BMJ Open

The effect of combined resistance exercise training and vitamin D3 supplementation on musculoskeletal health and function in older adults: A systematic review and meta-analysis

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2016-014619
Article Type:	Research
Date Submitted by the Author:	07-Oct-2016
Complete List of Authors:	Antoniak, Anneka; University of Birmingham, School of Sports, Exericse and Rehabilitation Sciences Greig, Carolyn; School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK; MRC-Arthritis Research UK Centre for Musculoskeletal Ageing Research
Primary Subject Heading:	Geriatric medicine
Secondary Subject Heading:	Nutrition and metabolism, Sports and exercise medicine
Keywords:	Physiology < BASIC SCIENCES, GERIATRIC MEDICINE, NUTRITION & DIETETICS, Bone diseases < ORTHOPAEDIC & TRAUMA SURGERY, Musculoskeletal disorders < ORTHOPAEDIC & TRAUMA SURGERY, SPORTS MEDICINE

The effect of combined resistance exercise training and vitamin D3 supplementation on musculoskeletal health and function in older adults: A systematic review and meta-analysis

AUTHORS
Anneka E Antoniak[1] and Carolyn A Greig[1,2]

[1]School of Sport, Exercise and Rehabilitation Sciences, [2]MRC-Arthritis Research UK Centre for Musculoskeletal Ageing and Health, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK

Corresponding author
Anneka E Antoniak
School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK
Email: aea423@bham.ac.uk
Telephone number: +44 121 371 3234

Keywords: Vitamin D, exercise, older adults, randomised controlled trial, systematic review

Word count: 4227

Number of figures: 14

Number of tables: 9

Number of references: 52

Number of supplementary files: 9

ABSTRACT

Objectives

In older adults there is a blunted responsiveness to resistance training and reduced muscle hypertrophy compared with younger adults. There is evidence that both exercise training and vitamin D supplementation may benefit musculoskeletal health in older adults, and it is plausible that in combination their effects may be additive. The aim of this systematic review was to evaluate the effectiveness of combined resistance exercise training and vitamin D3 supplementation on musculoskeletal health in older adults.

Data sources

A comprehensive search of electronic databases, including Science Direct, MedLine, PubMed, Google Scholar and Cochrane Central Register of Controlled Trials (Cochrane CENTRAL accessed by Wiley Science)**.**

Results

7 studies were included, with a total of 792 participants aged 65 years or over (or mean age ≥65 years). Studies were categorized into two groups; group 1 compared vitamin D3 supplementation and exercise training versus exercise alone, group 2 compared vitamin D3 supplementation and exercise training versus vitamin D3 supplementation alone. Meta-analyses for group 1 found muscle strength of the lower limb to be significantly improved within the intervention group (0.98, 95% CI 0.73, 1.24, p<0.001); all other outcomes showed small but non-significant positive effects for the intervention group. The SPPB, TUG, muscle strength of the lower limb and hip BMD all showed significantly greater improvements in the intervention group for group 2 comparisons.

Conclusions

This review provides tentative support for the additive effect of resistance exercise and vitamin D3 supplementation for the improvement of muscle strength in older adults. For other aspects of musculoskeletal function, such as SPPB and TUG, no additional benefit beyond exercise was shown. Further evidence is required to draw firm conclusions or make explicit recommendations regarding combined exercise and vitamin D3 supplementation.

Strengths and Limitations of this study

- To the best of our knowledge this study represents the first review evaluating the combined effects of vitamin D3 supplementation and exercise in older adults
- Generally, outcome measure data could be graded as representing moderate quality
- Only seven studies were found to be eligible for inclusion, highlighting the lack of literature available on the topic
- The inclusion of one high risk study was deemed necessary due to the lack of eligible studies

INTRODUCTION

Sarcopenia, originally defined as the age related loss of muscle mass[1], now also encompasses low muscle strength and/or muscle function[2]. The efficacy of resistance training in preventing or alleviating age-related musculoskeletal loss is well established; cited as the most promising intervention for improving symptoms of sarcopenia[3].

Clear evidence exists demonstrating an association between resistance exercise training (RET) and muscle hypertrophy, which is maintained in older age[3-5]. However, in older adults there is a blunted responsiveness to RET in comparison with younger adults; a blunted muscle protein synthetic rate in response to a single bout of resistance exercise has been reported[6], and others demonstrate a reduction in muscle hypertrophy in comparison to younger adults[7-10]. This 'anabolic resistance' may be due to changes in gene expression and anabolic signalling; an attenuated anabolic hormone response to resistance exercise is observed in comparison to younger adults[11].

Losses in muscle strength are associated with losses in functional ability, independence and increases in frailty, falls, and disability in older adults [12-15]; therefore, there may be merit associated with a combination of interventions to boost responsiveness of older muscle to resistance exercise and combat anabolic resistance.

Vitamin D3 supplementation in humans has been shown to positively influence musculoskeletal health in older adults: increases in relative number and cross-sectional area (CSA) of muscle fibres (type II in particular) has been reported[16-18], and muscle strength increased and fall rates decreased after treatment with vitamin D3[17]. Vitamin D receptor (VDR) concentration significantly increased with vitamin D3 supplementation[18]; conversely, supplementation conferred no benefits on strength, functioning and balance[19-21]. Moreover, a systematic review examining the effects of vitamin D3 supplementation in vitamin D replete adults aged over 18 years found no significant effect on grip or proximal lower limb muscle strength; however, pooled data including vitamin D deficient participants (serum 25(OH)D <25 nmol.l^{-1}) demonstrated a large effect on hip muscle strength[22].

There is conflicting evidence surrounding the efficacy of vitamin D3 supplementation alone or in combination with exercise on musculoskeletal health, with no clear consensus regarding the management or prevention of sarcopenia. Although epidemiological data suggest a relationship between vitamin D3 and muscle weakness[23], this association is not well understood, and evidence in published literature is lacking and contradictory. Considering the beneficial effects of both RET and vitamin D3 on muscle tissue, it is plausible an additive effect would exist if combined, optimizing the potential for healthy ageing muscle[24]. Thus, the aim of this study was to assess the combined effect of RET and vitamin D3 supplementation on musculoskeletal health in older adults.

MATERIALS AND METHODS

A systematic review of peer-reviewed literature relating to the effect of RET and vitamin D3 supplementation on musculoskeletal health in older adults was conducted in accordance with a study protocol registered on the PROSPERO database (record number CRD42015020157). The protocol was informed by the Cochrane Handbook for Systematic Reviews of Interventions[25], and reporting conformed to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement[26].

Eligibility Criteria

Randomized controlled trials were sought for this study. Journal studies included: (1) male and/or female participants (aged ≥65 years or mean age ≥ 65 years) (2) enlisted RET and vitamin D3 supplementation (studies utilising vitamin D3 and calcium supplementation were included) (3) included measures of muscle strength, function, muscle power, body composition, serum vitamin D/calcium status or quality of life (4) compared results with a control group (sedentary/usual care/no vitamin D3 supplementation). Articles were excluded if participants were supplemented with additional protein or any supplement/medication with a known anabolic effect on muscle tissue.

Search methods for identification of studies

Articles published before March 2016 were included. A computerised search of Science Direct, MedLine, PubMed, Google Scholar and Cochrane Central Register of Controlled Trials (Cochrane CENTRAL accessed by Wiley Science) databases was conducted. Table 1 shows the Medline search strategy, devised by AEA and LH.

Data items and collection

Data were extracted independently by 2 reviewers (AEA and ASA) using a standardised data extraction sheet; any disagreements were discussed and resolved with a third person (CAG). The inter-rater reliability was assessed using Cohen's Kappa[27]. Data items including general information, participant characteristics and details of the intervention were extracted. For key outcomes the definition used by the authors, methodology, results, mean differences and the presence/absence of statistical significance were reported.

Risk of bias analysis

2 reviewers (AEA and CAG) independently assessed the validity of included studies, with provisions for moderation from a third reviewer. The Cochrane Collaboration's tool for assessing risk of bias was utilised, as described in the Cochrane Handbook for Systematic Reviews of Interventions[25]; the use of scales for assessment is explicitly discouraged[28,29]. Pre-specified consensus points were devised and agreed by reviewers to ensure consistency. It was acknowledged that by nature of design, blinding of participants and personnel would be difficult in certain studies; therefore grading was based on the likelihood that outcome measures were influenced by the potential lack of blinding[25].

Grading the quality of evidence

The Grading of Recommendation, Assessment, Development, and Evaluation (GRADE) handbook[30] was used to evaluate the quality of evidence of outcomes assessed within the meta-analyses. The GRADE approach utilises systematically produced questions to reach conclusions on degree of confidence in the estimate of the effect. GRADE assesses patient important outcomes across five areas; risk of bias, inconsistency, indirectness, imprecision and publication bias, and grades outcomes as demonstrating high, moderate, low or very low quality of evidence.

RESULTS

Study selection:

7 studies were included within the review; Agergaard et al., 2015[31], Bunout et al., 2006[32], Drey et al., 2011[33], Gianoudis et al., 2014[34], Jessup et al., 2003[35], Uusi-Rasi et al., 2015[21], and Verschueren et al., 2011[36]; the study flow diagram is presented in Figure 1.

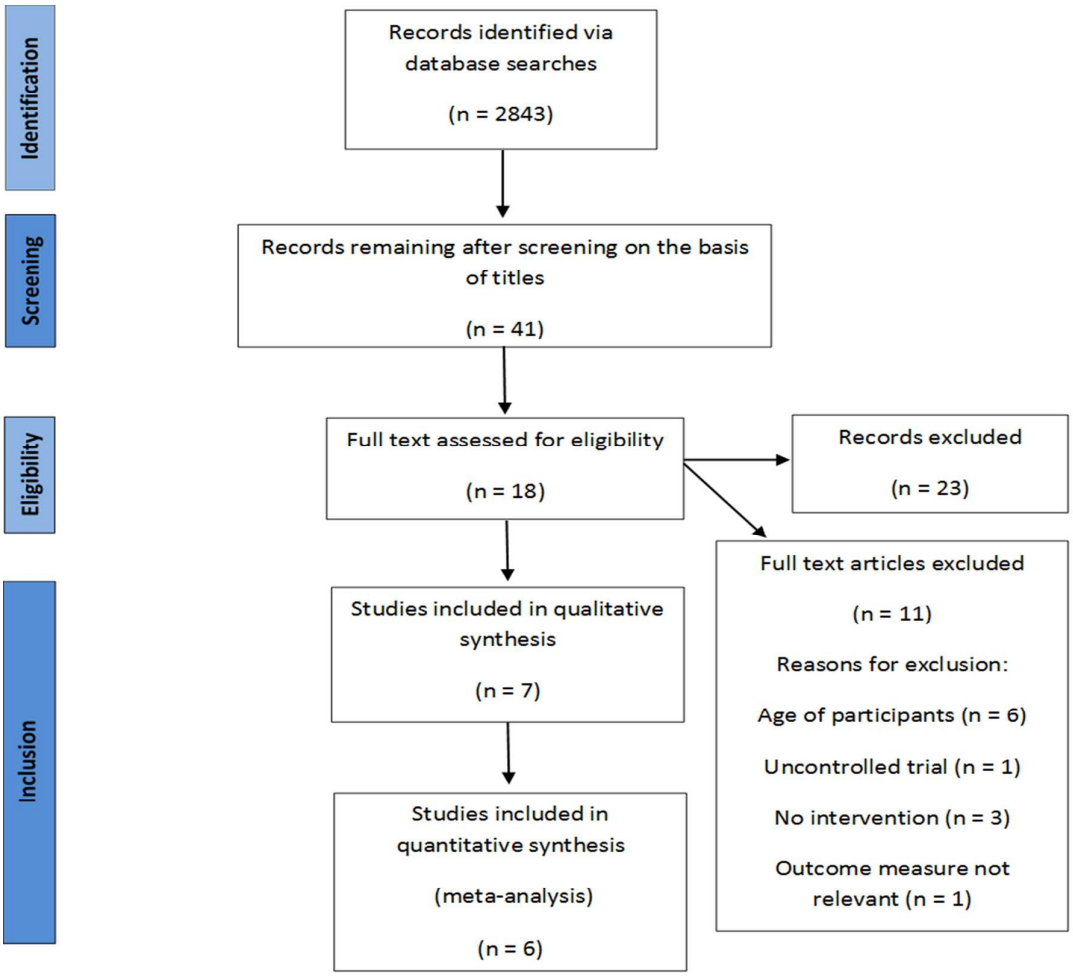

Figure 1: Study flow chart

Upon reading full text articles, it became clear that there were 2 separate groups of interventions; studies in which all participants took part in RET and the intervention arm was supplemented with vitamin D3; studies in which all participants were supplemented with vitamin D3 and the intervention arm took part in RET; and studies using a combination of the 2 interventions (Table 2).

Table 2: Study demographics

Author, year	N	Mean age (y)	Study design	Exercise protocol	Vitamin D3 protocol	Duration
Group 1: All participants exercised, intervention group received vitamin D supplementation						
Agergaard et al., (2015)	17	66.9	RCT	RET 3x per week	1920 IU D3 + 800mg Ca/day Or 800mg Ca/day	16 weeks
Group 2: All participants received vitamin D supplementation, intervention group exercised						
Drey et al., (2011)	42	77	RCT	RET 2x 60 mins per week	>20 ng/ml = 1000 IU D3/day <20 ng/ml = 2000 IU D3/day	12 weeks
Gianoudis et al., (2014)	150	67	RCT	HV-PRT 3x per week	1000 IU D3 & 700mg Ca/day	12 months
Jessup et al., (2003)	18	69	RCT Parallel	RET 3x 60-90 mins per week	400 IU D3 & 1000 mg Ca/day	32 weeks
Verschueren et al., (2011)	103	79	RCT	WBV 3x per week	High-dose = 1600 IU Conventional dose = 800 IU D3/day	6 months
Assigned to Group 1 & 2: Participants took part in a combination of exercise and vitamin D interventions						
Bunout et al., (2006)	92	77	RCT	RET 2x 1.5h per week	400 IU D3 + 800mg Ca/day Or 800mg Ca/day	9 months
Uusi-Rasi et al., (2015)	370	74	RCT	RET 2x/week for 12 months 1x/week for next 12 months	800 IU D3/day	2 years

*RCT: Randomized Controlled Trial, RET: Resistance Exercise Training, IU: International Units, Ca: Calcium

Study demographics

7 eligible studies included a total of 792 participants of mean age 72.8 years (Table 2). Of these, 1 included only males[31] and 3 included only females[21,35,36]. All studies included healthy participants living independently, except for 2 studies; [35] included participants living within a retirement community and [36] included institutionalized participants living in nursing homes, service flats or cloistered communities.

Interventions

Studies assigned to group 1 included Agergaard et al., 2015[31]; Bunout et al., 2006[32] and Uusi-Rasi et al., 2015[21]. In group 1, all participants took part in RET; incorporating a warm-up and strengthening exercises utilising commercial weight machines[21,31] or Thera-bands[31]. 2 studies included balance challenging aspects[21,32]. All studies included supervised, progressive exercise sessions; progression was monitored by a 5 rep max (RM) test[31], Borg scale[32] or metabolic equivalents (METs)[21]. Total number of sessions delivered ranged from 36[31]to 156[21], over a duration of 16 weeks[31] to 24 months[21]. All administered a vitamin D3 supplement, orally in tablet form; doses ranged from 400IU [32] to 1920 IU[31] per day; in 2 studies participants were supplemented with 800mg calcium per day[31,32] and 1 study supplemented the control group with a placebo[21].

6 studies assigned to group 2 included; Bunout et al., 2006[32], Drey et al., 2011[33] Gianoudis et al., 2014[34], Jessup et al., 2003[35], Uusi-Rasi et al., 2015[21] and Verschueren et al., 2011[36]. Within group 2, all participants took a vitamin D3 supplement, orally in tablet form. Doses ranged from 400 IU[32,35] to 2000 IU[33] per day; 1 study monitored serum 25(OH)D at baseline to determine supplement dosage[33]. In 4 studies[32,34-36] all participants were supplemented with calcium; doses ranged from 700mg[34] to 1000mg[35,36] per day. The intervention group took part in RET. Studies utilised machine weights and pulleys[21,33-35], Thera-bands[32], weighted vests[35] and Whole Body Vibration (WBV) machines[36] for resistance. 5 studies included balance challenging aspects[21,32-35]. All studies employed supervised, progressive exercise sessions monitored via a Borg scale[32-34], addition of weights to weighted vests[35], estimation of METs or individual ability[36]. Total number of sessions delivered ranged from 24[33] to 156[21], over a duration of 12 weeks[33] to 24 months[21]. Note that 2 studies included comparators which allowed allocation to both groups [21,32].

Outcome measures

All outcomes are listed in Table 3. Group 1 studies had few outcomes in common; however, all measured muscle strength[21,31,32]; isometric knee extensor strength was measured using a strain gauge[21,31] and isometric quadriceps strength was measured using a quadriceps table[32]. Hand grip strength was measured using a hand grip dynamometer[32]. Magnetic resonance imaging (MRI) was used to measure the CSA of the quadriceps[31,37], whilst[32] analysed fat and lean mass using dual-energy X-ray absorptiometry (DXA). 2 studies measured timed-up and go (TUG), hip and spine bone mineral density (BMD)[21,32]. 1 study analysed fibre type and muscle quality[31].

Of group 2 studies,[21,32,34,36] assessed lower limb strength, and[32,35] measured grip strength. Muscle power was measured as sit-to-stand transfer power[33] and the stair climb test[34]. The short physical performance battery (SPPB) was assessed by[32,34], and the TUG by[21,32,34]. BMD of the hip[21,32,34-36] and spine[21,32,34,35] were measured using DXA. Lean mass was measured using DXA[32-34]and X-ray computed tomography (CT)[36]. Balance was assessed via the Romberg ratio[32], four square step test[34], an AccuSway platform[35] and backwards walking[21]. Other outcomes included endurance (12-minute walk[32]), the 30 second sit-to-stand test[34], normal walking speed and the 5-time chair stand test[21].

Table 3: Summary of included study outcome measures and significant results

Author, year	Outcome measures		Significant results
Agergaard et al., 2015	Muscle strength Muscle CSA Muscle quality	Isometric knee extensor (strain gauge) MRI of quadriceps muscle (6mm thick) Muscle strength/CSA	Muscle strength – Increased (p<0.0001) but no between-group difference Muscle CSA – Increased (p=0.001) but no between-group difference Muscle quality – N/S
Bunout et al., 2006	Muscle strength Muscle function BMD Body sway Endurance	Quadriceps (table) & hand grip strength (dynamometer) SPPB, TUG Hip & spine (DXA) Romberg ratio Distance walked in 12 minutes	Muscle strength – Increased with exercise (p<0.001), no effect of vit D Muscle function – SPPB (p=0.002) no effect of vit D, TUG (p=0.004) > with vit D BMD – Hip increased with vit D, decreased without (p=0.006). Spine was N/S Body sway – Lower with vit D than without (p=0.05) Endurance – N/S
Drey et al., 2011	Muscle power Muscle function Body composition	Lower limb sit-to-stand transfer power (force plate) SPPB, SF-LLFDI aLM (DXA)	Muscle power - Increased with vit D intake (p=0.017) Muscle function – SPPB increased with exercise (p=0.009), SF-LLFDI was N/S Body composition – aLM was N/S
Gianoudis et al., 2014	Muscle strength Muscle power Muscle function BMD Body composition Dynamic balance	Lower limbs (bilateral leg press) and back (seated row) Timed stair climb test 30 second sit-to-stand test, TUG Hip & spine (DXA) Total body lean & fat mass (DXA) Four Square Step Test	Muscle strength- Exercise increased strength by +3% (p<0.05) Muscle power – Exercise increased power by +5% (p<0.05) Muscle function – Exercise improved Sit-to-stand by +16% (p<0.001). TUG -N/S BMD – Exercise increased hip & spine BMD by +0.1% (p<0.05) Body composition – Lean & fat mass – N/S Dynamic balance – Exercise improved by +6% (p<0.01)
Jessup et al., 2003	Muscle strength BMD Body sway	Hand grip (dynamometer), mean of 8 tests (stack machine) Hip & spine (DXA) AccuSway force platform	Muscle strength – increased with exercise (p=0.0156). No effect of vit D BMD hip – increase with exercise (p=0.00001), increase with vit D (p=0.016) Spine – increase with exercise (p=0.0094), vit D supplementation N/S Body sway – N/S
Uusi-Rasi et al., 2015	Muscle strength Muscle function BMD Dynamic balance	Max isometric leg extensor strength at a knee angle of 110° SPPB, TUG Hip & spine (BMD) Backwards walking	Muscle strength – increased with exercise (p<0.001). Vit D supplementation N/S Muscle function – SPPB = N/S. TUG decreased in vit D + no exercise group (p=0.01) BMD – Hip – Vit D maintained BMD (p=0.02) as did exercise (p=0.01). Spine – N/S Dynamic balance – Improved with exercise (placebo: p=0.001, vit D: p=0.03). No additive effect of vit D
Verschueren et al., 2011	Muscle strength BMD Muscle mass	Isometric & dynamic knee extensor strength Hip (DXA) Mass of upper leg (Multi-slice CT)	Muscle strength – Isometric: N/S. Dynamic: improved in all groups. Vit D=no effect BMD – Improved in all groups. No difference between training of vit D groups Muscle mass – N/S

*CSA: Cross-sectional Area, MRI: Magnetic Resonance Imaging, ELISA: Enzyme-linked Immunosorbent Assay, BMD: Bone Mineral Density, SPPB: Short Physical Performance Battery, TUG: Timed Up and Go, DXA: Duel-energy X-ray Absorptiometry, SF-LLFDI: Short Form of the Late Life Function and Disability Instrument, aLM: appendicular Lean Mass, QoL: Quality of Life, Multi-slice CT: Multi-slice X-ray Computed Tomography

Risk of bias within studies

For all studies, a high proportion of components were assigned an unclear risk of bias due to insufficient information and the unknown effect on study outcome measures. Many studies reported insufficient information on concealment and blinding procedures, or whether procedures were in place in the event of unblinding. In total, 6 studies were judged to have an unclear risk of bias[21,31-33,35,36]. Component 1 was assessed as having a low risk of bias for all studies. 1 study was assessed as having an overall high risk of bias[34] due to component 5, as no data were entered into the analyses for participants with missing data.

GRADE analysis

The GRADE summary of findings table for groups 1 and 2 are shown in Tables 5 and 6.

Within group 1, all studies were evaluated as moderate quality of evidence; no serious risk of bias was detected. Due to the nature of the studies included within this review, no serious indirectness was detected; all outcomes were measured directly without the use of a surrogate. Publication bias was not detected, and due to the number of studies included, it was not possible to produce funnel plots for any outcomes. Reasons for downgrading the quality of evidence included serious inconsistency due to substantial heterogeneity, and serious imprecision due to confidence intervals crossing the line of no effect.

Within group 2 studies, 5 outcomes were graded as high to moderate quality of evidence (SPPB, TUG, muscle strength of the lower limb, hand grip strength and BMD of the hip). Remaining outcomes were graded as low or very low quality, meaning that one could have little or very little confidence in the effect estimate. Common reasons for downgrading outcomes included a combination of serious risk of bias (due to the inclusion of study[34]), serious imprecision or serious inconsistency.

Results of individual studies and synthesis of results

Results of the 2 groups of studies are reported separately. Within each group, there were outcomes unsuitable for quantitative synthesis, due to a lack of studies with common outcomes or aspects of studies too dissimilar for comparison; therefore, a narrative analysis was utilised.

Quantitative synthesis

Outcomes compared for group 1 included muscle strength of the lower limb, TUG and BMD of the hip and spine (Figures 2-5). Only muscle strength of the lower limb was found to be significant, with a large effect size in favour of the intervention group (2.69, 95% CI 0.95, 4.42. p = 0.002).

Group 2 comparisons included the SPPB, TUG, muscle strength of the lower limb, hand grip strength, weight, lean mass, fat mass and the BMD of the hip and spine (Figures 6-14). Of these outcomes, hand grip strength, weight, lean mass, fat mass and the BMD of the spine were found to be non-significant. However, SPPB score was more improved in the intervention group (1.09, 95% CI 0.15, 2.03. p = 0.02), with a significant and large effect. Similarly, TUG was significantly reduced within the intervention group (-1.57, 95% CI -2.50, -0.64. p = 0.0010). The results of the quantitative analysis also supported the combined intervention for muscle strength of the lower limb (2.69, 95% CI 0.95, 4.42). p = 0.002), and BMD of the hip (0.04, 95% CI 0.01, 0.06. p = 0.002).

Qualitative synthesis

Referring to the narrative synthesis guidelines provided by the Cochrane Consumers and Communication Review Group[38], it was appropriate to apply 2 steps listed; developing a preliminary synthesis and exploring the relationships within and between studies. To develop a primary synthesis, results were systematically tabulated to identify patterns across studies (Tables 7-9). Exploring the relationships between and within studies for group 1, the control group in study[31]demonstrated a significant percentage increase in CSA of the quadriceps from baseline in comparison to the intervention group (+8.46% versus +4.94%, $p < 0.05$).

Comparing primary outcomes for group 2, the percentage increase in isometric knee extensor strength for study[36] was greater in the intervention group (+3.01% versus +0.11%), although not statistically significant. Muscle power was compared in studies[33] and[34], expressed as sit-to-stand transfer power and functional stair climbing muscle power respectively. Both studies reported a significant percentage increase in muscle power within the intervention groups, and smaller, non-significant increases within the control groups (sit-to-stand transfer power intervention group +8.00% versus +2.61%, p = 0.017; functional stair climbing muscle power intervention group +10.51% versus +7.32%, $p < 0.05$).

The 30 second sit-to-stand test showed significant favourable results for the combined intervention of exercise and vitamin D3 (+10.40% versus +6.20%, p<0.05). Although normal walking speed, 5-time chair stand time and the 12-minute walk test were further improved within the control groups, this did not achieve statistical significance. The four square step test, body sway and backwards walking were significantly more improved in the intervention groups. Only Romberg ratio showed the greatest improvement within the control group; Romberg ratio was decreased in comparison with the intervention group, although the results were non-significant (+2.8% versus -0.60%).

For group 2 secondary outcomes, small and non-significant gains in appendicular lean mass were demonstrated in the intervention group of study[33]. In study[36], muscle mass of the upper limb decreased non-significantly in both the intervention and control groups, although to a lesser extent in the intervention group. BMD of the hip was gained in both groups, although by a higher percentage in the control group; both trends were non-significant.

In summary, meta-analyses for group 1 found muscle strength of the lower limb to be significantly improved within the intervention group (0.98, 95% CI 0.73, 1.24, p<0.001). All other outcomes showed small but non-significant positive effects for the intervention group. The SPPB, TUG, muscle strength of the lower limb and hip BMD all showed significantly greater improvements in the intervention group for group 2 comparisons. The narrative analysis revealed significant differences in body composition, muscle power, muscle function and balance. A significant percentage increase in quadriceps CSA was observed in the control group of study[31]. The combined intervention of RET and vitamin D3 supplementation resulted in a greater percentage increase in muscle strength and power, and a greater improvement in the 30 second sit-to-stand test, the four square step test, body sway and backwards walking. However, vitamin D3 supplementation alone resulted in a greater improvement in normal walking speed, 5-time chair stand time, the 12-minute walk test and Romberg ratio.

DISCUSSION

The aim of this systematic review was to assess the combined effect of RET and vitamin D3 supplementation on musculoskeletal health in older adults. Only 7 studies were eligible for inclusion, with a total of 792 participants, highlighting the lack of available literature on the topic. Studies were categorised into 2 groups; studies in which all participants took part in RET and the intervention group was supplemented with vitamin D3, or studies in which all participants were supplemented with vitamin D3 and the intervention group took part in RET. 2 studies were categorized into both group 1 and group 2.

Quantitative analysis

Data analysis conducted for this review included meta-analyses and narrative reviews. Meta-analyses for group 1 included muscle strength of the lower limb, TUG and BMD of both the hip and spine. Evidence of additional benefit was shown for all outcomes within the intervention group; however, the effect size was small and non-significant for TUG and BMD of the hip and spine. Muscle strength of the lower limb was the only significant outcome of group 1, with a large effect size observed within the intervention group (0.98, 95% CI 0.73, 1.24. p<0.00001). Although numerous studies have demonstrated the beneficial effect of RET on muscle strength in older adults[3-5], this result provides evidence that vitamin D3 supplementation may enhance these effects in older adults. Skeletal muscle myopathies associated with vitamin D deficiency are well documented[39], and symptoms of significant muscle weakness are reversed with treatment of the deficiency[40]. A systematic review and meta-analysis reported a gain in lower extremity strength with vitamin D supplementation only in vitamin D deficient older adults; no effect was observed in replete adults[22]. Similarly, no effect of vitamin D3 supplementation on isometric quadriceps strength was demonstrated after 6 months in vitamin D replete older adults[41]. Interestingly, although the studies included within group 1[21,31,32] did not specify serum 25(OH)D levels as inclusion/exclusion criteria, baseline and post-intervention serum 25(OH)D were within the 'sufficient' range (>30nmol.L^{-1}). A greater increase of muscle strength in

replete older adults represents a novel finding of this review. Preliminary support for combined vitamin D supplementation and RET was demonstrated in a 3 month longitudinal study examining the effect of serum 25(OH)D and exercise training on functional performance in older men and women aged 65 years and over. No significant improvements in function were reported in participants with lower serum 25(OH)D (<47.5 nmol.L^{-1}), however higher serum 25(OH)D (>67.5 nmol.L^{-1}) was associated with greatest improvements in functionality and muscle strength[42].

This finding must be considered within the context of the risk of bias and GRADE analyses. The risk of bias analysis showed an overall unclear risk of bias for the included studies, and the GRADE analysis concluded that the evidenced was of moderate quality; however, serious inconsistency due to moderate heterogeneity (I^2 = 70%) was detected. This heterogeneity may have been due to the differing duration of interventions (12 weeks to 24 months), differences between measurement methodologies, differences between exercise regimens (although all adopted progressive RET), doses of vitamin D3 (400 IU to 1920 IU per day), or may indicate that these studies were unsuitable for comparison.

Significant effects for the SPPB, TUG, muscle strength of the lower limb, and the BMD of the hip were observed within the intervention groups of group 2 studies; unsurprisingly, RET was found to have a positive influence. In a recent systematic review and meta-analysis, exercise significantly increased SPPB score and decreased TUG time, with large effect sizes (1.87 and -2.47 respectively[43]); similar results are reported within this review. Vitamin D is a regulator of BMD, proliferating calcium and phosphate absorption in the intestine and acting directly on bone cells[44]. Vitamin D has previously been shown to influence BMD, fracture rate and risk[45]; studies of patients who have sustained a hip fracture typically demonstrated low serum vitamin D (≤30.0 nmol.L^{-1};[46]). Supplementation of vitamin D and calcium has been shown to significantly decrease the rate of bone loss in the hip and spine[47]. GRADE analyses for these outcomes concluded the quality of evidence to be high (SPPB and TUG) or moderate (muscle strength of the lower limb and BMD of the hip).

Closer examination of the control groups within significant outcomes for group 2 was undertaken to evaluate the effect of vitamin D3 supplementation alone. Intriguingly, although the intervention groups (RET and vitamin D3 supplementation) showed evidence of benefit in number of outcomes, the control groups (vitamin D3 supplementation alone) showed mixed, or even negative impacts on the same outcomes. SPPB score was decreased post-intervention compared with baseline by 0.30% and 0.50% in the control groups of studies[32] and[33] respectively. Muscle strength of the lower limb and BMD of the hip showed mixed results for the intervention groups, with some studies reporting small increases and others reporting small losses (non-significant). Previous reports of the effect of vitamin D supplementation on muscle strength and physical functioning are mixed; the InCHIANTI study of people aged 65 years or over reported a significant association between serum 25(OH)D <25nmol.L^{-1} and SPPB score[48]. Similarly, a large prospective cohort of older adults aged 65 years or over found those with low (<25nmol.L^{-1}) 25(OH)D were significantly more likely to experience losses in grip strength and higher rates of appendicular lean mass loss compared to those with higher (>50 nmol.L^{-1}) 25(OH)D[23]. Conversely, another large, prospective study found no association between serum

25(OH)D, walking speed and time for repeated chair stands[49]. The TUG test time was actually significantly increased within the control group of study[32], and increased by a smaller, non-significant amount in study[21]. Again, participants included in studies[32] and [21] had sufficient serum 25(OH)D levels, indicating that supplementation in replete older adults may not confer additional benefits to neuromuscular function unless combined with exercise.

Narrative analysis

Studies in group 1[21,31,32] had few body composition outcomes in common, therefore a narrative analysis was conducted. The CSA of the quadriceps was analysed within study[31], and results showed that although the intervention group did experience a +4.94%, increase from baseline, the control group (not supplemented with vitamin D3) actually showed a significantly higher increase in quadriceps CSA (+8.46%, $p<0.05$).

These results do not provide evidence for the additive effects of combined exercise training and vitamin D3. Other study groups have reported changes in muscle CSA consequent to RET which are both smaller[8,50] and comparable[51] to those reported in study[31]. Interestingly, study[31] also assessed "muscle quality" (muscle strength/CSA); although non-significant, the intervention group improved their muscle quality to a greater degree than the control group (+9.61% versus +0.66% change from baseline). The intervention and control groups both increased their muscle strength to a similar degree, and there was no significant difference between these changes; however, the control group (as previously mentioned) demonstrated a larger increase in their muscle CSA. This shows that the gains in muscle strength in the intervention group surpassed the improvements made in muscle CSA, indicative of an increased functionality of the muscle to produce force; conceptually more relevant in combatting the effects of sarcopenia than muscle size and strength alone[52].

Results of the narrative analysis for group 2 showed that the combined intervention of RET and vitamin D3 supplementation was significantly more beneficial than vitamin D3 supplementation alone for sit-to-stand transfer power, functional stair climbing muscle power, 30 second sit-to-stand, 5-time chair stand, the four square step test, body sway and backwards walking. The control groups also showed benefits although to a lesser degree; the only significant improvement for the control group was for the TUG in study[32] ($p=0.0006$). Only body sway was negatively affected by vitamin D3 supplementation, although the within group change was non-significant. Other outcomes of interest included normal walking speed, the distance walked in 12 minutes and Romberg ratio, in which the control groups made the most improvement, although not significantly.

Limitations

Few published studies were eligible for inclusion within this review, although this serves to highlight the knowledge gap with respect to this topic. The inclusion of a high risk study was deemed necessary due to the lack of available literature, although this had a negative effect on the perceived quality of evidence for the outcomes in which it was reported. Generally, outcome measure data could be graded as representing

moderate quality, although there were several outcome measures graded as low or very low quality, due to the high variability of participant numbers, duration of interventions, exercise methodologies or differing vitamin D3 doses and period of supplementation employed within the studies. Furthermore, data produced from meta-analyses including study[21] may have been skewed due to the high weighting assigned for this study as a result of the large number of participants recruited.

Of the individual studies included within this review, none reported inclusion/exclusion criterion for vitamin D status, and although at baseline serum vitamin D was not significantly different between the groups in 5 studies[21,31-33,36], 2 studies reported no data for serum vitamin D pre or post-intervention[34,35]. Additionally, analysis methods used within 5 studies included did not account for confounding factors[31-34,36], and participants were not stratified on the basis of any characteristics in 3 studies[21,31,35], although these were single-sex studies. Unfortunately, several outcome measures were unsuitable for inclusion within the qualitative analysis due to differing measurement methodologies utilised or too few outcome measures in common.

CONCLUSION

This review provides tentative support for the additive effect of combined RET and vitamin D3 supplementation for the improvement of muscle strength in older adults. For other aspects of musculoskeletal function, such as SPPB and TUG, no additional benefit beyond that gained from exercise training was found. This review showed no evidence of benefit of vitamin D3 supplementation alone, however, few studies were identified during the literature search, highlighting that further evidence is required to draw any firm conclusions or make explicit recommendations regarding vitamin D3 supplementation for musculoskeletal health and function in older adults.

ACKNOWLEDGEMENTS

We thank Lynn Harris (LH) for her help formulating the search strategy, Asma Alrushud (AA) for her help with data extraction and the National Osteoporosis Society for supporting Anneka Antoniak (AEA).

FOOTNOTES

Contributors AEA has planned, conducted and written the report for this study. CAG has been involved in all stages, particularly in critically reviewing and approving the final draft of the report. AA was involved in the search for literature and data extraction stage. LH assisted in formulating the search strategy.

Funding This research received no grant from any funding agency in the public, commercial or not-for-profit sectors. AEA is supported and funded by the National Osteoporosis Society via the Linda Edwards Memorial PhD Studentship.

Competing interests None declared.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement This publication is supported by multiple datasets, which are openly available at locations cited in the reference section. Additional data for this article have been provided as supplementary files. There is no additional unpublished data.

REFERENCES

1. Rosenberg IH. Summary comments. *The American journal of clinical nutrition* 1989;50(5):1231-33.
2. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age and Ageing* 2010 doi: 10.1093/ageing/afq034
3. Kosek DJ, Kim J-s, Petrella JK, et al. Efficacy of 3 days/wk resistance training on myofiber hypertrophy and myogenic mechanisms in young vs. older adults. *Journal of applied physiology* 2006;101(2):531-44.
4. Henwood TR, Taaffe DR. Improved physical performance in older adults undertaking a short-term programme of high-velocity resistance training. *Gerontology* 2005;51(2):108-15.
5. Stewart V, Saunders D, Greig C. Responsiveness of muscle size and strength to physical training in very elderly people: a systematic review. *Scandinavian journal of medicine & science in sports* 2014;24(1):e1-e10.
6. Kumar V, Selby A, Rankin D, et al. Age-related differences in the dose–response relationship of muscle protein synthesis to resistance exercise in young and old men. *The Journal of physiology* 2009;587(1):211-17.
7. Greig C, Gray C, Rankin D, et al. Blunting of adaptive responses to resistance exercise training in women over 75y. *Experimental gerontology* 2011;46(11):884-90.
8. Häkkinen K, Kallinen M, Izquierdo M, et al. Changes in agonist-antagonist EMG, muscle CSA, and force during strength training in middle-aged and older people. *Journal of Applied Physiology* 1998;84(4):1341-49.
9. Raue U, Slivka D, Minchev K, et al. Improvements in whole muscle and myocellular function are limited with high-intensity resistance training in octogenarian women. *Journal of applied physiology* 2009;106(5):1611-17.
10. Welle S, Totterman S, Thornton C. Effect of age on muscle hypertrophy induced by resistance training. *J Gerontol A Biol Sci Med Sci* 1996;51 doi: 10.1093/gerona/51A.6.M270
11. Hameed M, Orrell R, Cobbold M, et al. Expression of IGF-I splice variants in young and old human skeletal muscle after high resistance exercise. *The Journal of physiology* 2003;547(1):247-54.
12. Roubenoff R. Sarcopenia: a major modifiable cause of frailty in the elderly. *The journal of nutrition, health & aging* 1999;4(3):140-42.
13. Janssen I, Heymsfield SB, Ross R. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *Journal of the American Geriatrics Society* 2002;50(5):889-96.
14. Visser M, Kritchevsky SB, Goodpaster BH, et al. Leg muscle mass and composition in relation to lower extremity performance in men and women aged 70 to 79: the health, aging and body composition study. *Journal of the American Geriatrics Society* 2002;50(5):897-904.
15. Landi F, Liperoti R, Russo A, et al. Sarcopenia as a risk factor for falls in elderly individuals: results from the ilSIRENTE study. *Clinical nutrition* 2012;31(5):652-58.
16. Sørensen O, Lund B, Saltin B, et al. Myopathy in bone loss of ageing: improvement by treatment with 1α-hydroxycholecalciferol and calcium. *Clinical Science* 1979;56(2):157-61.
17. Sato Y, Iwamoto J, Kanoko T, et al. Low-dose vitamin D prevents muscular atrophy and reduces falls and hip fractures in women after stroke: a randomized controlled trial. *Cerebrovasc Dis* 2005;20 doi: 10.1159/000087203
18. Ceglia L, Niramitmahapanya S, Silva MM, et al. A randomized study on the effect of vitamin D supplementation on skeletal muscle morphology and vitamin D receptor concentration in older women. *J Clin Endocrinol Metab* 2013;98 doi: 10.1210/jc.2013-2820
19. Latham NK, Anderson CS, Lee A, et al. A randomized, controlled trial of quadriceps resistance exercise and vitamin D in frail older people: the Frailty Interventions Trial in Elderly Subjects (FITNESS). *J Am Geriatr Soc* 2003;51 doi: 10.1046/j.1532-5415.2003.51101.x

20. Lips P, Binkley N, Pfeifer M, et al. Once-weekly dose of 8400 IU vitamin D3 compared with placebo: effects on neuromuscular function and tolerability in older adults with vitamin D insufficiency. *The American journal of clinical nutrition* 2010;91(4):985-91.
21. Uusi-Rasi K, Patil R, Karinkanta S, et al. Exercise and vitamin D in fall prevention among older women: a randomized clinical trial. *JAMA internal medicine* 2015;175(5):703-11.
22. Stockton K, Mengersen K, Paratz JD, et al. Effect of vitamin D supplementation on muscle strength: a systematic review and meta-analysis. *Osteoporosis international* 2011;22(3):859-71.
23. Visser M, Deeg DJ, Lips P. Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam. *The Journal of Clinical Endocrinology & Metabolism* 2003;88(12):5766-72.
24. Robinson S, Cooper C, Aihie Sayer A. Nutrition and sarcopenia: a review of the evidence and implications for preventive strategies. *Journal of aging research* 2012;2012
25. Higgins JPT, Green S, editors. *Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]*: The Cochrane Collarboration, 2011.
26. Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Annals of internal medicine* 2009;151(4):264-69.
27. Hsu LM, Field R. Interrater agreement measures: Comments on Kappan, Cohen's Kappa, Scott's π, and Aickin's α. *Understanding Statistics* 2003;2(3):205-19.
28. Moher D, Olkin I. Meta-analysis of randomized controlled trials: a concern for standards. *JAMA* 1995;274(24):1962-64.
29. Moher D, Altman D, Liberati A, et al. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). *The Cochrane Collaboration Methods Groups Newsletter* 1996:6.
30. Schünemann H, Brozek J, Oxman A. GRADE handbook for grading quality of evidence and strength of recommendation. Version 3.2 [updated March 2009]. The GRADE Working Group, 2009. *www cc-i ms net/gradepro* 2009
31. Agergaard J, Trøstrup J, Uth J, et al. Does vitamin-D intake during resistance training improve the skeletal muscle hypertrophic and strength response in young and elderly men? – a randomized controlled trial. *Nutrition & Metabolism* 2015;12(1):1-14. doi: 10.1186/s12986-015-0029-y
32. Bunout D, Barrera G, Leiva L, et al. Effects of vitamin D supplementation and exercise training on physical performance in Chilean vitamin D deficient elderly subjects. *Exp Gerontol* 2006;41 doi: 10.1016/j.exger.2006.05.001
33. Drey M, Zech A, Freiberger E, et al. Effects of strength training versus power training on physical performance in prefrail community-dwelling older adults. *Gerontology* 2011;58(3):197-204.
34. Gianoudis J, Bailey CA, Ebeling PR, et al. Effects of a Targeted Multimodal Exercise Program Incorporating High-Speed Power Training on Falls and Fracture Risk Factors in Older Adults: A Community-Based Randomized Controlled Trial. *Journal of Bone and Mineral Research* 2014;29(1):182-91. doi: 10.1002/jbmr.2014
35. Jessup JV, Horne C, Vishen R, et al. Effects of exercise on bone density, balance, and self-efficacy in older women. *Biological Research for nursing* 2003;4(3):171-80.
36. Verschueren SM, Bogaerts A, Delecluse C, et al. The effects of whole-body vibration training and vitamin D supplementation on muscle strength, muscle mass, and bone density in institutionalized elderly women: A 6-month randomized, controlled trial. *Journal of bone and mineral research* 2011;26(1):42-49.
37. Aagaard P, Andersen JL, Dyhre-Poulsen P, et al. A mechanism for increased contractile strength of human pennate muscle in response to strength training: changes in muscle architecture. *J Physiol* 2001;534 doi: 10.1111/j.1469-7793.2001.t01-1-00613.x
38. Ryan.R. 'Cochrane Consumers and Communication Review Group: data synthesis and analysis': Cochrane Consumers and Communication Review Group; 2013 [Available from: http://cccrg.cochrane.org accessed July 2016.

39. SMITH R, STERN G. Myopathy, osteomalacia and hyperparathyroidism. *Brain* 1967;90:593-602.
40. Glerup H, Mikkelsen K, Poulsen L, et al. Commonly recommended daily intake of vitamin D is not sufficient if sunlight exposure is limited. *Journal of internal medicine* 2000;247(2):260-68.
41. Grady D, Halloran B, Cummings S, et al. 1, 25-Dihydroxyvitamin D3 and Muscle Strength in the Elderly: A Randomized Controlled Trial*. *The Journal of Clinical Endocrinology & Metabolism* 1991;73(5):1111-17.
42. Okuno J, Tomura S, Fukasaku T, et al. [Examination of effects of alfacalcidol vitamin D supplement and renal function on improvement in the physical fitness of pre-frail elderly persons attending a nursing care prevention class]. *Nihon Ronen Igakkai zasshi Japanese journal of geriatrics* 2010;48(6):691-98.
43. Giné-Garriga M, Roqué-Fíguls M, Coll-Planas L, et al. Physical exercise interventions for improving performance-based measures of physical function in community-dwelling, frail older adults: a systematic review and meta-analysis. *Archives of physical medicine and rehabilitation* 2014;95(4):753-69. e3.
44. Lips P, Hosking D, Lippuner K, et al. The prevalence of vitamin D inadequacy amongst women with osteoporosis: an international epidemiological investigation. *Journal of internal medicine* 2006;260(3):245-54.
45. Brincat M, Gambin J, Brincat M, et al. The role of vitamin D in osteoporosis. *Maturitas* 2015;80(3):329-32. doi: 10.1016/j.maturitas.2014.12.018
46. LeBoff MS, Kohlmeier L, Hurwitz S, et al. Occult vitamin D deficiency in postmenopausal US women with acute hip fracture. *Jama* 1999;281(16):1505-11.
47. Tang BM, Eslick GD, Nowson C, et al. Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. *The Lancet* 2007;370(9588):657-66.
48. Houston DK, Cesari M, Ferrucci L, et al. Association between vitamin D status and physical performance: the InCHIANTI study. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* 2007;62(4):440-46.
49. Verreault R, Semba RD, Volpato S, et al. Low serum vitamin D does not predict new disability or loss of muscle strength in older women. *Journal of the American Geriatrics Society* 2002;50(5):912-17.
50. Trappe S, Williamson D, Godard M. Maintenance of whole muscle strength and size following resistance training in older men. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* 2002;57(4):B138-B43.
51. Ferri A, Scaglioni G, Pousson M, et al. Strength and power changes of the human plantar flexors and knee extensors in response to resistance training in old age. *Acta Physiol Scand* 2003;177 doi: 10.1046/j.1365-201X.2003.01050.x
52. Fragala MS, Kenny AM, Kuchel GA. Muscle Quality in Aging: a Multi-Dimensional Approach to Muscle Functioning with Applications for Treatment. *Sports Medicine* 2015;45(5):641-58. doi: 10.1007/s40279-015-0305-z

Table 1: Example Ovid MEDLINE search, to be adapted for other databases

1	Aging/
2	Exp aged/
3	(65 adj2 (years or age* or old*))
4	(old* adj (adult* or people or person* or population* or men or women))
5	(elder* or senior* or geriatric* or ?enarian or ag?ing)
6	((age* or aging or old* or elder*) adj1 (musc*))
7	1 or 2 or 3 or 4 or 5 or 6
8	Vitamin D/
9	(cholecalciferol* or calciferol* or ergocalciferol*)
10	(supplements or dietary supplements)
11	((vitamin D* or cholecalciferol or calciferol* OR ergocalciferol) adj supplementation
12	8 or 9 or 10 or 11
13	Muscle Development/
14	Muscle, Skeletal/
15	(Skeletal muscle adj2 (atrophy or sarcopenia or wasting or loss or deterioration))
16	Muscle Strength/
17	(skeletal muscle mass or size or fibres or fibers or area)
18	(musc* adj2 (function* or power or strength))
19	(musc* adj2 (grow* or hypertrophy or size or mass or csa or cross sectional area or volume))
20	Body Composition/
21	(lean adj3 mass)
22	(protein adj2 (turnover or synthesis or breakdown))
23	(nitrogen adj2 (balance or turnover or synthesis or breakdown or retention or loss or retain*))
24	Sarcopenia/
25	13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24
26	Exp exercise/
27	(resistance exercise or resistance exercise training)
28	((resistance or strength or weight or cardio or aerobic) adj3 (train* or condition* or exercise* or lift*))
29	(physical adj3 (activit* or exercise* or train* or exertion* or endurance* or therap* or conditioning or fitness))
30	(exercise adj3 (train* or intervention* or protocol* or program* or therap* or regim* or activit*))
31	26 or 27 or 28 or 29 or 30
32	7 and 12 and 25 and 31
33	Limit 32 to humans
34	Remove duplicates from 33

Table 4: Summary of risk of bias analysis for each included study

Author, year	Components of risk of bias							Summary	Comments on high risk components
	1	2	3	4	5	6	7		
Agergaard et al., (2015)	L	U	L	L	U	L	L	**High (0)** Unclear (2) Low (5)	N/A
Bunout et al., (2006)	L	U	U	U	U	U	U	**High (0)** Unclear (6) Low (1)	N/A
Drey et al., (2011)	L	L	U	U	L	L	U	**High (0)** Unclear (3) Low (4)	N/A
Gianoudis et al., (2014)	L	U	U	U	H	L	L	**High (1)** Unclear (3) Low (3)	One high risk component, 5 ITT analysis utilised, but no data entered for participants with missing data
Jessup et al., (2003)	L	U	U	U	U	U	L	**High (0)** Unclear (5) Low (2)	N/A
Uusi-Rasi et al., (2015)	L	U	U	U	U	L	L	**High (0)** Unclear (4) Low (3)	N/A
Verschueren et al., (2011)	L	U	U	U	U	L	L	**High (0)** Unclear (4) Low (3)	N/A

* Risk of bias domains of assessment. 1: Random sequence generation, 2: Allocation concealment, 3: Blinding of participants and personnel, 4: Blinding of outcome assessment, 5: Incomplete outcome data, 6: Selective reporting, 7: Other sources of bias.
Judgements possible: H – High risk of bias, U – Unclear risk of bias, L – Low risk of bias

Table 5: GRADE analysis of group 1 measurement outcomes included in the quantitative synthesis

Quality Assessment								Summary of Findings			
Outcome	**Included studies (design)**	**ROB**	**Inconsistency**	**No serious Indirectness**	**Imprecision**	**Publication bias**	**Groups (Intervention/ control)**	**Effect size (direction)**	**Significance**	**95% CI**	**Quality**
Muscle strength (lower limb)	[1,2,6] (RCT)	No serious ROB	Serious inconsistency (substantial heterogeneity)	No serious indirectness	No serious imprecision	Undetected^	131/135	0.98 (Intervention)	$p<0.00001$	(0.73, 1.24)	⊕⊕⊕o Moderate
TUG	[2,6] (RCT)	No serious ROB	No serious inconsistency	No serious indirectness	Serious imprecision (CIs cross line of no effect/ OIS not reached)	Undetected^	124/125	0.37 (Intervention)	$p= 0.37$	(-0.68,0.26)	⊕⊕⊕o Moderate
BMD (hip)	[2,6] (RCT)	No serious ROB	No serious inconsistency	No serious indirectness	Serious imprecision (CIs cross line of no effect/ OIS not reached)	Undetected^	124/125	0.02 (Intervention)	$p= 0.15$	(-0.01,0.05)	⊕⊕⊕o Moderate
BMD (spine)	[2,6] (RCT)	No serious ROB	No serious inconsistency	No serious indirectness	Serious imprecision (CIs cross line of no effect/ OIS not reached)	Undetected^	124/125	0.02 (Intervention)	$p = 0.41$	(-0.03,0.07)	⊕⊕⊕o Moderate

*ROB: Risk of Bias; TUG: Timed Up and Go; RCT: Randomized Controlled Trial; CI: Confidence Interval; BMD: Bone Mineral Density; OIS: Optimum Information Size.
^Insufficient data to produce funnel plots. GRADE scoring: ⊕⊕⊕⊕ High; ⊕⊕⊕o Moderate; ⊕⊕oo Low; ⊕ooo Very low

Table 6: GRADE analysis of group 2 measurement outcomes included in the quantitative synthesis.

	Quality Assessment						Summary of Findings				
Outcome	**Included studies (design)**	**ROB**	**Inconsistency**	**Indirectness**	**Imprecision**	**Publication bias**	**Groups (intervention/ control)**	**Effect size (direction)**	**Significance**	**95% CI**	**Quality**
SPPB	[2,3] (RCT)	No serious ROB	No serious inconsistency	No serious indirectness	No serious imprecision	Undetected^	45/46	1.09 (Intervention)	$p = 0.02$	(0.15,2.03)	⊕⊕⊕⊕ High
TUG	[2,6] (RCT)	No serious ROB	No serious inconsistency	No serious indirectness	No serious imprecision	Undetected^	124/126	-1.57 (Intervention)	$p = 0.001$	(-2.50, -0.64)	⊕⊕⊕⊕ High
Muscle strength (lower limb)	[2,6] (RCT)	No serious ROB	Serious inconsistency (substantial heterogeneity)	No serious indirectness	No serious imprecision	Undetected^	124/126	2.69 (Intervention)	$p = 0.002$	(0.96,4.42)	⊕⊕⊕○ Moderate
Hand grip strength	[2,5] (RCT)	No serious ROB	No serious inconsistency	No serious indirectness	Serious imprecision (CI cross line of no effect, OIS not reached)	Undetected^	31/33	0.85 (Intervention)	$p = 0.55$	(-1.93,3.63)	⊕⊕⊕○ Moderate
Weight	[2,4,5] (RCT)	Serious ROB ([4] was evaluated as high risk for incomplete outcome data)	No serious inconsistency	No serious indirectness	Serious imprecision (CI cross line of no effect, OIS not reached)	Undetected^	112/114	-0.12 (Intervention)	$p = 0.37$	(-0.38,0.14)	⊕⊕○○ Low
Lean mass	[2,4] (RCT)	Serious ROB ([4] was evaluated as high risk for incomplete outcome data)	No serious inconsistency	No serious indirectness	Serious imprecision (CI cross line of no effect, OIS not reached)	Undetected^	103/105	0.02 (Intervention)	$p = 0.98$	(-1.31,1.35)	⊕⊕○○ Low
Fat mass	[2,4] (RCT)	Serious ROB ([4] was evaluated as high risk for incomplete outcome data)	No serious inconsistency	No serious indirectness	Serious imprecision (CI cross line of no effect, OIS not reached)	Undetected^	103/105	-0.39 (Intervention)	$p = 0.76$	(-2.82, 2.05)	⊕⊕○○ Low
BMD (hip)	[2,4,5,6] (RCT)	Serious ROB ([4] was evaluated as high risk for incomplete outcome data)	No serious inconsistency	No serious indirectness	No serious imprecision	Undetected^	124/126	0.04 (Intervention)	$p = 0.002$	(0.01,0.06)	⊕⊕⊕○ Moderate
BMD (spine)	[2,4,5,6] (RCT)	Serious ROB ([4] was evaluated as high risk for incomplete outcome data)	Serious inconsistency (substantial heterogeneity)	No serious indirectness	Serious imprecision (CI cross line of no effect, OIS not reached)	Undetected^	124/126	0.02 (Intervention)	$p = 0.24$	(-0.001,0.05)	⊕○○○ Very low

^Insufficient data to produce funnel plots. GRADE scoring: ⊕⊕⊕⊕ High; ⊕⊕⊕○ Moderate; ⊕⊕○○ Low; ⊕○○○ Very low

Figures 2-5: Meta-analyses for Group 1 outcome measures

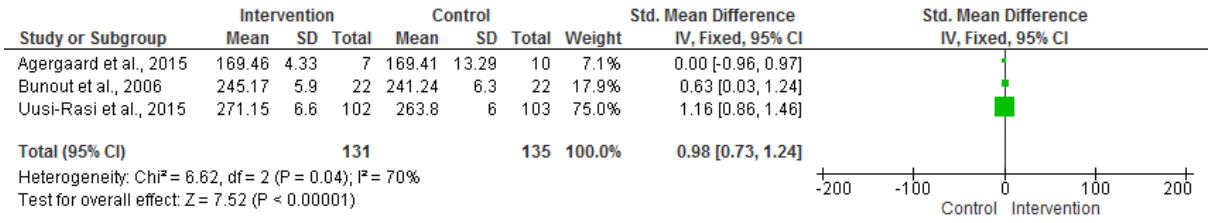

Figure 2: Group 1 analysis of muscle strength of the lower limb

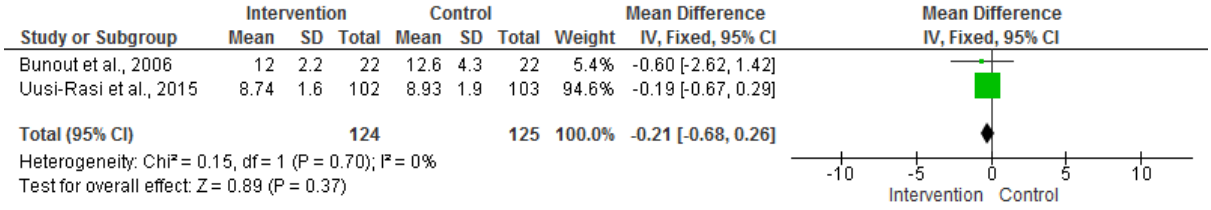

Figure 3: Group 1 analysis of the TUG test

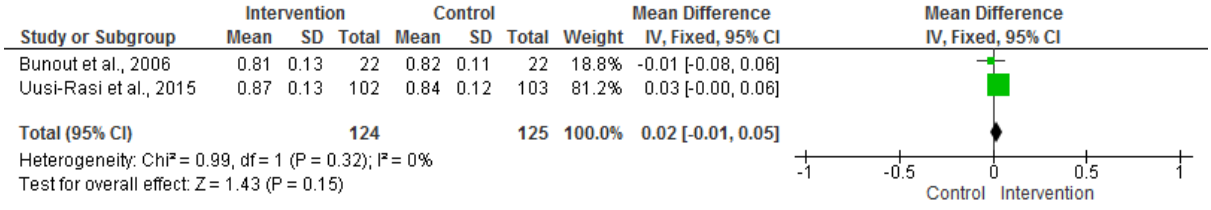

Figure 4: Group 1 analysis of BMD of the hip

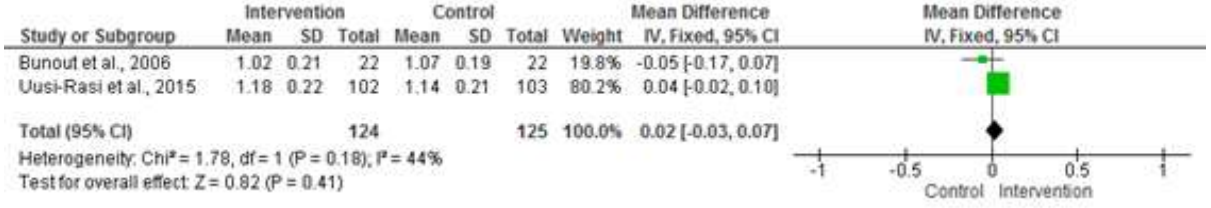

Figure 5: Group 1 analysis of BMD of the spine

Figures 6-14: Meta-analyses for Group 2 outcome measures

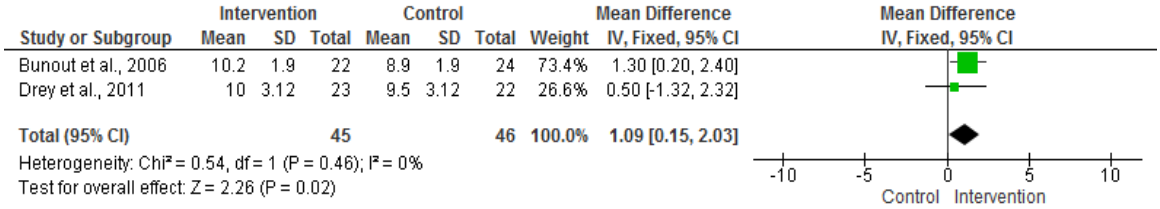

Figure 6: Group 2 analysis of the SPPB test

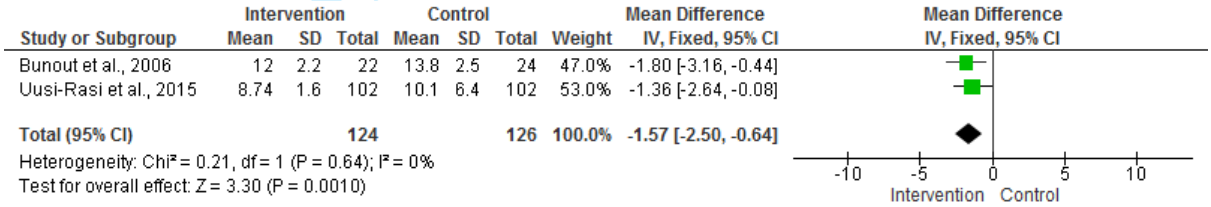

Figure 7: Group 2 analysis of the TUG test

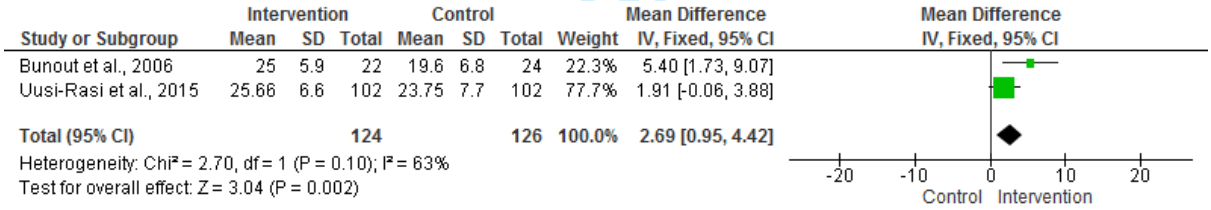

Figure 8: Group 2 analysis of the muscle strength of the lower limb

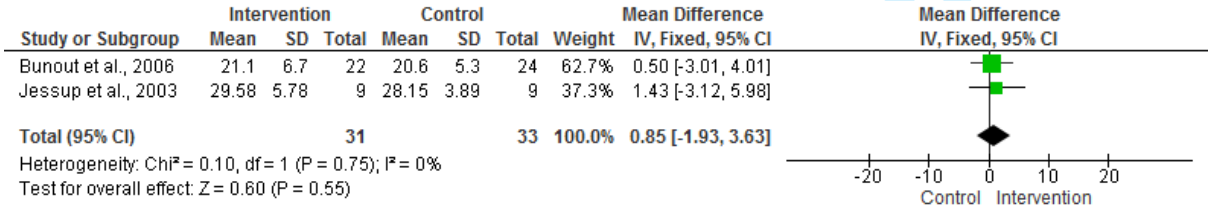

Figure 9: Group 2 analysis of hand grip strength

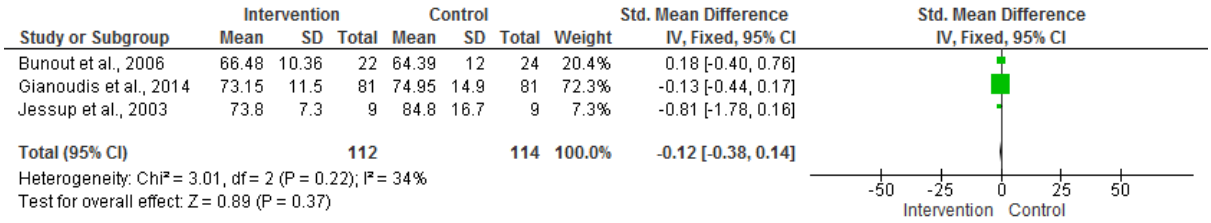

Figure 10: Group 2 analysis of total body weight

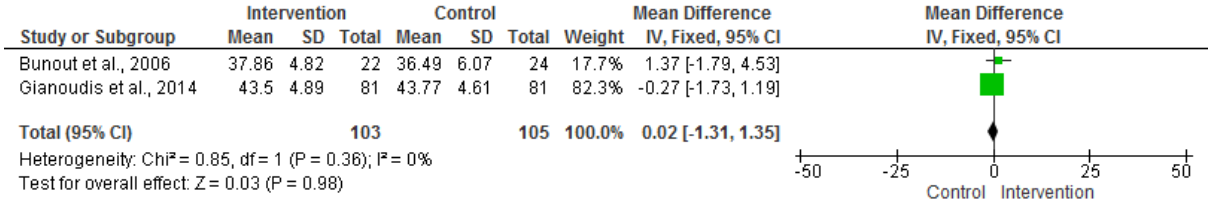

Figure 11: Group 2 analysis of lean mass

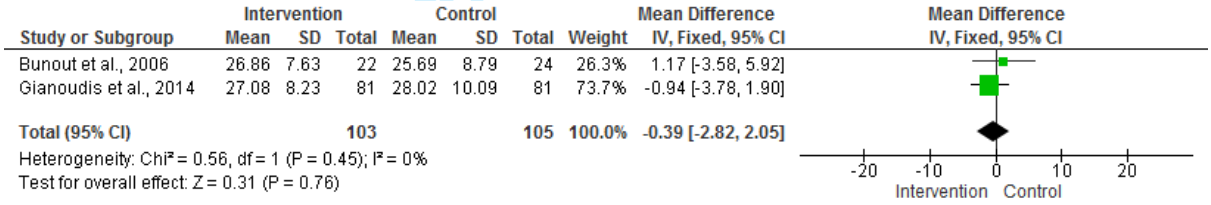

Figure 12: Group 2 analysis of fat mass

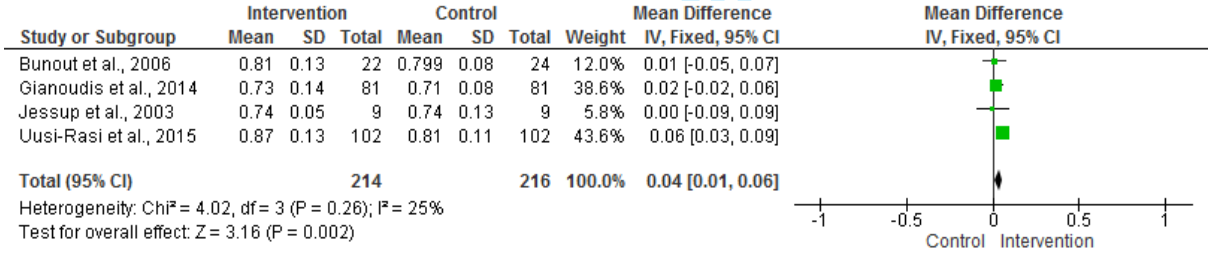

Figure 13: Group 2 analysis of BMD of the hip

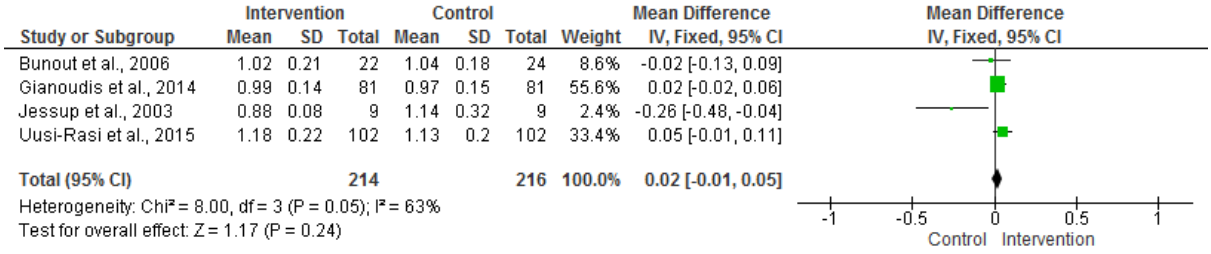

Figure 14: Group 2 analysis of BMD of the spine

Table 7: Narrative analysis summary of findings for group 1 secondary outcome measures

Category	Outcome measure	Assessment point	Study	Intervention group % change from baseline			Control group % change from baseline		
				M	SD	N	M	SD	N
Body composition	CSA of quadriceps muscles (cm^2)	16 weeks	Agergaard et al., 2015	+4.94	5.28	7	+8.46*	6.80	10

Table 8: Narrative analysis summary of findings for group 2 primary outcome measures

Category	Outcome	Assessment point	Study	Intervention group % change from baseline			Control group % change from baseline		
				M	SD	N	M	SD	N
Muscle strength	Isometric knee extensor strength (Nm)	6 months	Verschueren et al., 2011	+3.01	2.67	28	+0.11	3.18	28
Muscle power	Sit-to-stand transfer power (W)	12 weeks	Drey et al., 2011	+8.99*	5.51	23	+2.61	2.49	22
	Functional stair climbing muscle power (W)	12 months	Gianoudis et al., 2014	+10.40*	13.00	81	+6.20	12.70	81
Muscle function	30 second sit-to-stand (n.stands)	12 months	Gianoudis et al., 2014	+18.30*	23.60	81	+2.70	17.2	81
	5-time chair stand time (s)	24 months	Uusi-Rasi et al., 2015	-6.95	2.50	102	-3.49	3.30	102
	Normal walking speed (m/s)	24 months	Uusi-Rasi et al., 2015	-1.80	0.20	102	-3.30	0.21	102
	Endurance: 12-minute walk (m)	9 months	Bunout et al., 2006	+8.80	17.60	22	+20.90	27.70	24
Balance	Romberg ratio (%)	9 months	Bunout et al., 2006	+2.80	33.80	22	-0.60	35.80	24
	Four square step test (s)	12 months	Gianoudis et al., 2014	-12.00*	14.10	81	-5.20	14.90	81
	Body sway (cm)	32 weeks	Jessup et al., 2003	-26.39*	0.52	9	+2.90	0.49	9
	Backwards walking (% able to complete)	24 months	Uusi-Rasi et al., 2015	+25.47*	13.59	102	+9.48	15.58	102

Table 9: Narrative analysis summary of findings for group 2 secondary outcomes

Category	Outcome measure	Assessment point	Study	Intervention group % change from baseline			Control group % change from baseline		
				M	SD	N	M	SD	N
Body composition	Appendicular lean mass (Kg)	12 weeks	Drey et al., 2011	+1.65	0.71	23	+0.00	0.87	22
	Muscle mass of upper limb (cm^3)	6 months	Verschueren et al., 2011	-0.16	0.57	28	-0.25	0.38	28
	BMD of hip (g/cm^2)	6 months	Verschueren et al., 2011	+0.71	0.42	28	+0.99	0.51	28

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	4
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	Supplementary file 1
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	4
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	4
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	5
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	4
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	4

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	Supplementary file 2
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	5 Supplementary files 3-4
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	5-6
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	7-10
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	Supplementary file 2
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	10
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Supplementary files 5-6
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	Supplementary file 2
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	Supplementary files 3-4
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	1-16
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	16
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	16
FUNDING			

PRISMA 2009 Checklist

Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	17

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(6): e1000097. doi:10.1371/journal.pmed1000097

For more information, visit: **www.prisma-statement.org**.

Page 2 of 2

BMJ Open

The effect of combined resistance exercise training and vitamin D3 supplementation on musculoskeletal health and function in older adults: A systematic review and meta-analysis

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2016-014619.R1
Article Type:	Research
Date Submitted by the Author:	10-Feb-2017
Complete List of Authors:	Antoniak, Anneka; University of Birmingham, School of Sports, Exericse and Rehabilitation Sciences Greig, Carolyn; School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK; MRC-Arthritis Research UK Centre for Musculoskeletal Ageing Research
Primary Subject Heading:	Geriatric medicine
Secondary Subject Heading:	Nutrition and metabolism, Sports and exercise medicine
Keywords:	Physiology < BASIC SCIENCES, GERIATRIC MEDICINE, NUTRITION & DIETETICS, Bone diseases < ORTHOPAEDIC & TRAUMA SURGERY, Musculoskeletal disorders < ORTHOPAEDIC & TRAUMA SURGERY, SPORTS MEDICINE

The effect of combined resistance exercise training and vitamin D3 supplementation on musculoskeletal health and function in older adults: A systematic review and meta-analysis

AUTHORS
Anneka E Antoniak[1] and Carolyn A Greig[1,2]

[1]School of Sport, Exercise and Rehabilitation Sciences, [2]MRC-Arthritis Research UK Centre for Musculoskeletal Ageing and Health, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK

Corresponding author
Anneka E Antoniak
School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK
Email: aea423@bham.ac.uk
Telephone number: +44 121 371 3234

Keywords: Vitamin D, exercise, older adults, randomized controlled trial, systematic review

Word count: 4662

Number of figures: 14

Number of tables: 9

Number of references: 54

Number of colour images: 4

ABSTRACT

Objectives

In older adults there is a blunted responsiveness to resistance training and reduced muscle hypertrophy compared with younger adults. There is evidence that both exercise training and vitamin D supplementation may benefit musculoskeletal health in older adults, and it is plausible that in combination their effects may be additive. The aim of this systematic review was to evaluate the effectiveness of combined resistance exercise training and vitamin D3 supplementation on musculoskeletal health in older adults.

Data sources

A comprehensive search of electronic databases, including Science Direct, MedLine, PubMed, Google Scholar and Cochrane Central Register of Controlled Trials (Cochrane CENTRAL accessed by Wiley Science). Eligible studies were randomized controlled trials including men and women (aged ≥65 years or mean age ≥ 65 years); enlisting resistance exercise training (RET) and vitamin D3 supplementation; including outcomes of muscle strength, function, muscle power, body composition, serum vitamin D/calcium status or quality of life (4) comparing results with a control group. The review was informed by a pre-registered protocol (http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42015020157).

Results

7 studies including a total of 792 participants were identified. Studies were categorized into two groups; group 1 compared vitamin D3 supplementation and exercise training versus exercise alone (describing the additive effect of vitamin D3 supplementation when combined with resistance exercise training) and group 2 compared vitamin D3 supplementation and exercise training versus vitamin D3 supplementation alone (describing the additive effect of resistance exercise training when combined with vitamin D3 supplementation).
Meta-analyses for group 1 found muscle strength of the lower limb to be significantly improved within the intervention group (0.98, 95% CI 0.73, 1.24, p<0.001); all other outcomes showed small but non-significant positive effects for the intervention group. The Short Physical Performance Battery (SPPB), Timed Up and Go (TUG), muscle strength of the lower limb and femoral neck Bone Mineral Density (BMD) showed significantly greater improvements in the intervention group for group 2 comparisons.

Conclusions

This review provides tentative support for the additive effect of resistance exercise and vitamin D3 supplementation for the improvement of muscle strength in older adults. For other functional variables, such as SPPB and TUG, no additional benefit beyond exercise was shown. Further evidence is required to draw firm conclusions or make explicit recommendations regarding combined exercise and vitamin D3 supplementation.

Strengths and Limitations of this study

- To the best of our knowledge this study represents the first review evaluating the combined effects of vitamin D3 supplementation and exercise in older adults
- Generally, outcome measure data could be graded as representing moderate quality
- Only seven studies were found to be eligible for inclusion, highlighting the lack of literature available on the topic
- The inclusion of one high risk study was deemed necessary due to the lack of eligible studies

INTRODUCTION

Sarcopenia, originally defined as the age related loss of muscle mass[1], now also encompasses low muscle strength and/or muscle function[2]. The efficacy of resistance training in preventing or alleviating age-related musculoskeletal loss is well established; cited as the most promising intervention for improving symptoms of sarcopenia[3].

Clear evidence exists demonstrating an association between resistance exercise training (RET) and muscle hypertrophy, which is maintained in older age[3-5]. However, in older adults there is a blunted responsiveness to RET in comparison with younger adults; a blunted muscle protein synthetic rate in response to a single bout of resistance exercise has been reported[6], and others demonstrate a reduction in muscle hypertrophy in comparison to younger adults[7-10]. This 'anabolic resistance' may be due to changes in gene expression and anabolic signalling; an attenuated anabolic hormone response to resistance exercise is observed in comparison to younger adults[11].

Losses in muscle strength are associated with losses in functional ability, independence and increases in frailty, falls, and disability in older adults [12-15]; therefore, there may be merit associated with a combination of interventions to boost responsiveness of older muscle to resistance exercise and combat anabolic resistance.

Vitamin D3 supplementation in humans has been shown to positively influence musculoskeletal health in older adults: increases in relative number and cross-sectional area (CSA) of muscle fibres (type II in particular) has been reported[16-18], and muscle strength increased and fall rates decreased after treatment with vitamin D3[17]. Vitamin D receptor (VDR) concentration significantly increased with vitamin D3 supplementation[18]; conversely, supplementation conferred no benefits on strength, functioning and balance[19-21]. Moreover, a systematic review examining the effects of vitamin D3 supplementation in vitamin D replete adults aged over 18 years found no significant effect on grip or proximal lower limb muscle strength; however, pooled data including vitamin D deficient participants (serum 25(OH)D <25 nmol.l^{-1}) demonstrated a large effect on hip muscle strength[22].

There is conflicting evidence surrounding the efficacy of vitamin D3 supplementation alone or in combination with exercise on musculoskeletal health, with no clear consensus regarding the management or prevention of sarcopenia. Although epidemiological data suggest a relationship between vitamin D3 and muscle weakness[23], this association is not well understood, and evidence in published literature is lacking and contradictory. Considering the beneficial effects of both RET and vitamin D3 on muscle tissue, it is plausible an additive effect would exist if combined, optimizing the potential for healthy ageing muscle[24]. Thus, the aim of this study was to assess the combined effect of RET and vitamin D3 supplementation on musculoskeletal health in older adults.

MATERIALS AND METHODS

A systematic review of peer-reviewed literature relating to the effect of RET and vitamin D3 supplementation on musculoskeletal health in older adults was conducted in accordance with a study protocol registered on the PROSPERO database (record number CRD42015020157). The protocol was informed by the Cochrane Handbook for Systematic Reviews of Interventions[25], and reporting conformed to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement[26].

Eligibility Criteria

Randomized controlled trials were sought for this study. Journal studies included: (1) male and/or female participants (aged ≥65 years or mean age ≥ 65 years) (2) enlisted RET and vitamin D3 supplementation (studies utilising vitamin D3 and calcium supplementation were included) (3) included measures of muscle strength, function, muscle power, body composition, serum vitamin D/calcium status or quality of life (4) compared results with a control group (sedentary/usual care/no vitamin D3 supplementation). Articles were excluded if participants were supplemented with additional protein or any supplement/medication with a known anabolic effect on muscle tissue.

Search methods for identification of studies

Articles published before March 2016 were included. A computerised search of Science Direct, MedLine, PubMed, Google Scholar and Cochrane Central Register of Controlled Trials (Cochrane CENTRAL accessed by Wiley Science) databases was conducted. Table 1 shows the Medline search strategy, devised by AEA and LH.

Table 1: Example Ovid MEDLINE search, to be adapted for other databases

1	Aging/
2	Exp aged/
3	(65 adj2 (years or age* or old*))
4	(old* adj (adult* or people or person* or population* or men or women))
5	(elder* or senior* or geriatric* or ?enarian or ag?ing)
6	((age* or aging or old* or elder*) adj1 (musc*))
7	1 or 2 or 3 or 4 or 5 or 6
8	Vitamin D/
9	(cholecalciferol* or calciferol* or ergocalciferol*)
10	(supplements or dietary supplements)
11	((vitamin D* or cholecalciferol or calciferol* OR ergocalciferol) adj supplementation
12	8 or 9 or 10 or 11
13	Muscle Development/
14	Muscle, Skeletal/
15	(Skeletal muscle adj2 (atrophy or sarcopenia or wasting or loss or deterioration))
16	Muscle Strength/
17	(skeletal muscle mass or size or fibres or fibers or area)
18	(musc* adj2 (function* or power or strength))
19	(musc* adj2 (grow* or hypertrophy or size or mass or csa or cross sectional area or volume))
20	Body Composition/
21	(lean adj3 mass)
22	(protein adj2 (turnover or synthesis or breakdown))
23	(nitrogen adj2 (balance or turnover or synthesis or breakdown or retention or loss or retain*))
24	Sarcopenia/
25	13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24
26	Exp exercise/
27	(resistance exercise or resistance exercise training)
28	((resistance or strength or weight or cardio or aerobic) adj3 (train* or condition* or exercise* or lift*))
29	(physical adj3 (activit* or exercise* or train* or exertion* or endurance* or therap* or conditioning or fitness))
30	(exercise adj3 (train* or intervention* or protocol* or program* or therap* or regim* or activit*))
31	26 or 27 or 28 or 29 or 30
32	7 and 12 and 25 and 31
33	Limit 32 to humans
34	Remove duplicates from 33

Data items and collection

Data were extracted independently by 2 reviewers (AEA and ASA) using a standardised data extraction sheet; any disagreements were discussed and resolved with a third person (CAG). The inter-rater reliability assessed using Cohen's Kappa, was found to be excellent (86% agreement)[27]. Data items including general information, participant characteristics and details of the intervention were extracted. For key outcomes, the definition used by the authors, methodology, results, mean differences and the presence/absence of statistical significance were reported.

Risk of bias analysis

2 reviewers (AEA and CAG) independently assessed the validity of included studies, with provisions for moderation from a third reviewer. The Cochrane Collaboration's tool for assessing risk of bias was utilised, as described in the Cochrane Handbook for Systematic Reviews of Interventions[25]; the use of scales for assessment is explicitly discouraged[28,29]. Pre-specified consensus points were devised and agreed by reviewers to ensure consistency. It was acknowledged that by nature of design, blinding of participants and personnel would be difficult in certain studies; therefore grading was based on the likelihood that outcome measures were influenced by the potential lack of blinding[25].

Grading the quality of evidence

The Grading of Recommendation, Assessment, Development, and Evaluation (GRADE) handbook[30] was used to evaluate the quality of evidence of outcomes assessed within the meta-analyses. The GRADE approach utilises systematically produced questions to reach conclusions on degree of confidence in the estimate of the effect. GRADE assesses patient important outcomes across five areas; risk of bias, inconsistency, indirectness, imprecision and publication bias, and grades outcomes as demonstrating high, moderate, low or very low quality of evidence.

RESULTS

Study selection:

7 studies were included within the review; Agergaard et al., 2015[31], Bunout et al., 2006[32], Drey et al., 2011[33], Gianoudis et al., 2014[34], Jessup et al., 2003[35], Uusi-Rasi et al., 2015[21], and Verschueren et al., 2011[36]; the study flow diagram is presented in Figure 1.

Upon reading full text articles, it became clear that there were 2 separate groups of interventions; group 1, in which all participants took part in RET and the intervention arm was supplemented with vitamin D3 (describing the additive effect of vitamin D3 supplementation when combined with resistance exercise training), group 2 in which all participants were supplemented with vitamin D3 and the intervention arm took part in RET (describing the additive effect of resistance exercise training when combined with vitamin D3 supplementation); and studies using a combination of the 2 interventions (Table 2).

Table 2: Study demographics

Author, year	N included in analyses	Mean age (y)	Sex (M:F)	Study design	Intervention group protocol	Control group protocol	Duration
	Group 1: All participants exercised, intervention group received vitamin D supplementation						
Agergaard et al., 2015[31]	17	66.9	17:0	RCT	RET 3x per week & 1920 IU D3 + 800mg Ca/day	RET 3x per week & 800mg Ca/day	16 weeks
	Group 2: All participants received vitamin D supplementation, intervention group exercised						
Drey et al., 2011[33]	45	77	13:32	RCT	RET 2x 60 mins per week & >20 ng/ml = 1000 IU D3/day <20 ng/ml = 2000 IU D3/day	Sedentary & >20 ng/ml = 1000 IU D3/day <20 ng/ml = 2000 IU D3/day	12 weeks
Gianoudis et al.,2014[34]	162	67	119:43	RCT	HV-PRT 3x per week & 1000 IU D3 + 700mg Ca/day	Sedentary & 1000 IU D3 + 700mg Ca/day	12 months
Jessup et al., 2003[35]	18	69	0:18	RCT Parallel	RET 3x 60-90 mins per week & 400 IU D3 + 1000 mg Ca/day	Sedentary & 400 IU D3 + 1000 mg Ca/day	32 weeks
Verschueren et al., 2011[36]	111	79	0:111	RCT	WBV 3x per week & High-dose = 1600 IU Or Conventional dose = 800 IU D3/day + 1000mg Ca/day	Sedentary & High-dose = 1600 IU Or Conventional dose = 800 IU D3/day + 1000mg Ca/day	6 months
	Assigned to Group 1 & 2: Participants took part in a combination of exercise and vitamin D interventions						
Bunout et al., 2006[32]	92	77	9:83	RCT	RET 2x 1.5h per week Or sedentary & 400 IU D3 + 800mg Ca/day	RET 2x 1.5h per week Or sedentary & 800mg Ca/day	9 months
Uusi-Rasi et al., 2015[21]	409	74	0:409	RCT	RET 2x/week for 12 months, 1x/week for next 12 months Or sedentary & 800 IU D3/day	RET 2x/week for 12 months, 1x/week for next 12 months Or sedentary & Placebo/day	2 years

*RCT: Randomized Controlled Trial, RET: Resistance Exercise Training, IU: International Units, Ca: Calcium, HV-PRT: High-Velocity Progressive Resistance Training

Study demographics

7 eligible studies included a total of 792 participants of mean age 72.8 years (Table 2). Of these, 1 included only males[31] and 3 included only females[21,35,36]. All studies included healthy participants living independently, except for 2 studies; [35] included participants living within a retirement community and [36] included institutionalized participants living in nursing homes, service flats or cloistered communities.

Interventions

Studies assigned to group 1 included Agergaard et al., 2015[31]; Bunout et al., 2006[32] and Uusi-Rasi et al., 2015[21]. In group 1, all participants took part in RET; incorporating a warm-up and strengthening exercises utilising commercial weight machines[21,31] or Thera-bands[31]. 2 studies included balance challenging aspects[21,32]. All studies included supervised, progressive exercise sessions; progression was monitored by a 5 rep max (RM) test[31], Borg scale[32] or metabolic equivalents (METs)[21]. Total number of sessions delivered ranged from 36[31]to 156[21], over a duration of 16 weeks[31] to 24 months[21]. All administered a vitamin D3 supplement, orally in tablet form; doses ranged from 400IU [32] to 1920 IU[31] per day; in 2 studies participants were supplemented with 800mg calcium per day[31,32] and 1 study supplemented the control group with a placebo[21].

6 studies assigned to group 2 included; Bunout et al., 2006[32], Drey et al., 2011[33] Gianoudis et al., 2014[34], Jessup et al., 2003[35], Uusi-Rasi et al., 2015[21] and Verschueren et al., 2011[36]. Within group 2, all participants took a vitamin D3 supplement, orally in tablet form. Doses ranged from 400 IU[32,35] to 2000 IU[33] per day; 1 study monitored serum 25(OH)D at baseline to determine supplement dosage[33]. In 4 studies[32,34-36] all participants were supplemented with calcium; doses ranged from 700mg[34] to 1000mg[35,36] per day. The intervention group took part in RET. Studies utilised machine weights and pulleys[21,33-35], Thera-bands[32], weighted vests[35] and Whole Body Vibration (WBV) machines[36] for resistance. 5 studies included balance challenging aspects[21,32-35]. All studies employed supervised, progressive exercise sessions monitored via a Borg scale[32-34], addition of weights to weighted vests[35], estimation of METs or individual ability[36]. Total number of sessions delivered ranged from 24[33] to 156[21], over a duration of 12 weeks[33] to 24 months[21]. Note that 2 studies included comparators which allowed allocation to both groups [21,32].

Outcome measures

All outcomes are listed in Table 3. Group 1 studies had few outcomes in common; however, all measured muscle strength[21,31,32]; isometric knee extensor strength was measured using a strain gauge[21,31] and isometric quadriceps strength was measured using a quadriceps table[32]. Hand grip strength was measured using a hand grip dynamometer[32]. Magnetic resonance imaging (MRI) was used to measure the CSA of the quadriceps[31,37], whilst[32] analysed fat and lean mass using dual-energy X-ray absorptiometry (DXA). 2 studies measured timed-up and go (TUG), femoral neck and spine bone mineral density (BMD)[21,32]. 1 study analysed fibre type and muscle quality[31].

Of group 2 studies,[21,32,34,36] assessed lower limb strength, and[32,35] measured grip strength. Muscle power was measured as sit-to-stand transfer power[33] and the stair climb test[34]. The short physical performance battery (SPPB) was assessed by[32,34], and the TUG by[21,32,34]. BMD of the femoral neck[21,32,34-36] and spine[21,32,34,35] were measured using DXA. Lean mass was measured using DXA[32-34]and X-ray computed tomography (CT)[36]. Balance was assessed via the Romberg ratio[32], four square step test[34], an AccuSway platform[35] and backwards walking[21]. Other outcomes included endurance (12-minute walk[32]), the 30 second sit-to-stand test[34], normal walking speed and the 5-time chair stand test[21].

Table 3: Summary of included study outcome measures and significant results

Author, year	Outcome measures		Significant results
Aagergaard et al., 2015[31]	Muscle strength Muscle CSA Muscle quality	Isometric knee extensor (strain gauge) MRI of quadriceps muscle (6mm thick) Muscle strength/CSA	Muscle strength –**no between-group difference** Muscle CSA –**no between-group difference** Muscle quality – N/S
Bunout et al., 2006[32]	Muscle strength Muscle function BMD Body sway Endurance	Quadriceps (table) & hand grip strength (dynamometer) SPPB, TUG Femoral neck & spine (DXA) Romberg ratio Distance walked in 12 minutes	Muscle strength – Increased with exercise (p<0.001), no effect of vit D Muscle function – SPPB increased with exercise (p=0.002) no effect of vit D, TUG: Increased in both groups (p=0.004) BMD – Femoral neck increased with vit D, decreased without (p=0.006). Spine was N/S Body sway – Lower with vit D than without (p=0.05) Endurance – N/S
Dhesy et al., 2011[33]	Muscle power Muscle function Body composition	Lower limb sit-to-stand transfer power (force plate) SPPB, SF-LLFDI aLM (DXA)	Muscle power - Increased with vit D intake (p=0.017) Muscle function – SPPB increased with exercise (p=0.009), SF-LLFDI was N/S Body composition – aLM was N/S
Gianoudis et al., 2014[34]	Muscle strength Muscle power Muscle function BMD Body composition Dynamic balance	Lower limbs (bilateral leg press) Timed stair climb test 30 second sit-to-stand test, TUG Femoral neck & spine (DXA) Total body lean & fat mass (DXA) Four Square Step Test	Muscle strength- Intervention increased strength relative to controls (p<0.001) Muscle power – Intervention increased power relative to controls (p<0.05) Muscle function – Intervention improved Sit-to-stand relative to controls (p<0.05). TUG – **No between group difference** BMD -Intervention increased femoral neck relative to controls (p<0.05). Spine - Intervention increased relative to controls (p<0.05). Body composition – Lean & fat mass – N/S Dynamic balance – Intervention increased relative to **controls** (p<0.05).
Jessup et al., 2003[35]	Muscle strength BMD Body sway	Hand grip (dynamometer), mean of 8 tests (stack machine) Femoral neck & spine (DXA) AccuSway force platform	Muscle strength – increased with intervention (p=0.0156). BMD femoral neck – increase with intervention (p=0.00001). Spine – **No between group difference** Body sway – Significantly reduced in intervention group (p=0.0027)
Ousi-Rasi et al., 2015[21]	Muscle strength Muscle function BMD Dynamic balance	Max isometric leg extensor strength at a knee angle of 110° SPPB, TUG Femoral neck & spine (BMD) Backwards walking	Muscle strength – increased with exercise (p<0.001). Vit D supplementation N/S Muscle function – SPPB = N/S. TUG – vitamin D without exercise increased relative to placebo without exercise (p=0.01) BMD – Femoral neck – Vit D maintained BMD (p=0.02) as did exercise (p=0.01). Spine – N/S Dynamic balance – Improved with exercise (placebo: p=0.001, vit D: p=0.03). No additive effect of vit D
Verschueren et al., 2011[36]	Muscle strength BMD Muscle mass	Isometric & dynamic knee extensor strength Femoral neck (DXA) Mass of upper leg (Multi-slice CT)	Muscle strength – Isometric: N/S. Dynamic: N/S. Vit D=no effect BMD – Improved in all groups. **No between group difference.** Muscle mass – N/S

*CSA: Cross-sectional Area, MRI: Magnetic Resonance Imaging, ELISA: Enzyme-linked Immunosorbent Assay, BMD: Bone Mineral Density, SPPB: Short Physical Performance Battery, TUG: Timed Up and Go, DXA: Duel-energy X-ray Absorptiometry, SF-LLFDI: Short Form of the Late Life Function and Disability Instrument, aLM: appendicular Lean Mass, QoL: Quality of Life, Multi-slice CT: Multi-slice X-ray Computed Tomography

Risk of bias within studies

The risk of bias analyses are displayed within Table 4. For all studies, a high proportion of components were assigned an unclear risk of bias due to insufficient information and the unknown effect on study outcome measures. Many studies reported insufficient information on concealment and blinding procedures, or whether procedures were in place in the event of unblinding. In total, 6 studies were judged to have an unclear risk of bias[21,31-33,35,36]. Component 1 was assessed as having a low risk of bias for all studies. 1 study was assessed as having an overall high risk of bias[34] due to component 5, as no data were entered into the analyses for participants with missing data.

Table 4: Summary of risk of bias analysis for each included study

Author, year	Components of risk of bias							Summary	Comments on high risk components
	1	2	3	4	5	6	7		
Agergaard et al., (2015)[31]	L	U	L	L	U	L	L	**High (0)** Unclear (2) Low (5)	N/A
Bunout et al., (2006)[32]	L	U	U	U	U	U	U	**High (0)** Unclear (6) Low (1)	N/A
Drey et al., (2011)[33]	L	L	U	U	L	L	U	**High (0)** Unclear (3) Low (4)	N/A
Gianoudis et al., (2014)[34]	L	U	U	U	H	L	L	**High (1)** Unclear (3) Low (3)	One high risk component, 5 ITT analysis utilised, but no data entered for participants with missing data
Jessup et al., (2003)[35]	L	U	U	U	U	U	L	**High (0)** Unclear (5) Low (2)	N/A
Uusi-Rasi et al., (2015)[21]	L	U	U	U	U	L	L	**High (0)** Unclear (4) Low (3)	N/A
Verschueren et al., (2011)[36]	L	U	U	U	U	L	L	**High (0)** Unclear (4) Low (3)	N/A

* Risk of bias domains of assessment. 1: Random sequence generation, 2: Allocation concealment, 3: Blinding of participants and personnel, 4: Blinding of outcome assessment, 5: Incomplete outcome data, 6: Selective reporting, 7: Other sources of bias. Judgements possible: H – High risk of bias, U – Unclear risk of bias, L – Low risk of bias

GRADE analysis

The GRADE summary of findings table for groups 1 and 2 are shown in Tables 5 and 6.

Within group 1, all studies were evaluated as moderate quality of evidence; no serious risk of bias was detected. Due to the nature of the studies included within this review, no serious indirectness was detected; all outcomes were measured directly without the use of a surrogate. Publication bias was not detected, and due to the number of studies included, it was not possible to produce funnel plots for any outcomes. Although publication bias was "not detected", it is difficult to conclude that there was a complete absence of bias since studies with significant results are more likely to be published than those reporting null or non-significant results[25] Published, peer-reviewed articles were included in this review, since the Cochrane Handbook for Systematic Reviews of Interventions further suggests that the inclusion of unpublished studies may introduce additional bias, as these studies have not been strengthened by the peer-review process and may be of lower methodological quality[25]. Reasons for downgrading the quality of evidence included serious inconsistency due to substantial heterogeneity, and serious imprecision due to confidence intervals crossing the line of no effect.

Within group 2 studies, 5 outcomes were graded as high to moderate quality of evidence (SPPB, TUG, muscle strength of the lower limb, hand grip strength and BMD of the femoral neck). Remaining outcomes were graded as low or very low quality, meaning that one could have little or very little confidence in the effect estimate. Common reasons for downgrading outcomes included a combination of serious risk of bias (due to the inclusion of study[34]), serious imprecision or serious inconsistency.

Table 5: GRADE analysis of group 1 measurement outcomes included in the quantitative synthesis

Quality Assessment							Summary of Findings				
Outcome	Included studies (design)	ROB	Inconsistency	No serious Indirectness	Imprecision	Publication bias	Groups (Intervention /control)	Effect size (direction)	Significance	95% CI	Quality
Muscle strength (lower limb)	[21,31,32] (RCT)	No serious ROB	Serious inconsistency (substantial heterogeneity)	No serious indirectness	No serious imprecision	Undetected^	131/135	0.98 (Intervention)	$p<0.00001$	(0.73, 1.24)	⊕⊕⊕o Moderate
TUG	[21,32] (RCT)	No serious ROB	No serious inconsistency	No serious indirectness	Serious imprecision (CIs cross line of no effect/ OIS not reached)	Undetected^	124/125	0.37 (Intervention)	$p= 0.37$	(-0.68,0.26)	⊕⊕⊕o Moderate
BMD (Femoral neck)	[21,32] (RCT)	No serious ROB	No serious inconsistency	No serious indirectness	Serious imprecision (CIs cross line of no effect/ OIS not reached)	Undetected^	124/125	0.02 (Intervention)	$p= 0.15$	(-0.01,0.05)	⊕⊕⊕o Moderate
BMD (spine)	[21,32] (RCT)	No serious ROB	No serious inconsistency	No serious indirectness	Serious imprecision (CIs cross line of no effect/ OIS not reached)	Undetected^	124/125	0.02 (Intervention)	$p = 0.41$	(-0.03,0.07)	⊕⊕⊕o Moderate

*ROB: Risk of Bias; TUG: Timed Up and Go; RCT: Randomized Controlled Trial; CI: Confidence Interval; BMD: Bone Mineral Density; OIS: Optimum Information Size.
^Insufficient data to produce funnel plots. GRADE scoring: ⊕⊕⊕⊕ High; ⊕⊕⊕o Moderate; ⊕⊕oo Low; ⊕ooo Very low

Table 6: GRADE analysis of group 2 measurement outcomes included in the quantitative synthesis.

Quality Assessment							Summary of Findings				
Outcome	**Included studies (design)**	**ROB**	**Inconsistency**	**Indirectness**	**Imprecision**	**Publication bias**	**Groups (intervention/ control)**	**Effect size (direction)**	**Significance**	**95% CI**	**Quality**
SPPB	[32,33] (RCT)	No serious ROB	No serious inconsistency	No serious indirectness	No serious imprecision	Undetected^	45/46	1.09 (Intervention)	$p = 0.02$	(0.15,2.03)	⊕⊕⊕⊕ High
TUG	[21,32 (RCT)	No serious ROB	No serious inconsistency	No serious indirectness	No serious imprecision	Undetected^	124/126	-1.57 (Intervention)	$p = 0.001$	(-2.50, -0.64)	⊕⊕⊕⊕ High
Muscle strength (lower limb)	[21,32] (RCT)	No serious ROB	Serious inconsistency (substantial heterogeneity)	No serious indirectness	No serious imprecision	Undetected^	124/126	2.69 (Intervention)	$p = 0.002$	(0.96,4.42)	⊕⊕⊕o Moderate
Hand grip strength	[32,35] (RCT)	No serious ROB	No serious inconsistency	No serious indirectness	Serious imprecision (CI cross line of no effect, OIS not reached)	Undetected^	31/33	0.85 (Intervention)	$p = 0.55$	(-1.93,3.63)	⊕⊕⊕o Moderate
Weight	[32,34,35] (RCT)	Serious ROB ([4] was evaluated as high risk for incomplete outcome data)	No serious inconsistency	No serious indirectness	Serious imprecision (CI cross line of no effect, OIS not reached)	Undetected^	112/114	-0.12 (Intervention)	$p = 0.37$	(-0.38,0.14)	⊕⊕oo Low
Lean mass	[32,34] (RCT)	Serious ROB ([4] was evaluated as high risk for incomplete outcome data)	No serious inconsistency	No serious indirectness	Serious imprecision (CI cross line of no effect, OIS not reached)	Undetected^	103/105	0.02 (Intervention)	$p = 0.98$	(-1.31,1.35)	⊕⊕oo Low
Fat mass	[32,34] (RCT)	Serious ROB ([4] was evaluated as high risk for incomplete outcome data)	No serious inconsistency	No serious indirectness	Serious imprecision (CI cross line of no effect, OIS not reached)	Undetected^	103/105	-0.39 (Intervention)	$p = 0.76$	(-2.82, 2.05)	⊕⊕oo Low
BMD (femoral neck)	[21,32,34,35] (RCT)	Serious ROB ([4] was evaluated as high risk for incomplete outcome data)	No serious inconsistency	No serious indirectness	No serious imprecision	Undetected^	124/126	0.04 (Intervention)	$p = 0.002$	(0.01,0.06)	⊕⊕⊕o Moderate
BMD (spine)	[21,32,34,35] (RCT)	Serious ROB ([4] was evaluated as high risk for incomplete outcome data)	Serious inconsistency (substantial heterogeneity)	No serious indirectness	Serious imprecision (CI cross line of no effect, OIS not reached)	Undetected^	124/126	0.02 (Intervention)	$p = 0.24$	(-0.001,0.05)	⊕ooo Very low

^Insufficient data to produce funnel plots. GRADE scoring: ⊕⊕⊕⊕ High; ⊕⊕⊕o Moderate; ⊕⊕oo Low; ⊕ooo Very low

Results of individual studies and synthesis of results

Results of the 2 groups of studies are reported separately. Qualitative syntheses were conducted for studies with similar interventions and outcomes measures using RevMan 5.3 software. Study outcomes reporting results in the same units were pooled using a fixed-effect meta-analysis. Effect sizes are expressed as percentage mean differences or standardized mean differences (when outcomes were measured utilising different methods), with 95% confidence intervals. Higher weighting was assigned to studies with smaller standard deviations and a larger sample size[25]. Analyses were completed from extracted data; where necessary data were estimated from statistics or figures, or requested from the authors of the article. Heterogeneity was assessed via the chi squared test (Figures 2-14 and Tables 5-6). One article[36] was not included in any of the quantitative analyses since the exercise intervention modality was considered to be too dissimilar to compare with the other included articles. Within each group, there were outcomes unsuitable for quantitative synthesis, due to a lack of studies with common outcomes or aspects of studies too dissimilar for comparison; therefore, a narrative analysis was utilised.

Quantitative synthesis

Outcomes compared for group 1 included muscle strength of the lower limb, TUG and BMD of the femoral neck and spine (Figures 2-5). Only muscle strength of the lower limb was found to be significant, with a large effect size in favour of the intervention group (Figure 2. 2.69, 95% CI 0.95, 4.42. $p = 0.002$).

Group 2 comparisons included the SPPB (Figure 6), TUG (Figure 7), muscle strength of the lower limb (Figure 8), hand grip strength (Figure 9), weight (Figure 10), lean mass (Figure 11), fat mass (Figure 12), BMD of the femoral neck (Figure 13) and spine (Figure 14). Of these outcomes, hand grip strength, weight, lean mass, fat mass and the BMD of the spine were found to be non-significant. However, SPPB score was more improved in the intervention group (1.09, 95% CI 0.15, 2.03. $p = 0.02$), with a significant and large effect. Similarly, TUG was significantly reduced within the intervention group (-1.57, 95% CI -2.50, -0.64. $p = 0.0010$). The results of the quantitative analysis also supported the combined intervention for muscle strength of the lower limb (2.69, 95% CI 0.95, 4.42). $p = 0.002$), and BMD of the femoral neck (0.04, 95% CI 0.01, 0.06. $p = 0.002$).

Qualitative synthesis

Referring to the narrative synthesis guidelines provided by the Cochrane Consumers and Communication Review Group[38], it was appropriate to apply 2 steps listed; developing a preliminary synthesis and exploring the relationships within and between studies. To develop a primary synthesis, results were systematically tabulated to identify patterns across studies (Tables 7-9). Exploring the relationships between and within studies for group 1, the control group in study[31]demonstrated a significant percentage increase in CSA of the quadriceps from baseline in comparison to the intervention group (+8.46% versus +4.94%, $p < 0.05$).

Table 7: Narrative analysis summary of findings for group 1 secondary outcome measures

Category	Outcome measure	Assessment point	Study	Intervention group % change from baseline			Control group % change from baseline		
				M	SD	N	M	SD	N
Body composition	CSA of quadriceps muscles (cm^2)	16 weeks	Agergaard et al., 2015[31]	+4.94	5.28	7	+8.46*	6.80	10

Group 1 studies compared vitamin D3 supplementation and exercise training versus exercise alone

Comparing primary outcomes for group 2, the percentage increase in isometric knee extensor strength for study[36] was greater in the intervention group (+3.01% versus +0.11%), although not statistically significant. Muscle power was compared in studies[33] and[34], expressed as sit-to-stand transfer power and functional stair climbing muscle power respectively. Both studies reported a significant percentage increase in muscle power within the intervention groups, and smaller, non-significant increases within the control groups (sit-to-stand transfer power intervention group +8.00% versus +2.61%, $p = 0.017$; functional stair climbing muscle power intervention group +10.51% versus +7.32%, $p < 0.05$).

The 30 second sit-to-stand test showed significant favourable results for the combined intervention of exercise and vitamin D3 (+10.40% versus +6.20%, p<0.05). Within study[21], normal walking speed and the 5-time chair stand time deteriorated non-significantly in both groups. The 12-minute walk test in study[32] was further improved within the control group, although this did not achieve statistical significance. The four-square step test, body sway and backwards walking were significantly more improved in the intervention groups. Only Romberg ratio showed the greatest improvement within the control group; Romberg ratio was decreased in comparison with the intervention group, although the results were non-significant (+2.8% versus -0.60%).

Table 8: Narrative analysis summary of findings for group 2 primary outcome measures

Category	Outcome	Assessment point	Study	Intervention group % change from baseline			Control group % change from baseline		
				M	SD	N	M	SD	N
Muscle strength	Isometric knee extensor strength (Nm)	6 months	Verschueren et al., 2011[36]	+3.01	2.67	28	+0.11	3.18	28
Muscle power	Sit-to-stand transfer power (W)	12 weeks	Drey et al., 2011[33]	+8.99*	5.51	23	+2.61	2.49	22
	Functional stair climbing muscle power (W)	12 months	Gianoudis et al., 2014[34]	+10.40*	13.00	81	+6.20	12.70	81
Muscle function	30 second sit-to-stand (n.stands)	12 months	Gianoudis et al., 2014[34]	+18.30*	23.60	81	+2.70	17.2	81
	5-time chair stand time (s)	24 months	Uusi-Rasi et al., 2015[21]	-6.95	2.50	102	-3.49	3.30	102
	Normal walking speed (m/s)	24 months	Uusi-Rasi et al., 2015[21]	-1.80	0.20	102	-3.30	0.21	102
	Endurance: 12-minute walk (m)	9 months	Bunout et al., 2006[32]	+8.80	17.60	22	+20.90	27.70	24
Balance	Romberg ratio (%)	9 months	Bunout et al., 2006[32]	+2.80	33.80	22	-0.60	35.80	24
	Four square step test (s)	12 months	Gianoudis et al., 2014[34]	-12.00*	14.10	81	-5.20	14.90	81
	Body sway (cm)	32 weeks	Jessup et al., 2003[35]	-26.39*	0.52	9	+2.90	0.49	9
	Backwards walking (% able to complete)	24 months	Uusi-Rasi et al., 2015[21]	+25.47*	13.59	102	+9.48	15.58	102

Group 2 compared vitamin D3 supplementation and exercise training versus vitamin D3 supplementation alone

For group 2 secondary outcomes, small and non-significant gains in appendicular lean mass were demonstrated in the intervention group of study[33]. In study[36], muscle mass of the upper limb decreased non-significantly in both the intervention and control groups, although to a lesser extent in the intervention group. BMD of the femoral neck was gained in both groups, although by a higher percentage in the control group; both trends were non-significant.

Table 9: Narrative analysis summary of findings for group 2 secondary outcomes

Category	Outcome measure	Assessment point	Study	Intervention group % change from baseline			Control group % change from baseline		
				M	SD	N	M	SD	N
Body composition	Appendicular lean mass (Kg)	12 weeks	Drey et al., 2011[33]	+1.65	0.71	23	+0.00	0.87	22
	Muscle mass of upper limb (cm^3)	6 months	Verschueren et al., 2011[36]	-0.16	0.57	28	-0.25	0.38	28
	BMD of femoral neck (g/cm^2)	6 months	Verschueren et al., 2011[36]	+0.71	0.42	28	+0.99	0.51	28

Group 2 compared vitamin D3 supplementation and exercise training versus vitamin D3 supplementation alone

In summary, meta-analyses for group 1 found muscle strength of the lower limb to be significantly improved within the intervention group (0.98, 95% CI 0.73, 1.24, $p<0.001$). All other outcomes showed small but non-significant positive effects for the intervention group. The SPPB, TUG, muscle strength of the lower limb and femoral neck BMD all showed significantly greater improvements in the intervention group for group 2 comparisons.

The narrative analysis revealed significant differences in body composition, muscle power, muscle function and balance. A significant percentage increase in quadriceps CSA was observed in the control group of study[31]. The combined intervention of RET and vitamin D3 supplementation resulted in a greater percentage increase in muscle strength and power, and a greater improvement in the 30 second sit-to-stand test, the four-square step test, body sway and backwards walking. However, vitamin D3 supplementation alone resulted in a greater improvement in the 12-minute walk test and Romberg ratio.

DISCUSSION

The aim of this systematic review was to assess the combined effect of RET and vitamin D3 supplementation on musculoskeletal health in older adults. Only 7 studies were eligible for inclusion, with a total of 792 participants, highlighting the lack of available literature on the topic. Studies were categorised into 2 groups; studies in which all participants took part in RET and the intervention group was supplemented with vitamin D3, or studies in which all participants were supplemented with vitamin D3 and the intervention group took part in RET. 2 studies were categorized into both group 1 and group 2.

Quantitative analysis

Data analysis conducted for this review included meta-analyses and narrative reviews. Meta-analyses for group 1 included muscle strength of the lower limb, TUG and BMD of both the femoral neck and spine. Evidence of additional benefit was shown for all outcomes within the intervention group; however, the effect size was small and non-significant for TUG and BMD of the femoral neck and spine. Muscle strength of the lower limb was the only significant outcome of group 1, with a large effect size observed within the intervention group (0.98, 95% CI 0.73, 1.24. p<0.00001). Although numerous studies have demonstrated the beneficial effect of RET on muscle strength in older adults[3-5], this result provides evidence that vitamin D3 supplementation may enhance these effects in older adults. Skeletal muscle myopathies associated with vitamin D deficiency are well documented[39], and symptoms of significant muscle weakness are reversed with treatment of the deficiency[40]. A systematic review and meta-analysis reported a gain in lower extremity strength with vitamin D supplementation only in vitamin D deficient older adults; no effect was observed in replete adults[22]. Similarly, no effect of vitamin D3 supplementation on isometric quadriceps strength was demonstrated after 6 months in vitamin D replete older adults[41]. Interestingly, although the studies included within group 1[21,31,32] did not specify serum 25(OH)D levels as inclusion/exclusion criteria, baseline and post-intervention serum 25(OH)D were within the 'sufficient' range (>30nmol.L^{-1}). A greater increase of muscle strength in replete older adults represents a novel finding of this review. Preliminary support for combined vitamin D supplementation and RET was demonstrated in a 3-month longitudinal study examining the effect of serum 25(OH)D and exercise training on functional performance in older men and women aged 65 years and over. No significant improvements in function were reported in participants with lower serum 25(OH)D (<47.5 nmol.L^{-1}), however higher serum 25(OH)D (>67.5 nmol.L^{-1}) was associated with greatest improvements in functionality and muscle strength[42].

This finding must be considered within the context of the risk of bias and GRADE analyses. The risk of bias analysis showed an overall unclear risk of bias for the included studies, and the GRADE analysis concluded that the evidenced was of moderate quality; however, serious inconsistency due to moderate heterogeneity (I^2 = 70%) was detected. This heterogeneity may have been due to the differing duration of interventions (12 weeks to 24 months), differences between measurement methodologies, differences between exercise regimens

(although all adopted progressive RET), doses of vitamin D3 (400 IU to 1920 IU per day), or may indicate that these studies were unsuitable for comparison.

Significant effects for the SPPB, TUG, muscle strength of the lower limb, and the BMD of the femoral neck were observed within the intervention groups of group 2 studies; unsurprisingly, RET was found to have a positive influence. In a recent systematic review and meta-analysis, exercise significantly increased SPPB score and decreased TUG time, with large effect sizes (1.87 and -2.47 respectively[43]); similar results are reported within this review. Vitamin D is a regulator of BMD, proliferating calcium and phosphate absorption in the intestine and acting directly on bone cells[44]. Vitamin D has previously been shown to influence BMD, fracture rate and risk[45]; studies of patients who have sustained a hip fracture typically demonstrated low serum vitamin D (≤30.0 $nmol.L^{-1}$;[46]). Supplementation of vitamin D and calcium has been shown to significantly decrease the rate of bone loss in the hip and spine[47]. GRADE analyses for these outcomes concluded the quality of evidence to be high (SPPB and TUG) or moderate (muscle strength of the lower limb and BMD of the femoral neck).

Closer examination of the control groups within significant outcomes for group 2 was undertaken to evaluate the effect of vitamin D3 supplementation alone. Intriguingly, although the intervention groups (RET and vitamin D3 supplementation) showed evidence of benefit in number of outcomes, the control groups (vitamin D3 supplementation alone) showed mixed, or even negative impacts on the same outcomes. SPPB score was decreased post-intervention compared with baseline by 0.30% and 0.50% in the control groups of studies[32] and[33] respectively. Muscle strength of the lower limb and BMD of the femoral neck showed mixed results for the intervention groups, with some studies reporting small increases and others reporting small losses (non-significant). Previous reports of the effect of vitamin D supplementation on muscle strength and physical functioning are mixed; the InCHIANTI study of people aged 65 years or over reported a significant association between serum 25(OH)D <25$nmol.L^{-1}$ and SPPB score[48]. Similarly, a large prospective cohort of older adults aged 65 years or over found those with low (<25$nmol.L^{-1}$) 25(OH)D were significantly more likely to experience losses in grip strength and higher rates of appendicular lean mass loss compared to those with higher (>50 $nmol.L^{-1}$) 25(OH)D[23]. Conversely, another large, prospective study found no association between serum 25(OH)D, walking speed and time for repeated chair stands[49]. The TUG test time increased in all groups of study [32], and was significantly increased in the vitamin D without exercise group in study p=0.01) [21]. Again, participants included in studies[32] and [21] had sufficient serum 25(OH)D levels, indicating that supplementation in replete older adults may not confer additional benefits to neuromuscular function unless combined with exercise.

Narrative analysis

Studies in group 1[21,31,32] had few body composition outcomes in common, therefore a narrative analysis was conducted. The CSA of the quadriceps was analysed within study[31], and results showed that although the intervention group did experience a +4.94%, increase from baseline, the control group (not supplemented with vitamin D3) actually showed a significantly higher increase in quadriceps CSA (+8.46%, $p<0.05$).

These results do not provide evidence for the additive effects of combined exercise training and vitamin D3. Other study groups have reported changes in muscle CSA consequent to RET which are both smaller[8,50] and comparable[51] to those reported in study[31]. Interestingly, study[31] also assessed "muscle quality" (muscle strength/CSA); although non-significant, the intervention group improved their muscle quality to a greater degree than the control group (+9.61% versus +0.66% change from baseline), f indicating an increased functionality of the muscle to produce force; conceptually more relevant in combatting the effects of sarcopenia than muscle size and strength alone[52].

Results of the narrative analysis for group 2 showed that the combined intervention of RET and vitamin D3 supplementation was significantly more beneficial than vitamin D3 supplementation alone for sit-to-stand transfer power, functional stair climbing muscle power, 30 second sit-to-stand, 5-time chair stand, the four-square step test, body sway and backwards walking. Only body sway was negatively affected by vitamin D3 supplementation, although the within group change was non-significant. Other outcomes of interest included normal walking speed, which deteriorated in both groups, the distance walked in 12 minutes and Romberg ratio, in which the control groups made the most improvement, although not significantly.

Limitations

Few published studies were eligible for inclusion within this review, although this serves to highlight the knowledge gap with respect to this topic. The inclusion of a high-risk study was deemed necessary due to the lack of available literature, although this had a negative effect on the perceived quality of evidence for the outcomes in which it was reported. Generally, outcome measure data could be graded as representing moderate quality, although there were several outcome measures graded as low or very low quality, due to the high variability of participant numbers, duration of interventions, exercise methodologies or differing vitamin D3 doses and period of supplementation employed within the studies. Furthermore, data produced from meta-analyses including study[21] may have been skewed due to the high weighting assigned for this study as a result of the large number of participants recruited.

Of the individual studies included within this review, none reported inclusion/exclusion criterion for vitamin D status, and although at baseline serum vitamin D was not significantly different between the groups in 5 studies[21,31-33,36], 2 studies reported no data for serum vitamin D pre or post-intervention[34,35]. Additionally, analysis methods used within 5 studies included did not account for confounding factors[31-34,36], and participants were not stratified on the basis of any characteristics in 3 studies[21,31,35], although

these were single-sex studies. Unfortunately, several outcome measures were unsuitable for inclusion within the qualitative analysis due to differing measurement methodologies utilised or too few outcome measures in common. A recent systematic review and meta-analysis investigating the effects of vitamin D on neuromuscular remodelling following exercise or injury similarly found few eligible studies and high levels of heterogeneity due to methodological differences, resulting in the authors to suggest more high quality evidence is needed to reach a result that is conclusive [53].

CONCLUSION

This review provides tentative support for the additive effect of combined RET and vitamin D3 supplementation for the improvement of muscle strength in older adults. For other aspects of musculoskeletal function, such as SPPB and TUG, no additional benefit beyond that gained from exercise training was found. This review showed no evidence of benefit of vitamin D3 supplementation alone, however, few studies were identified during the literature search, highlighting that further evidence is required to draw any firm conclusions or make explicit recommendations regarding vitamin D3 supplementation for musculoskeletal health and function in older adults.

Our recommendations to enable future studies to definitively answer questions regarding the additive effects of the combined vitamin D3 supplementation and RET include; common outcomes relevant to the condition studied, for example the SPPB, 400m walk and gait speed are recommended to assess physical performance[54], which would allow for a more detailed assessment of results. Additionally, exercise interventions of similar durations would allow for a more accurate comparison between studies; it has been suggested that interventions with older adults should be of a minimum duration of 3 months to obtain significant differences in relevant outcomes [54]. Reporting of confounding factors would allow for adjustment of results via the use of covariates; for example, objective measures of physical activity using accelerometers, baseline serum vitamin D3 status and participant characteristics, which may bias the participant pool. Separate analysis of male and female participants, or the addition of sex as a covariate in any analysis models would help to address sex-related differences in performance. Regarding study design, four-armed RCT studies are best placed to answer combined effects research questions; i.e. exercise intervention, vitamin D intervention, both exercise and vitamin D, neither exercise nor vitamin D (true control). A true control group was lacking from a number of the included studies within this review.

ACKNOWLEDGEMENTS

We thank Lynn Harris (LH) for her help formulating the search strategy, Asma Alrushud (AA) for her help with data extraction and the National Osteoporosis Society for supporting Anneka Antoniak (AEA).

FOOTNOTES

Contributors AEA has planned, conducted and written the report for this study. CAG has been involved in all stages, particularly in critically reviewing and approving the final draft of the report. AA was involved in the search for literature and data extraction stage. LH assisted in formulating the search strategy.

Funding This research received no grant from any funding agency in the public, commercial or not-for-profit sectors. AEA is supported and funded by the National Osteoporosis Society via the Linda Edwards Memorial PhD Studentship.

Competing interests None declared.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement This publication is supported by multiple datasets, which are openly available at locations cited in the reference section. Additional data for this article have been provided as supplementary files. There is no additional unpublished data.

REFERENCES

1. Rosenberg IH. Summary comments. *The American journal of clinical nutrition* 1989;50(5):1231-33.
2. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age and Ageing* 2010 doi: 10.1093/ageing/afq034
3. Kosek DJ, Kim J-s, Petrella JK, et al. Efficacy of 3 days/wk resistance training on myofiber hypertrophy and myogenic mechanisms in young vs. older adults. *Journal of applied physiology* 2006;101(2):531-44.
4. Henwood TR, Taaffe DR. Improved physical performance in older adults undertaking a short-term programme of high-velocity resistance training. *Gerontology* 2005;51(2):108-15.
5. Stewart V, Saunders D, Greig C. Responsiveness of muscle size and strength to physical training in very elderly people: a systematic review. *Scandinavian journal of medicine & science in sports* 2014;24(1):e1-e10.
6. Kumar V, Selby A, Rankin D, et al. Age-related differences in the dose–response relationship of muscle protein synthesis to resistance exercise in young and old men. *The Journal of physiology* 2009;587(1):211-17.
7. Greig C, Gray C, Rankin D, et al. Blunting of adaptive responses to resistance exercise training in women over 75y. *Experimental gerontology* 2011;46(11):884-90.
8. Häkkinen K, Kallinen M, Izquierdo M, et al. Changes in agonist-antagonist EMG, muscle CSA, and force during strength training in middle-aged and older people. *Journal of Applied Physiology* 1998;84(4):1341-49.
9. Raue U, Slivka D, Minchev K, et al. Improvements in whole muscle and myocellular function are limited with high-intensity resistance training in octogenarian women. *Journal of applied physiology* 2009;106(5):1611-17.
10. Welle S, Totterman S, Thornton C. Effect of age on muscle hypertrophy induced by resistance training. *J Gerontol A Biol Sci Med Sci* 1996;51 doi: 10.1093/gerona/51A.6.M270
11. Hameed M, Orrell R, Cobbold M, et al. Expression of IGF-I splice variants in young and old human skeletal muscle after high resistance exercise. *The Journal of physiology* 2003;547(1):247-54.
12. Roubenoff R. Sarcopenia: a major modifiable cause of frailty in the elderly. *The journal of nutrition, health & aging* 1999;4(3):140-42.
13. Janssen I, Heymsfield SB, Ross R. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *Journal of the American Geriatrics Society* 2002;50(5):889-96.
14. Visser M, Kritchevsky SB, Goodpaster BH, et al. Leg muscle mass and composition in relation to lower extremity performance in men and women aged 70 to 79: the health, aging and body composition study. *Journal of the American Geriatrics Society* 2002;50(5):897-904.
15. Landi F, Liperoti R, Russo A, et al. Sarcopenia as a risk factor for falls in elderly individuals: results from the ilSIRENTE study. *Clinical nutrition* 2012;31(5):652-58.
16. Sørensen O, Lund B, Saltin B, et al. Myopathy in bone loss of ageing: improvement by treatment with 1α-hydroxycholecalciferol and calcium. *Clinical Science* 1979;56(2):157-61.
17. Sato Y, Iwamoto J, Kanoko T, et al. Low-dose vitamin D prevents muscular atrophy and reduces falls and hip fractures in women after stroke: a randomized controlled trial. *Cerebrovasc Dis* 2005;20 doi: 10.1159/000087203
18. Ceglia L, Niramitmahapanya S, Silva MM, et al. A randomized study on the effect of vitamin D supplementation on skeletal muscle morphology and vitamin D receptor concentration in older women. *J Clin Endocrinol Metab* 2013;98 doi: 10.1210/jc.2013-2820
19. Latham NK, Anderson CS, Lee A, et al. A randomized, controlled trial of quadriceps resistance exercise and vitamin D in frail older people: the Frailty Interventions Trial in Elderly Subjects (FITNESS). *J Am Geriatr Soc* 2003;51 doi: 10.1046/j.1532-5415.2003.51101.x

20. Lips P, Binkley N, Pfeifer M, et al. Once-weekly dose of 8400 IU vitamin D3 compared with placebo: effects on neuromuscular function and tolerability in older adults with vitamin D insufficiency. *The American journal of clinical nutrition* 2010;91(4):985-91.
21. Uusi-Rasi K, Patil R, Karinkanta S, et al. Exercise and vitamin D in fall prevention among older women: a randomized clinical trial. *JAMA internal medicine* 2015;175(5):703-11.
22. Stockton K, Mengersen K, Paratz JD, et al. Effect of vitamin D supplementation on muscle strength: a systematic review and meta-analysis. *Osteoporosis international* 2011;22(3):859-71.
23. Visser M, Deeg DJ, Lips P. Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam. *The Journal of Clinical Endocrinology & Metabolism* 2003;88(12):5766-72.
24. Robinson S, Cooper C, Aihie Sayer A. Nutrition and sarcopenia: a review of the evidence and implications for preventive strategies. *Journal of aging research* 2012;2012
25. Higgins JPT, Green S, editors. *Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]*: The Cochrane Collarboration, 2011.
26. Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Annals of internal medicine* 2009;151(4):264-69.
27. Hsu LM, Field R. Interrater agreement measures: Comments on Kappan, Cohen's Kappa, Scott's π, and Aickin's α. *Understanding Statistics* 2003;2(3):205-19.
28. Moher D, Olkin I. Meta-analysis of randomized controlled trials: a concern for standards. *JAMA* 1995;274(24):1962-64.
29. Moher D, Altman D, Liberati A, et al. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). *The Cochrane Collaboration Methods Groups Newsletter* 1996:6.
30. Schünemann H, Brozek J, Oxman A. GRADE handbook for grading quality of evidence and strength of recommendation. Version 3.2 [updated March 2009]. The GRADE Working Group, 2009. *www cc-i ms net/gradepro* 2009
31. Agergaard J, Trøstrup J, Uth J, et al. Does vitamin-D intake during resistance training improve the skeletal muscle hypertrophic and strength response in young and elderly men? – a randomized controlled trial. *Nutrition & Metabolism* 2015;12(1):1-14. doi: 10.1186/s12986-015-0029-y
32. Bunout D, Barrera G, Leiva L, et al. Effects of vitamin D supplementation and exercise training on physical performance in Chilean vitamin D deficient elderly subjects. *Exp Gerontol* 2006;41 doi: 10.1016/j.exger.2006.05.001
33. Drey M, Zech A, Freiberger E, et al. Effects of strength training versus power training on physical performance in prefrail community-dwelling older adults. *Gerontology* 2011;58(3):197-204.
34. Gianoudis J, Bailey CA, Ebeling PR, et al. Effects of a Targeted Multimodal Exercise Program Incorporating High-Speed Power Training on Falls and Fracture Risk Factors in Older Adults: A Community-Based Randomized Controlled Trial. *Journal of Bone and Mineral Research* 2014;29(1):182-91. doi: 10.1002/jbmr.2014
35. Jessup JV, Horne C, Vishen R, et al. Effects of exercise on bone density, balance, and self-efficacy in older women. *Biological Research for nursing* 2003;4(3):171-80.
36. Verschueren SM, Bogaerts A, Delecluse C, et al. The effects of whole-body vibration training and vitamin D supplementation on muscle strength, muscle mass, and bone density in institutionalized elderly women: A 6-month randomized, controlled trial. *Journal of bone and mineral research* 2011;26(1):42-49.
37. Aagaard P, Andersen JL, Dyhre-Poulsen P, et al. A mechanism for increased contractile strength of human pennate muscle in response to strength training: changes in muscle architecture. *J Physiol* 2001;534 doi: 10.1111/j.1469-7793.2001.t01-1-00613.x
38. Ryan.R. 'Cochrane Consumers and Communication Review Group: data synthesis and analysis': Cochrane Consumers and Communication Review Group; 2013 [Available from: http://cccrg.cochrane.org accessed July 2016.

39. SMITH R, STERN G. Myopathy, osteomalacia and hyperparathyroidism. *Brain* 1967;90:593-602.
40. Glerup H, Mikkelsen K, Poulsen L, et al. Commonly recommended daily intake of vitamin D is not sufficient if sunlight exposure is limited. *Journal of internal medicine* 2000;247(2):260-68.
41. Grady D, Halloran B, Cummings S, et al. 1, 25-Dihydroxyvitamin D3 and Muscle Strength in the Elderly: A Randomized Controlled Trial*. *The Journal of Clinical Endocrinology & Metabolism* 1991;73(5):1111-17.
42. Okuno J, Tomura S, Fukasaku T, et al. [Examination of effects of alfacalcidol vitamin D supplement and renal function on improvement in the physical fitness of pre-frail elderly persons attending a nursing care prevention class]. *Nihon Ronen Igakkai zasshi Japanese journal of geriatrics* 2010;48(6):691-98.
43. Giné-Garriga M, Roqué-Fíguls M, Coll-Planas L, et al. Physical exercise interventions for improving performance-based measures of physical function in community-dwelling, frail older adults: a systematic review and meta-analysis. *Archives of physical medicine and rehabilitation* 2014;95(4):753-69. e3.
44. Lips P, Hosking D, Lippuner K, et al. The prevalence of vitamin D inadequacy amongst women with osteoporosis: an international epidemiological investigation. *Journal of internal medicine* 2006;260(3):245-54.
45. Brincat M, Gambin J, Brincat M, et al. The role of vitamin D in osteoporosis. *Maturitas* 2015;80(3):329-32. doi: 10.1016/j.maturitas.2014.12.018
46. LeBoff MS, Kohlmeier L, Hurwitz S, et al. Occult vitamin D deficiency in postmenopausal US women with acute hip fracture. *Jama* 1999;281(16):1505-11.
47. Tang BM, Eslick GD, Nowson C, et al. Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. *The Lancet* 2007;370(9588):657-66.
48. Houston DK, Cesari M, Ferrucci L, et al. Association between vitamin D status and physical performance: the InCHIANTI study. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* 2007;62(4):440-46.
49. Verreault R, Semba RD, Volpato S, et al. Low serum vitamin D does not predict new disability or loss of muscle strength in older women. *Journal of the American Geriatrics Society* 2002;50(5):912-17.
50. Trappe S, Williamson D, Godard M. Maintenance of whole muscle strength and size following resistance training in older men. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* 2002;57(4):B138-B43.
51. Ferri A, Scaglioni G, Pousson M, et al. Strength and power changes of the human plantar flexors and knee extensors in response to resistance training in old age. *Acta Physiol Scand* 2003;177 doi: 10.1046/j.1365-201X.2003.01050.x
52. Fragala MS, Kenny AM, Kuchel GA. Muscle Quality in Aging: a Multi-Dimensional Approach to Muscle Functioning with Applications for Treatment. *Sports Medicine* 2015;45(5):641-58. doi: 10.1007/s40279-015-0305-z
53. Minshull C, Biant LC, Ralston SH, et al. A Systematic Review of the Role of Vitamin D on Neuromuscular Remodelling Following Exercise and Injury. *Calcified tissue international.* 2016; 1;98(5):426-37.
54. Cruz-Jentoft AJ, Landi F, Schneider SM, et al. Prevalence of and interventions for sarcopenia in ageing adults: a systematic review. Report of the International Sarcopenia Initiative (EWGSOP and IWGS). *Age and ageing* 2014;43(6):748-59.

1

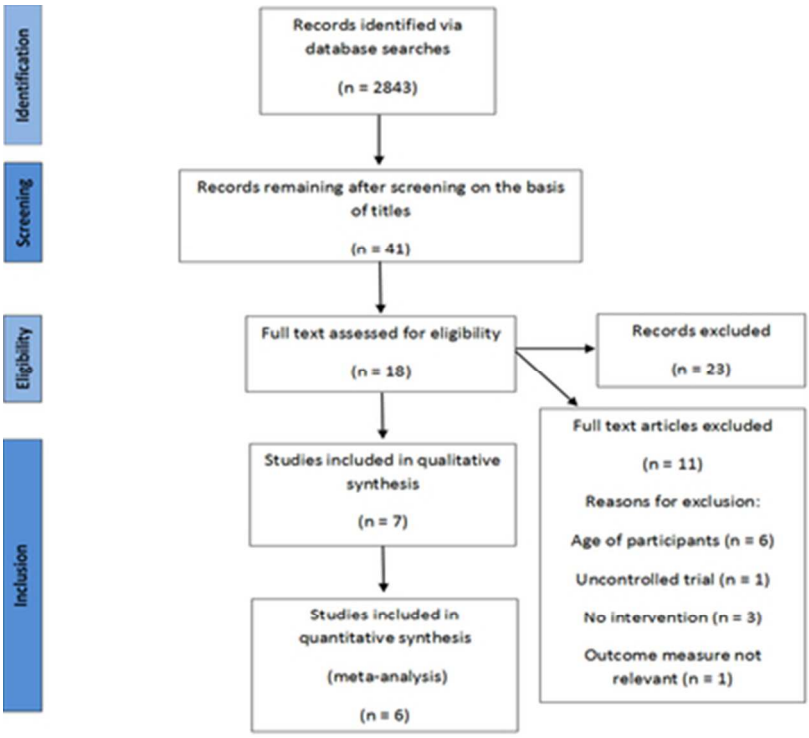

Figure 1: Study flow chart

34x30mm (300 x 300 DPI)

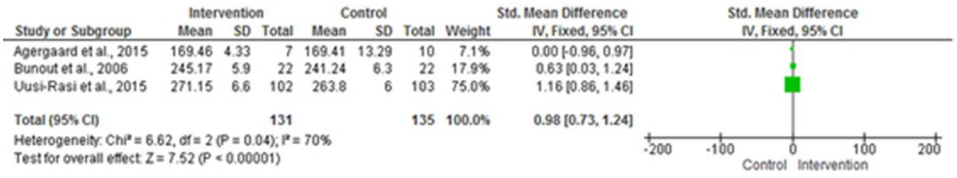

Figure 2: Group 1 analysis of muscle strength of the lower limb

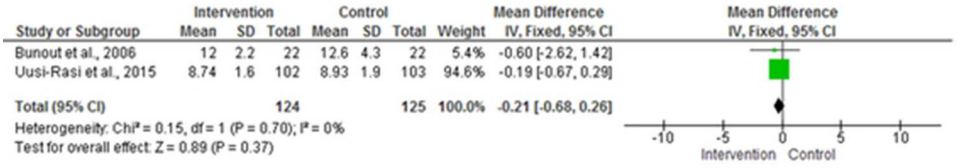

Figure 3: Group 1 analysis of the TUG test

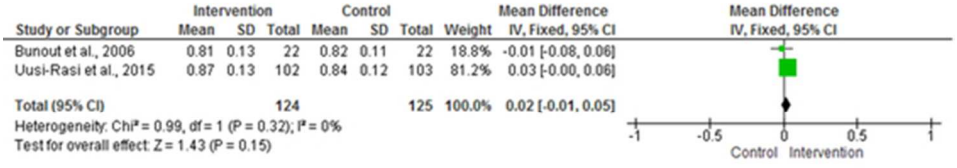

Figure 4: Group 1 analysis of BMD of the femoral neck

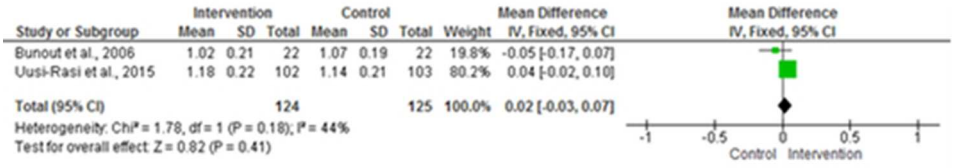

Figure 5: Group 1 analysis of BMD of the spine

Figures 2-5: Meta-analyses for Group 1 outcome measures

47x60mm (300 x 300 DPI)

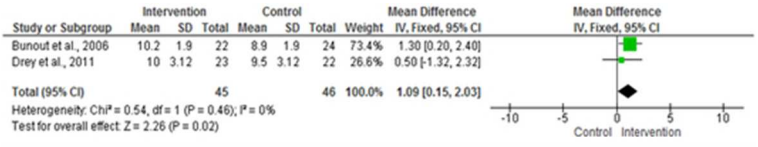

Figure 6: Group 2 analysis of the SPPB test

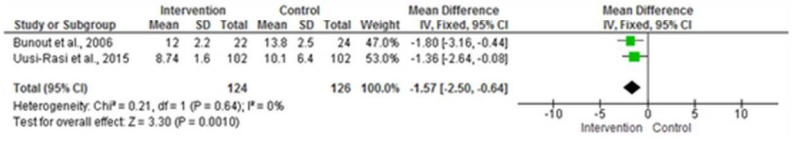

Figure 7: Group 2 analysis of the TUG test

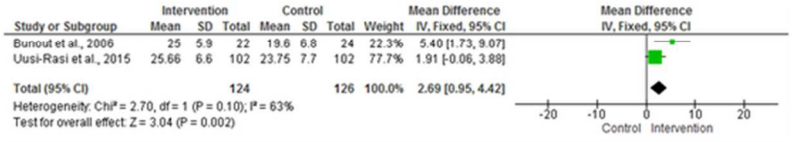

Figure 8: Group 2 analysis of the muscle strength of the lower limb

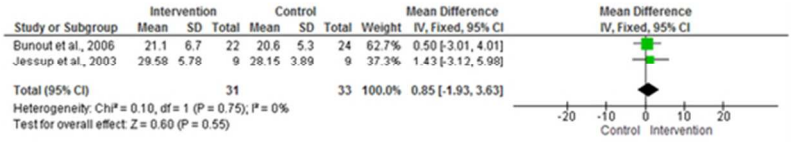

Figure 9: Group 2 analysis of hand grip strength

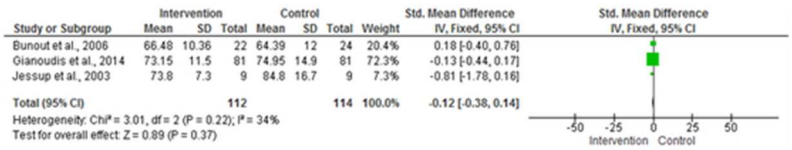

Figure 10: Group 2 analysis of total body weight

Figures 6-10: Meta-analyses for Group 2 outcome measures

47x71mm (300 x 300 DPI)

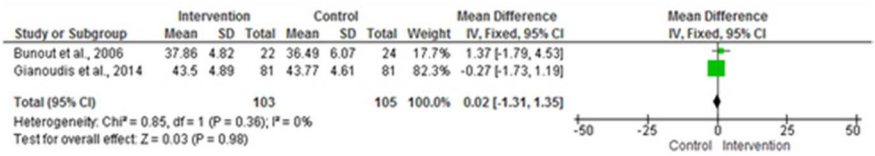

Figure 11: Group 2 analysis of lean mass

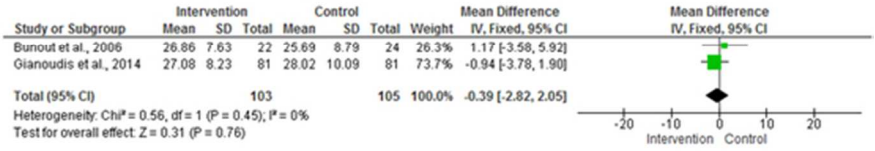

Figure 12: Group 2 analysis of fat mass

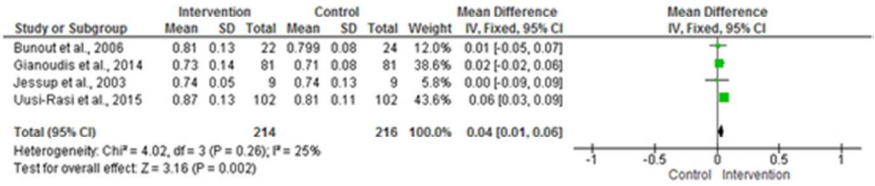

Figure 13: Group 2 analysis of BMD of the femoral neck

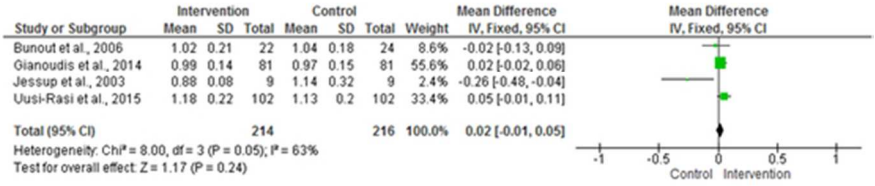

Figure 14: Group 2 analysis of BMD of the spine

Figures 11-14: Meta-analyses for Group 2 outcome measures

47x64mm (300 x 300 DPI)

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	4
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	15
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	15

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	11
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	12-18
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Image 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	6-7
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	11
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Image 2-3
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	11
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	12-18
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	19
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	21-22
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	22
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	23

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed100009

BMJ Open

The effect of combined resistance exercise training and vitamin D3 supplementation on musculoskeletal health and function in older adults: A systematic review and meta-analysis

Journal:	*BMJ Open*
Manuscript ID	bmjopen-2016-014619.R2
Article Type:	Research
Date Submitted by the Author:	27-Mar-2017
Complete List of Authors:	Antoniak, Anneka; University of Birmingham, School of Sports, Exericse and Rehabilitation Sciences Greig, Carolyn; School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK; MRC-Arthritis Research UK Centre for Musculoskeletal Ageing Research
Primary Subject Heading:	Geriatric medicine
Secondary Subject Heading:	Nutrition and metabolism, Sports and exercise medicine
Keywords:	Physiology < BASIC SCIENCES, GERIATRIC MEDICINE, NUTRITION & DIETETICS, Bone diseases < ORTHOPAEDIC & TRAUMA SURGERY, Musculoskeletal disorders < ORTHOPAEDIC & TRAUMA SURGERY, SPORTS MEDICINE

The effect of combined resistance exercise training and vitamin D3 supplementation on musculoskeletal health and function in older adults: A systematic review and meta-analysis

AUTHORS
Anneka E Antoniak[1] and Carolyn A Greig[1,2]

[1]School of Sport, Exercise and Rehabilitation Sciences, [2]MRC-Arthritis Research UK Centre for Musculoskeletal Ageing and Health, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK

Corresponding author
Anneka E Antoniak
School of Sport, Exercise and Rehabilitation Sciences, University of Birmingham, Edgbaston, Birmingham, B15 2TT, UK
Email: aea423@bham.ac.uk
Telephone number: +44 121 371 3234

Keywords: Vitamin D, exercise, older adults, randomized controlled trial, systematic review

Word count: 4662

Number of figures: 14

Number of tables: 9

Number of references: 53

ABSTRACT

Objectives

In older adults there is a blunted responsiveness to resistance training and reduced muscle hypertrophy compared with younger adults. There is evidence that both exercise training and vitamin D supplementation may benefit musculoskeletal health in older adults, and it is plausible that in combination their effects may be additive. The aim of this systematic review was to evaluate the effectiveness of combined resistance exercise training and vitamin D3 supplementation on musculoskeletal health in older adults.

Data sources

A comprehensive search of electronic databases, including Science Direct, MedLine, PubMed, Google Scholar and Cochrane Central Register of Controlled Trials (Cochrane CENTRAL accessed by Wiley Science). Eligible studies were randomized controlled trials including men and women (aged ≥65 years or mean age ≥ 65 years); enlisting resistance exercise training (RET) and vitamin D3 supplementation; including outcomes of muscle strength, function, muscle power, body composition, serum vitamin D/calcium status or quality of life (4) comparing results with a control group. The review was informed by a pre-registered protocol (http://www.crd.york.ac.uk/PROSPERO/display_record.asp?ID=CRD42015020157).

Results

7 studies including a total of 792 participants were identified. Studies were categorized into two groups; group 1 compared vitamin D3 supplementation and exercise training versus exercise alone (describing the additive effect of vitamin D3 supplementation when combined with resistance exercise training) and group 2 compared vitamin D3 supplementation and exercise training versus vitamin D3 supplementation alone (describing the additive effect of resistance exercise training when combined with vitamin D3 supplementation).
Meta-analyses for group 1 found muscle strength of the lower limb to be significantly improved within the intervention group (0.98, 95% CI 0.73, 1.24, $p<0.001$); all other outcomes showed small but non-significant positive effects for the intervention group. The Short Physical Performance Battery (SPPB), Timed Up and Go (TUG), muscle strength of the lower limb and femoral neck Bone Mineral Density (BMD) showed significantly greater improvements in the intervention group for group 2 comparisons.

Conclusions

This review provides tentative support for the additive effect of resistance exercise and vitamin D3 supplementation for the improvement of muscle strength in older adults. For other functional variables, such as SPPB and TUG, no additional benefit beyond exercise was shown. Further evidence is required to draw firm conclusions or make explicit recommendations regarding combined exercise and vitamin D3 supplementation.

Strengths and Limitations of this study

- To the best of our knowledge this study represents the first review evaluating the combined effects of vitamin D3 supplementation and exercise in older adults
- Generally, outcome measure data could be graded as representing moderate quality
- Only seven studies were found to be eligible for inclusion, highlighting the lack of literature available on the topic
- The inclusion of one high risk study was deemed necessary due to the lack of eligible studies

INTRODUCTION

Sarcopenia, originally defined as the age related loss of muscle mass[1], now also encompasses low muscle strength and/or muscle function[2]. The efficacy of resistance training in preventing or alleviating age-related musculoskeletal loss is well established; cited as the most promising intervention for improving symptoms of sarcopenia[3].

Clear evidence exists demonstrating an association between resistance exercise training (RET) and muscle hypertrophy, which is maintained in older age[3-5]. However, in older adults there is a blunted responsiveness to RET in comparison with younger adults; a blunted muscle protein synthetic rate in response to a single bout of resistance exercise has been reported[6], and others demonstrate a reduction in muscle hypertrophy in comparison to younger adults[7-10]. This 'anabolic resistance' may be due to changes in gene expression and anabolic signalling; an attenuated anabolic hormone response to resistance exercise is observed in comparison to younger adults[11].

Losses in muscle strength are associated with losses in functional ability, independence and increases in frailty, falls, and disability in older adults [12-15]; therefore, there may be merit associated with a combination of interventions to boost responsiveness of older muscle to resistance exercise and combat anabolic resistance.

Vitamin D3 supplementation in humans has been shown to positively influence musculoskeletal health in older adults: increases in relative number and cross-sectional area (CSA) of muscle fibres (type II in particular) has been reported[16-18], and muscle strength increased and fall rates decreased after treatment with vitamin D3[17]. Vitamin D receptor (VDR) concentration significantly increased with vitamin D3 supplementation[18]; conversely, supplementation conferred no benefits on strength, functioning and balance[19-21]. Moreover, a systematic review examining the effects of vitamin D3 supplementation in vitamin D replete adults aged over 18 years found no significant effect on grip or proximal lower limb muscle strength; however, pooled data including vitamin D deficient participants (serum 25(OH)D <25 nmol.l^{-1}) demonstrated a large effect on hip muscle strength[22].

There is conflicting evidence surrounding the efficacy of vitamin D3 supplementation alone or in combination with exercise on musculoskeletal health, with no clear consensus regarding the management or prevention of sarcopenia. Although epidemiological data suggest a relationship between vitamin D3 and muscle weakness[23], this association is not well understood, and evidence in published literature is lacking and contradictory. Considering the beneficial effects of both RET and vitamin D3 on muscle tissue, it is plausible an additive effect would exist if combined, optimizing the potential for healthy ageing muscle[24]. Thus, the aim of this study was to assess the combined effect of RET and vitamin D3 supplementation on musculoskeletal health in older adults.

MATERIALS AND METHODS

A systematic review of peer-reviewed literature relating to the effect of RET and vitamin D3 supplementation on musculoskeletal health in older adults was conducted in accordance with a study protocol registered on the PROSPERO database (record number CRD42015020157). The protocol was informed by the Cochrane Handbook for Systematic Reviews of Interventions[25], and reporting conformed to the Preferred Reporting Items for Systematic Reviews and Meta-analysis (PRISMA) statement[26].

Eligibility Criteria

Randomized controlled trials were sought for this study. Journal studies included: (1) male and/or female participants (aged ≥65 years or mean age ≥ 65 years) (2) enlisted RET and vitamin D3 supplementation (studies utilising vitamin D3 and calcium supplementation were included) (3) included measures of muscle strength, function, muscle power, body composition, serum vitamin D/calcium status or quality of life (4) compared results with a control group (sedentary/usual care/no vitamin D3 supplementation). Articles were excluded if participants were supplemented with additional protein or any supplement/medication with a known anabolic effect on muscle tissue.

Search methods for identification of studies

Articles published before March 2016 were included. A computerised search of Science Direct, MedLine, PubMed, Google Scholar and Cochrane Central Register of Controlled Trials (Cochrane CENTRAL accessed by Wiley Science) databases was conducted. Table 1 shows the Medline search strategy, devised by AEA and LH.

Table 1: Example Ovid MEDLINE search, to be adapted for other databases

1	Aging/
2	Exp aged/
3	(65 adj2 (years or age* or old*))
4	(old* adj (adult* or people or person* or population* or men or women))
5	(elder* or senior* or geriatric* or ?enarian or ag?ing)
6	((age* or aging or old* or elder*) adj1 (musc*))
7	1 or 2 or 3 or 4 or 5 or 6
8	Vitamin D/
9	(cholecalciferol* or calciferol* or ergocalciferol*)
10	(supplements or dietary supplements)
11	((vitamin D* or cholecalciferol or calciferol* OR ergocalciferol) adj supplementation
12	8 or 9 or 10 or 11
13	Muscle Development/
14	Muscle, Skeletal/
15	(Skeletal muscle adj2 (atrophy or sarcopenia or wasting or loss or deterioration))
16	Muscle Strength/
17	(skeletal muscle mass or size or fibres or fibers or area)
18	(musc* adj2 (function* or power or strength))
19	(musc* adj2 (grow* or hypertrophy or size or mass or csa or cross sectional area or volume))
20	Body Composition/
21	(lean adj3 mass)
22	(protein adj2 (turnover or synthesis or breakdown))
23	(nitrogen adj2 (balance or turnover or synthesis or breakdown or retention or loss or retain*))
24	Sarcopenia/
25	13 or 14 or 15 or 16 or 17 or 18 or 19 or 20 or 21 or 22 or 23 or 24
26	Exp exercise/
27	(resistance exercise or resistance exercise training)
28	((resistance or strength or weight or cardio or aerobic) adj3 (train* or condition* or exercise* or lift*))
29	(physical adj3 (activit* or exercise* or train* or exertion* or endurance* or therap* or conditioning or fitness))
30	(exercise adj3 (train* or intervention* or protocol* or program* or therap* or regim* or activit*))
31	26 or 27 or 28 or 29 or 30
32	7 and 12 and 25 and 31
33	Limit 32 to humans
34	Remove duplicates from 33

Data items and collection

Data were extracted independently by 2 reviewers (AEA and ASA) using a standardised data extraction sheet; any disagreements were discussed and resolved with a third person (CAG). The inter-rater reliability assessed using Cohen's Kappa, was found to be excellent (86% agreement)[27]. Data items including general information, participant characteristics and details of the intervention were extracted. For key outcomes, the definition used by the authors, methodology, results, mean differences and the presence/absence of statistical significance were reported.

Risk of bias analysis

2 reviewers (AEA and CAG) independently assessed the validity of included studies, with provisions for moderation from a third reviewer. The Cochrane Collaboration's tool for assessing risk of bias was utilised, as described in the Cochrane Handbook for Systematic Reviews of Interventions[25]; the use of scales for assessment is explicitly discouraged[28,29]. Pre-specified consensus points were devised and agreed by reviewers to ensure consistency. It was acknowledged that by nature of design, blinding of participants and personnel would be difficult in certain studies; therefore grading was based on the likelihood that outcome measures were influenced by the potential lack of blinding[25].

Grading the quality of evidence

The Grading of Recommendation, Assessment, Development, and Evaluation (GRADE) handbook[30] was used to evaluate the quality of evidence of outcomes assessed within the meta-analyses. The GRADE approach utilises systematically produced questions to reach conclusions on degree of confidence in the estimate of the effect. GRADE assesses patient important outcomes across five areas; risk of bias, inconsistency, indirectness, imprecision and publication bias, and grades outcomes as demonstrating high, moderate, low or very low quality of evidence.

RESULTS

Study selection:

7 studies were included within the review; Agergaard et al., 2015[31], Bunout et al., 2006[32], Drey et al., 2011[33], Gianoudis et al., 2014[34], Jessup et al., 2003[35], Uusi-Rasi et al., 2015[21], and Verschueren et al., 2011[36]; the study flow diagram is presented in Figure 1.

Upon reading full text articles, it became clear that there were 2 separate groups of interventions; group 1, in which all participants took part in RET and the intervention arm was supplemented with vitamin D3 (describing the additive effect of vitamin D3 supplementation when combined with resistance exercise training), group 2 in which all participants were supplemented with vitamin D3 and the intervention arm took part in RET (describing the additive effect of resistance exercise training when combined with vitamin D3 supplementation); and studies using a combination of the 2 interventions (Table 2).

Table 2: Study demographics

Author, year	N included in analyses	Mean age (y)	Sex (M:F)	Study design	Intervention group protocol	Control group protocol	Duration
	Group 1: All participants exercised, intervention group received vitamin D supplementation						
Agergaard et al., 2015[31]	17	66.9	17:0	RCT	RET 3x per week & 1920 IU D3 + 800mg Ca/day	RET 3x per week & 800mg Ca/day	16 weeks
	Group 2: All participants received vitamin D supplementation, intervention group exercised						
Drey et al., 2011[33]	45	77	13:32	RCT	RET 2x 60 mins per week & >20 ng/ml = 1000 IU D3/day <20 ng/ml = 2000 IU D3/day	Sedentary & >20 ng/ml = 1000 IU D3/day <20 ng/ml = 2000 IU D3/day	12 weeks
Gianoudis et al.,2014[34]	162	67	119:43	RCT	HV-PRT 3x per week & 1000 IU D3 + 700mg Ca/day	Sedentary & 1000 IU D3 + 700mg Ca/day	12 months
Jessup et al., 2003[35]	18	69	0:18	RCT Parallel	RET 3x 60-90 mins per week & 400 IU D3 + 1000 mg Ca/day	Sedentary & 400 IU D3 + 1000 mg Ca/day	32 weeks
Verschueren et al., 2011[36]	111	79	0:111	RCT	WBV 3x per week & High-dose = 1600 IU Or Conventional dose = 800 IU D3/day + 1000mg Ca/day	Sedentary & High-dose = 1600 IU Or Conventional dose = 800 IU D3/day + 1000mg Ca/day	6 months
	Assigned to Group 1 & 2: Participants took part in a combination of exercise and vitamin D interventions						
Bunout et al., 2006[32]	92	77	9:83	RCT	RET 2x 1.5h per week Or sedentary & 400 IU D3 + 800mg Ca/day	RET 2x 1.5h per week Or sedentary & 800mg Ca/day	9 months
Uusi-Rasi et al., 2015[21]	409	74	0:409	RCT	RET 2x/week for 12 months, 1x/week for next 12 months Or sedentary & 800 IU D3/day	RET 2x/week for 12 months, 1x/week for next 12 months Or sedentary & Placebo/day	2 years

*RCT: Randomized Controlled Trial, RET: Resistance Exercise Training, IU: International Units, Ca: Calcium, HV-PRT: High-Velocity Progressive Resistance Training

Study demographics

7 eligible studies included a total of 792 participants of mean age 72.8 years (Table 2). Of these, 1 included only males[31] and 3 included only females[21,35,36]. All studies included healthy participants living independently, except for 2 studies; [35] included participants living within a retirement community and [36] included institutionalized participants living in nursing homes, service flats or cloistered communities.

Interventions

Studies assigned to group 1 included Agergaard et al., 2015[31]; Bunout et al., 2006[32] and Uusi-Rasi et al., 2015[21]. In group 1, all participants took part in RET; incorporating a warm-up and strengthening exercises utilising commercial weight machines[21,31] or Thera-bands[31]. 2 studies included balance challenging aspects[21,32]. All studies included supervised, progressive exercise sessions; progression was monitored by a 5 rep max (RM) test[31], Borg scale[32] or metabolic equivalents (METs)[21]. Total number of sessions delivered ranged from 36[31]to 156[21], over a duration of 16 weeks[31] to 24 months[21]. All administered a vitamin D3 supplement, orally in tablet form; doses ranged from 400IU [32] to 1920 IU[31] per day; in 2 studies participants were supplemented with 800mg calcium per day[31,32] and 1 study supplemented the control group with a placebo[21].

6 studies assigned to group 2 included; Bunout et al., 2006[32], Drey et al., 2011[33] Gianoudis et al., 2014[34], Jessup et al., 2003[35], Uusi-Rasi et al., 2015[21] and Verschueren et al., 2011[36]. Within group 2, all participants took a vitamin D3 supplement, orally in tablet form. Doses ranged from 400 IU[32,35] to 2000 IU[33] per day; 1 study monitored serum 25(OH)D at baseline to determine supplement dosage[33]. In 4 studies[32,34-36] all participants were supplemented with calcium; doses ranged from 700mg[34] to 1000mg[35,36] per day. The intervention group took part in RET. Studies utilised machine weights and pulleys[21,33-35], Thera-bands[32], weighted vests[35] and Whole Body Vibration (WBV) machines[36] for resistance. 5 studies included balance challenging aspects[21,32-35]. All studies employed supervised, progressive exercise sessions monitored via a Borg scale[32-34], addition of weights to weighted vests[35], estimation of METs or individual ability[36]. Total number of sessions delivered ranged from 24[33] to 156[21], over a duration of 12 weeks[33] to 24 months[21]. Note that 2 studies included comparators which allowed allocation to both groups [21,32].

Outcome measures

All outcomes are listed in Table 3. Group 1 studies had few outcomes in common; however, all measured muscle strength[21,31,32]; isometric knee extensor strength was measured using a strain gauge[21,31] and isometric quadriceps strength was measured using a quadriceps table[32]. Hand grip strength was measured using a hand grip dynamometer[32]. Magnetic resonance imaging (MRI) was used to measure the CSA of the quadriceps[31], whilst[32] analysed fat and lean mass using dual-energy X-ray absorptiometry (DXA). 2 studies measured timed-up and go (TUG), femoral neck and spine bone mineral density (BMD)[21,32]. 1 study analysed fibre type and muscle quality[31].

Of group 2 studies,[21,32,34,36] assessed lower limb strength, and[32,35] measured grip strength. Muscle power was measured as sit-to-stand transfer power[33] and the stair climb test[34]. The short physical performance battery (SPPB) was assessed by[32,34], and the TUG by[21,32,34]. BMD of the femoral neck[21,32,34-36] and spine[21,32,34,35] were measured using DXA. Lean mass was measured using DXA[32-34]and X-ray computed tomography (CT)[36]. Balance was assessed via the Romberg ratio[32], four square step test[34], an AccuSway platform[35] and backwards walking[21]. Other outcomes included endurance (12-minute walk[32]), the 30 second sit-to-stand test[34], normal walking speed and the 5-time chair stand test[21].

Table 3: Summary of included study outcome measures and significant results

Author, year	Outcome measures		Significant results
Aagergaard et al., 2015[31]	Muscle strength Muscle CSA Muscle quality	Isometric knee extensor (strain gauge) MRI of quadriceps muscle (6mm thick) Muscle strength/CSA	Muscle strength –**no between-group difference** Muscle CSA –**no between-group difference** Muscle quality – N/S
Bunout et al., 2006[32]	Muscle strength Muscle function BMD Body sway Endurance	Quadriceps (table) & hand grip strength (dynamometer) SPPB, TUG Femoral neck & spine (DXA) Romberg ratio Distance walked in 12 minutes	Muscle strength – Increased with exercise (p<0.001), no effect of vit D Muscle function – SPPB increased with exercise (p=0.002) no effect of vit D, TUG: Increased in both groups (p=0.004) BMD – Femoral neck increased with vit D, decreased without (p=0.006). Spine was N/S Body sway – Lower with vit D than without (p=0.05) Endurance – N/S
Dhesy et al., 2011[33]	Muscle power Muscle function Body composition	Lower limb sit-to-stand transfer power (force plate) SPPB, SF-LLFDI aLM (DXA)	Muscle power - Increased with vit D intake (p=0.017) Muscle function – SPPB increased with exercise (p=0.009), SF-LLFDI was N/S Body composition – aLM was N/S
Gianoudis et al., 2014[34]	Muscle strength Muscle power Muscle function BMD Body composition Dynamic balance	Lower limbs (bilateral leg press) Timed stair climb test 30 second sit-to-stand test, TUG Femoral neck & spine (DXA) Total body lean & fat mass (DXA) Four Square Step Test	Muscle strength- Intervention increased strength relative to controls (p<0.001) Muscle power – Intervention increased power relative to controls (p<0.05) Muscle function – Intervention improved Sit-to-stand relative to controls (p<0.05). TUG – **No between group difference** BMD -Intervention increased femoral neck relative to controls (p<0.05). Spine - Intervention increased relative to controls (p<0.05). Body composition – Lean & fat mass – N/S Dynamic balance – Intervention increased relative to **controls** (p<0.05).
Jessup et al., 2003[35]	Muscle strength BMD Body sway	Hand grip (dynamometer), mean of 8 tests (stack machine) Femoral neck & spine (DXA) AccuSway force platform	Muscle strength – increased with intervention (p=0.0156). BMD femoral neck – increase with intervention (p=0.00001). Spine – **No between group difference** Body sway – Significantly reduced in intervention group (p=0.0027)
Celi-Rasi et al., 2015[21]	Muscle strength Muscle function BMD Dynamic balance	Max isometric leg extensor strength at a knee angle of 110° SPPB, TUG Femoral neck & spine (BMD) Backwards walking	Muscle strength – increased with exercise (p<0.001). Vit D supplementation N/S Muscle function – SPPB = N/S. TUG – vitamin D without exercise increased relative to placebo without exercise (p=0.01) BMD – Femoral neck – Vit D maintained BMD (p=0.02) as did exercise (p=0.01). Spine – N/S Dynamic balance – Improved with exercise (placebo: p=0.001, vit D: p=0.03). No additive effect of vit D
Verschueren et al., 2011[36]	Muscle strength BMD Muscle mass	Isometric & dynamic knee extensor strength Femoral neck (DXA) Mass of upper leg (Multi-slice CT)	Muscle strength – Isometric: N/S. Dynamic: N/S. Vit D=no effect BMD – Improved in all groups. **No between group difference.** Muscle mass – N/S

*CSA: Cross-sectional Area, MRI: Magnetic Resonance Imaging, ELISA: Enzyme-linked Immunosorbent Assay, BMD: Bone Mineral Density, SPPB: Short Physical Performance Battery, TUG: Timed Up and Go, DXA: Duel-energy X-ray Absorptiometry, SF-LLFDI: Short Form of the Late Life Function and Disability Instrument, aLM: appendicular Lean Mass, QoL: Quality of Life, Multi-slice CT: Multi-slice X-ray Computed Tomography

Risk of bias within studies

The risk of bias analyses are displayed within Table 4. For all studies, a high proportion of components were assigned an unclear risk of bias due to insufficient information and the unknown effect on study outcome measures. Many studies reported insufficient information on concealment and blinding procedures, or whether procedures were in place in the event of unblinding. In total, 6 studies were judged to have an unclear risk of bias[21,31-33,35,36]. Component 1 was assessed as having a low risk of bias for all studies. 1 study was assessed as having an overall high risk of bias[34] due to component 5, as no data were entered into the analyses for participants with missing data.

Table 4: Summary of risk of bias analysis for each included study

Author, year	Components of risk of bias							Summary	Comments on high risk components
	1	2	3	4	5	6	7		
Agergaard et al., (2015)[31]	L	U	L	L	U	L	L	**High (0)** Unclear (2) Low (5)	N/A
Bunout et al., (2006)[32]	L	U	U	U	U	U	U	**High (0)** Unclear (6) Low (1)	N/A
Drey et al., (2011)[33]	L	L	U	U	L	L	U	**High (0)** Unclear (3) Low (4)	N/A
Gianoudis et al., (2014)[34]	L	U	U	U	H	L	L	**High (1)** Unclear (3) Low (3)	One high risk component, 5 ITT analysis utilised, but no data entered for participants with missing data
Jessup et al., (2003)[35]	L	U	U	U	U	U	L	**High (0)** Unclear (5) Low (2)	N/A
Uusi-Rasi et al., (2015)[21]	L	U	U	U	U	L	L	**High (0)** Unclear (4) Low (3)	N/A
Verschueren et al., (2011)[36]	L	U	U	U	U	L	L	**High (0)** Unclear (4) Low (3)	N/A

* Risk of bias domains of assessment. 1: Random sequence generation, 2: Allocation concealment, 3: Blinding of participants and personnel, 4: Blinding of outcome assessment, 5: Incomplete outcome data, 6: Selective reporting, 7: Other sources of bias. Judgements possible: H – High risk of bias, U – Unclear risk of bias, L – Low risk of bias

GRADE analysis

The GRADE summary of findings table for groups 1 and 2 are shown in Tables 5 and 6.

Within group 1, all studies were evaluated as moderate quality of evidence; no serious risk of bias was detected. Due to the nature of the studies included within this review, no serious indirectness was detected; all outcomes were measured directly without the use of a surrogate. Publication bias was not detected, and due to the number of studies included, it was not possible to produce funnel plots for any outcomes. Although publication bias was "not detected", it is difficult to conclude that there was a complete absence of bias since studies with significant results are more likely to be published than those reporting null or non-significant results[25] Published, peer-reviewed articles were included in this review, since the Cochrane Handbook for Systematic Reviews of Interventions further suggests that the inclusion of unpublished studies may introduce additional bias, as these studies have not been strengthened by the peer-review process and may be of lower methodological quality[25]. Reasons for downgrading the quality of evidence included serious inconsistency due to substantial heterogeneity, and serious imprecision due to confidence intervals crossing the line of no effect.

Within group 2 studies, 5 outcomes were graded as high to moderate quality of evidence (SPPB, TUG, muscle strength of the lower limb, hand grip strength and BMD of the femoral neck). Remaining outcomes were graded as low or very low quality, meaning that one could have little or very little confidence in the effect estimate. Common reasons for downgrading outcomes included a combination of serious risk of bias (due to the inclusion of study[34]), serious imprecision or serious inconsistency.

Table 5: GRADE analysis of group 1 measurement outcomes included in the quantitative synthesis

Quality Assessment							Summary of Findings				
Outcome	Included studies (design)	ROB	Inconsistency	No serious Indirectness	Imprecision	Publication bias	Groups (Intervention /control)	Effect size (direction)	Significance	95% CI	Quality
Muscle strength (lower limb)	[21,31,32] (RCT)	No serious ROB	Serious inconsistency (substantial heterogeneity)	No serious indirectness	No serious imprecision	Undetected^	131/135	0.98 (Intervention)	$p<0.00001$	(0.73, 1.24)	⊕⊕⊕o Moderate
TUG	[21,32] (RCT)	No serious ROB	No serious inconsistency	No serious indirectness	Serious imprecision (CIs cross line of no effect/ OIS not reached)	Undetected^	124/125	0.37 (Intervention)	$p= 0.37$	(-0.68,0.26)	⊕⊕⊕o Moderate
BMD (Femoral neck)	[21,32] (RCT)	No serious ROB	No serious inconsistency	No serious indirectness	Serious imprecision (CIs cross line of no effect/ OIS not reached)	Undetected^	124/125	0.02 (Intervention)	$p= 0.15$	(-0.01,0.05)	⊕⊕⊕o Moderate
BMD (spine)	[21,32] (RCT)	No serious ROB	No serious inconsistency	No serious indirectness	Serious imprecision (CIs cross line of no effect/ OIS not reached)	Undetected^	124/125	0.02 (Intervention)	$p = 0.41$	(-0.03,0.07)	⊕⊕⊕o Moderate

*ROB: Risk of Bias; TUG: Timed Up and Go; RCT: Randomized Controlled Trial; CI: Confidence Interval; BMD: Bone Mineral Density; OIS: Optimum Information Size.
^Insufficient data to produce funnel plots. GRADE scoring: ⊕⊕⊕⊕ High; ⊕⊕⊕o Moderate; ⊕⊕oo Low; ⊕ooo Very low

Table 6: GRADE analysis of group 2 measurement outcomes included in the quantitative synthesis.

Quality Assessment							Summary of Findings				
Outcome	**Included studies (design)**	**ROB**	**Inconsistency**	**Indirectness**	**Imprecision**	**Publication bias**	**Groups (intervention/ control)**	**Effect size (direction)**	**Significance**	**95% CI**	**Quality**
SPPB	[32,33] (RCT)	No serious ROB	No serious inconsistency	No serious indirectness	No serious imprecision	Undetected^	45/46	1.09 (Intervention)	$p = 0.02$	(0.15,2.03)	⊕⊕⊕⊕ High
TUG	[21,32 (RCT)	No serious ROB	No serious inconsistency	No serious indirectness	No serious imprecision	Undetected^	124/126	-1.57 (Intervention)	$p = 0.001$	(-2.50, -0.64)	⊕⊕⊕⊕ High
Muscle strength (lower limb)	[21,32] (RCT)	No serious ROB	Serious inconsistency (substantial heterogeneity)	No serious indirectness	No serious imprecision	Undetected^	124/126	2.69 (Intervention)	$p = 0.002$	(0.96,4.42)	⊕⊕⊕o Moderate
Hand grip strength	[32,35] (RCT)	No serious ROB	No serious inconsistency	No serious indirectness	Serious imprecision (CI cross line of no effect, OIS not reached)	Undetected^	31/33	0.85 (Intervention)	$p = 0.55$	(-1.93,3.63)	⊕⊕⊕o Moderate
Weight	[32,34,35] (RCT)	Serious ROB ([4] was evaluated as high risk for incomplete outcome data)	No serious inconsistency	No serious indirectness	Serious imprecision (CI cross line of no effect, OIS not reached)	Undetected^	112/114	-0.12 (Intervention)	$p = 0.37$	(-0.38,0.14)	⊕⊕oo Low
Lean mass	[32,34] (RCT)	Serious ROB ([4] was evaluated as high risk for incomplete outcome data)	No serious inconsistency	No serious indirectness	Serious imprecision (CI cross line of no effect, OIS not reached)	Undetected^	103/105	0.02 (Intervention)	$p = 0.98$	(-1.31,1.35)	⊕⊕oo Low
Fat mass	[32,34] (RCT)	Serious ROB ([4] was evaluated as high risk for incomplete outcome data)	No serious inconsistency	No serious indirectness	Serious imprecision (CI cross line of no effect, OIS not reached)	Undetected^	103/105	-0.39 (Intervention)	$p = 0.76$	(-2.82, 2.05)	⊕⊕oo Low
BMD (femoral neck)	[21,32,34,35] (RCT)	Serious ROB ([4] was evaluated as high risk for incomplete outcome data)	No serious inconsistency	No serious indirectness	No serious imprecision	Undetected^	124/126	0.04 (Intervention)	$p = 0.002$	(0.01,0.06)	⊕⊕⊕o Moderate
BMD (spine)	[21,32,34,35] (RCT)	Serious ROB ([4] was evaluated as high risk for incomplete outcome data)	Serious inconsistency (substantial heterogeneity)	No serious indirectness	Serious imprecision (CI cross line of no effect, OIS not reached)	Undetected^	124/126	0.02 (Intervention)	$p = 0.24$	(-0.001,0.05)	⊕ooo Very low

^Insufficient data to produce funnel plots. GRADE scoring: ⊕⊕⊕⊕ High; ⊕⊕⊕o Moderate; ⊕⊕oo Low; ⊕ooo Very low

Results of individual studies and synthesis of results

Results of the 2 groups of studies are reported separately. Qualitative syntheses were conducted for studies with similar interventions and outcomes measures using RevMan 5.3 software. Study outcomes reporting results in the same units were pooled using a fixed-effect meta-analysis. Effect sizes are expressed as percentage mean differences or standardized mean differences (when outcomes were measured utilising different methods), with 95% confidence intervals. Higher weighting was assigned to studies with smaller standard deviations and a larger sample size[25]. Analyses were completed from extracted data; where necessary data were estimated from statistics or figures, or requested from the authors of the article. Heterogeneity was assessed via the chi squared test (Figures 2-14 and Tables 5-6). One article[36] was not included in any of the quantitative analyses since the exercise intervention modality was considered to be too dissimilar to compare with the other included articles. Within each group, there were outcomes unsuitable for quantitative synthesis, due to a lack of studies with common outcomes or aspects of studies too dissimilar for comparison; therefore, a narrative analysis was utilised.

Quantitative synthesis

Outcomes compared for group 1 included muscle strength of the lower limb, TUG and BMD of the femoral neck and spine (Figures 2-5). Only muscle strength of the lower limb was found to be significant, with a large effect size in favour of the intervention group (Figure 2. 0.98, 95% CI 0.73, 1.24. $p < 0.00001$).

Group 2 comparisons included the SPPB (Figure 6), TUG (Figure 7), muscle strength of the lower limb (Figure 8), hand grip strength (Figure 9), weight (Figure 10), lean mass (Figure 11), fat mass (Figure 12), BMD of the femoral neck (Figure 13) and spine (Figure 14). Of these outcomes, hand grip strength, weight, lean mass, fat mass and the BMD of the spine were found to be non-significant. However, SPPB score was more improved in the intervention group (1.09, 95% CI 0.15, 2.03. $p = 0.02$), with a significant and large effect. Similarly, TUG was significantly reduced within the intervention group (-1.57, 95% CI -2.50, -0.64. $p = 0.0010$). The results of the quantitative analysis also supported the combined intervention for muscle strength of the lower limb (2.69, 95% CI 0.95, 4.42). $p = 0.002$), and BMD of the femoral neck (0.04, 95% CI 0.01, 0.06. $p = 0.002$).

Qualitative synthesis

Referring to the narrative synthesis guidelines provided by the Cochrane Consumers and Communication Review Group[37], it was appropriate to apply 2 steps listed; developing a preliminary synthesis and exploring the relationships within and between studies. To develop a primary synthesis, results were systematically tabulated to identify patterns across studies (Tables 7-9). Exploring the relationships between and within studies for group 1, the control group in study[31]demonstrated a significant percentage increase in CSA of the quadriceps from baseline in comparison to the intervention group (+8.46% versus +4.94%, $p < 0.05$).

Table 7: Narrative analysis summary of findings for group 1 secondary outcome measures

Category	Outcome measure	Assessment point	Study	Intervention group % change from baseline			Control group % change from baseline		
				M	SD	N	M	SD	N
Body composition	CSA of quadriceps muscles (cm^2)	16 weeks	Agergaard et al., 2015[31]	+4.94	5.28	7	+8.46*	6.80	10

Group 1 studies compared vitamin D3 supplementation and exercise training versus exercise alone

Comparing primary outcomes for group 2, the percentage increase in isometric knee extensor strength for study[36] was greater in the intervention group (+3.01% versus +0.11%), although not statistically significant. Muscle power was compared in studies[33] and[34], expressed as sit-to-stand transfer power and functional stair climbing muscle power respectively. Both studies reported a significant percentage increase in muscle power within the intervention groups, and smaller, non-significant increases within the control groups (sit-to-stand transfer power intervention group +8.00% versus +2.61%, $p = 0.017$; functional stair climbing muscle power intervention group +10.51% versus +7.32%, $p < 0.05$).

The 30 second sit-to-stand test showed significant favourable results for the combined intervention of exercise and vitamin D3 (+10.40% versus +6.20%, $p<0.05$). Within study[21], normal walking speed declined in both groups and the 5-time chair stand time was improved non-significantly in both groups. The 12-minute walk test in study[32] was further improved within the control group, although this did not achieve statistical significance. The four-square step test, body sway and backwards walking were significantly more improved in the intervention groups. Only Romberg ratio showed the greatest improvement within the control group; Romberg ratio was decreased in comparison with the intervention group, although the results were non-significant (+2.8% versus -0.60%).

Table 8: Narrative analysis summary of findings for group 2 primary outcome measures

Category	Outcome	Assessment point	Study	Intervention group % change from baseline			Control group % change from baseline		
				M	SD	N	M	SD	N
Muscle strength	Isometric knee extensor strength (Nm)	6 months	Verschueren et al., 2011[36]	+3.01	2.67	28	+0.11	3.18	28
Muscle power	Sit-to-stand transfer power (W)	12 weeks	Drey et al., 2011[33]	+8.99*	5.51	23	+2.61	2.49	22
	Functional stair climbing muscle power (W)	12 months	Gianoudis et al., 2014[34]	+10.40*	13.00	81	+6.20	12.70	81
Muscle function	30 second sit-to-stand (n.stands)	12 months	Gianoudis et al., 2014[34]	+18.30*	23.60	81	+2.70	17.2	81
	5-time chair stand time (s)	24 months	Uusi-Rasi et al., 2015[21]	-6.95	2.50	102	-3.49	3.30	102
	Normal walking speed (m/s)	24 months	Uusi-Rasi et al., 2015[21]	-1.80	0.20	102	-3.30	0.21	102
	Endurance: 12-minute walk (m)	9 months	Bunout et al., 2006[32]	+8.80	17.60	22	+20.90	27.70	24
Balance	Romberg ratio (%)	9 months	Bunout et al., 2006[32]	+2.80	33.80	22	-0.60	35.80	24
	Four square step test (s)	12 months	Gianoudis et al., 2014[34]	-12.00*	14.10	81	-5.20	14.90	81
	Body sway (cm)	32 weeks	Jessup et al., 2003[35]	-26.39*	0.52	9	+2.90	0.49	9
	Backwards walking (% able to complete)	24 months	Uusi-Rasi et al., 2015[21]	+25.47*	13.59	102	+9.48	15.58	102

Group 2 compared vitamin D3 supplementation and exercise training versus vitamin D3 supplementation alone

For group 2 secondary outcomes, small and non-significant gains in appendicular lean mass were demonstrated in the intervention group of study[33]. In study[36], muscle mass of the upper limb decreased non-significantly in both the intervention and control groups, although to a lesser extent in the intervention group. BMD of the femoral neck was gained in both groups, although by a higher percentage in the control group; both trends were non-significant.

Category	Outcome measure	Assessment point	Study	Intervention group % change from baseline			Control group % change from baseline		
				M	SD	N	M	SD	N
Body composition	Appendicular lean mass (Kg)	12 weeks	Drey et al., 2011[33]	+1.65	0.71	23	+0.00	0.87	22
	Muscle mass of upper limb (cm^3)	6 months	Verschueren et al., 2011[36]	-0.16	0.57	28	-0.25	0.38	28
	BMD of femoral neck (g/cm^2)	6 months	Verschueren et al., 2011[36]	+0.71	0.42	28	+0.99	0.51	28

Table 9: Narrative analysis summary of findings for group 2 secondary outcomes

Group 2 compared vitamin D3 supplementation and exercise training versus vitamin D3 supplementation alone

In summary, meta-analyses for group 1 found muscle strength of the lower limb to be significantly improved within the intervention group (0.98, 95% CI 0.73, 1.24, p<0.001). All other outcomes showed small but non-significant positive effects for the intervention group. The SPPB, TUG, muscle strength of the lower limb and femoral neck BMD all showed significantly greater improvements in the intervention group for group 2 comparisons.

The narrative analysis revealed significant differences in body composition, muscle power, muscle function and balance. A significant percentage increase in quadriceps CSA was observed in the control group of study[31]. The combined intervention of RET and vitamin D3 supplementation resulted in a greater percentage increase in muscle strength and power, and a greater improvement in the 30 second sit-to-stand test, the four-square step test, body sway and backwards walking. However, vitamin D3 supplementation alone resulted in a greater improvement in the 12-minute walk test and Romberg ratio.

DISCUSSION

The aim of this systematic review was to assess the combined effect of RET and vitamin D3 supplementation on musculoskeletal health in older adults. Only 7 studies were eligible for inclusion, with a total of 792 participants, highlighting the lack of available literature on the topic. Studies were categorised into 2 groups; studies in which all participants took part in RET and the intervention group was supplemented with vitamin D3, or studies in which all participants were supplemented with vitamin D3 and the intervention group took part in RET. 2 studies were categorized into both group 1 and group 2.

Quantitative analysis

Data analysis conducted for this review included meta-analyses and narrative reviews. Meta-analyses for group 1 included muscle strength of the lower limb, TUG and BMD of both the femoral neck and spine. Evidence of additional benefit was shown for all outcomes within the intervention group; however, the effect size was small and non-significant for TUG and BMD of the femoral neck and spine. Muscle strength of the lower limb was the only significant outcome of group 1, with a large effect size observed within the intervention group (0.98, 95% CI 0.73, 1.24. p<0.00001). Although numerous studies have demonstrated the beneficial effect of RET on muscle strength in older adults[3-5], this result provides evidence that vitamin D3 supplementation may enhance these effects in older adults. Skeletal muscle myopathies associated with vitamin D deficiency are well documented[38], and symptoms of significant muscle weakness are reversed with treatment of the deficiency[39]. A systematic review and meta-analysis reported a gain in lower extremity strength with vitamin D supplementation only in vitamin D deficient older adults; no effect was observed in replete adults[22]. Similarly, no effect of vitamin D3 supplementation on isometric quadriceps strength was demonstrated after 6 months in vitamin D replete older adults[40]. Interestingly, although the studies included within group 1[21,31,32] did not specify serum 25(OH)D levels as inclusion/exclusion criteria, baseline and post-intervention serum 25(OH)D were within the 'sufficient' range (>30nmol.L^{-1}). A greater increase of muscle strength in replete older adults represents a novel finding of this review. Preliminary support for combined vitamin D supplementation and RET was demonstrated in a 3-month longitudinal study examining the effect of serum 25(OH)D and exercise training on functional performance in older men and women aged 65 years and over. No significant improvements in function were reported in participants with lower serum 25(OH)D (<47.5 nmol.L^{-1}), however higher serum 25(OH)D (>67.5 nmol.L^{-1}) was associated with greatest improvements in functionality and muscle strength[41].

This finding must be considered within the context of the risk of bias and GRADE analyses. The risk of bias analysis showed an overall unclear risk of bias for the included studies, and the GRADE analysis concluded that the evidenced was of moderate quality; however, serious inconsistency due to moderate heterogeneity (I^2 = 70%) was detected. This heterogeneity may have been due to the differing duration of interventions (12 weeks

to 24 months), differences between measurement methodologies, differences between exercise regimens (although all adopted progressive RET), doses of vitamin D3 (400 IU to 1920 IU per day), or may indicate that these studies were unsuitable for comparison.

Significant effects for the SPPB, TUG, muscle strength of the lower limb, and the BMD of the femoral neck were observed within the intervention groups of group 2 studies; unsurprisingly, RET was found to have a positive influence. In a recent systematic review and meta-analysis, exercise significantly increased SPPB score and decreased TUG time, with large effect sizes (1.87 and -2.47 respectively[42]); similar results are reported within this review. Vitamin D is a regulator of BMD, proliferating calcium and phosphate absorption in the intestine and acting directly on bone cells[43]. Vitamin D has previously been shown to influence BMD, fracture rate and risk[44]; studies of patients who have sustained a hip fracture typically demonstrated low serum vitamin D (≤30.0 $nmol.L^{-1}$;[45]). Supplementation of vitamin D and calcium has been shown to significantly decrease the rate of bone loss in the hip and spine[46]. GRADE analyses for these outcomes concluded the quality of evidence to be high (SPPB and TUG) or moderate (muscle strength of the lower limb and BMD of the femoral neck).

Closer examination of the control groups within significant outcomes for group 2 was undertaken to evaluate the effect of vitamin D3 supplementation alone. Intriguingly, although the intervention groups (RET and vitamin D3 supplementation) showed evidence of benefit in number of outcomes, the control groups (vitamin D3 supplementation alone) showed mixed, or even negative impacts on the same outcomes. SPPB score was decreased post-intervention compared with baseline by 0.30% and 0.50% in the control groups of studies[32] and[33] respectively. Muscle strength of the lower limb and BMD of the femoral neck showed mixed results for the intervention groups, with some studies reporting small increases and others reporting small losses (non-significant). Previous reports of the effect of vitamin D supplementation on muscle strength and physical functioning are mixed; the InCHIANTI study of people aged 65 years or over reported a significant association between serum 25(OH)D <25$nmol.L^{-1}$ and SPPB score[47]. Similarly, a large prospective cohort of older adults aged 65 years or over found those with low (<25$nmol.L^{-1}$) 25(OH)D were significantly more likely to experience losses in grip strength and higher rates of appendicular lean mass loss compared to those with higher (>50 $nmol.L^{-1}$) 25(OH)D[23]. Conversely, another large, prospective study found no association between serum 25(OH)D, walking speed and time for repeated chair stands[48]. The TUG test time increased in all groups of study [32], and was significantly increased in the vitamin D without exercise group in study p=0.01) [21]. Again, participants included in studies[32] and[21] had sufficient serum 25(OH)D levels, indicating that supplementation in replete older adults may not confer additional benefits to neuromuscular function unless combined with exercise.

Narrative analysis

Studies in group 1[21,31,32] had few body composition outcomes in common, therefore a narrative analysis was conducted. The CSA of the quadriceps was analysed within study[31], and results showed that although the intervention group did experience a +4.94%, increase from baseline, the control group (not supplemented with vitamin D3) actually showed a significantly higher increase in quadriceps CSA (+8.46%, $p<0.05$).

These results do not provide evidence for the additive effects of combined exercise training and vitamin D3. Other study groups have reported changes in muscle CSA consequent to RET which are both smaller[8,49] and comparable[50] to those reported in study[31]. Interestingly, study[31] also assessed "muscle quality" (muscle strength/CSA); although non-significant, the intervention group improved their muscle quality to a greater degree than the control group (+9.61% versus +0.66% change from baseline), indicating an increased functionality of the muscle to produce force; conceptually more relevant in combatting the effects of sarcopenia than muscle size and strength alone[51].

Results of the narrative analysis for group 2 showed that the combined intervention of RET and vitamin D3 supplementation was significantly more beneficial than vitamin D3 supplementation alone for sit-to-stand transfer power, functional stair climbing muscle power, 30 second sit-to-stand, 5-time chair stand, the four-square step test, body sway and backwards walking. Only body sway was negatively affected by vitamin D3 supplementation, although the within group change was non-significant. Other outcomes of interest included normal walking speed, which deteriorated in both groups, the distance walked in 12 minutes and Romberg ratio, in which the control groups made the most improvement, although not significantly.

Limitations

Few published studies were eligible for inclusion within this review, although this serves to highlight the knowledge gap with respect to this topic. The inclusion of a high-risk study was deemed necessary due to the lack of available literature, although this had a negative effect on the perceived quality of evidence for the outcomes in which it was reported. Generally, outcome measure data could be graded as representing moderate quality, although there were several outcome measures graded as low or very low quality, due to the high variability of participant numbers, duration of interventions, exercise methodologies or differing vitamin D3 doses and period of supplementation employed within the studies. Furthermore, data produced from meta-analyses including study[21] may have been skewed due to the high weighting assigned for this study as a result of the large number of participants recruited.

Of the individual studies included within this review, none reported inclusion/exclusion criterion for vitamin D status, and although at baseline serum vitamin D was not significantly different between the groups in 5 studies[21,31-33,36], 2 studies reported no data for serum vitamin D pre or post-intervention[34,35].

Additionally, analysis methods used within 5 studies included did not account for confounding factors[31-34,36], and participants were not stratified on the basis of any characteristics in 3 studies[21,31,35], although these were single-sex studies. Unfortunately, several outcome measures were unsuitable for inclusion within the qualitative analysis due to differing measurement methodologies utilised or too few outcome measures in common. A recent systematic review and meta-analysis investigating the effects of vitamin D on neuromuscular remodelling following exercise or injury similarly found few eligible studies and high levels of heterogeneity due to methodological differences, resulting in the authors to suggest more high quality evidence is needed to reach a result that is conclusive[52].

CONCLUSION

This review provides tentative support for the additive effect of combined RET and vitamin D3 supplementation for the improvement of muscle strength in older adults. For other aspects of musculoskeletal function, such as SPPB and TUG, no additional benefit beyond that gained from exercise training was found. This review showed no evidence of benefit of vitamin D3 supplementation alone, however, few studies were identified during the literature search, highlighting that further evidence is required to draw any firm conclusions or make explicit recommendations regarding vitamin D3 supplementation for musculoskeletal health and function in older adults.

Our recommendations to enable future studies to definitively answer questions regarding the additive effects of the combined vitamin D3 supplementation and RET include; common outcomes relevant to the condition studied, for example the SPPB, 400m walk and gait speed are recommended to assess physical performance[53], which would allow for a more detailed assessment of results. Additionally, exercise interventions of similar durations would allow for a more accurate comparison between studies; it has been suggested that interventions with older adults should be of a minimum duration of 3 months to obtain significant differences in relevant outcomes[53]. Reporting of confounding factors would allow for adjustment of results via the use of covariates; for example, objective measures of physical activity using accelerometers, baseline serum vitamin D3 status and participant characteristics, which may bias the participant pool. Separate analysis of male and female participants, or the addition of sex as a covariate in any analysis models would help to address sex-related differences in performance. Regarding study design, four-armed RCT studies are best placed to answer combined effects research questions; i.e. exercise intervention, vitamin D intervention, both exercise and vitamin D, neither exercise nor vitamin D (true control). A true control group was lacking from a number of the included studies within this review.

ACKNOWLEDGEMENTS

We thank Lynn Harris (LH) for her help formulating the search strategy, Asma Alrushud (AA) for her help with data extraction and the National Osteoporosis Society for supporting Anneka Antoniak (AEA).

FOOTNOTES

Contributors AEA has planned, conducted and written the report for this study. CAG has been involved in all stages, particularly in critically reviewing and approving the final draft of the report. AA was involved in the search for literature and data extraction stage. LH assisted in formulating the search strategy.

Funding This research received no grant from any funding agency in the public, commercial or not-for-profit sectors. AEA is supported and funded by the National Osteoporosis Society via the Linda Edwards Memorial PhD Studentship.

Competing interests None declared.

Provenance and peer review Not commissioned; externally peer reviewed.

Data sharing statement This publication is supported by multiple datasets, which are openly available at locations cited in the reference section. Additional data for this article have been provided as supplementary files. There is no additional unpublished data.

REFERENCES

1. Rosenberg IH. Summary comments. *The American journal of clinical nutrition* 1989;50(5):1231-33.
2. Cruz-Jentoft AJ, Baeyens JP, Bauer JM, et al. Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age and Ageing* 2010 doi: 10.1093/ageing/afq034
3. Kosek DJ, Kim J-s, Petrella JK, et al. Efficacy of 3 days/wk resistance training on myofiber hypertrophy and myogenic mechanisms in young vs. older adults. *Journal of applied physiology* 2006;101(2):531-44.
4. Henwood TR, Taaffe DR. Improved physical performance in older adults undertaking a short-term programme of high-velocity resistance training. *Gerontology* 2005;51(2):108-15.
5. Stewart V, Saunders D, Greig C. Responsiveness of muscle size and strength to physical training in very elderly people: a systematic review. *Scandinavian journal of medicine & science in sports* 2014;24(1):e1-e10.
6. Kumar V, Selby A, Rankin D, et al. Age-related differences in the dose–response relationship of muscle protein synthesis to resistance exercise in young and old men. *The Journal of physiology* 2009;587(1):211-17.
7. Greig C, Gray C, Rankin D, et al. Blunting of adaptive responses to resistance exercise training in women over 75y. *Experimental gerontology* 2011;46(11):884-90.
8. Häkkinen K, Kallinen M, Izquierdo M, et al. Changes in agonist-antagonist EMG, muscle CSA, and force during strength training in middle-aged and older people. *Journal of Applied Physiology* 1998;84(4):1341-49.
9. Raue U, Slivka D, Minchev K, et al. Improvements in whole muscle and myocellular function are limited with high-intensity resistance training in octogenarian women. *Journal of applied physiology* 2009;106(5):1611-17.
10. Welle S, Totterman S, Thornton C. Effect of age on muscle hypertrophy induced by resistance training. *J Gerontol A Biol Sci Med Sci* 1996;51 doi: 10.1093/gerona/51A.6.M270
11. Hameed M, Orrell R, Cobbold M, et al. Expression of IGF-I splice variants in young and old human skeletal muscle after high resistance exercise. *The Journal of physiology* 2003;547(1):247-54.
12. Roubenoff R. Sarcopenia: a major modifiable cause of frailty in the elderly. *The journal of nutrition, health & aging* 1999;4(3):140-42.
13. Janssen I, Heymsfield SB, Ross R. Low relative skeletal muscle mass (sarcopenia) in older persons is associated with functional impairment and physical disability. *Journal of the American Geriatrics Society* 2002;50(5):889-96.
14. Visser M, Kritchevsky SB, Goodpaster BH, et al. Leg muscle mass and composition in relation to lower extremity performance in men and women aged 70 to 79: the health, aging and body composition study. *Journal of the American Geriatrics Society* 2002;50(5):897-904.
15. Landi F, Liperoti R, Russo A, et al. Sarcopenia as a risk factor for falls in elderly individuals: results from the ilSIRENTE study. *Clinical nutrition* 2012;31(5):652-58.
16. Sørensen O, Lund B, Saltin B, et al. Myopathy in bone loss of ageing: improvement by treatment with 1α-hydroxycholecalciferol and calcium. *Clinical Science* 1979;56(2):157-61.
17. Sato Y, Iwamoto J, Kanoko T, et al. Low-dose vitamin D prevents muscular atrophy and reduces falls and hip fractures in women after stroke: a randomized controlled trial. *Cerebrovasc Dis* 2005;20 doi: 10.1159/000087203
18. Ceglia L, Niramitmahapanya S, Silva MM, et al. A randomized study on the effect of vitamin D supplementation on skeletal muscle morphology and vitamin D receptor concentration in older women. *J Clin Endocrinol Metab* 2013;98 doi: 10.1210/jc.2013-2820

19. Latham NK, Anderson CS, Lee A, et al. A randomized, controlled trial of quadriceps resistance exercise and vitamin D in frail older people: the Frailty Interventions Trial in Elderly Subjects (FITNESS). *J Am Geriatr Soc* 2003;51 doi: 10.1046/j.1532-5415.2003.51101.x
20. Lips P, Binkley N, Pfeifer M, et al. Once-weekly dose of 8400 IU vitamin D3 compared with placebo: effects on neuromuscular function and tolerability in older adults with vitamin D insufficiency. *The American journal of clinical nutrition* 2010;91(4):985-91.
21. Uusi-Rasi K, Patil R, Karinkanta S, et al. Exercise and vitamin D in fall prevention among older women: a randomized clinical trial. *JAMA internal medicine* 2015;175(5):703-11.
22. Stockton K, Mengersen K, Paratz JD, et al. Effect of vitamin D supplementation on muscle strength: a systematic review and meta-analysis. *Osteoporosis international* 2011;22(3):859-71.
23. Visser M, Deeg DJ, Lips P. Low vitamin D and high parathyroid hormone levels as determinants of loss of muscle strength and muscle mass (sarcopenia): the Longitudinal Aging Study Amsterdam. *The Journal of Clinical Endocrinology & Metabolism* 2003;88(12):5766-72.
24. Robinson S, Cooper C, Aihie Sayer A. Nutrition and sarcopenia: a review of the evidence and implications for preventive strategies. *Journal of aging research* 2012;2012
25. Higgins JPT, Green S, editors. *Cochrane Handbook for Systematic Reviews of Interventions Version 5.1.0 [updated March 2011]*: The Cochrane Collarboration, 2011.
26. Moher D, Liberati A, Tetzlaff J, et al. Preferred reporting items for systematic reviews and meta-analyses: the PRISMA statement. *Annals of internal medicine* 2009;151(4):264-69.
27. Hsu LM, Field R. Interrater agreement measures: Comments on Kappan, Cohen's Kappa, Scott's π, and Aickin's α. *Understanding Statistics* 2003;2(3):205-19.
28. Moher D, Olkin I. Meta-analysis of randomized controlled trials: a concern for standards. *JAMA* 1995;274(24):1962-64.
29. Moher D, Altman D, Liberati A, et al. PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses). *The Cochrane Collaboration Methods Groups Newsletter* 1996:6.
30. Schünemann H, Brozek J, Oxman A. GRADE handbook for grading quality of evidence and strength of recommendation. Version 3.2 [updated March 2009]. The GRADE Working Group, 2009. *www cc-i ms net/gradepro* 2009
31. Agergaard J, Trøstrup J, Uth J, et al. Does vitamin-D intake during resistance training improve the skeletal muscle hypertrophic and strength response in young and elderly men? – a randomized controlled trial. *Nutrition & Metabolism* 2015;12(1):1-14. doi: 10.1186/s12986-015-0029-y
32. Bunout D, Barrera G, Leiva L, et al. Effects of vitamin D supplementation and exercise training on physical performance in Chilean vitamin D deficient elderly subjects. *Exp Gerontol* 2006;41 doi: 10.1016/j.exger.2006.05.001
33. Drey M, Zech A, Freiberger E, et al. Effects of strength training versus power training on physical performance in prefrail community-dwelling older adults. *Gerontology* 2011;58(3):197-204.
34. Gianoudis J, Bailey CA, Ebeling PR, et al. Effects of a Targeted Multimodal Exercise Program Incorporating High-Speed Power Training on Falls and Fracture Risk Factors in Older Adults: A Community-Based Randomized Controlled Trial. *Journal of Bone and Mineral Research* 2014;29(1):182-91. doi: 10.1002/jbmr.2014
35. Jessup JV, Horne C, Vishen R, et al. Effects of exercise on bone density, balance, and self-efficacy in older women. *Biological Research for nursing* 2003;4(3):171-80.
36. Verschueren SM, Bogaerts A, Delecluse C, et al. The effects of whole-body vibration training and vitamin D supplementation on muscle strength, muscle mass, and bone density in institutionalized elderly women: A 6-month randomized, controlled trial. *Journal of bone and mineral research* 2011;26(1):42-49.
37. Ryan.R. 'Cochrane Consumers and Communication Review Group: data synthesis and analysis': Cochrane Consumers and Communication Review Group; 2013 [Available from: http://cccrg.cochrane.org accessed July 2016.

38. SMITH R, STERN G. Myopathy, osteomalacia and hyperparathyroidism. *Brain* 1967;90:593-602.
39. Glerup H, Mikkelsen K, Poulsen L, et al. Commonly recommended daily intake of vitamin D is not sufficient if sunlight exposure is limited. *Journal of internal medicine* 2000;247(2):260-68.
40. Grady D, Halloran B, Cummings S, et al. 1, 25-Dihydroxyvitamin D3 and Muscle Strength in the Elderly: A Randomized Controlled Trial*. *The Journal of Clinical Endocrinology & Metabolism* 1991;73(5):1111-17.
41. Okuno J, Tomura S, Fukasaku T, et al. [Examination of effects of alfacalcidol vitamin D supplement and renal function on improvement in the physical fitness of pre-frail elderly persons attending a nursing care prevention class]. *Nihon Ronen Igakkai zasshi Japanese journal of geriatrics* 2010;48(6):691-98.
42. Giné-Garriga M, Roqué-Fíguls M, Coll-Planas L, et al. Physical exercise interventions for improving performance-based measures of physical function in community-dwelling, frail older adults: a systematic review and meta-analysis. *Archives of physical medicine and rehabilitation* 2014;95(4):753-69. e3.
43. Lips P, Hosking D, Lippuner K, et al. The prevalence of vitamin D inadequacy amongst women with osteoporosis: an international epidemiological investigation. *Journal of internal medicine* 2006;260(3):245-54.
44. Brincat M, Gambin J, Brincat M, et al. The role of vitamin D in osteoporosis. *Maturitas* 2015;80(3):329-32. doi: 10.1016/j.maturitas.2014.12.018
45. LeBoff MS, Kohlmeier L, Hurwitz S, et al. Occult vitamin D deficiency in postmenopausal US women with acute hip fracture. *Jama* 1999;281(16):1505-11.
46. Tang BM, Eslick GD, Nowson C, et al. Use of calcium or calcium in combination with vitamin D supplementation to prevent fractures and bone loss in people aged 50 years and older: a meta-analysis. *The Lancet* 2007;370(9588):657-66.
47. Houston DK, Cesari M, Ferrucci L, et al. Association between vitamin D status and physical performance: the InCHIANTI study. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* 2007;62(4):440-46.
48. Verreault R, Semba RD, Volpato S, et al. Low serum vitamin D does not predict new disability or loss of muscle strength in older women. *Journal of the American Geriatrics Society* 2002;50(5):912-17.
49. Trappe S, Williamson D, Godard M. Maintenance of whole muscle strength and size following resistance training in older men. *The Journals of Gerontology Series A: Biological Sciences and Medical Sciences* 2002;57(4):B138-B43.
50. Ferri A, Scaglioni G, Pousson M, et al. Strength and power changes of the human plantar flexors and knee extensors in response to resistance training in old age. *Acta Physiol Scand* 2003;177 doi: 10.1046/j.1365-201X.2003.01050.x
51. Fragala MS, Kenny AM, Kuchel GA. Muscle Quality in Aging: a Multi-Dimensional Approach to Muscle Functioning with Applications for Treatment. *Sports Medicine* 2015;45(5):641-58. doi: 10.1007/s40279-015-0305-z
52. Minshull C, Biant LC, Ralston SH, et al. A Systematic Review of the Role of Vitamin D on Neuromuscular Remodelling Following Exercise and Injury. *Calcified tissue international.* 2016; 1;98(5):426-37.
53. Cruz-Jentoft AJ, Landi F, Schneider SM, et al. Prevalence of and interventions for sarcopenia in ageing adults: a systematic review. Report of the International Sarcopenia Initiative (EWGSOP and IWGS). *Age and ageing* 2014;43(6):748-59.

1

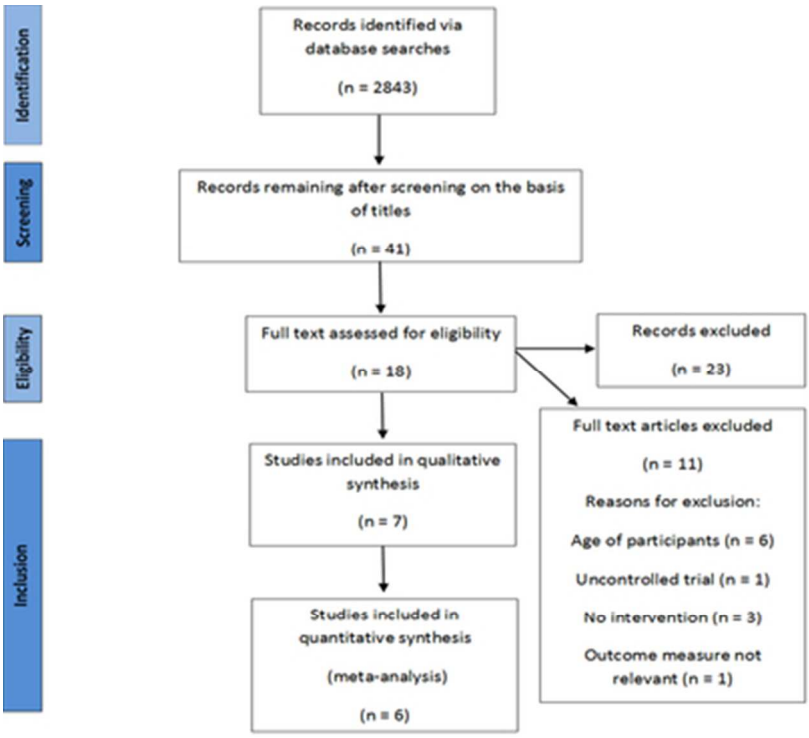

Figure 1: Study flow chart

34x30mm (300 x 300 DPI)

Group 1 analysis of muscle strength of the lower limb

11x2mm (300 x 300 DPI)

Group 1 analysis of the TUG test

10x1mm (300 x 300 DPI)

Group 1 analysis of BMD of the femoral neck

10x1mm (300 x 300 DPI)

Group 1 analysis of BMD of the spine

10x1mm (300 x 300 DPI)

Group 2 analysis of the SPPB test

10x2mm (300 x 300 DPI)

Group 2 analysis of the TUG test

10x1mm (300 x 300 DPI)

Group 2 analysis of muscle strength of the lower limb

10x1mm (300 x 300 DPI)

Group 2 analysis of hand grip strength

10x1mm (300 x 300 DPI)

Group 2 analysis of total body weight

11x2mm (300 x 300 DPI)

Group 2 analysis of lean mass

10x1mm (300 x 300 DPI)

Group 2 analysis of fat mass

10x1mm (300 x 300 DPI)

Group 2 analysis of BMD of the femoral neck

12x2mm (300 x 300 DPI)

Group 2 analysis of BMD of the spine

12x2mm (300 x 300 DPI)

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	1
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	2
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	3
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	3
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	4
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	4
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	4
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	5
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	4
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	6
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	6
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	6
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	15
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I^2) for each meta-analysis.	15

PRISMA 2009 Checklist

Section/topic	#	Checklist item	Reported on page #
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	11
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	12-18
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	Image 1
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	6-7
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	11
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	Image 2-3
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	11
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	12-18
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	19
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	21-22
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	22
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	23

From: Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. PLoS Med 6(7): e1000097. doi:10.1371/journal.pmed100009