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REVIEW

Effects of combined protein and exercise interventions on bone health in middle-aged and older adults — A systematic literature review and meta-analysis of randomized controlled trials

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Abstract

Purpose Osteoporosis has become a global public health concern making prevention and treatment essential to reduce severe consequences for individuals and health systems. This systematic review with meta-analysis aimed to determine the effects of combined protein and exercise interventions compared to (a) exercise alone and (b) protein alone on bone mineral content (BMC) or density (BMD) in middle-aged and older adults.

Methods We systematically searched Medline, CINAHL, CENTRAL, Web of Science, and SPORTDiscus until 24th January 2023. Pairwise random-effects meta-analyses were performed to calculate weighted mean differences (WMD) with 95% confidence intervals (95% CI). We evaluated risk of bias (Cochrane RoB2) and certainty of evidence (CoE; GRADE). If pooling was not possible, the results were summarized descriptively.

Results For the comparison of combined protein supplementation and exercise vs. exercise alone, no meta-analysis for BMD (2 RCTs) was possible. For BMC, little to no intervention effect was found (WMD 0.03 kg; 95% CI − 0.00 to 0.05; 4 RCTs; IG = 97/CG = 98; $I^2 = 58.4\%$). In a sensitivity analysis, restricted to combined milk-protein supplementation and exercise, the result remained similar (0.01 kg; 95% CI − 0.01 to 0.03; 4 RCTs; IG = 71/CG = 71; $I^2 = 0.0\%$; low CoE). For the comparison of combined protein and exercise interventions vs. protein alone, no RCT on BMC was identified; the results on total or regional BMD (2 RCTs) were inconclusive.

Conclusion Based on our findings, no robust conclusions can be drawn on whether combining protein and exercise interventions is more beneficial for bone health than one component alone. Sufficiently powered studies with longer duration are required to clarify these questions (CRD42022334026).

Keywords Bone health · Protein and exercise interventions · Randomized controlled trials

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Introduction

Aging in combination with intrinsic (e.g. genetics and hormones) and extrinsic factors (e.g. nutrition and physical activity) contributes to bone mass loss, increasing the risk of osteoporosis and fractures [1]. Osteoporosis is one of the most common metabolic bone diseases worldwide, affecting about 18.3% of the population [2]. The prevalence is particularly high among older women (35.3%) [2]. In 2017, using data from six European countries, approximately 1 million quality-adjusted life-years were lost due to fragility fractures [3]. Among older people with osteoporotic vertebral fractures, 1-year mortality ranges from 20 to 27%, while 34 to 50% of patients are discharged from hospital to care facilities [4]. Moreover, osteoporotic vertebral fractures reduce health-related quality of life [5]. With demographic shifts, the number of people aged 50

years or older at risk of osteoporosis-related fractures is projected to double globally by 2040 [6]. In 2019, the total direct costs of fragility fractures in the EU 27 + 2 countries were estimated at 56.9 billion Euros [7]. Thus, osteoporosis poses not only a medical but also an economic and social burden, underscoring the importance of prevention and treatment to mitigate severe consequences for individuals and health systems. Bone mineral density progressively declines starting in the fourth decade of life, with an accelerated decrease in women during menopause [8]. Osteoporosis prevention aims to slow the rate of bone loss and should, therefore, be initiated earlier, targeting middle-aged individuals already [9]. Research has demonstrated that falling is not exclusively an issue for older adults, and middle age has been identified as a critical life stage for fall prevention [10]. Exercise training is considered an integral component in the prevention and treatment of osteoporosis, as well as in reducing the risk of falls and fractures [11, 12]. Mechanical loading through exercise stimulates osteogenesis by promoting osteoblast activity, thereby enhancing bone mineral density and bone strength. This adaptive response is particularly pronounced in high-impact activities and is most effective when initiated during early childhood, with benefits persisting across the lifespan [13]. Several meta-analyses indicate that exercise training effectively improves bone mineral density in postmenopausal women [14, 15], men [16] and older adults [17].

Approximately 50% of bone volume and one-third of bone mass are composed of protein [18]. In addition to serving as a substrate for bone matrix formation, protein intake is relevant for the production of insulin-like growth factor 1 (IGF-1), a hormone that promotes bone and muscle growth [19, 20]. Several studies suggest that a protein intake exceeding the recommended dietary allowance (RDA) may increase bone mineral density and content [21, 22]. However, a recent umbrella review of systematic reviews examining prospective studies in the general adult population rated the certainty of evidence regarding the association or effect of total, animal or plant proteins on bone mineral density and fracture risk, except for hip fractures, as insufficient [20].

Combinations of exercise and protein interventions are frequently recommended for optimizing body composition [23, 24], due to potential synergistic effects of exercise-induced bone (re-)modelling and the provision of protein as a substrate for bone synthesis [19]. However, the evidence regarding their impact on bone health in middle-aged and older adults remains heterogeneous [25–30]. To the best of our knowledge, systematic reviews directly comparing the combined effects of these interventions with the solitary effects of either exercise or protein interventions are lacking.

Therefore, we performed a systematic review with meta-analysis to summarize and evaluate the effects of combined protein and exercise interventions compared to (a) exercise or (b) protein alone on bone mass parameters in middle-aged and older adults.

Methods

This systematic review was conducted within the framework of the (German) National guideline on exercise training for fracture prevention (AWMF-registry number: 183–002) and is reported according to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) [31] and its extension for searching [32]. The protocol of the study was prospectively registered with the International Prospective Register of Systematic Reviews (PROSPERO; CRD42022334026) [33].

Data sources and searches

We conducted a comprehensive search in five databases (Medline via Ovid, CINAHL via Ebsco Host, Cochrane Central Register of Controlled Trials (CENTRAL) via Wiley, Web of Science via Clarivate and SPORTDiscus via Ebsco Host) from their inceptions until 24th January 2023. The search combined blocks focusing on "protein", "exercise" and "bone" and "RCTs" (except for CENTRAL). No language restrictions were applied. The search strategy for each database can be found in Table SI1. Additionally, reference lists of included studies were screened manually to identify further potentially relevant articles.

Study eligibility criteria

We included studies in this systematic review fulfilling the criteria displayed in Table 1.

Study selection

Identified references were saved in Endnote, and duplicates were removed according to the method described by Bramer [34]. Two reviewers (JW and EK) independently screened titles, abstracts and full texts for eligibility. All disagreements were resolved by discussion.

Data extraction

Using a standardized data extraction form, one reviewer (JW) extracted data from included RCTs. A second reviewer (EK) checked and verified the extracted data. Conflicts were resolved by discussion.

We extracted data on study characteristics (e.g. author, publication year, country, setting, study duration, sample size, conflict of interest and study funding), participants' characteristics (e.g. age, sex and health status), intervention characteristics (e.g. content, dose, adherence and control group) and outcomes (e.g. definition, measurement,

Table 1 Definition of the research question and the eligibility criteria based on the PICOS (population, intervention, comparator, outcome and study design) scheme

Population	Middle-aged and older adults	Included: people aged ≥ 45 years as this age is commonly used in medical databases such as Medline to categorize "middle-aged" People without any medical conditions except for obesity, sarcopenia, frailty, previous or current fractures Excluded: other pre-existing medical conditions
Intervention	Combined protein and exercise interventions	Included: exercise — any planned, structured and repetitive movement to improve or maintain physical fitness, for example, aerobic, resistance and balance training, according to the definition of the American College of Sports Medicine [33]. Interventions could focus on weight-bearing or non-weight-bearing exercises or both. Physically supported methods, such as electrical muscle stimulation and vibration training, when combined with gross motor movements and performed upright Protein — high protein diets (RDA > 0.8 g/kg KG/d), products fortified with protein or amino acids as well as protein and amino acid supplementation. If supplements contained further nutrients that could affect bone health, i.e. vitamin D, the comparator needed to receive the same dose of these nutrients. Excluded: weight loss interventions
Comparator	(a) exercise alone (b) protein alone	(a) and (b):[+] see specification under *intervention* Excluded: control groups receiving no intervention
Outcomes	Bone health parameters	Primary: bone mineral density/content (both site-specific and total body) measured by DXA or other devices Secondary: incident fractures or osteoporosis* Excluded: bone turnover markers and hormones
Study design	(Quasi) randomized controlled trials	Parallel, cluster and cross-over designs

* As our search did not identify any RCTs reporting on these secondary outcomes, the results of this work refer to the primary outcome only

[+]Evidence was synthesized and meta-analyzed separately for comparators (a) and (b)

baseline and follow up values and/or change values of bone health parameters). If data was only presented in figures, one reviewer (EK) extracted the data with the WebPlotDigitizer tool (Version 4.6; https://automeris.io/WebPlotDigitizer/index.html), and a second reviewer (JW) cross-checked the results. If relevant data was missing, we contacted the corresponding author of the primary studies. In case of no response, the request was repeated after 2 weeks.

Risk of bias in individual studies

The risk of bias was assessed with Cochrane's RoB 2 tool for individually randomized controlled studies [35] or cluster-randomized clinical trials [36]. Sources of bias were identified by assessing the randomization process, deviations from intended interventions, missing outcome data, measurement of the outcome and selection of the reported results [35] and were rated as 'low,' 'some concerns' or 'high' risk of bias [35]. Two reviewers (JW and EK) conducted the assessment independently, and disagreements were resolved by discussion or the help of a third reviewer (DS).

Data synthesis

Data were synthesized narratively if studies were judged too heterogeneous for pooling in a meta-analysis due to issues related to outcome measurement, sample characteristics or interventions, or when outcomes were reported by single studies only.

If data quality and quantity were sufficient, pairwise, random-effects meta-analyses were conducted with the R software using the metafor package [37]. Continuous outcome data were synthesized using weighted mean differences (WMDs) with 95% confidence intervals (95% CI). The Cochran Q test was used to evaluate variability between studies, and the level of heterogeneity was assessed with the I^2 statistic. Tests for funnel plot asymmetry were not applied as the number of studies included in the meta-analysis was below ten [38]. Instead funnel plot symmetry was inspected visually.

Subgroup and sensitivity analyses

We conducted a predefined subgroup analysis based on sex. Further pre-specified subgroup analyses by age (< 65 vs. ≥ 65 years) and type of exercise (e.g. resistance, aerobic and multi-component) were not conducted as mean ages of all studies included in the meta-analysis were ≥ 65 years, and exercise protocols were similar between trials. Similarly, for the type of protein intervention (e.g. protein-rich diet, fortified foods, protein supplements and amino acid supplements), we did not conduct the preplanned subgroup analysis; instead a sensitivity analysis

was performed since the protein intervention of one RCT distinctly differed from other trials.

In addition, a meta-analysis was conducted for different durations of the intervention (12 and 24 weeks).

Assessment of the certainty of evidence

For outcomes that were meta-analyzed, we evaluated the certainty of evidence according to the Grading of Recommendations, Assessment, Development, and Evaluation (GRADE) approach [39]. Two reviewers (EK and JW) independently assessed the certainty of evidence, and disagreements were resolved by discussion. The domains risk of bias, inconsistency [40], indirectness, imprecision [41] and publication bias were used to downgrade the certainty of evidence. We used the null effect to assess imprecision. GRADE classifies the certainty of evidence into four levels: high, moderate, low and very low. Evidence profiles were created using GRADEpro GDT software (https://www.gradepro.org/).

Results

Study selection

Of the 4661 articles identified, 733 duplicates were removed. Titles and abstracts of the remaining 3928 articles were screened for eligibility, and 70 full-text articles were assessed with regard to the inclusion and exclusion criteria. Of these, ten RCTs were included in this review (Fig. 1). A list of excluded full-texts with the respective reasons for exclusion is given in Table SI2. We contacted authors of four included RCTs [42–45] as relevant outcome data were not reported, and we received the respective information from three [43–45].

Study and participants' characteristics

Table 2 shows the study and participants` characteristics of included studies. With the exception of one cluster randomized controlled trial, studies were randomized at the individual level. Sample sizes ranged from $n = 28$ [44] to $n = 112$ participants [25]. The mean age ranged from 54 [29]

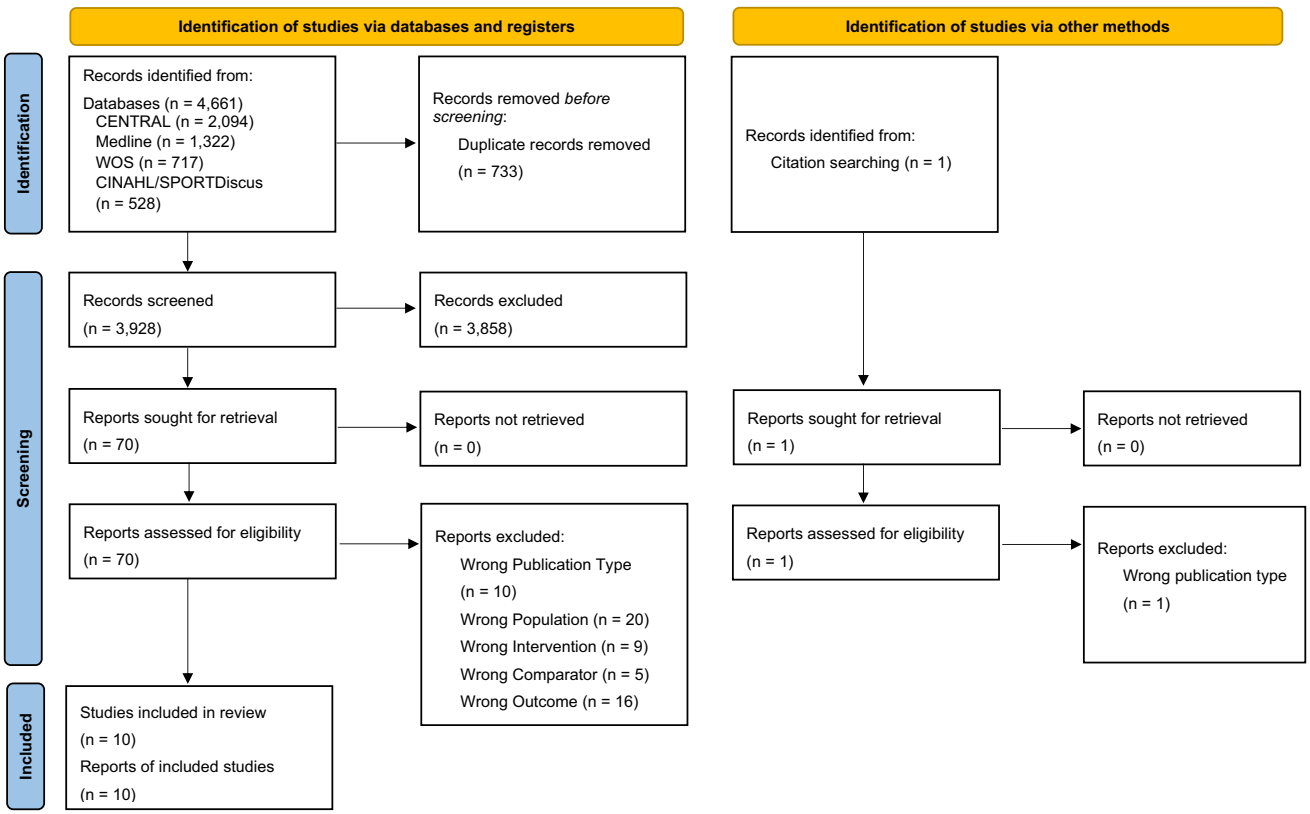

Fig. 1 PRISMA Flow chart. From: Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ 2021;372:n71. https://doi.org/10.1136/bmj.n71. For more information, visit: http://www.prisma-statement.org/

Table 2 Studies' and participants' characteristics

Study author (country, year)	RCT design	Setting	Sample size (*n* analyzed)	Female %	Age *mean* (*SD*)	Health status/characteristics	Topic*
Amasene (Spain, 2019)	Parallel Single-blind Placebo-controlled	Community Post-hospitalization	A1: 21 (15) A2: 20 (13)	A1: 46.7 A2: 53.8	A1: 82.9 (5.6) A2: 81.7 (6.5)	Sarcopenic	1
Daly (Australia, 2014)	Cluster	Self-care retirement villages	A1: 53 (48) A2: 47 (43)	A1: 100 A2: 100	A1: 72.1 (6.4) A2: 73.6 (7.7)	Postmenopausal	1
De Azevedo Bach (Brazil, 2022)	Parallel Double-blind Placebo-controlled	Community	A1: 18 (15) A2: 18 (16)	A1: 66.7 A2: 75.0	A1: 66.9 (4.3) A2: 65.8 (5.0)	Healthy, older	1
Evans (USA, 2007)	Parallel Double-blind Placebo-controlled	NR	Total: 61 (43) A1: (10) A2: (12) A3: (11) A4: (10)	A1: 100 A2: 100 A3: 100 A4: 100	A1: 63.5 (4.8) A2: 62.8 (5.3) A3: 62.5 (5.3) A4: 59.7 (5.2)	Postmenopausal	2
Fernandes (Brazil, 2018)	Parallel Double-blind Placebo-controlled	Community	A1: 16 A2: 16	A1: 100 A2: 100	A1: 67.3 (4.1) A2: 67.8 (4.0)	Physically independent	1
Leenders (Netherlands, 2013)	Parallel Double-blind Placebo-controlled	Community	A1: 30 (27) A2: 30 (26)	A1: 44 A2: 46	A1: W: 72 (2) M: 70 (1) A2: W: 69 (1) M: 70 (1)	Living independently	1
Roschel (Brazil, 2021)	Parallel Double-blind Placebo-controlled	Community	A1: 45 (39) A2: 22 (20) A3: 45 (41)	A1: 49 A2: 100 A3: 49	A1: W: 72 (6) M: 73 (8) A2: W: 72 (6) A3: W: 73 (6) M: 72 (5)	Pre-frail or frail	1
Shenoy (India, 2014)	Parallel	NR	A1: 20 A2: 20	A1: 100 A2: 100	A1: 54.6 (5.2) A2: 54.1 (6.9)	Postmenopausal Osteopenic or osteoporotic	2
Verdijk (Netherlands, 2009)	Parallel Double-blind Placebo-controlled	Community	A1: 14 (13) A2: 14 (13)	A1: 0 A2: 0	A1: 72 (2) A2: 72 (2)	Living independently	1

A study arm, *M* men, *W* women, **Topic 1* additional effect of protein on exercise, *Topic 2* additional effect of exercise on protein

to 82 years [42]. Two studies included only male participants [26, 44], and four included only female participants [29, 45–47], with three studies conducted in postmenopausal women [29, 46, 47]. Regarding health status, studies included participants with sarcopenia [42], osteopenia/osteoporosis [29], pre-frailty/frailty [25] and poor muscular strength/physical performance [26].

Information on funding sources of included RCTs and declarations of potential competing interests of study authors can be found in Table SI3.

Intervention characteristics

Eight RCTs investigated the effects of combined protein and exercise interventions compared to exercise alone [25, 26, 42–46, 48] (Table 3) and two RCTs compared to protein alone (Table 3). Intervention duration was 12 weeks [26, 29, 42, 44, 45, 48], 16 weeks [25, 46], 24 weeks [43] or 39 weeks [47]. Nine RCTs performed supervised, progressive resistance training (2–3 times per week) for the upper and lower body [25, 26, 29, 42, 43, 45, 46, 48] or the lower body only [44], while one RCT performed moderate-to-vigorous continuous endurance training (3 times per week) [47]. Adherence to training interventions was described in six RCTs [25, 26, 43–46] and was reported to be ≥ 90% in three RCTs [43, 44, 48]. Protein interventions comprised supplements based on milk proteins (i.e. whey [25, 42, 45, 48], casein [44], milk protein concentrate [43], milk protein isolate [47]), soy [25, 29, 47] or collagen peptides [26]. All these trials were placebo-controlled [25, 26, 29, 42–45, 47, 48]. The amount of the supplements offered ranged from 10 to 40 g/d. One study investigated the effects of 220 g

Table 3 Description of interventions for RCTs investigating combined protein and exercise interventions compared to (a) exercise alone and (b) protein alone on bone health in middle-aged and older adults

Author, year	Duration (wk)	Protein intervention(s)	Control	(a) Exercise co-intervention	Adherence to the intervention
Amasene (2019)	12	**Whey supplement:** - B-lactoglobulin (20 g/bottle) - L-Leucine (3 g/bottle) - 150 ml - 2×/wk after training	**Placebo supplement:** - Maltodextrin (23 g/bottle) - Hydroxyethylcellulose (0.200 g/bottle) - 150 ml - 2×/wk after training	**Resistance training:** - 1 h/session, 2×/wk - Supervised - Progressive (50–70% of 1-RM) - 2 sets per exercise Content: - Warm up - Strengthening of upper and lower limbs (arm-curl, knee extension, standing knee flexion, side hip raise, standing hip extension and chair stand) - Dynamic balance - Cool down	1 Participant refused to take the supplement, no further information
Daly (2014)	16	**Lean red meat:** - 220 g (raw weight) of veal, lamb or beef cuts) prepared by participants - Distributed over 2 meals/day at 6 days/wk - Individual counselling	**High carbohydrates:** - Usual diet + ≥ 1 serving of rice/pasta - 1×daily - Individual counselling	**Resistance and balance-agility training:** - 45–60 min/session, 2×/wk - Supervised - Progressive - First 2 wks: 3 sets of 12 repetitions; thereafter 3 sets of 8–12 repetitions Content: - Warm up (rhythmic exercise) - Resistance training (included squats, lunges, box step-ups, leg extensions, standing leg curls, hip abductions, calf raises, shoulder press, upright row, bicep curls, wall pushups and triceps kickback) - Balance-agility exercises - Cool down	Assessment: RT: attendance to exercise classes (daily exercise cards completed and checked after each session) Meat consumption: compliance calendar to record daily consumption (collected 1/month) Carbohydrates: self-report calendar Degree: RT: 74% no difference between groups Meat consumption: 81% Carbohydrates: 100%

Table 3 (continued)

De Azevedo Bach (2022)	12	**Whey supplement:** - 20 g whey protein isolate - Diluted in 100 ml water - 2×/day (after breakfast and dinner)	**Placebo supplement:** - 20 g of maltodextrin - Diluted in 100 ml water - 2×/day (after breakfast and dinner	**Resistance training:** - 2×/wk - Supervised, group based (6–8 persons) - Progressive (3 wks: 2 sets of 12–15 RM, 3 wks: 3 sets of 10–12 RM, 3 wks: 4 sets 8–10 RM, 3 wks: 4 sets of 6–8 RM) - Training load increased from 2.5 to 5.0 kg whenever participants were able to perform more repetitions than prescribed Content: - Chest press, bilateral knee extension, lat pull-down, bilateral elbow extension, hip abduction, bilateral elbow flexion and hip adduction	Assessment: RT: NR Supplement: collection of empty packages, recording of consumption, 3d dietary records Degree: RT: A1: 100%, A2: 99% Supplement: A1: 97.23%; A2: 97.1% Protein intake (g/kg BW/d, pre, post) A1: 1.1 ± 0.3; 1.5 ± 0.4 A2: 1.3 ± 0.4; 1.3 ± 0.5
Fernandes (2018)	12	**Whey supplement:** - 35 g of hydrolysed whey protein - Diluted in 200 ml water - 3×/wk	**Placebo supplement:** - 35 g of maltodextrin - Diluted in 200 ml water - 3×/wk	**Resistance training:** - 3×/wk - Supervised - Progressive (training weight was adjusted on a weekly basis using the weight test for repetition maximum) - 3 sets of 8–12 RM Content: - Whole body training (chest press, horizontal leg press, seated row, knee extension, preacher curl, leg curl, triceps pushdown and seated calf raises)	Assessment: RT + Supplement intake: Supervision Degree: Protein intake (g/kg BW/day, pre, post): A1: 0.85 ± 0.1; 1.4 ± 0.1 A2: 0.81 ± 0.1; 0.87 ± 0.1

Table 3 (continued)

Leenders (2013)	24	**Protein supplement:** - 15 g of protein (milk protein concentrate); 80% of casein and 20% of whey protein - 0.5 g of fat, 7.13 g of lactose and 0.42 g of calcium - 250 ml - 1 × daily	**Placebo supplement:** - No protein or fat, 7.13 g lactose and 0.42 g calcium - 250 ml - 1 × daily	**Resistance training:** - 3 × /wk - Supervised - Progressive (1 RM was increased in the first 4 wks of training from 60% (10–15 repetitions/set) to 75% (8–10 repetitions/set), from week 5 onwards: 8 repetitions at 75–80%) Content: - Warm-up -Leg press, leg extension, chest press, horizontal row in every session; vertical lat pull, abdominals, biceps curl and triceps extension were alternated between subsequent sessions - Cool down	Assessment: RT: Supervision Protein: 3-day dietary record Degree: RT: 90% ± 1% (no difference between groups) Protein: Increase of protein intake by 0.24 (w) and 0.18 g/kg BW/day (m) in the supplement group
Roschel (2021)	16	**Whey supplement:** - 15 g of whey mixed in 150 ml of water - 2 × daily OR **Soy supplement:** - 15 g of soy mixed in 150 ml of water - 2 × daily	**Placebo supplement:** - 15 g of corn starch mixed in 150 ml of water - 2 × daily	**Resistance training:** - 2 × /wk - Supervised - Progressive (ranged from 2 sets at 50% 1 RM (first 4 wks of training) to 4 sets at 70% 1-RM (last 4 wks of training) for each exercise) Content: - Exercises for the main muscle groups (inclined leg press, leg extension, horizontal bench press, shoulder press and lat pull down)	Assessment: RT: training log of each exercise session Supplement: Record of supplement intake (time and day); return of supplement containers each month and weighing the remaining supplement in each container Degree: Protein intake (g/kg KG/day, pre, post): A1: 0.88 (0.75, 1.00); 1.19 (1.05, 1.33) A2: 0.91 (0.78, 1.04); 1.26 (1.2, 1.4) A3: 0.81 (0.68, 0.95); 0.89 (0.75, 1.03)

Table 3 (continued)

Verdijk (2009)	12	**Protein supplement:** - 10 g protein as casein hydrolysate - As 250 ml beverage - 2×per session (during warm up and cooling down)	**Placebo supplement:** - Water (250 ml) - 2×per session (during warm up and cooling down)	**Resistance training:** - 3×/wk - Supervised - Progressive (from 60% of 1 RM (10–15 repetitions in each set) to 75–80% of 1 RM (8–10 repetitions) within the first 4 wks; from wk 5: 8 repetitions at 75–80%) - 4 sets per exercise Content: - Warm up - Leg press, leg-extension - Cool down	Assessment: RT: attendance in sessions Protein: supplement given before/after exercise session Degree: 35 ± 1 of the 36 sessions (no difference between groups)
Zdzieblik (2015)	12	**Collagen supplement:** - 15 g/day (molecular weight of approximately 3 kDa, included 2.7% leucine) - In 250 ml water - 1×daily	**Placebo supplement:** - Silicon dioxide - In 250 ml water - 1×daily	**Resistance training:** - 3×/wk, 60 min - Supervised - Progressive (wks 1–4: 15 repetitions, wks 5–9: 10 repetitions, wks 10–12: 8 repetitions; 4 s/repetition) Content: - All larger muscle groups (pull down, leg press, bench press, back press, etc.)	Assessment: Supplement: collection of unused supplement; daily records about the timing of ingestion Exercise: exclusion if missing > 10% of the training sessions Degree: 7 dropouts due to incompliance with training protocol Supplement: NR
Author, Year	**Duration (wk)**	**Exercise Intervention**	**Control**	**(b) Protein Co-Intervention**	**Adherence to the intervention**
Evans (2007)	39	**Endurance exercise:** - 3×/wk (increased until 45 min/session) - Supervised - Progressive (intensity of 55 to 60% of peak aerobic capacity (VO_2 peak) and progressed gradually to 75 to 80% of VO_2 peak within 4 to 6 weeks) Content: - Different exercise modes were used (e.g. a four-lane 17-lap per mile indoor track, treadmills, rowing ergometers, and stair-climbing ergo-meters) – Individualized on the basis of participant preferences	**No exercise** - Maintain usual physical activities	**Soy** (2 groups): - 25.6 g of protein and 91.2 mg aglycone units of isoflavones - Daily Or **Milk protein isolate** (2 groups): - 25.6 g of protein and 0 mg aglycone units of isoflavones - Daily	Assessment: Protein: food records Degree: Significant increase of protein intake in all groups (+ 15 to + 23 g/day) Adherence to the supplement: "~ 90%"

Table 3 (continued)

Shenoy (2014)	12	**Resistance training:** - 4 × /wk (40–50 min/session) - Supervised - Progressive (1 set of 15 repetitions at 40–50% of 1 RM until 3 sets of 8–12 repetitions at 60–80% of 1 RM) Content: Step up and down, prone leg curl, wrist leg curl, biceps curl, seated and supine triceps extension, standing calf/heel raise, wall push-up and knee flexion/extension shoulder press	**No exercise**	**Soy isolate protein:** - 40 g of soy protein powder/day mixed in 200 ml –250 ml milk twice a day	Assessment: Exercise: NR Protein: 24-h-recall method twice (before beginning and at the end of three months) Degree: No information regarding soy protein intake

A arm, *BW* body weight, *d* day, *wk* week, *min* minutes, *RM* repetition maximum, *RT* resistance training, *NR* not reported

lean red meat intake as protein source compared to a control diet high in carbohydrates [46]. Adherence to the protein interventions was described in one study by percentage of consumed product (81%) [46], in three studies by protein intake assessment (increase in intervention groups) [25, 43, 45], and in two studies by both measures [47, 48]. The other studies did not report on adherence.

Outcome characteristics

All but one study measured bone health using a DXA device. Of these, six RCTs [26, 42–45, 48] reported bone mineral content, the only outcome judged appropriate for pooling in a meta-analysis. Total body and site-specific bone mineral density were assessed in three RCTs [25, 46, 47] with DXA (total body ($n = 2$) and regions of the proximal femur ($n = 1$–3)). One study used ultrasound densitometry and reported speed of sound as measure of bone mineral density at the radius and tibia [29].

Risk of bias

Out of the nine parallel-designed RCTs, none was judged to have an overall low risk of bias (Fig. 2a). Six RCTs were identified to show "some concerns" [25, 26, 43–45, 48], and three studies were rated as "high risk of bias" [29, 42, 47] due to "bias arising from the randomization process", "bias due to missing outcome data" or "bias due to measurement of the outcome" [49, 50]. For the cluster-randomized study (Fig. 2b), we detected an overall "high risk of bias" due to "bias arising from the timing of identification and recruitment of individual participants in relation to timing of randomization".

Effects of combined protein and exercise interventions versus exercise alone

Bone mineral content

Six RCTs investigated whether combining protein and exercise interventions is more effective than exercise alone on total body bone mineral content [26, 42–45, 48]. As Amasene et al. [42] did not present effect estimates and data could not be obtained from the authors, the study was not included in the meta-analysis (Table SI4). Based on the meta-analysis, combining protein and exercise interventions showed no to little effect on bone mass compared to exercise alone (*WMD* 0.03 kg; 95% *CI* − 0.00 to 0.05; 5 RCTs; $IG = 97/CG = 98$; $p = 0.050$; $I^2 = 58.4\%$) (Fig. 3).

Subgroup analysis by sex did not show any notable changes in effects on bone mineral content between men (*WMD* 0.02 kg; 95% *CI* − 0.01 to 0.05; 4 RCTs; $IG = 59/CG = 58$; $p = 0.008$, $I^2 = 65.2\%$) and women (*WMD*

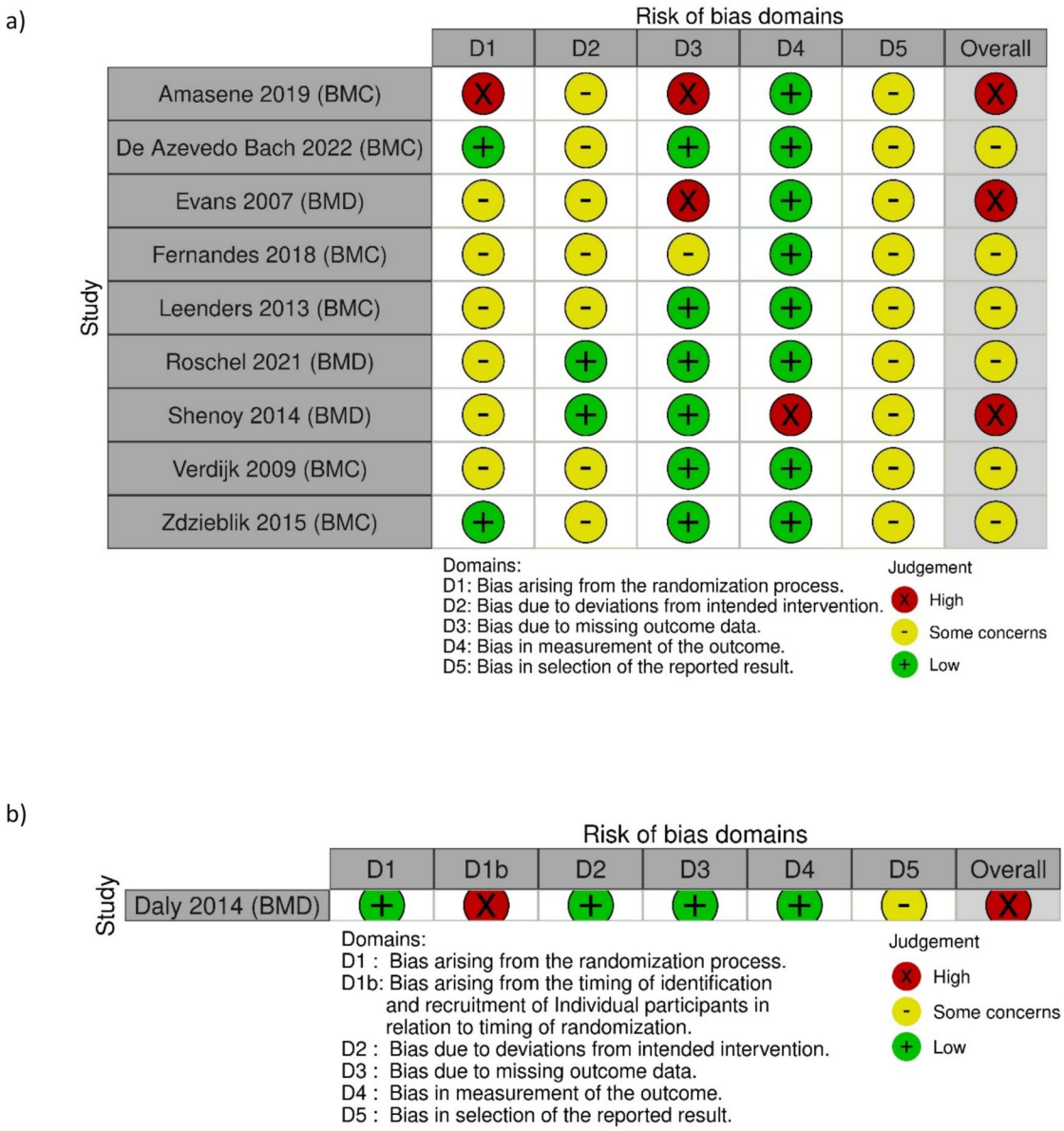

Fig. 2 Summary of risk of bias assessment with RoB 2 for each relevant outcome of included RCTs with **a** individual randomization ($n=9$) and **b** cluster randomization ($n=1$); Abbreviations: BMC, bone mineral content; BMD bone mineral density

0.01 kg; 95% *CI* − 0.02 to 0.04; 3 RCTs; $IG=38/CG=40$; $p=0.102$, $I^2=56.1\%$) (Fig. SI1).

Due to the level of heterogeneity identified in the main analysis, we conducted a sensitivity analysis, excluding the study by Zdziebelik et al. [26] as the protein intervention differed from other trials. Based on low certainty of evidence (*WMD* 0.01 kg; 95% *CI* − 0.01 to 0.03; 4 RCTs; $IG=71/CG=71$; $p=0.903$, $I^2=0.0\%$), combined milk-derived protein and exercise interventions may not affect bone mineral content compared to exercise alone (Fig. 4 and Table 4). The funnel plot indicated no signs of publication bias (Fig. SI2).

Meta-analysis based on intervention duration (12 weeks [26, 43–45, 48] and 24 weeks [43]) did not show differences in results (12 weeks: *WMD* 0.02 kg; 95% *CI* − 0.01 to 0.05; 5 RCTs; $IG=97/CG=98$; $p=0.001$, $I^2=70.9\%$; 24 weeks: *WMD* 0.02 kg; 95% *CI* − 0.02 to 0.06; 1 RCT; $IG=27/CG=26$) (Fig. SI3).

Bone mineral density

Two of the included RCTs investigated the effects of combined protein and exercise interventions compared to exercise alone on bone mineral density [25, 46]. As the RCTs distinctly differed in the intervention approach (Table 2),

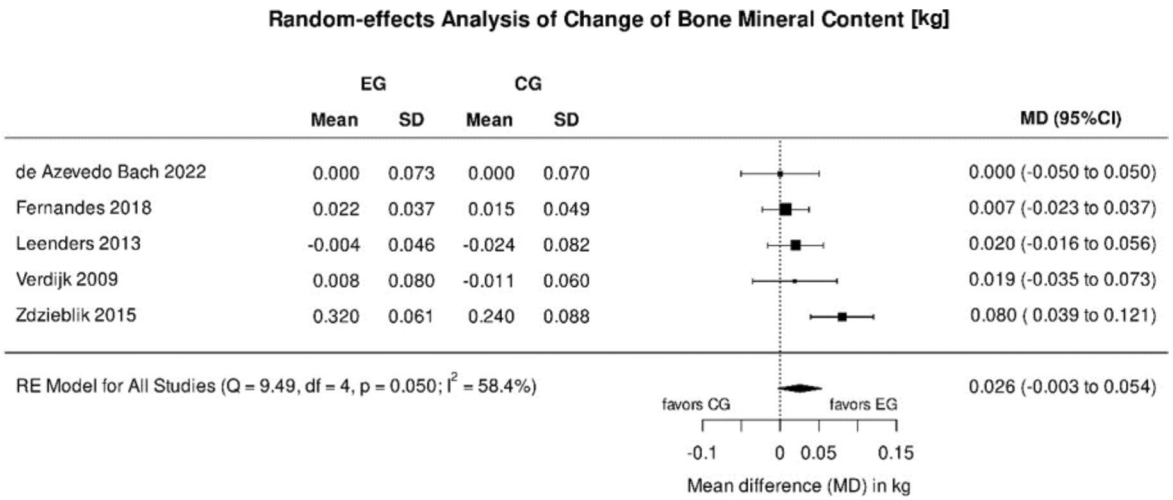

Fig. 3 Forest plot of meta-analysis summarizing mean differences with 95% confidence intervals for changes in bone mineral content by combined protein and exercise interventions (*EG*) compared to exercise alone (*CG*)

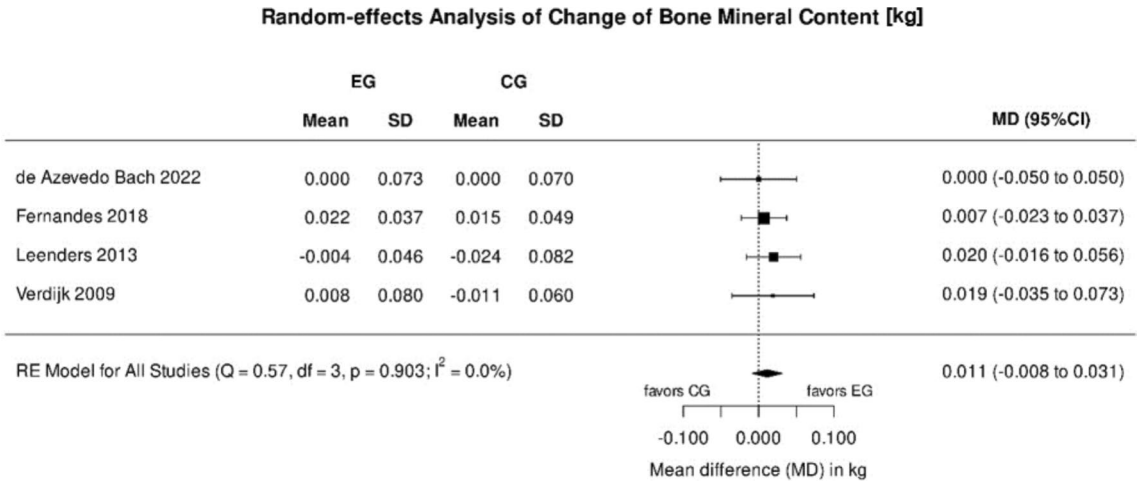

Fig. 4 Forest plot of sensitivity analysis summarizing weighted mean differences with 95% confidence intervals for changes in bone mineral content by combined milk-derived protein and exercise interventions (*EG*) compared to exercise alone (*CG*)

the results were summarized descriptively (Table SI4). Daly et al. compared the consumption of a high protein diet based on lean red meat to a low protein, high carbohydrate diet in addition to resistance and balance agility training [46] and did not find any group × time interaction effects on changes in bone mineral density of the femoral neck, total hip and lumbar spine following 16 weeks of intervention [46]. Roschel et al. investigated the effect of supervised and progressive resistance training (2 times per week for 16 weeks) in combination with 15 g of whey or soy supplementation or placebo twice daily on bone mineral density of the whole body, the femoral neck and the lumbar spine but observed no significant intervention effects [25].

Effects of combined protein and exercise interventions versus protein alone

Bone mineral content

We did not identify any RCTs comparing combined protein and exercise interventions versus protein alone regarding bone mineral content.

Bone mineral density

Two RCTs focused on the effects of combined protein and exercise interventions compared to protein alone on bone mineral density [29, 47] (Table 3). As these studies differed

Table 4 GRADE assessment on the meta-analysis of combined milk-derived protein and exercise interventions compared to exercise alone on bone mineral content (kg)

Certainty assessment							Summary of findings		
Participants (studies) follow-up	Risk of bias	Inconsistency	Indirectness	Imprecision	Publication bias	Overall certainty of evidence	Participants		Anticipated absolute effects
							With exercise	With milk protein + exercise	Risk difference with protein + exercise
142 (4 RCTs)	Serious[a]	Not serious	Not serious	Serious[b]	None	⨁⨁◯◯ Low	72	71	MD **0.01 kg higher** (0.01 lower to 0.03 higher)

CI confidence interval, *MD* weighted mean difference

[a]All studies were rated as "some concerns"

[b]The 95% confidence interval of the pooled effect crossed the line of no effect

distinctly in the exercise interventions and the regions of bone mineral density measurement, no meta-analysis was conducted, and the results were only descriptively summarized (Table SI5). Shenoy et al. investigated the effect of supervised and progressive resistance training in combination with a soy protein supplement compared to the supplement alone after 12 weeks [29] and showed improvements in bone strength of the radius and the tibia (assessed by ultrasound and reported as measure of bone mineral density) in both groups, with more pronounced gains in the combined group. Evans et al. investigated a milk or soy protein intervention with or without supervised moderate-to-vigorous intensity continuous endurance training for 39 weeks and did not find any group differences in the relative change in bone mineral density of the whole body, the lumbar spine and the proximal femur [47].

Discussion

To our knowledge, this is the first systematic review specifically focusing on the effects of combining protein and exercise interventions compared (a) exercise and (b) protein alone on bone mineral content or density in middle-aged and older adults. In summary, protein supplementation (mainly milk-derived) combined with resistance exercise may not improve bone mineral content compared to resistance exercise alone. For bone mineral density, no meta-analysis was possible, but limited study results indicate no effects. For the comparison of combined protein and exercise interventions with protein alone, no studies investigating bone mineral content were identified. Findings regarding bone mineral density were inconclusive due to the small number of studies with inconsistent results and must be interpreted with caution given their high bias risk.

Comparison with other studies

A recently published systematic review summarizing RCTs on milk-derived protein supplementation in relation to bone health indices in adults found an increase in insulin-like growth factor-1 (IGF-1) concentrations but no effects on bone mineral density (in various anatomical regions) and other bone formation markers [51]. The review also comprised five RCTs [25, 27, 28, 52, 53] with exercise training as co-intervention; four of those were not included in our review due to younger age and intervention products containing other ingredients than protein. Similar to our results, the studies did not find any additional effects of combined protein and exercise interventions compared to exercise alone on bone mineral content or density. Of note is the 9-month RCT by Wright et al. (2017) which investigated different doses of whey protein supplementation (0, 20, 40, or 60 g/day) in addition to exercise in middle-aged adults with overweight and obesity. This study did not show any beneficial dose–response relationship with bone quantity [28]. Several other systematic reviews compared the effects of different protein sources on bone health suggesting that soy protein regardless of its isoflavone content may not be superior to animal-based protein in improving bone quantity [51, 54]. In our review, due to the low number of included studies using plant-based (soy) protein supplements, a subgroup analysis based on protein source was not possible. However, similar to the RCTs using milk-derived supplements, those using

soy were not effective in improving bone mass or density compared to exercise training alone [25, 29, 47]. The study by Zdzieblik (2015) supplementing collagen peptides was the only study in our meta-analysis showing an additional effect compared to exercise training on bone mineral content (MD 0.08 (0.04 to 0.12)) [26]. Further evidence from two RCTs in postmenopausal women with reduced bone mineral density not exercising suggests also improvements in bone mineral density and content, respectively, after a 12-month supplementation of 5 g collagen peptides [55, 56].

Regarding exercise training, several systematic reviews indicate positive effects on bone health [14–17] that do not remarkably differ regarding type of exercise. In contrast to these results, most of the studies included in our review did not show any within-group changes in bone mineral content and density, respectively, by exercise training.

Potential reasons explaining null-findings

Several factors may account for the null findings of this systematic review. First, most of the included RCTs assessed bone health as a secondary or exploratory outcome. Therefore, these studies may have been underpowered to detect group differences. A meta-analysis was only feasible for the comparison of combined protein and exercise interventions versus exercise alone on total body bone mineral content. Site-specific measurements of bone mineral density (e.g. lumbar spine or femoral neck) were reported in only three studies [25, 46, 47] and could not be pooled due to heterogeneity in the interventions. However, these site-specific measures are considered more sensitive for diagnosing osteoporosis and predicting fracture risk [57–59]. Moreover, the adaptation to treatment is thought to vary by region, as bones subjected to the stress and strains of training are more likely to exhibit increased density [12]. Additionally, the intervention durations of included studies (mainly 12 weeks) may have been too short to observe significant changes in bone quantity. Bone resorption normally requires 30–40 days (median) [60], followed by approximately 150 days of bone formation [60]. Thus, measurable changes can be detected only after roughly 27 to 28 weeks [60].

Secondly, exercise training generally did not result in increased bone mineral content or density, despite most included studies employing progressive and dynamic resistance training protocols with at least two sessions per week, a regimen considered favourable for its osteoanabolic potential [61]. In addition to the short intervention duration, this lack of effect may be attributed to training dose aspects such as strain magnitude and rate [61]. Moreover, adherence to exercise training in the included RCTs was primarily assessed based on session attendance. However, this measure may inadequately reflect the actual performance during training sessions.

Thirdly, the composition and dose of protein supplements varied across studies. Even though most supplements were based on milk-derived proteins, their effects may differ due to variations in amino acid composition and digestibility [62]. Furthermore, adherence to protein supplementation was not well-controlled in all included studies potentially influencing the results. Nonetheless, a lack of effect attributable to increased protein intake in the control groups seems unlikely. All RCTs included in the meta-analysis comparing combined protein and exercise interventions with exercise alone were placebo-controlled [26, 43–45, 48]. Four of the five RCTs reported protein intakes [43–45, 48], and no differences in pre- and post-intervention protein intake were observed in the exercise alone groups, whereas intervention groups consistently demonstrated an increase.

Fourthly, habitual protein intake must be taken into account. None of the included studies specifically targeted people with low or insufficient habitual protein intake. Six of the included trials reported mean baseline protein intakes ranging from 0.81 to 1.3 g/kg BW/day [25, 43–46, 48], while four provided no such information. Expert groups suggest an optimal protein intake of 1.0–1.2 g/kg BW/day for healthy older adults, with higher values for those suffering from acute or chronic illness [63, 64]. A recent umbrella review concluded that a beneficial effect of protein intake above 1.0 g/kg BW/day on bone health, particularly hip fracture risk, in adults aged 65 years and older cannot be excluded [20]. While most participants in our review were older people, those with low baseline protein intake may have received insufficient supplementation to surpass the thres-hold of 1 g/kg BW/day. Conversely, a saturable dose–response relationship may exist, whereby people with already high baseline protein intakes do not further benefit. The meta-analysis by Morton et al. demonstrated that protein supplementation beyond a total protein intake of 1.62 g/kg/day did not yield further resistance training-induced gains in fat-free mass in healthy adults [65].

Fifthly, an interaction between protein and calcium intake may influence outcomes [66]. Some studies have observed positive associations of protein intake with fracture risk and total bone mineral density only in participants with sufficient calcium intake [67]. In our review, seven of the included RCTs neither controlled for nor assessed calcium intake [26, 29, 42, 44–46, 48]; thus, potential confounding cannot be ruled out. Interactions between protein and calcium intake need further investigation.

Strength and limitations

This systematic review has several strengths and limitations that need to be considered. Among the strengths are the use of state-of-the-art methods to evaluate risk of bias and certainty of evidence and the inclusion of unpublished data that

were requested from study authors. As limitation, the low number of included studies needs to be acknowledged precluding the performance of several meta-analyses, most of the pre-planned subgroup analyses, and the testing for funnel plot asymmetry to evaluate publication bias. Sample sizes of included studies were small which may have contributed to a lack of power to detect differences and led to imprecise study results. Moreover, the heterogeneous intervention protocols — especially regarding the protein supplementation — and the short intervention durations may have affected the results. Reporting of different outcome measures (bone mineral content vs. density in various regions) limited the pooling of study results. Furthermore, as none of the included RCTs assessed fractures or osteoporosis incidence, no conclusions on these secondary outcomes could be drawn. Lastly, only limited data were available on the precision and standard error of DXA measurements in included studies.

Conclusions

Based on our findings, no robust conclusions can be drawn regarding whether combined protein and exercise interventions are more beneficial for bone health than (a) exercise or (b) protein alone in middle-aged and older adults. While most RCTs included focused on comparisons between combined protein and exercise interventions and exercise alone, data on the effects of combined interventions versus protein supplementation alone were insufficient. This restricted the ability to draw comprehensive conclusions about the distinct or synergistic effects of these interventions on bone health. Future research should prioritize RCTs that directly compare combined protein and exercise interventions against each component alone. Additionally, this approach could provide valuable insights into optimal strategies for maintaining or improving bone health in middle-aged and older populations.

Although these findings do not support specific evidence-based recommendations, they highlight a critical research gap, as only few studies addressing these research questions were identified and the need for well-designed and sufficiently powered studies to inform future guidelines. Nevertheless, it is important to acknowledge that both sufficient protein intake and regular exercise are crucial for maintaining bone health.

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Data availability This manuscript makes use of publicly available data from published studies; therefore, no original data are available for sharing. Relevant extracted data from the data sheet which included study and participant characteristics as well as study results are provided in Tables 2 and 3 as well as Tables SI4 and SI5.

Declarations

Conflicts of interest None.

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