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Title page

Review: Supplemental protein from dairy products increases body weight and vitamin D improves physical performance in older adults: a systematic review and meta-analysis.

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Abbreviation list

BW	body weight
EAA	essential amino acids
FFM	fat-free mass
FM	fat mass
HGS	hand grip strength
IU	international units
LBM	lean body mass
LM	lean mass
SMD	standard mean difference
TUG	timed up and go

Abstract

The purpose of this systematic review and meta-analysis was to assess the effectiveness of dairy components on nutritional status and physical fitness in older adults, as evidence for efficacy of the supplementation of these components is inconclusive. Scopus and MEDLINE were searched. Main inclusion criteria for articles were: double-blind, randomized placebo-controlled trials including participants aged ≥55 years who received dairy components or a placebo. Outcome measures were nutrient status (body weight and BMI) and physical fitness (body composition, muscle strength, and physical performance). Thirty-six trials with 4,947participants were included. Most trials investigated protein and vitamin D supplementation and showed no effect on the outcomes. Meta-analysis on the effect of protein on body weight showed a significant increase in mean difference (MD) of 1.13 kg (95% CI: 0.59 to 1.67). This effect increased by selecting trials with study a duration of six months in which less nourished and physically fit participants were included. Trials where the participants were (pre-) frail, inactive older adults or when supplementing ≥20 grams of protein/day, tended to increase Lean Body Mass (LBM). Only small significant effects of vitamin D supplementation on Timed Up and GO (TUG) (MD -0.75 seconds; 95% CI: –1.44 to -0.07) were determined. This effect increased when vitamin D doses ranged between 400-1000 IU. Additional large RCT trials of ≥six months are needed regarding the effect of dairy components containing an adequate amount of vitamin D (400-1000 IU) and/or protein (≥20 grams) on nutritional status and physical fitness in malnourished or frail older adults.

Keywords

Aged, Protein, Vitamin D, nutritional status, Physical fitness

1. Introduction

Malnutrition in older adults is considered to be a serious complication of illness and is associated with increased risks of mortality, longer hospital stays, frequent readmissions [1], and a lower quality of life [2]. Malnutrition can result from starvation, disease, or advanced ageing (e.g. >80 years) and can be experienced alone or in a combination [3]. Moreover, malnutrition can influence the physical fitness, that exists out of body composition, muscle function, and physical function [4]. A decrease in muscle and physical function can pose difficulties in daily activities such as eating [5]. The comprehensive healthcare costs for malnourished institutionalized and community-dwelling older adults in Europe are considerably higher than for well-nourished older adults [6]. Therefore, prevention of malnutrition in the elderly population is an important strategy for maintaining their quality of life and saving on these costs.

Older adults are among the high risk groups for malnutrition [7] for at least two reasons. First, they may have decreased food intake due to “anorexia of ageing”, i.e., a physiologic loss of appetite [8,9] or nutrition impact symptoms caused by illness or medical treatment. For example, difficulties with chewing and swallowing, pain, alterations in taste [10], medication that decreases appetite or increases nutrient losses, polypharmacy, dependency on help for consuming meals, and decreased thirst response [11,12] are frequently reported. Second, older adults are more prone to the onset of chronic disease which can activate chronic inflammation [13,14] and consequently result in loss of muscle mass.

For older adults, protein requirements can be increased in various situations. For example, when a loss of muscle mass occurs, a high intake of protein is necessary to stimulate muscle synthesis [15–17]. In addition to increased protein require-

ments, energy requirements may be decreased in older adults mainly due to diminished physical activity and decreased basal metabolic rate during ageing. Nutrient requirements may also possibly increase for older adults [18] such as, discussed for vitamin D [19]. Therefore, this population experiencing unintended weight or appetite loss have an increased risk for nutrient deficiencies.

Dairy products can contribute to satisfying the macronutrient and micronutrient insufficiencies in the diets of older adults. Various dairy components have been shown to enhance maintenance of bone mass and muscle mass [20–22]. Studies supporting the beneficial effects of milk or dairy products on bone health indicate a significant inverse association between dairy food intake and bone turnover markers and a positive association with bone mineral content [23]. Qualitative evidence is also available for the role of vitamin D and dairy consumption in promoting maximal bone health from childhood through young to late adolescence [24]. In addition to appropriate dietary calcium intake, sufficient serum vitamin D levels are important for skeletal health [25,26]. Vitamin D also stimulates gene expression by involvement in cell development, differentiation, and growth as well as stimulating muscle protein synthesis, and improving strength and balance [27,28].

Milk protein consists of whey with a high amount of leucine which has been suggested as being responsible for the enhancing ability to stimulate muscle protein anabolism [20,21], which is confirmed by a meta-analysis when combined with physical activity [22]. According to the digestible indispensable amino acid score (DIAAS) of the Food and Agricultural Organization [29], dairy proteins score among the highest in quality even though the presence of leucine was not yet taken into account in this scoring [28]. In a statement from the European Society for Clinical and Economic Aspects of Osteoporosis and Osteoarthritis (ESCEO), the pathways through which

dietary protein influences muscle synthesis are either via the activation of mTOR and aromatic amino acids, or via an increase in serum IGF-I. IGF-I can directly affect both muscle synthesis, but also indirectly via vitamin D, as increases in serum IGF-I also stimulates the renal production of 1,25(OH)D3 (Fig. 1) [30–33]. Via the vitamin D receptors in muscle tissue, 1,25(OH)D3 contributes to improved balance and physical function. Supplementation has a greater effect on fall risk than on muscle strength [34]. In addition to containing protein, vitamin D, and calcium, (fortified) dairy products are rich in nutrients that are essential for good bone health including zinc, vitamin B12, potassium, and phosphorus [35].

Although some evidence for the role of dairy components in physical performance and bone health seems apparent, there is insufficient knowledge about the effect of dairy components on body composition and muscle strength in older adults. One review states that dairy component protein has a positive effect on muscle mass and strength and that low levels of 25(OH)D are associated with low muscle performance and postural instability. Additionally, it is stated that the supply of both vitamin D and dietary protein could have a beneficial effect on muscle mass and strength, but mechanisms of interaction remain unclear [30]. Another systematic review of two Randomized Controlled Trials (RCTs) about sarcopenic obese older adults found no effect from 15 gram of protein (through cheese consumption) and no effect from a high-speed circuit resistance training intervention [36]. To the best of our knowledge, there has been no systematic review and meta-analysis that investigated the effectiveness of dairy components on nutritional status and physical fitness in healthy older adults, much less in frail or malnourished older adults. Moreover, the quality of most studies is disputable due to the lack of comparison with a placebo and poor randomization, concealment of allocation, and blinding. Therefore, the purpose of

this systematic review and meta-analysis was: 1) to review medium-to-high quality trials in order to assess the effectiveness of dairy or dairy components on the nutritional status and physical fitness in older adults aged 55 years or older and 2) to identify the most effective treatment based on target groups, intervention duration, and doses, as a basis for future practical recommendations. Our hypothesis is that dairy or dairy components have a beneficial effect on nutritional status and physical fitness in older adults.

2. Approach

This systematic review and meta-analyses were performed in accordance to the Preferred Reporting Items for Systematic Reviews and Meta-analyses(PRISMA) guidelines [37].

2.1. Search strategy

Relevant studies were searched in the electronic databases of Scopus and PubMed until March 2, 2016. No limits were established for the date of publication and for PubMed, however, the filter humans was activated. A combination of Medical Subject Headings (MeSH) and Boolean operators were used. The most significant key terms that were employed included: aged AND Malnutrition AND “Dairy product*” OR Vitamin D* AND “Body weight” OR “physical perform*” OR Muscles.

2.2. Inclusion and exclusion criteria

Double-blind, randomized placebo-controlled trials were included when using at least one of the following components present in dairy as an intervention agent: protein,

amino acids, calcium, zinc, selenium, iodine, potassium, phosphorus, magnesium, vitamin B2, vitamin B3, vitamin B12, vitamin K, or vitamin D as supplementation in addition to the habitual intake. Sources of the intervention components were not restrictively dairy-based, however, they should be present in dairy as a component. Studies with a nutritional intervention in addition to exercise were also included. Studies based upon only dietary advice were excluded. The control group was required to receive a placebo tablet or capsule or a 'regular' food product that was compared to the intervention substance. Studies that included 'usual practice' instead of a placebo in the control group were excluded. Furthermore, studies of men and women aged 55 years and older who did not have having any type of kidney dysfunction requiring adherence to a protein restricted diet were included in this systematic review and meta-analysis. All inclusion and exclusion criteria are indicated in Table 1.

2.3. Article eligibility

The title and abstract of every record that was retrieved based on a selection of relevant keywords were screened for relevance and eligibility by one reviewer. In addition, reference lists of review articles and articles that underwent full-text selection were screened for other relevant articles. Full-text articles were further assessed for eligibility based on the inclusion and exclusion criteria by two reviewers independently. In the event of uncertainty regarding eligibility of the article or unclear results due to incomplete information, the authors of the article were contacted for additional information. If no clarification was provided after the contact, the trial was excluded

from further review, however, when the author provided data, the meta-analysis was adjusted accordingly.

2.4. Data extraction

Two reviewers extracted the data of the eligible full text articles on the study characteristics, i.e., mean age, location of study, the amount, type, and duration of intervention, and outcome measurements. When the two reviewers did not agree on a decision, one of the co-authors was asked for an opinion. Final decisions were made by consensus based on the best argument.

2.5. Quality assessment

The quality of the methodology of the RCTs was also assessed by the two reviewers using the standardized assessment protocol from the Cochrane Collaboration with a score system [38]. A total of 11 criteria were assessed for each study in order to test the internal and external validity (Table 2). Per criteria, a maximum of three points and a minimum of one point could be scored where the maximum score indicated a low risk of bias. Trials that scored the highest points per criteria were selected for an additional meta-analysis that we refer to as a sensitivity analysis.

2.6. Primary outcome measures

The two main outcome measurements investigated in this systematic review were nutritional status including body weight and BMI and physical fitness, i.e., body com-

position, muscle strength, and physical performance. Physical performance includes combinations of walking speed, chair stand, TUG, and balance time [39–43].

2.7. Meta-, subgroup-, sensitivity analysis and funnel plots

Meta-, subgroup-, sensitivity analyses and funnel plots were performed when at least three studies per outcome measure were found. The data for meta-analyses were either directly extracted from the article as mean difference (MD) values with accompanied standard deviation for the intervention and control group or calculated separately for both by subtracting the post-test mean from the baseline mean. Standard deviations of the mean differences not provided by a report were estimated with the use of a within-subject correlation coefficient of 0.5 [44]. Robustness of this assumption was tested by applying sensitivity analyses on lower (0.3) and higher (0.8) correlation coefficients as well. Meta-analyses were performed in Reman (version 5.3), and data are reported as estimated pooled mean differences with 95% CIs. A p-value of 0.05 or smaller is considered significant for a treatment to be effective in changing the outcome. The sample size of participants was indicated by n while the number of included trials in the meta-analyses was defined as N. For the outcomes ‘physical performance’, ‘walking capacity’, and ‘leg strength’, the standardized mean difference (SMD) was used as the summary statistic in the meta-analyses. SMD was utilized instead of MD when all of the trials assessed the same outcome but measured it by different methods [44]. The SMD expresses the size of the intervention effect per trial relative to the variability observed in that trial [44]. All other results were reported as mean ± SD unless stated otherwise.

We did not restrict the literature search for gender, but recorded this variable as a pre-specified factor for subgroup analyses when applicable. In addition, sub-

group analyses were performed on study duration in months; composition of the intervention component; intervention dose; several target populations; and exercise training. Sensitivity analyses were conducted based on the risk of bias assessment by only including trials in the analysis that scored the highest points for each risk of bias on each assessment question, which indicates a low risk of bias.

3. Results

From an initial number of 3,475 articles identified in the search, 36 were included in the systematic review and, of these, 19 were suitable for meta-analyses (Fig. 2). In total, the 36 trials comprised a total number of 4,947 randomized participants ranging from 18 to 1,471 per trial (Table 3). Included trials were carried out in Australia, Brazil, Chili, Finland, Iceland, Ireland, Italy, Japan, the Netherlands, Spain, Sweden, Switzerland, and the USA. The trials were conducted in the following settings: various types of senior citizen care facilities (N=8), hospitals (N=1), fall clinics (N=1), outpatient clinics (N=1), or in the community (N=25).

A substantial variety of types of nutritional supplementation was employed in the trials selected for this systematic review. We did not locate any articles regarding the effect of cheese, ice cream, yoghurt, buttermilk, kefir, cultured milk products, selenium, iodine, vitamin B2, vitamin B12, phosphorus, potassium, magnesium, vitamin K, Vitamin B3, or zinc. Eighteen trials provided vitamin D supplementation [39,42,43,45–59] of which one provided the intervention group with an extremely low amount of vitamin D of 0.5 µg/day [46]. For this reason, the trial was excluded from all analyses. Fifteen trials used protein as an intervention [40,41,60–72]. A complete overview of all of the included trials and their intervention components is shown in Table 3.

Six trials used a factorial design, comparing a nutrition intervention with an exercise-type intervention, using a control activity for exercise (such as watching films, reading, singing and conversation [61,73], visits or phone calls [42], or a social program). In five other trials, participants received a resistance-type exercise training in addition to protein supplementation or a placebo [40,62–64,67].

3.1. Risk of Bias

Only eight of the 36 trials confirmed proper concealment of allocation, i.e., using sealed envelopes or computer allocation [40–42,50,51,54,62,73]. The quality of the trials was lowest for the criteria blinding of outcome assessors and treatment providers. Details on quality assessment per study are shown in Table 4.

3.2. Compliance

In 18 trials, compliance was reported to be good (≥80%), and the other 18 trials did not report on compliance rate.

3.3. Nutritional status

One trial investigated the effect of whey protein drinks [60] on nutritional status as assessed by the Mini Nutritional Assessment (MNA) but did not show a significant improvement in MNA score.

Fourteen trials investigated the effect of dairy components on body weight; two trials did not publish the summarized data for body weight [42,69], therefore,

could not be included in the meta-analysis. Three out of 14 trials ascertained a significant increase in body weight [40,60,69]. An overview of trials included in the systematic review and meta-analysis is depicted in Table 3.

Eight trials with either protein or amino acid intervention were included in the meta-analysis on body weight [40,41,60,63–65,67,72]. A significant pooled MD in body weight was found between the protein and placebo group (n=418; MD 1.13 kg; 95% CI: 0.59 to 1.67; I2=0%; p<0.0001) (Fig.3).

Restricting the meta-analyses to trials wherin a mixture of amino acids as supplementation was provided or excluding trials in which one specific amino acid was given also resulted in a significant body weight increase (n= 330; MD 2.16 kg; 95% CI: 0.93 to 3.38; I^2=0%; p=0.0006) [40,41,60,63,64]. This was also found when restricting to trials with a duration of at least six months (n=264; MD 2.09 kg; 95% CI: 0.88 to 3.29; I^2=0%; p=0.0007) [40,41,60,63,65]. Subgroup analyses on four trials that included participants who were at risk of malnutrition, malnourisment [60], or (pre-)frailty [40,41], and those who were bed rest patients and those older than 70, resulted in a substantial increase in body weight with a MD of 1.16 kg; 95% CI: 0.62 to 1.71; I^2=9%; p=0.0001. Restricting to the five trials in which ≥20 grams of protein per day was supplemented also resulted in a significant increase of body weight (MD 1.55 kg; 95%: 0.75 to 2.35; I^2=0%; p=0.0001).

Finally, restricting to trials that included an exercise training component to both the intervention and control groups or when restricting to trials with sufficient blinding according to the risk of bias assessment resulted in a significant increase of body weight (n=179; MD: 0.78 kg; 95% CI: 0.06 to 1.51; I^2=0%; p=0.03, n=235; and MD: 1.18; 95% CI: 0.55 to 1.82; I^2=31%; p=0.0003, respectively). The funnel plot, subgroup and sensitivity analyses are shown in Supplemental Figure S1. As indicat-

ed in Table 3, nine trials investigated the effect of dairy components on BMI. One out of nine trials reported a significant increase in BMI [69]. In this trial, an amino acid mixture [69] was provided as an intervention.

3.4 Physical fitness

Only one out of 14 trials determined a significant increase in LBM (Table 3). In that study, protein drinks in combination with resistance-type exercise training were compared to a placebo drink combined with exercise training in frail older adults [40]. Compared to the other 14 trials, this trial did not specifically differ in intervention duration or dose of the supplementation except that they included (pre)frail older adults in their trial while the other studies included well-nourished older adults.

A meta-analysis was performed on eight trials (n=474) that investigated protein supplementation [40,62–66,72,74] but did not show an effect in LBM (MD 0.03 kg; 95% CI: -0.33 to 0.39; I^2=0%; p=0.87) (Fig.4). Restriction to an intervention duration of at least six months resulted in the inclusion of four trials [40,41,63,65] but did not show a significant increase in LBM (n=258; MD 0.40 kg; 95% CI: -1.59 to 2.40; I^2=0%; p=0.69). Restriction to trials in which ≥20 grams of protein supplementation was given per day, did not result in a significant increase in LBM (n= 221; MD of 0.60 kg; 95% CI: -0.09 to 1.29; I^2=0%; p=0.09) [40,41,63,72]. When including trials with (pre-) frail older adults [40,41] and compulsorily inactive older adults [72], the increase of LBM was inconsequential (n=141; MD: 0.61 kg; 95% CI: -0.09 to 1.31; I^2=0%; p=0.09). Finally, subgroup analyses including trials that intervened with protein supplementation as well as exercise showed no beneficial effect (n=303; MD -0.18 kg; 95% CI: -0.61 to 0.26; I^2=0%; p=0.42) [40,62–64].

None of the four trials investigating the effect of protein on regional leg LBM showed a significant difference between the intervention and placebo group. One trial investigated the effect of ß-Hydroxy-Methyl butyrate (HMB), arginine, and lysine supplementation [70] on FFM but failed to find a significant effect. One trial investigated the effect of vitamin D on regional FFM, but the summarized data and result were not published [45]. Another study investigated the effect of six months of supplementation with a protein drink on skeletal muscle index but did not show a considerable difference between the intervention and placebo group [60].

Thirteen trials investigated the effect of dairy components or vitamin D on total or regional fat mass. One out of the eight studies showed a significant increase in favor of protein supplementation on fat mass and found a substantial increase in favor of protein [40]. One trial providing calcium supplementation reported a significant decrease in fat mass in the active absorbable algal calcium (AAACa) group (but not in the calcium carbonate group) compared to the placebo group [75]. Trials investigating the effect of leucine supplementation on leg fat mass [65,66] did not show a meaningful difference between the intervention and placebo groups over time. The one trial investigating the effect of vitamin D on regional fat mass did not publish the summarized data on regional fat mass but reported that the change in fat mass was not significant [45].

One out of the three trials investigated the effect of dairy components on fat mass on calf, arm, waist, hip, trunk, and limb circumference or waist-to-hip ratio [42,67,70] and revealed a significant effect of HMB, arginine, and lysine on calf, trunk and limb circumference [70].

Five trials investigated the effect of protein supplementation and vitamin D on the fiber cross-sectional area (Table 3). A significant increase in total (combined type

I and type II) fiber cross-sectional area (FCSA) was found in only one trial in which vitamin D3 was supplemented [39]. Another trial confirmed the effect of vitamin D2 on type II fiber diameter (mean percentage change of 96.5%±26.7%; -22.5%±6.7%, $p<0.0001$, respectively). The other three trials investigated the effect of protein supplementation but did not show a significant effect [41,65,66].

Table 3 indicates that 20 trials investigated the effect of dairy components and vitamin D on leg strength. Two of the 20 trials reported a significant increase in (left) leg strength in participants receiving vitamin D3 supplementation. These trials were different from the other studies as they included long-stay geriatric care residents who were given 800 IU per day [51] instead of 400 IU or high doses of vitamin D3 on monthly basis (150,000 IU once a month during the first two months followed by 90,000 IU once a month for the last four months) [50]; a greater number of participants (n=242) [52] and a longer study duration of 12 months [52].

Six trials that investigated vitamin D supplementation were included in the meta-analyses [42,48,49,52,57] which showed no significant effect of vitamin D on leg strength (n= 735; SMD 0.09; 95% CI: -0.05 to 0.24; I^2=0%; p=0.22, Fig. 5).Selecting trials with vitamin D doses between 1000 to 4000 IU did not result in an indication of a significant increase of leg strength (n=345; SMD 0.04; 95% CI -0.17 to 0.26; I^2=1%; p=0.69). When restricting to trials with a study duration of at least six months, no significant increase of leg strength was found (n=711; SMD 0.08; 95% CI -0.07 to 0.22; I^2=0%; p= 0.31). When excluding studies with high loss to follow up, a significant increase in leg strength did not occur with a vitamin D supplementation (n=380; SMD 0.08; 95% CI: -0.07 to 0.22; I^2=0%; p=0.31) [42,49,52]. With a restriction to trials with comparable baseline characteristics in the intervention and con-

trol groups, an increase in leg strength also did not occur with a vitamin D supplementation (n=581; SMD 0.13; 95% CI: -0.07 to 0.33; I^2=0%; p=0.19) [42,48,52,57].

For the meta-analysis on six trials in which protein supplementation took place [40,41,62–65], there was no significant effect on leg strength (n=417; SMD 0.05; 95% CI: -0.14 to 0.24; I^2=0%; p=0.60, Fig. 6). Furthermore, no significant effect on leg strength was found after limiting to four trials that used six months of protein supplementation (n= 193; SMD 0.05; 95% CI: -0.20 to 0.30; I^2=0%; p=0.68) [40,41,63,65] nor when restricting to trials with protein supplementation of at least 20 gram per day (n=193; SMD 0.05; 95% CI -0.23 to 0.34; I^2=0%; p=0.71).

Two trials investigated the effect of vitamin D3 and calcium on ankle dorsiflexion, hip abductor, hip extensor hip adductor [57], and hip flexor [50,57]. One trial did not publish the data or only reported the significance of the results [50]. One trial showed a significant increase in hip extensor (vitamin D group: 5.2±0.7 kg versus placebo group: 3.1±0.8 SE kg,) and hip adductor strength (vitamin D group: 3.4±0.5 and placebo group: 2.1±0.6) when compared to the lowest tertile for hip extensor (≤11 kg) and for hip adductor (≤12 kg) [57].

Eight trials studied the effect of dairy components and vitamin D on hand grip strength (Table 3). One trial did not publish the summarized data or significance for hand grip strength [71]. Four trials with vitamin D intervention were included in order to perform a meta-analysis with the outcome hand grip strength [42,48,49]. Two trials studied a daily dose of 400 IU vitamin D3 [42], and the other trial studied the effect of 1000 IU vitamin D3 [49]. For one trial, we compared vitamin D and no training with a placebo and no training (Bunout 2006-A) but also vitamin D and training with a placebo and training (Bunout 2006-B) [42].

Meta-analysis showed a non-significant MD of 0.40 kg; 95% CI: -1.11 to 1.92; I^2=0%; p= 0.60 (Fig. 7) (n=222 participants). For the sensitivity analysis, a meta-analysis was performed separately for trials with a low loss to follow-up, however, no significant increase of hand grip strength due to vitamin D supplementation was found (MD 0.52 kg; 95% CI: -12 to 2.15; I^2=0%; p=0.54).

Table 3 depicts that seven trials investigated the effect of dairy components and vitamin D on physical performance. Two of the seven trials studying the effect of protein drinks or vitamin D found a significant effect when compared to the placebo group [41,54]. These two trials differed with the other trials by including paticipants who were vitamin D deficient and attending a fall clinic or (pre-)frail older adults [40,54]. The other eight trials did not find significant improvement in physical performance.

A total of four trials supplying vitamin D were included in the meta-analysis (Fig. 8). No effect from vitamin D supplementation on physical performance was found (SMD 0.12; 95% CI: -0.07 to 0.30; I^2=57%; p=0.22). A subgroup analysis was performed based on restricting to trials that gave vitamin D3 supplementation. However, no significant effect was determined (SMD 0.12; 95% CI: -0.07 to 0.31; I^2=67%; p=0.21). The funnel plot on physical performance showed a small asymmetry, indicating possible weak publication bias (Supplemental figure S8).

Ten trials investigated the effect of dairy components and vitamin D on walking capacity (Table 3). One study investigated with participants walking 50 feet but did not publish the summarized data or results [54]. Only one of the 15 remaining trials showed a significant increase in walking capacity as measured by the six minute walking test [71]. This study investigated the effect of a 12 gram amino acid mixture (L-leucine, L-lysine, L-isoleucine, L-valine, L-threonine, L-cysteine, L-

401 histidine, L-phenylalanine, L-methionine, L-tyrosine, and L- tryptophan) per day
402 compared to a placebo in a healthy elderly population for three months. Meta-
403 analysis was performed on three trials that investigated the effect of vitamin D
404 supplementation on walking capacity (Fig.9). The SMD was 0.04; 95% CI: -0.17 to
405 0.24; I^2=0%; p=0.73. The funnel plot showed no signs of publiction bias.

406 Eight trials investigated the effect of dairy components or vitamin D on the
407 timed chair stands (Table 3). Two trials did not report the effect size or the p-value of
408 the duration of the chair stand [43,54]. Only one of the remaining seven trials
409 showed a significant change in the duration of the timed chair stand using chairs that
410 differed in height (36 cm, 47 cm and 53 cm). The test with the shortest chair showed
411 a significantly higher score for the intervention group compared to the placebo group.
412 This significant result was not found for the tallest chair [53]. The other trials did not
413 report the height of the chair.

414 Four trials investigated the effect of dairy components and vitamin D on bal-
415 ance (Table 3). Two trials did not report the summarized data or result [41,61]. The
416 remaining trials did not show a substantial difference between the intervention and
417 placebo group over time [49,73].

418 Table 3 shows that ten trials investigated the effect of dairy components or
419 vitamin D on TUG. However, in one trial, no data or description of significance was
420 provided (37). Only two of the remaining nine trials found a significant effect of vita-
421 min D or protein supplementation on TUG [57,70]. Six trials with vitamin D
422 supplementation were included in a meta-analysis [42,48,49,52,56,57]. These six
423 trials gave vitamin D3 [42,48,49,52] or vitamin D2 [56,57]. One trial used a 2x2 facto-
424 rial design with an exercise intervention and a control activity [42]. Meta-analysis
425 showed a significantly decreased MD of -0.75 seconds; 95% CI: –1.44 to -0.07

seconds; I^2=0%; p=0.03 (Fig. 10).

A significant effect on TUG was found after limiting to trials that used a daily vitamin D dose of 400-1000 IU (N=5; n=711; MD -0.81 seconds; 95% CI: -1.51 to -0.11; I^2=0%; p=0.02) [42,48,49,52,57] (Supplemental Figure S9). No significant effect was found after limiting the analysis to trials that included vitamin D3 as a supplement: MD -0.70; 95% CI: -1.52 to 0.12; I^2=0%; p=0.10 [42,48,49,52]. The sensitivity analysis was limited to trials with a low loss to follow-up [42,49,52,56]. It did not show a significant difference between intervention and placebo groups, MD -0.62; 95% CI: -1.44 to 0.20; I^2=0%; p=0.14.

4. Discussion

This systematic review and meta-analysis showed that the dairy components protein and vitamin D have beneficial effects for older adults. Protein supplementation increased body weight by 1.13 kg, and vitamin D supplementation increased physical fitness, measured as TUG improvement by 0.75 seconds. However, the majority of the trials found no effect on nutritional status or physical fitness from supplementation with dairy components. No studies were determined to examine the effects of specific dairy products on any of the investigated outcomes. Leg strength was the most frequently studied outcome, i.e., in 20 studies, followed by body weight in 14 studies. The least studied outcome was hip muscle strength (two studies).

The significant effect of protein supplementation on body weight found in this meta-analysis confirmed results from two earlier meta-analyses which showed an increase in body weight of 2.15 kg (95% CI: 1.80 to 2.49 [76] and 1.02 kg (95% CI: 0.19 to 1.85) [77] due to protein-energy and leucine supplementation. The greater

increase in body weight found in one meta-analysis [76] can be explained by the amount of protein-energy supplementation in the included studies wiich ranged from 175 to 1350 kcal/day compared to a maximal amount of 313 kcal/day provided in the trials included in our meta-analysis. This indicates that a larger amount of energy and protein is required in the event that more weight gain is warranted such as in the elderly that are more frail. When we restricted the current meta-analysis to studies that provided ≥20-grams protein per day, this resulted in an increased change in body weight (MD) from 1.13 kg to 1.55 kg. Substantial effects on body weight were also observed when restricting to studies of longer duration (MD 2.09 kg) or when a mixture of amino acids (MD 2.16 kg) was given. Similar results were found when restricting to studies including older adults >70 years of age, those at risk for malnutrition, or those who were already malnourished, (pre-) frail, or compulsory inactive (MD 1.16). When restricting to trials that combined protein supplementation and exercise training, a significant change in body weightwas found, although with a smaller effect size of 0.76 kg.

In our systematic review, no effect from protein supplementation on physical fitness, measured by body composition components (e.g. LBM), muscle strength, or physical performance was ascertained. A potential explanation of the absence of significant results of protein on body composition measures by meta-analyses might be poor compliance. Although most trials did not report on the compliance rate, we found that protein intake in 50% of the individual trials of the current meta-analysis did not significantly differ from baseline in the protein group at follow-up. This indicates that the compliance was low and that the effect of protein supplementation was not properly investigated. Moreover, in two trials, both the intervention and control groups had already complied with the daily protein intake guidelines [64,65] which

might not provide the opportunity to increase LBM by postprandial muscle protein synthesis. To achieve stimulation of muscle protein synthesis, an amount of 20-30 grams of protein per meal has been suggested as being required for sufficient stimulation of postprandial muscle synthesis [41,78]. This was partly confirmed in our systematic review; restriction to trials that supplemented ≥20 grams of protein per day or to trials which included (pre-) frail or compulsory inactive older adults resulted in a tendency towards an increase in LBM of 0.61 kg (p=0.09).

We investigated the effect of the supplementation of dairy components on FFM; however, a meta-analysis was not possible due to a low number of trials. In contrast to other meta-analyses and systematic reviews [22,79], we found no significant effect of protein supplementation on FFM. Cermak et al.[22] reported a pooled estimate of a 0.48 kg (95% CI: 0.10 to 0.85) increase in FFM due to protein supplementation during prolonged resistance-type exercise training in healthy older adults. This finding is in accordance with a review that states that resistance training in combination with dietary protein or amino acids or protein supplementation has a positive effect on muscle strength, muscle mass, and also physical function in older adults [17]. Cermak et al.[22]included studies that did not apply to our two inclusion criteria regarding age which were being younger (aged 18 years and older) and the obligation of using a placebo as a control [80]. Our stricter inclusion criteria hindered us in including an adequate number of trials for a meta-analysis with FFM as outcome and, therefore, sufficient power to detect any possible significant differences. The trial without a placebo [80] indicated a substantial increase in muscle mass and was also included in the other positive systematic review[79].

We found that the effect of vitamin D supplementation on TUG improved more profoundly when restricted to trials that provided a dose of 400-1000 IU (MD: -0.81

seconds; p=0.02). The actual proper dose of vitamin D supplementation is currently under discussion [81]. One trial found no effect of high monthly doses of 60.000 IU (2000 IU/d) vitamin D calcifediol on lower extremity function in older adults compared to a reference dose of 24.000 (800 IU/d) [81]. Their subgroup analysis even revealed that participants reaching elevated 25(OH)D levels between 111.8-247.3 nmol/L had the greatest chances of falling and had the most falls compared to participants with levels between 53.3-75.8 nmol/L [81]. Another systematic review states that a daily dose of at least 800 IU is required to improve muscle strength [82]. Unfortunately, there were too few trials to do a subgroup analysis on a specific daily dose such as 800 IU or doses of vitamin D higher than 1000 IU per day. However, according to our meta-analysis, a regimen of dosing towards a daily dose of 400 to 1000 IU seems to be beneficial with a small reduction in TUG.

Our systematic review and meta-analysis have several strengths and limitations. A major strength is the strict selection criteria for the trials which only allowed randomized controlled trials that used a placebo and that had applied double blinding to ensure a high level of evidence due to a lower risk of bias. A second strength is the small degree of heterogeneity, indicated by I^2, in the meta-analysis which makes comparison between trials reliable. Third, although the abstract and title selection were decided by one reviewer, the full text selection of articles was done carefully by two independent reviewers. Fourth, we performed funnel plots to assess possible publication bias and most of these showed no concern for it. The funnel plot on physical performance showed a small asymmetry, indicating possible weak publication bias. In addition to publication bias, we assessed the risk of bias per trial. Although the quality of studies could have been improved by more effective blinding of out-

525 come assessors and treatment providers, the quality of included trials scored rather
526 well since the inclusion criteria were strict.
527 The first limitation of our systematic review and meta-analysis is that we could
528 not draw a conclusion about the effect of specific dairy products on the outcomes of
529 nutrient status or physical fitness, because studies where the intervention existed out
530 of dairy products instead of only dairy components were not available. Second, the
531 majority of the trials did not publish the mean change with its accompanying stand-
532 ard deviation nor the correlation coefficient which describes the association between
533 baseline and final measurements across participants [44]. Another meta-analysis
534 estimated a correlation coefficient of 0.98 for FFM, and 0.70 (protein) and 0.80 (pla-
535 cebo) for one repetition maximum (1-RM) strength based on trials in healthy younger
536 (<50 yrs. old) and older participants (>50 yrs. old) [22]. We estimated the standard
537 deviation change, by using a conservative correlation coefficient of 0.5 [83]. Howev-
538 er, a sensitivity analysis of different correlation coefficients showed that using a cor-
539 relation coefficient of 0.8 resulted in a significantly increased LBM due to protein.
540 Similarly, a significant effect was found of vitamin D3 (excluding trials that supple-
541 mented vitamin D2) on TUG as well as when selecting trials that had a low loss of
542 follow-up when applying a correlation coefficient of 0.8. Therefore, we may have un-
543 derestimated these effects. It would be beneficial if future scientific papers present
544 the actual mean change with its accompanying standard deviations to enable more
545 meta-analyses that are accurate and allow for higher levels of scientific evidence. A
546 third limitation is that we included trials in which resistance-type exercise training
547 was given to both the nutritional intervention and the control groups in order to in-
548 crease the number of included trials and, therefore, statistical power. However, we
549 do not have information on the interaction of the nutritional intervention and the exer-

cise training. Therefore it is not possible to differentiate the effect between the nutritional intervention and interaction with the exercise component. However, a meta-analysis did not show an increase in heterogeneity with the trials that included an exercise component. A fourth limitation is the rather low number of studies included, especially for the meta-,subgroup, and sensitivity analysis. This hampered a robust effect estimate for older adults who were more vulnerable and gender specific conclusions whereas others stated that protein and vitamin D supplementation have a beneficial effect on target groups who were malnourished, vitamin D deficient, or frail [41,81,84]. The next limitation was the extensive number of outcome measurements used in included studies to examine body composition and physical fitness. Uniformity in outcome measurements is important for delivering high quality evidence based on meta-analyses, and it brings more focus to practical advice for the health care setting. Finally, we had a low response rate on our attempts to contact authors to request missing or additional details on their published data.

5. Conclusion

Most of the trials included in this systematic review showed no effect of dairy components that were provided in addition to the habitual intake on nutritional status and physical fitness. However, based on the current meta-analysis, we conclude that protein supplementation increases body weight. This effect was greater after selecting trials with a study duration of six months and when participants were at risk for malnutrition, malnourished, or (pre-)frailty, were bed rest patients, or were older than 70 years. The increase in body weight tended to be explained by differences in LBM but only when supplementing doses of protein higher than 20 g/d or when giving pro-

tein supplementation to (pre-) frail or compulsorily inactive older adults. In addition, the meta-analysis showed that vitamin D can improve physical performance as assessed by TUG in older adults. No other beneficial effect of dairy components on muscle strength, hand grip strength, overall physical performance, walking capacity, or balance could be substantiated in this systematic review and meta-analysis. Publication bias was nearly nonexistent, and heterogenity between trials was minimal.

6. Recommendations for future research

More double-blind, randomized placebo-controlled trials should be undertaken in frail or malnourished older adults instead of healthy community-dwelling older adults. The effect of additional dairy components seems to be of little clinical relevance in healthy older adults but appears to be higher older adults that are more vulnerable. Additionally, doses of 400-1000 IU of vitamin D supplementation seem beneficial for physical performance, but supplementation doses of 800 IU are hypothesized to be more beneficial than 400 IU for muscle strength [77]. Therefore, future research should focus on doses of approximately 800 IU in order to test this hypothesis. Finally, the combination of dairy components in one intervention should be studied to see if the combination of adequate vitamin D and protein is more effective than either of them alone.

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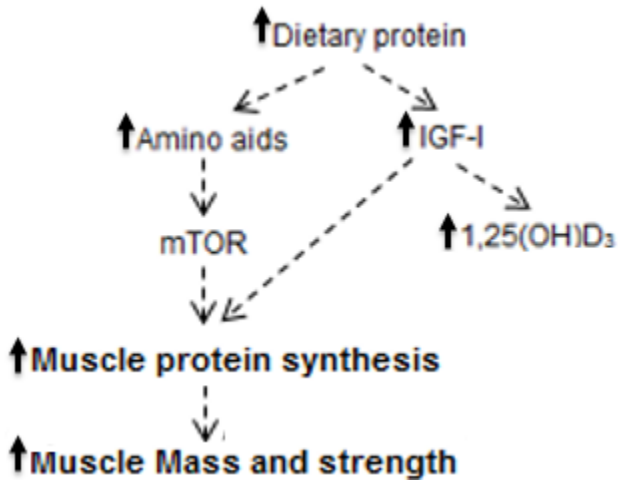


Fig. 1 Dietary protein increases serum levels of amino acids and IGF-I. Amino acids are the most potent activators of mTOR, that stimulate muscle protein synthesis and hence hypertrophy, and consequently muscle strength. Increases in serum IGF-I also stimulates the renal production of 1,25(OH)D3. IGF-I insulin-like growth factor-I; mTOR, mammalian target of rapamycin; 1,25(OH)D2D3, 1,25-dihydroxyvitamin-D3. Adopted from Bonjour et al. 2007 and Rizzoli et al. 2014 [30,31]

Table 1. Inclusion and exclusion criteria for including studies in the systematic review

	Inclusion	Exclusion
POPULATION	• Adults aged 55 years or older	• Animal studies • Children • Pregnancy or lactating • Athletes / body builders • Renal dysfunction
INTERVENTION	• Randomized double-blind, placebo-controlled trials • Dairy, or dairy specific components (not mandatory to be dairy-based) • Vitamin D	• Dietary counseling only • Nutritional supplementation products that also give other micronutrients that are not present in dairy • Regular or usual care as reference in case intervention consisted of supplement • Small amounts of intervention component where there could not be an expected effect based on outcomes relevant for this review and meta-analysis
OTHER	• Articles written in English • Full text articles only	• Reviews • Conference articles • Letters • Notes • Short surveys • Editorials • Book chapter • Duplicate publications

Table 2. Quality assessment criteria and scored risk of bias table.

A: Was the assigned treatment adequately concealed prior to allocation? 3= Method did not allow disclosure of assignment 2= Small but possible chance of disclosure of assignment 1= States random, but no description or quasi-randomized
B1: Were all randomized participants accounted for? 3 = Yes 2 = Partial description; some uncertainty 1 = Inadequate detail
B2: How many participants were lost to follow-up or otherwise excluded from the analysis? 3= 0-10% 2=>10-20% 1= >20%
C: Were the outcome assessors blinded to treatment status? 3= Effective action taken to blind assessors 2= Small or moderate chance of unblinding of assessors 1= Not mentioned or not possible
D: Were the treatment and control group comparable at entry? 3= Good comparability of groups, or confounding adjusted for in analysis 2= Confounding small; mentioned but not adjusted for 1= Large potential for confounding, or not discussed
E: Were the subjects blind to assignment status after allocation? 3= Effective action taken to blind subjects 2= Small or moderate chance of unblinding of subjects 1= Not possible, or not mentioned (unless double-blind), or possible, but not done
F: Were the treatment providers blind to assignment status? 3= Effective action taken to blind treatment providers 2= Small or moderate chance of unblinding of treatment providers 1= Not possible, or not mentioned, or possible, but not done
G: Were the inclusion and exclusion criteria clearly defined? 3= Clearly defined 2= Poorly defined 1= Not defined
H: Were the outcome measures used clearly defined? 3= Clearly defined 2= Poorly defined 1= Not defined
J: Was ascertainment of the outcomes reliable? 3= “Golden standard” was used to measure outcome 2= Validated method, but not the “golden standard” 1= Not possible, or not mentioned, or possible, but not done
K: Was the duration of surveillance clinically appropriate? 3= 6 months or more 2= 3 months to less than 6 months 1= Less than 3 months or not defined

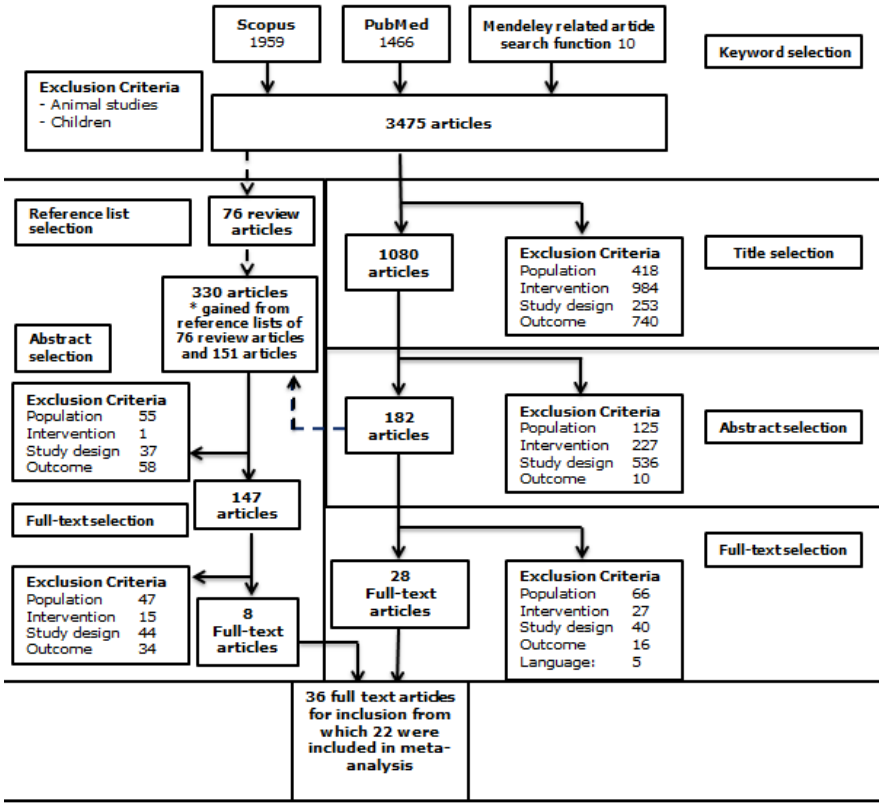


Fig. 2 Flow-chart of article selection for systematic reviewing and meta-analysis

Table 3: Overview of studies included in the systematic review and meta-analysis.

Reference (primary author and year)	Study **population; age; mean (range); place of study**	**Intervention**	**Compliance**	**Treatment duration**	**Outcome measurements**	**Effect on outcome**
Björkman 2012 [60]	103 men and women from a single center municipal nursing home; age: 83.5±8.2 y; Helsinki, Finland.	20 g protein in 4.5 dL of juice/day for test group. 45 kcal of energy, 11 g carbohydrates, 1 g fiber, and 40 mg of vitamin C/ dl in test drink and placebo drink without protein.	Intervention group: 81.8±18.1%, Control: 73.9±24.8% (P = 0.263)	6 months	HGS, Body Weight (BW), Fat-Free Mass (FFM), Mini Nutritional Assessment (MNA) and Improved MNA score.	Weight showed a higher mean change over 6 months-period compared to the control group. The mean change of FFM, and HGS between the two groups did not differ significantly.
Reid 2005 [85]	1471 healthy women who are ≥5y postmenopausal and ≥55y; Intervention: 74.2±4.2, placebo: 74.3±4.3.	1 g elemental calcium daily (in form of 2 tablets in morning and 3 tablets in the evening) as citrate or identical placebo.	Intervention: 78%; Control: 80% (= 0.26)	30 months	BW, BMI, Fat Mass (FM), and Lean Body Mass (LBM) (DXA).	Weight decreased in both groups, but the difference was not significant (P=-0.93). Fat and LBM did not show an effect of calcium.
Bunout 2006 [42]	48 healthy older adults living in the community; 76 ± 4 y; Chili.	Daily, vitamin D3 (vitamin D3)) 400 IU + 800 mg calcium or calcium alone as control.	92±3% in both intervention and control group.	9 months	Body composition: Lean Mass (LM), FM, arm circumference, waist circumference, hip circumference, HGS, general physical fitness (TUG) and physical performance (Short Physical Battery=SPPB).	Gait speed increased in supplementation group (whether trained or not) than in non-supplemented subjects (P = 0.02). No significant changes were found in weight, circumferences or body composition in any of the four groups.
Carlson 2011 [61]	94 people; 65-99 y with severe physical or cognitive impairments, and living in residential care facilities; Umeå, Northern Sweden.	2x2 factorial model exercise intervention compared with a control activity and an intake of a protein-enriched drink (200 ml) compared to a placebo. The intervention drink contained 7.4 g protein, 15.7 g carbohydrate, and 0.43 g fat; the placebo drink contained 0.2 g protein and 10.8 g carbohydrate. Subjects got the drink 5 times per two weeks.	Protein drink: 84%; placebo: 79. The protein Drink package was completely taken in on 82% and the placebo drink on 80%.	3 months	Body composition (FFM, Intra Cellular Water=ICW, BW), BMI and Berg Balance Scale.	No significant differences in ICW, muscle mass, BW, Berg Balance scale in the protein group compared to the placebo group after 3 months or 6 months. The within group analyses in people with MNA ≤17 showed no significant changes in ICW and BW either in exercise group (0.9 l, p = 0.410 vs -0.9 kg, p = 0.185) or in control group (0.3 l, p = 0.673 vs -0.5 kg, p = 0.548).

Ceglia 2013 [39]	21 ambulatory, community-dwelling, postmenopausal women 78±5yrs.	Daily oral vitamin D3 capsule (4000 IU) or matching placebo.	No information	4 months	SPPB	No changes in total SPPB score by group (P= 1.0).
Corless 1985 [53]	65 patients in the geriatric wards of four hospitals; intervention: 82.6 y (6.9) mean (SEM), placebo: 82.3 y (6.0); England.	9000 unit's vitamin D2 or identical looking placebo.	Intervention group showed no significant increase in serum vitamin D at follow-up, suggesting low compliance.	2 months	Muscle function score.	No significant differences were found for muscle function between the two groups before or at any time during the trial.
Dawson-Hughes 1991 [47]	246 postmenopausal women; intervention: 61.4±0.5 y, placebo: 61.9±0.5 y; USA.	400 IU vitamin D + 127 mg calcium (calcium phosphate) or placebo with 127 mg calcium. Additionally, all subjects received 250 mg elemental calcium (calcium citrate).	Compliance was 99%.	12 months	Fat%, LBM, and muscle strength.	There was no change or effect of vitamin D supplementation on whole-body fat or lean-tissue mass during the study. Muscle strength did not differ between the groups during both periods.
Dhesi 2004 [54]	123 elderly men and women attending a falls clinic and with 25OHD levels ≤12 µg/l and normal bone biochemistry; intervention group: 77.0±6.3 y, placebo group: 76.6±6.1 y; London, UK.	Single intramuscular injection of 600,000 IU vitamin D2.	High compliance due to single injection of vitamin D2.	6 months	Functional performance (Aggregate Functional Performance Time (AFPT)): 50 ft. walk, rising from a 42-cm high chair.	The placebo group showed a 6.6 s deterioration in AFPT over the trial period; treatment group improved by 2.0 s. This resulted in a significant difference in the change over 6 months between the two groups (P<0.01). Both groups showed a loss of strength over the trial period.
Gallagher 2013 [45]	STOP IT: 488 elderly women; 71±4 y; USA.	Twice daily, 0.25 mcg calcitriol, conjugated estrogens 0.625 mg daily, a combination of both or placebo.	No information.	3 years	Body composition: total and regional fat and LBM (DXA).	No significant effect of calcitriol on total body fat (p=0.572) but a significant decrease in LBM at the end of the 3-year study (p<0.0001) in both groups (placebo and calcitriol) was found. In the placebo group, there was a significant decrease in LBM (p< 0.0001) but not in total FM (p =0.628). There was no difference between the placebo and calcitriol groups in percent body FM (p = 0.410) or LBM (p = 0.921) at the end of the 3-year study.
Janssen 2010 [48]	59 women with serum 25OHD levels between 20-50 nmol/L, attending an outpatient clinic of the De-	Daily 400 IU vitamin D (vitamin D3) +500 mg/day calcium, or identical placebo tablets + calcium 500 mg/day.	59-100% (average 94.8%).	6 months	BW, HGS, Timed "Get Up and Go" test (TGUG) and habitual physical activity.	No significant difference was found between the two groups at 6 months in HGS. In the analysis of variance, with handgrip strength as outcome, the intervention did not affect muscle strength compared to placebo significantly.

	partment of Geriatric Medicine; intervention group: 82.4±6.4 y, placebo group: 79.2±6.7y; Utrecht.					
Kenny 2003 [49]	65 healthy, community-dwelling men; 76.7±4 y (65–87); Connecticut, USA.	Daily vitamin D3 (1,000 IU/d) + 500 mg calcium or a matching placebo pill with 500 mg calcium.	No information.	6 months	Handgrip strength, physical performance (ability to rise from a chair, static balance and the 8-foot walk, the timed ‘‘up and go’’ test, and the timed ‘‘supine to stand’’ test).	Time effects for handgrip strength and timed supine to stand, were seen, but there was no time-by-group effect for any of these measures.
Leenders 2011 [65]	57 elderly males with type 2 diabetes; 71±1 yr; Wageningen, The Netherlands.	2.5 g L-leucine or placebo after each main meal.	No information.	6 months	Body composition (DXA): BW, LM, FM, regional leg mean mass, leg FM, fat percentage.	Lean tissue mass did not change or differ between groups and any time point (0, 3 and 6 months). Also, no changes were found in body fat percentage.
Lips 2010 [43]	213 men and women with insufficient vitamin D serum values (≤50 but ≥15 nmol/L); placebo: 77.6±6.6 y, intervention: 78.5± 6.2 y; North America and Europe.	3 tablets once per week containing a placebo or 2800 IU vitamin D3. Only for those with a daily dietary calcium intake <1000 mg, 500 mg elemental calcium was prescribed.	No information.	4 months	SPPB: balance tests, gait speed test (timed 4-m walk) and timed rising from a chair and sitting for 5 repetitions.	After 16 weeks, SPPB did not differ significantly between treatment groups.
Moreira-Pfrimer 2009 [50]	51 institutionalized living in two different long-stay geriatric care (LSGC) units; median age: 77.6, range 62–94 years; São Paulo, Brazil.	Daily calcium plus monthly placebo (calcium/placebo group) or daily calcium plus oral vitamin D3 (150,000 IU once a month during the first 2 months, followed by 90,000 IU once a month for the last 4 months (calcium/vitamin D group).	High	6 months	Maximum isometric strength of hip flexors (SHF) and knee extensors (SKE).	The placebo group showed no improvement in SHF and SKE at 6 months ($p = 0.93$ and $p = 0.61$, respectively), SHF and SKE was increased in the intervention group ($p=0.0001$ and $p=0.0007$).
Fujita 2004 [75]	58 hospitalized elderly women; group A: 80±6 y, group B 83±6 y and group C: 79±9 y; Osaka, Japan.	Group A got 900 mg/day Ca supplement as AAA Ca, group B got 900 mg/day Ca supplement in de form CaCO3 and group C got the placebo. Participants were instructed to take two capsules after each meal	No information	2 years	Whole body mass, fat content, lean content measured by DXA and BW.	Whole body mass, lean content expressed as a percentage of whole body mass, remained the same in the three groups. However, increase of fat content was significantly decreased in group A compared to group C, but not in group B compared to group C.

		daily.				
Smedshaug 2007 [59]	60 nursing home residents; mean age of 85 y;	Intervention group: 5 ml cod liver oil p/d containing 10 mg vitamin D3; The control group received 5 ml cod liver oil p/d without vitamin D.	No information	1 year	Grip strength	Grip strength did not improve in the vitamin D group (0.4 kg increase) compared with the control group (1.6 kg increase) after 1 year vitamin D supplementation (p=0.22).
Solerte 2008 [69]	41 consecutive elderly outpatients with sarcopenia and reduced whole-body lean body mass; age range: 66–84 y; Italy.	The oral AA mixture and an isocaloric placebo were ingested as snacks at 10:00 AM and 5:00 PM. The AA preparation (70.6 kcal/day) contained 8 g/day of AAs (L-leucine, 2.5 g; L-lysine, 1.3 g; L-isoleucine, 1.25 g; L-valine, 1.25 g; L-threonine, 0.7 g; L-cysteine, 0.3 g; L-histidine, 0.3 g; L-phenylalanine, 0.2 g; L-methionine, 0.1 g; L-tyrosine, 0.06 g; and L-tryptophan, 0.04 g). The trial had a cross-over design.	No information	18 months	Mean whole-body mass, BMI, FM	Mean values of BMI significantly increased in the AAs group (p=0.05) and in the placebo group (p=0.01); whereas the total body FM remained unchanged throughout the study. Whole-body LBM increased significantly after six months and more consistently after 18 months of oral nutritional supplementation with AAs
Rosendahl 2006 [73]	86 elderly dependent in activities of daily living; intervention group: 82.9±6.4 y, placebo group 85.6±7.0 y; Umeå Sweden.	Milk-based nutrient drink (200 ml) that contained 7.4 g protein, 15.7 g carbohydrate, and 408 kJ per 100 g. The placebo drink (200 ml) contained 0.2 g protein, 10.8 g carbohydrate, and 191 kJ per 100 g. This study had a 2x2 factorial design with exercise as an intervention and a control activity.	The protein-enriched energy supplement: 82%; placebo drink: 78%. Package completely emptied: 80%.	6 months	Berg Balance Scale, 2.4 meters timed test, and modified chair-stand test.	Berg Balance Scale, self-paced and maximum gait speed were followed-up at three and six months. No interaction effects were seen between the exercise and nutrition interventions. There was a significant difference in self-paced gait speed in favor of the placebo group at six months compared to the nutrition intervention group. For other outcomes, there was no significant effect for both the intervention group and placebo group.
Sato 2005 [55]	96 elderly women who were hospitalized stroke patients with hemiplegia; Placebo	Daily dose of 1,000 IU vitamin D2 or placebo.	No information.	2 years	Muscle strength and muscle biopsy.	Increases in the relative number and size of type II muscle fibers and improved muscle strength was found in the vitamin D-treated group compared with the placebo group. Type I fibers was not dif-

	group: 74.2±4.1 y, Intervention group: 74.1±3.9 y; Japan.					ferent in the intervention group compared to the placebo group.
Tieland, van de Rest 2012 [41]	61 prefrail and frail elderly; placebo: 81± 1 y, intervention: 78±1 y; The Netherlands.	Daily, either 2 times beverages (250 mL) containing 15 g protein (milk protein concentrate, 7.1 g lactose, 0.5 g fat, and 0.4 g calcium), or a matching placebo containing no protein, 7.1 g lactose, and 0.4 g calcium.	92%	6 months	Skeletal muscle mass, FM, BW, HGS and physical performance (SPPB).	Skeletal muscle mass did not change in the intervention or placebo group during 6 months of intervention. Physical performance improved significantly in the intervention group and did not change in the placebo group. HGS did not significantly differ in the intervention group compared to the placebo group at the end of the intervention.
Witham 2010 [56]	91 elderly patients with systolic heart failure and 25-hydroxy vitamin D level of <50 nmol/L (20 ng/mL); placebo group: 80.6±5.7 y, intervention group: 78.8±5.6 y; UK.	Oral dose (100 000 IU vitamin D2 or placebo) was administered after baseline outcome measures and again after 10 weeks.	High	5 months	6-minute walk test, TUG-test and daily physical activity Levels.	The 6-minute walk test, TUG-test and daily activity did not significantly improve in the treatment group relative to placebo.
Zhu 2010 [57]	261 community-dwelling ambulant elderly women with serum 25-hydroxyvitamin D concentrations <24 ng/mL; placebo group: 77.0 ±4.8 y, intervention group: 76.8±4.2 y; Perth, Australia.	Vitamin D2 1,000 IU/d or identical placebo; calcium citrate (1 g calcium/d) in both groups	Vitamin D group: 86.7%; placebo group: 86.8%.	1 year	TUG-test.	In the lowest tertile, vitamin D improved TUAG more than calcium alone (P=0.05).
Bischoff 2003 [51]	62 elderly women in long-stay geriatric care; 85.3 y (63-99 y); Switzerland.	Daily 2 tablets containing 600 mg calcium carbonate and 400 IU vitamin D3 per tablet or 2 tablets containing 600 mg calcium carbonate.	Out of 89 participants, ten had a decreasing compliance.	3 months	TUG-test, and grip strength.	Musculoskeletal function improved significantly in CAL+D-group compared to CAL-group (No P-value for TUG was published).
Del Favero 2012 [68]	18 healthy older adults; Intervention group: 65±4 y, placebo group: 64±7 y; Sao Paolo, Brazil.	3.2 g beta-alanine (2 tablets x 800 mg given twice per day after lunch and dinner) or an identical placebo	100% (self-reported)	3 months	Muscle function test (timed stands and TUG-test) and physical functioning.	No significant changes between intervention and placebo group was observed for the timed stands and TUG-test after beta-alanine supplementation when compared to the placebo group.

Flakoll 2004 [70]	29 women recruited from senior citizen centers and adult assisted-living and care facilities; 81.1±1.8 y.	Orange drink, which contained HMB (Calcium HMB, 2 g), arginine (5 g), LYS (lysine Hall, 1.5 g) and ascorbic acid (0.5 g) in 8 oz. of water (HMB/ARG/LYS. In S1, the placebo group conceived: orange-flavored isocaloric drink of maltodextrin and ascorbic acid (0.5 g) in 8 oz. of water.	100%	3 months	Functionality ("get-up-and-go" performance test, body composition (body fat% and FFM, trunk circumference (abdomen and hip), limb circumference (arm, forearm, and thigh) and trunk circumference (abdomen and hip).	The HMB/ARG/LYS-supplemented subject's significantly improved get-up-and-go performance times compared to the placebo group. Although not statistically different, the HMB/ARG/LYS group had an increase in fat-free mass, whereas subjects taking placebo showed a decrease in fat-free mass. FM and percentage of body fat were unchanged in both groups.
Pfeifer 2009 [52]	242 healthy ambulatory elderly women and men; Bad Pyrmont, Germany and Graz, Austria.	Daily, one tablet containing 400 IU vitamin D3 and 500 mg of elemental calcium (calcium carbonate) or one tablet with 500 mg at breakfast and dinner together with the meals.	No information.	12 months	TUG	Significant improvements in timed-up-and-go test after 12 months in the calcium + vitamin D group.
Scognamiglio 2005 [71]	95 healthy elderly subjects with reduced physical activity; AA-group: 74±6 y, placebo group: 74± 5 y; Italy.	12 g of AAs plus and 12.21 g of glucose, or placebo containing 12.21 g of glucose at 10.00 a.m., 4.00 p.m. and 10.00 p.m. The composition of the AA mixture (g/day): L-leucine 3.8, L-lysine 2, L-isoleucine 1.9, L-valine 1.9, L-threonine 1.1, L-cysteine 0.4, L-histidine 0.4, L-phenylalanine 0.3, L-methionine 0.2, L-tyrosine 0.1, and L-tryptophan 0.1.	Intervention: 86%; placebo: 87%	3 months	BMI, 6-min walk test.	BMI did not change significantly with intervention. The 6-min walk distance increased significantly in the AA-group, but not in the placebo group.
Smidt 1991 [86]	80 healthy older adult's women selected from medical registration lists provided by the Southern Health Board of Ireland; Cork City, Republic of Ireland	Thiamin-supplemented (10 mg daily) or placebo.	95%	6 weeks	BW.	BW did not differ between the two groups during baseline and treatment, and there were no difference in mean values when comparing baseline and treatment for the placebo group. In the thiamin supplemented group, mean BW increased significantly during treatment compared to baseline values. Energy intake also increased significantly in the intervention group, but not in the placebo

						group.
Verhoeven 2009 [66]	29 healthy elderly men; 71±4 y; The Netherlands.	2.5 g leucine or a placebo with each main meal (breakfast, lunch, and dinner).	No information.	3 months	BW, whole-body and leg LBM, FM and leg fat.	Whole-body, leg FM, fat-free mass (DXA), did not differ between groups before the intervention. No changes in body composition or muscle mass were observed over time, and no significant differences were observed between groups.
Verdijk 2009 [64]	26 healthy elderly men;72±2 in both groups; The Netherlands	Resistance-type exercise training with protein supplement (3 sessions/ week, 20 grams protein per session) or placebo.	No information	3 months	BMI, BW, leg mean mass, LM and FM.	Leg muscle mass, BMI and BW did not significantly differ from protein to placebo group.
Tieland, Dirks, 2012 [40]	62 frail older adults 78±1 y; The Netherlands.	Resistance-type exercise training (2 sessions/week) with daily protein supplement of 2 times 15 grams protein or placebo.	No information.	6 months	BW, LBM, FM, HGS, SPPB, gait speed, chair rise.	LBM increased significantly in the protein group compared to placebo group. Physical performance increased in both groups, with no interaction effect of dietary intervention.
Arnarson 2013 [62]	141 apparently healthy older adults; intervention group: 73.3 ±6 y, placebo group: 74.6 ±5.8 y (range: 65-91); Capital area of Iceland.	20 gram of whey protein or isocaloric placebo that was given 3 times per week during resistance exercise training.	No information.	3 months	LM, appendicular skeletal muscle mass, TUG, 6 min walk for distance.	No significant difference was found between the protein and placebo group after three months for LM, appendicular skeletal muscle mass, TUG, 6 min walk for distance.
Chalé 2013 [63]	75 mobility-limited older adults; age range 70-85 y; USA.	Daily 40 gram of whey protein or an isocaloric placebo powder was given to subjects, both in combination with high-intensity resistance training.	No information.	6 months	Body mass, LM, FM, chair-rise time, SPPB, and 400m walk.	No outcomes scored significantly higher in the intervention group compared to the placebo group over time.
Trabal 2015 [67]	30 older adults; Age: Leucine group: 85±8 y, Control group: 4±4; Barcelona, Spain	10 g/d of L-leucine or placebo (maltodextrin) and both groups had resistance training (3 times/week)	Intervention: 80%; placebo: 94%.	3 months	Physical Performance Battery (PPB): balance test, 4m walking speed test, 5 times chair rise test, and the TUG-test, MNA, BW, BMI, waist circumference, triceps skin fold, and calf circumference.	Mid-upper arm muscle area and TUG significantly improved in the intervention group compared to the placebo group. For the other outcomes, no significant between-group differences were found.
Ferrando 2010 [72]	22 older adults; Age: control: 68±5 y, Intervention: 71±6.72 y;	3 times per day 15 g of EEA or placebo during constant bed rest (except	No information.	10 days	LM, leg LM, FM.	No significant effect of EAA on total or leg LM, FM, body mass.

	USA.	when toileting).				
Schürch 1998 [87]	82 orthopedic ward patients; 80.7±7.4 y;	550 mg/d calcium and one dose at baseline of vitamin D (200000 IU) for all patients and 20 g/d protein (90% milk proteins) or placebo. Both powders contained: vitamin A (1000 IU), vitamin K, (30 µg), vitamin C (20 mg), calcium (550 mg), magnesium (91 mg), phosphorus (429 mg), and sodium (228 mg).	No information.	6 months	Muscle strength, BW, LM, FM and HGS.	No significant between-group differences were found for biceps muscle strength, BW, LM, FM and hand grips strength.

Table 4. Risk of bias scores per trial.

Reference	A	B1	B2	C	D	E	F	H	J	K	L
Bjorkman 2012 [55]	2	2	3	3	3	3	3	3	3	3	3
Reid 2005 [80]	2	2	2	1	3	3	2	3	3	3	3
Bunout 2006 [37]	3	3	3	2	3	3	2	3	3	2	3
Carlsson 2011 [61]	2	3	2	1	3	2	1	3	3	2	2
Ceglia 2013 [39]	1	3	2	2	3	2	2	3	3	3	2
Dawson-Hughes 1991 [47]	2	2	2	2	3	3	2	2	3	2	3
Dhesi 2004 [54]	3	3	2	2	3	3	3	3	2	2	3
Gallagher 2013 [45]	2	3	3	2	3	3	3	3	3	1	3
Janssen 2010 [48]	2	2	2	2	3	3	2	2	3	2	3
Kenny 2003 [49]	2	3	3	2	2	3	2	2	2	2	3
Leenders 2011 [65]	2	3	3	2	3	3	2	3	3	3	3
Lips 2010 [43]	2	2	2	2	2	3	2	3	3	2	2
Moreira-Pfrimer 2009 [50]	3	3	2	2	3	3	2	3	3	2	3
Fujita 2004 [75]	2	1	1	2	3	3	2	2	2	3	3
Smedshaug 2007 [59]	2	2	3	2	3	3	2	3	3	2	3
Solerte 2008 [69]	2	1	1	1	1	2	1	1	2	3	3
Rosendahl 2006 [73]	3	2	2	2	2	3	2	2	3	2	3
Sato 2005 [55]	2	3	2	2	3	3	2	3	3	3	3
Tieland 2012 [41]	3	3	3	3	3	3	3	3	3	2	3
Witham 2010 [56]	2	3	3	3	3	3	3	3	2	2	2
Zhu 2010 [57]	2	2	2	3	3	3	3	3	3	2	3
Bischoff 2003 [51]	3	2	1	3	3	3	3	3	3	2	2
Del Favero 2012 [68]	2	3	3	2	3	3	2	3	3	2	2
Flakoll 2004 [70]	2	3	2	2	3	3	2	3	2	2	2
Pfeifer 2009 [52]	2	3	3	2	3	3	2	3	3	2	3
Scognamiglio 2005 [71]	2	2	3	2	3	3	2	3	3	2	2
Smidt 1991 [86]	2	3	3	2	3	3	2	3	3	2	1
Verhoeven 2009 [66]	2	3	3	2	3	3	2	2	2	2	2
Verdijk 2009 [64]	2	3	3	2	3	2	2	2	3	2	1
Tieland, Dirks 2012 [40]	3	3	2	3	3	3	3	3	3	2	3
Arnarson 2013 [62]	3	2	3	3	3	3	3	2	3	2	2
Chalé 2013 [63]	2	2	3	2	3	3	3	3	3	2	3
Trabal (2015) [67]	2	2	1	3	3	3	3	2	3	2	2
Ferrando (2010) [72]	2	2	2	1	3	3	1	3	3	2	1
Schürch (1998) [87]	2	2	1	2	3	3	2	3	3	2	3

Letters and scores are assigned to a quality assessment question and explanation of the score which are displayed in Table 2

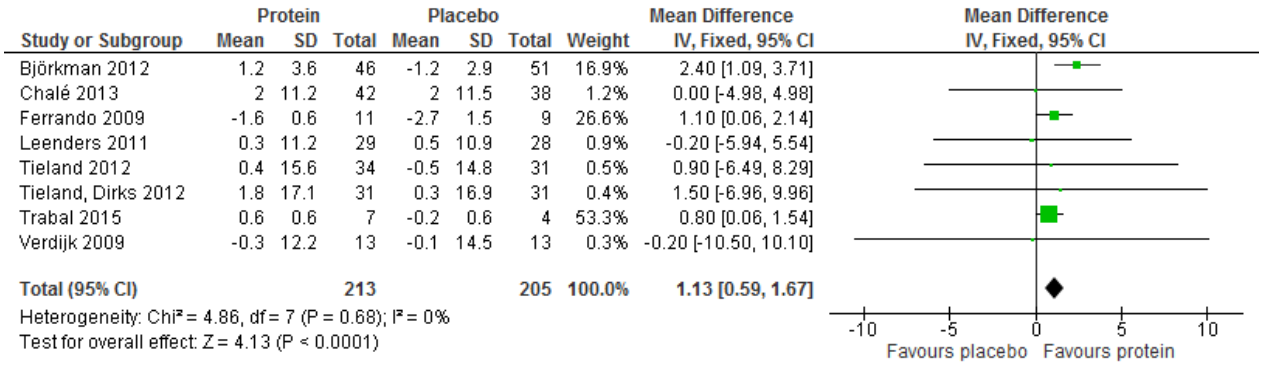


Fig.3 Meta-analysis of the effect of protein and amino acids on body weight (kg)

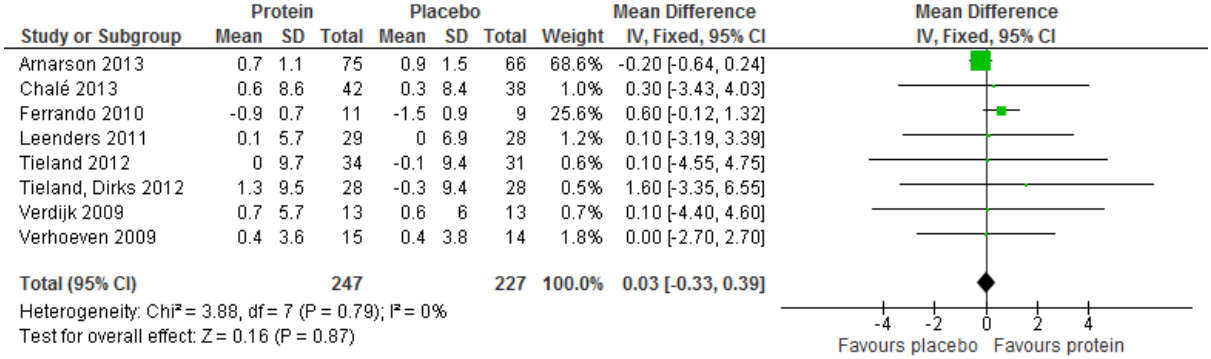
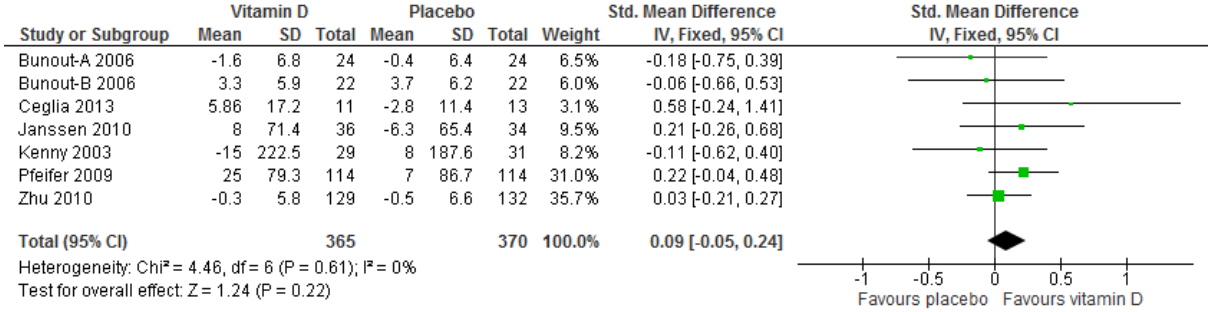


Fig. 4. Meta-analysis of the effect of protein supplementation (during ten days to six months) on lean body mass (kg)



Bunout-A= no training, Bunout-B= training

Fig. 5 Meta-analysis of the effect of vitamin D3 on leg strength

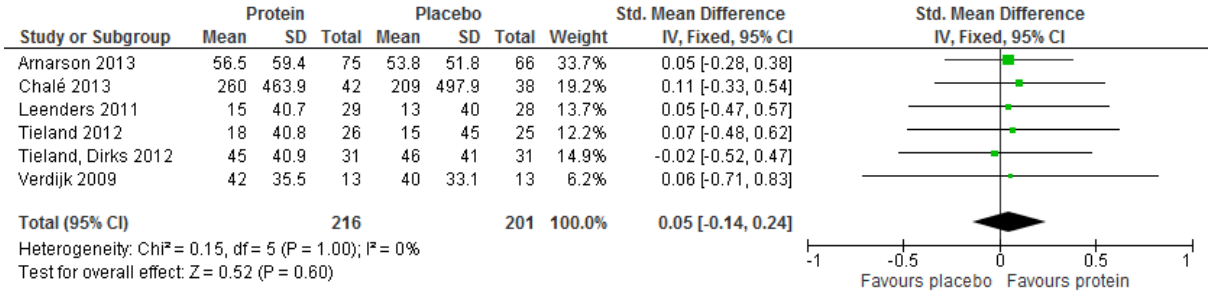
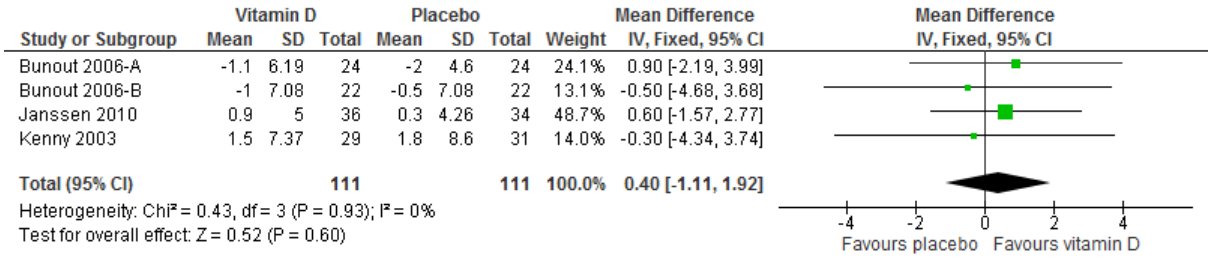
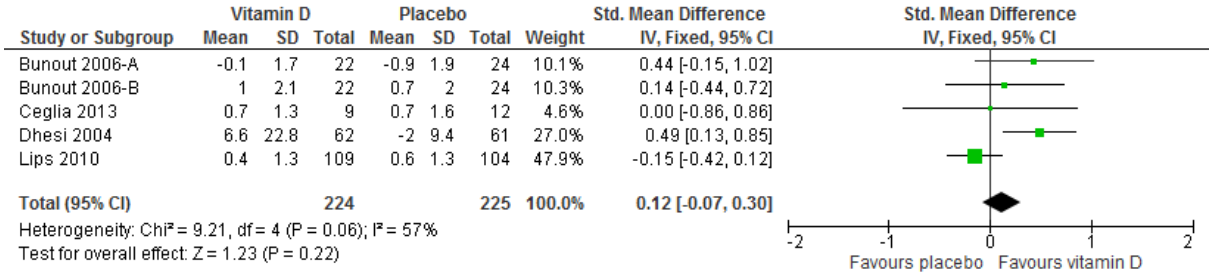


Fig. 6 Meta-analysis of the effect of protein on leg strength.



Bunout 2006-A: no exercise intervention in both groups; Bunout 2006-B: with exercise intervention in both groups.

Fig. 7 Meta-analysis of the effect of vitamin D on hand grip strength (in kg).



Bunout 2006-A: no exercise intervention in both groups; Bunout 2006-B: with exercise intervention in both groups.

Fig. 8 Meta-analysis of the effect of vitamin D on physical performance.

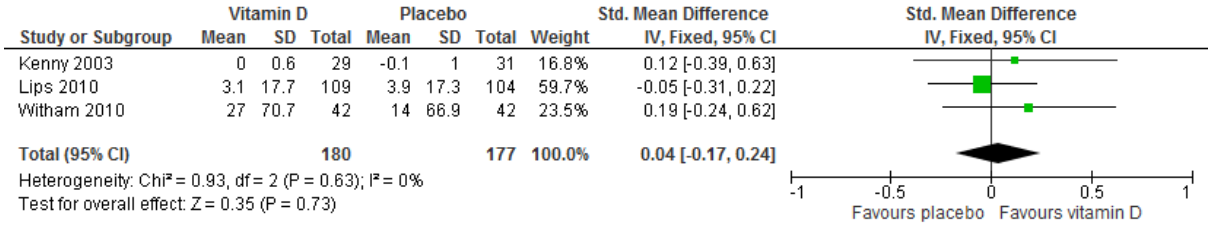
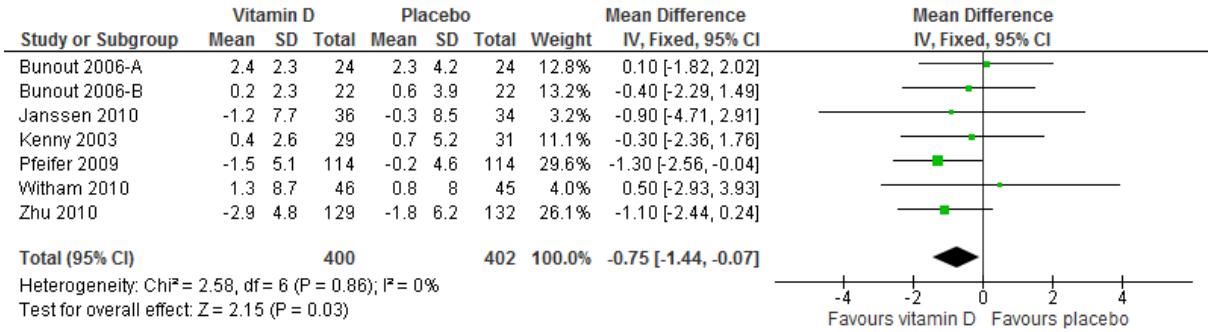


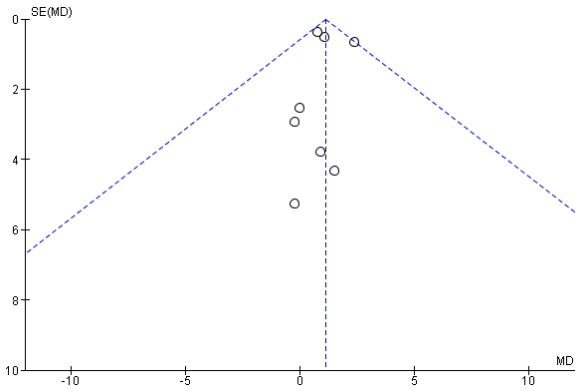
Fig. 9 Meta-analysis of the effect of vitamin D on walking capacity.



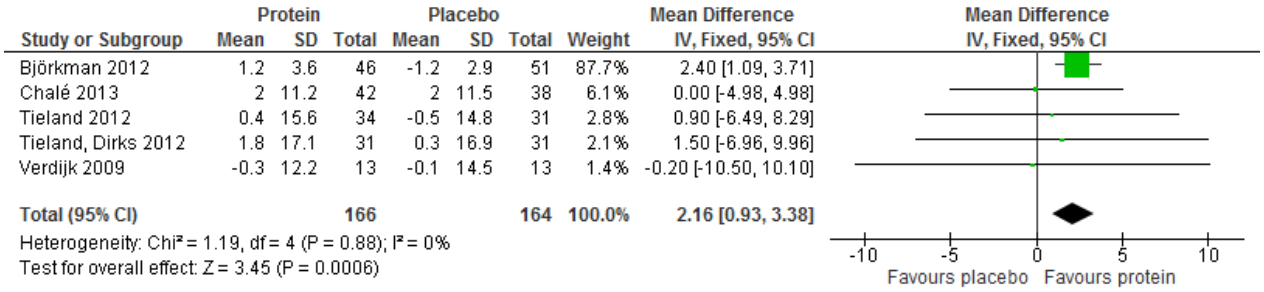
Bunout 2006-A: no exercise intervention in both groups; Bunout 2006-B: exercise intervention in both groups.

Fig. 10 Meta-analysis of the effect of vitamin D supplementation on TUG (s).

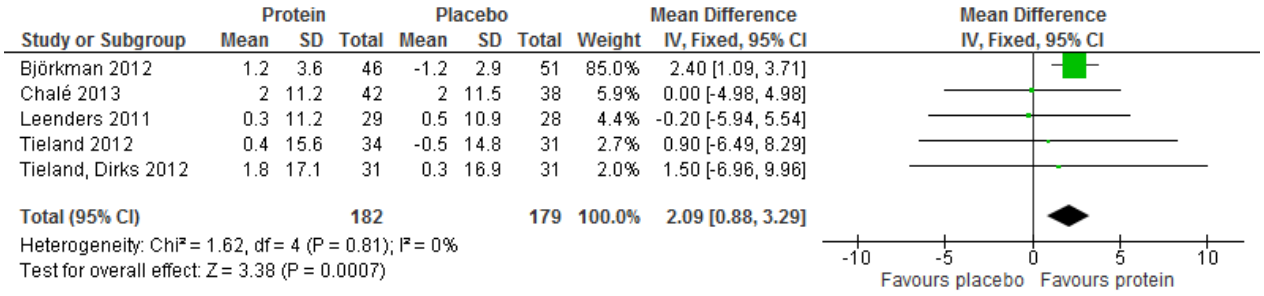
Supplemental Material



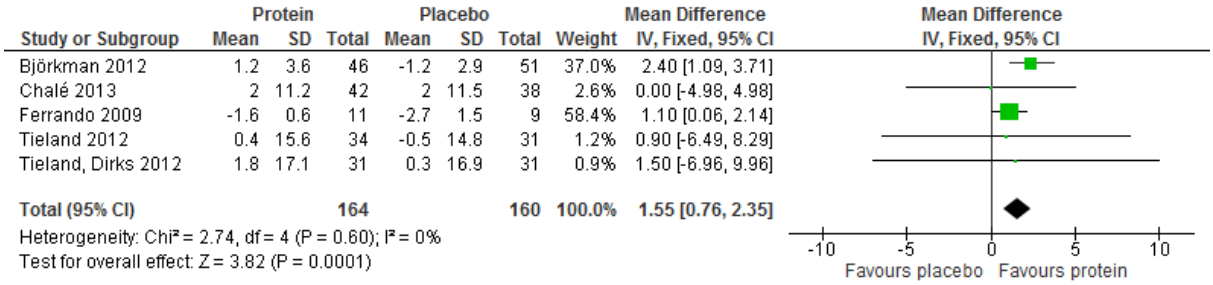
Supplemental Figure S1. Funnel plot of protein supplementation trials on body weight



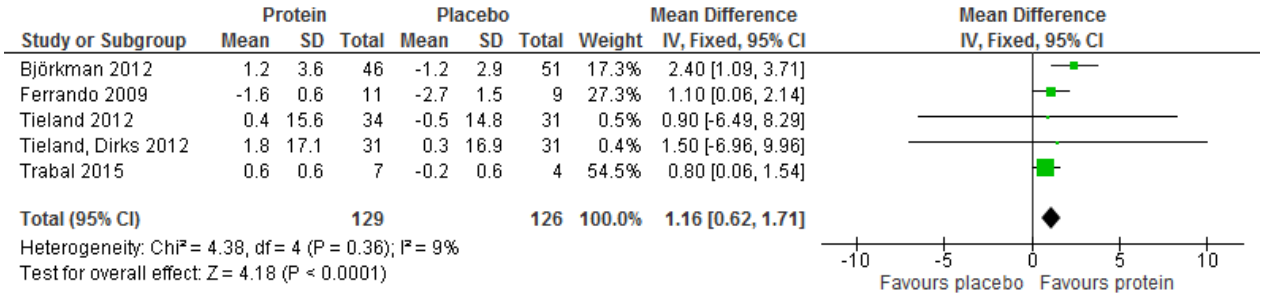
Supplemental figure S2. Subgroup analysis of the effect of protein supplementation on body weight.



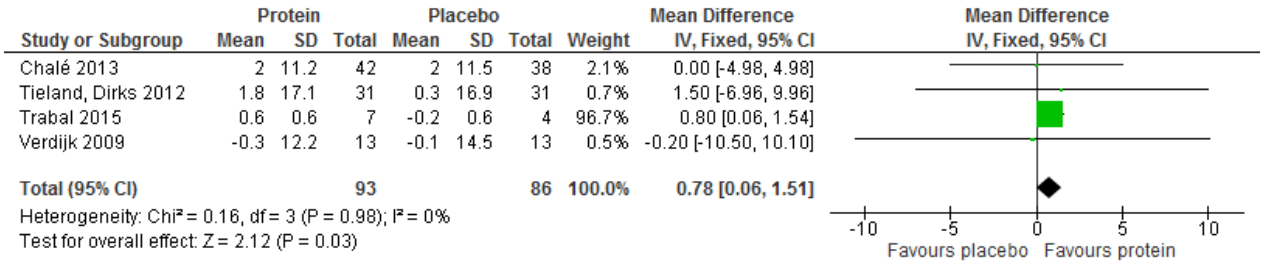
Supplemental figure S3. Subgroup analysis of the effect of 6 months of protein and L-leucine supplementation on body weight.



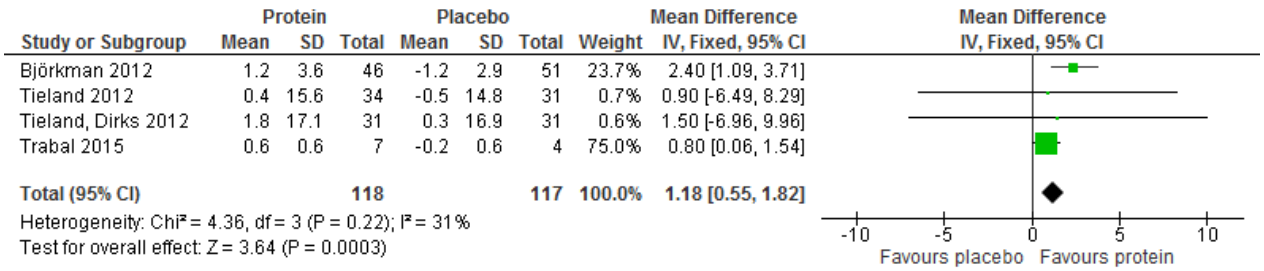
Supplemental figure S4. Subgroup analysis of the effect ≥20 grams protein supplementation per day.



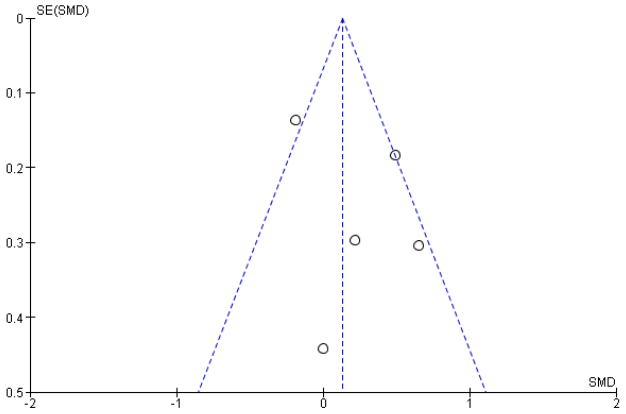
Supplemental figure S5. Subgroup analysis of the effect protein and L-leucine supplementation on body weight in "vulnerable older adults".



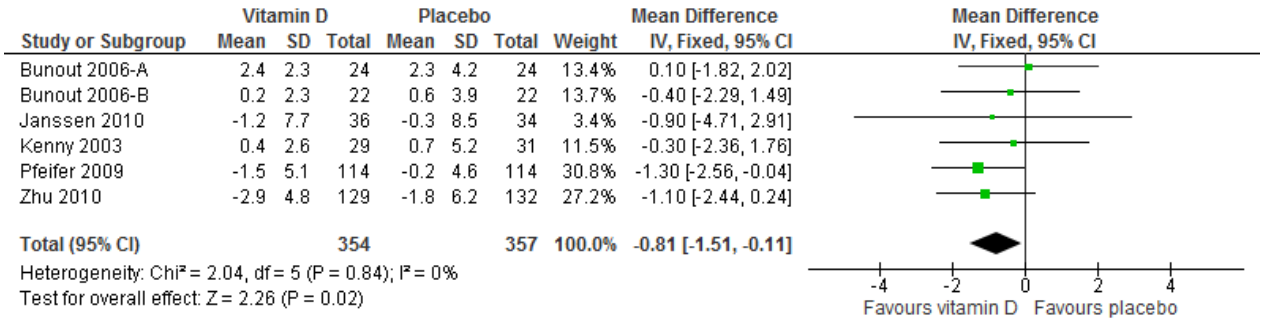
Supplemental figure S6. Subgroup analysis of trials that included exercise-training (in both protein groups as in placebo group) and that study the effect of protein supplementation on body weight.



Supplemental figure S7. Sensitivity analysis (blinding of outcome assessors and treatment providers) of the effect of protein/amino acid (AA) supplementation on body weight.



Supplemental figure S8. Funnel plot of the effect of vitamin D supplementation on physical performance.



Bunout-A= no training, Bunout-B= training

Supplemental figure S9. Subgroup-analysis of the effect 400-1000 IU vitamin D on TUG.