CIs on fat-free mass. Data were analyzed by stata 15. Squares are effect sizes. Vertical lines and diamonds indicate the overall measure of effects and confidence intervals. **Note:** Weights are from random-effects model.

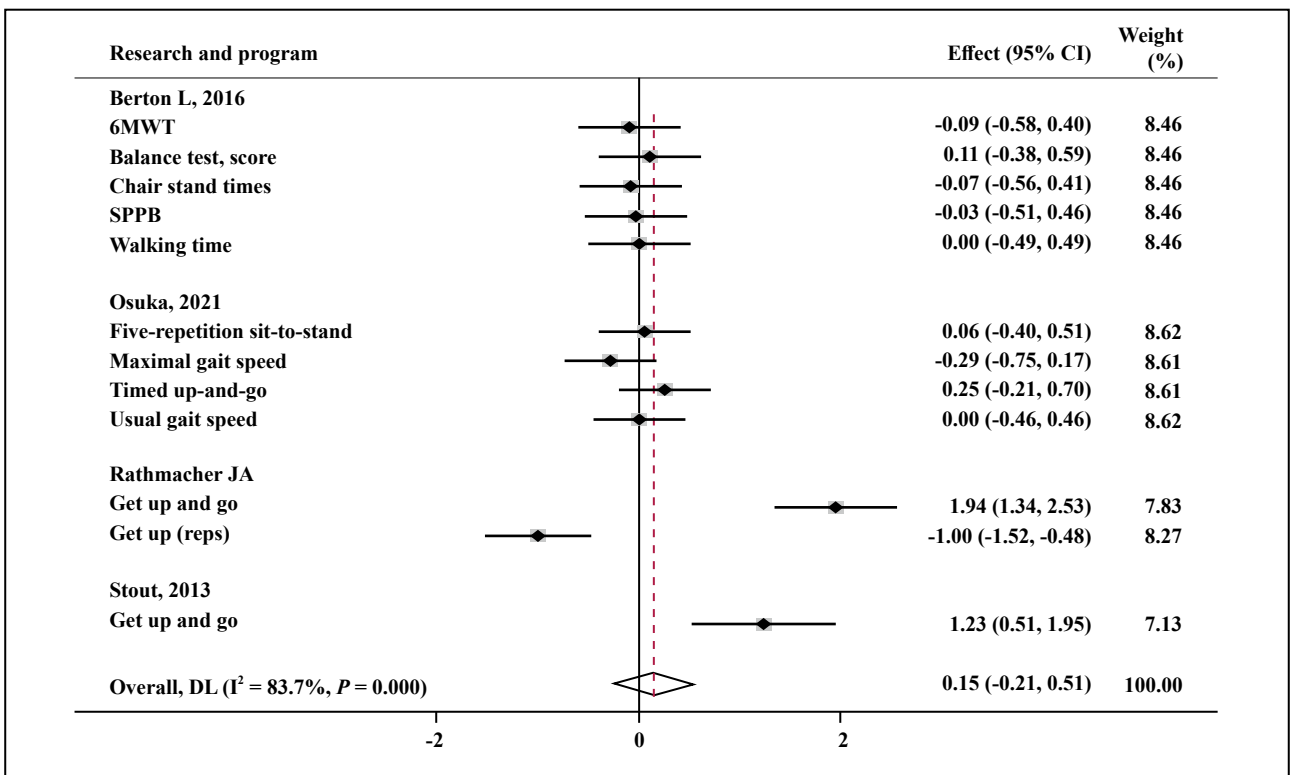


Figure 8. Forest plot of the results of a random-effects meta-analysis of HMB shown as pooled standard mean differences with 95% CIs on muscle function. Data were analyzed by stata 15. Squares are effect sizes. Vertical lines and diamonds indicate the overall measure of effects and confidence intervals. **Note:** Weights are from random-effects model.

HMB combined with resistance exercise resulted in segmental lower extremity FFM growth when compared with resistance exercise alone [21]. These different results may relate to the heterogeneity of the participants included and the interventions used. Although the additional effect of muscle gains from the combination of HMB and RET is unknown, drawing a final conclusion was too early, and more updated research should be included for evaluation. Our analysis, which included the latest clinical randomized controlled trial (RCT) studies, showed that HMB supplementation combined with RET significantly enhanced the muscle strength of the lower limbs in the elderly, but no positive effect was noted for muscle strength of the upper limbs, lean mass, FM, and muscle function during the entire study. Here, we found that in the elderly population, supplementing HMB on the basis of resistance training could achieve additional positive effects on muscle strength of lower limbs. This conclusion was similar to the previous study conducted among untrained lifters.

With the aging of the population intensifying, we conducted this study mainly for the elderly, who often suffer multiple chronic diseases. The synergy between HMB supplementation and RET in the elderly is not surprising. Physical inactivity and malnutrition, which interact with each other, are common conditions in older adults. Nutritional interventions are important for promoting physical activity. Exercise also helps improve appetite and promote nutrient absorption. Although a recent meta-analysis suggested that HMB supplementation in addition to physical exercise has a low impact on improving muscle strength in adults aged 50–80 years [22], we found preserved muscle strength in the intervention group compared with the gradual loss experienced in the control group. This might be due to the findings of our meta-analysis being mainly based on older adults who were untrained or who lightly exercised.

Although studies have shown that RET is an effective way to prevent frailty and sarcopenia, nutritional supplementation remains a safer, simpler, and more feasible intervention for older adults than RET. HMB has been widely recognized as a nutrient ingredient for the treatment of sarcopenia by activating the major signaling pathways leading to protein synthesis [23, 24]. In several preclinical models, HMB has been shown to improve MPS by activating mTOR. To explore the exact role of HMB intervention during RET treatment, we conducted subgroup analyses and found that HMB combined with RET mainly improved lower limb muscle strength rather than the upper limbs. Notably, the strength of the lower limb muscles is more important than the upper ones to improve the self-care ability and stability of the elderly. This novel result may provide optimized strategies to prevent frailty and sarcopenia. The findings of our analysis showed blunted effects of additional HMB supplementation in muscle mass. This result may be due to four reasons. First, the analysis included studies of healthy older adults who were probably not malnourished. The effect of the HMB inter-

vention may be diminished in older adults who habitually consume adequate nutrients. Second, in contrast to the young, the elderly may not achieve the expected effects of the conventional dose of HMB because of the degradation of digestion and absorption functions. In addition, because of the imbalance in muscle protein metabolism in older adults, long-term nutritional supplementation is needed, whereas short-term supplementation may not show obvious effects. Moreover, inflammation, immobilization, and chronic comorbidities could further lead to muscle loss. Thus, the different results may indicate that different populations respond differently to HMB supplementation combined with RET.

Through the results of this study, we confirmed that HMB supplementation combined with RET had a positive effect on the muscle improvement of the elderly living in the community, although this positive effect was limited to the muscle strength of the lower limbs. Among the elderly in the community, the molecular mechanism of HMB supplementation on the basis of RET to improve the muscle strength of the lower limbs needs to be further studied. Further research is needed to explore the appropriate timing, intensity, and prognosis of interventions.

Conclusion

The combination of HMB supplementation and RET in older people has an additional benefit for muscle strength, especially in the lower limbs, instead of muscle function and physical performance. Further studies are needed to demonstrate the mechanism.

Declarations

Authors' contributions: Made substantial contributions to the conception and design of the study and performed data analysis and interpretation: Guangqin Zou, Quan Wang, Xiang Lu, Wei Gao; Performed data acquisition, as well as providing administrative, technical, and material support: Guangqin Zou, Hua Wan, Wei Gao.

Availability of data and materials: Not applicable.

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Conflicts of interest: Wei Gao is a member of the Youth Editorial Board of *Aging Pathobiology and Therapeutics*. All authors declare no conflict of interest and were not involved in the journal's review or decisions related to this manuscript.

Ethical approval and consent to participate: Not applicable.

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