ΕΘΝΙΚΟ ΚΑΙ ΚΑΠΟΔΙΣΤΡΙΑΚΟ ΠΑΝΕΠΙΣΤΗΜΙΟ ΑΘΗΝΩΝ

ΙΑΤΡΙΚΗ ΣΧΟΛΗ

ΘΕΡΑΠΕΥΤΙΚΗ ΚΛΙΝΙΚΗ ΝΟΣ. ΑΛΕΞΑΝΔΡΑ

ΠΡΟΓΡΑΜΜΑ ΜΕΤΑΠΤΥΧΙΑΚΩΝ ΣΠΟΥΔΩΝ

«ΚΛΙΝΙΚΕΣ ΜΕΛΕΤΕΣ: ΣΧΕΔΙΑΣΜΟΣ ΚΑΙ ΕΚΤΕΛΕΣΗ»

MSc: "Clinical Trials: Design and Conduct"

Διευθυντής

Ευάγγελος Τέρπος, Καθηγητής Ιατρικής Σχολής ΕΚΠΑ

Συμπληρώματα Μικροθρεπτικών Συστατικών στο Σύνδρομο Ευθραυστότητας: Μια Συστηματική Ανασκόπηση και Μέτα-Ανάλυση Τυχαιοποιημένων Κλινικών Δοκιμών

Micronutrient Supplementation in Frailty: A Systematic Review and Meta-Analysis of Randomized Clinical Trials

Όνομα: Παρασκευάς Θεμιστοκλής

Αρ. μητρώου: 7450492100028

Επάγγελμα/ή Ιδιότητα: Ιατρός

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ΑΘΗΝΑ 2023

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ΠΡΟΛΟΓΟΣ

Η παρούσα διπλωματική εργασία αποτελεί μια προσπάθεια ανασκόπησης της τρέχουσας βιβλιογραφίας για ένα σύνηθες κλινικό ερώτημα, που πρόκειται να συνεχίσει να μας απασχολεί τις επόμενες δεκαετίες. Ακολουθώντας αυστηρά τις σύγχρονες κατευθυντήριες οδηγίες γύρω από των τομέα των συστηματικών ανασκοπήσεων όχι μόνο καταλήγουμε σε αριθμητικά συμπεράσματα, αλλά συγχρόνως χαρτογραφούμε τις ελλείψεις τις διαθέσιμης βιβλιογραφίας και συνεπώς της έρευνας που διεξάγεται παγκοσμίως. Τέλος, τονίζουμε τη σημασία της αξιολόγησης της ποιότητας των διαθέσιμων στοιχείων, κάτι που συχνά αγνοείται στην κλινική πράξη.

Θα ήθελα να ευχαριστήσω πρωτίστως τον επίκουρο καθηγητή και επιβλέποντα αυτής της διατριβής κύριο Θεόδωρο Σεργεντάνη για την συνεχή στήριξη και εμπιστοσύνη που μου προσέφερε, καθώς και τα υπόλοιπα μέλη της τριμελούς επιτροπής.

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ΠΙΝΑΚΑΣ ΠΕΡΙΟΧΟΜΕΝΩΝ

Micronutrient Supplementation in Frailty: A Systematic Review and Meta-Analysis of Randomized Controlled Trials

Περίληψη

Εισαγωγή: Τα άτομα με σύνδρομο ευθραυστότητας βρίσκονται σε κίνδυνο για αυξημένη νοσηρότητα και θνησιμότητα. Τα χαμηλά επίπεδα πολλών βιταμινών και μετάλλων έχουν συσχετιστεί με μεγαλύτερη επίπτωση και βαρύτητα του συνδρόμου ευθραυστότητας. Συχνά στην κλινική πράξη χρησιμοποιούνται από του στόματος συμπληρώματα χωρίς επαρκείς ενδείξεις.

Στόχοι: Να καθορίσουμε το αποτέλεσμα των συμπληρωμάτων μικροθρεπτικών συστατικών σε μέτρα σωματικής ευθραυστότητας, στην θνησιμότητα και άλλες εκβάσεις που σχετίζονται με τον ασθενή.

Μέθοδοι: Τον Σεπτέμβριο του 2022 πραγματοποιήθηκε μια συστηματική αναζήτηση στις βάσεις δεδομένων PubMed και Embase και συμπεριλήφθηκαν τυχαιοποιημένες κλινικές που διερεύνησαν την επίδραση της συμπλήρωσης μικροθρεπτικών συστατικών σε άτομα με σύνδρομο ευθραυστότητας. Η βεβαιότητα των διαθέσιμων στοιχείων (certainty of evidence) καθορίστηκε βάσει των κατευθυντήριων οδηγιών GRADE.

Αποτελέσματα: Συμπεριλάβαμε 18 μελέτες που περιγράφονται σε 30 δημοσιευμένα άρθρα. Όλες οι μελέτες αναφέρονταν σε συμπληρώματα βιταμίνης D, είτε σε πολυσυμπληρώματα που περιείχαν έναν αριθμό μικροθρεπτικών. Μόνο 7 από τις 18 μελέτες χρησιμοποίησαν μια καθιερωμένη κλίμακα ευθραυστότητας για την αξιολόγηση των συμμετεχόντων. Όσον αφορά τον κίνδυνο συστηματικού σφάλματος, η συνολική ποιότητα των μελετών ήταν μέτρια. Για τα συμπληρώματα βιταμίνης D, 7 μελέτες (2600 συμμετέχοντες) ανέφεραν την ολική θνησιμότητα (RR: 1.04, 95% CI: 0.83 to 1.31, I^2=35%) με μέτριο βεβαιότητα, ενώ μόνο μία μελέτη ανέφερε τις αλλαγές των επιπέδων ευθραυστότητας. Για τα πολυσυμπληρώματα, βρήκαμε 4 μελέτες (180 συμμετέχοντες) για την θνησιμότητα (δεν πραγματοποιήθηκε ποσοτική σύνθεση) και 2 μελέτες για τις αλλαγές των επιπέδων ευθραυστότητας (MD= -0.28, 95% CI: -0.71 to 0.16, I^2=0%) με πολύ χαμηλή βεβαιότητα και για τις δύο εκβάσεις.

Συμπεράσματα: Η λήψη συμπληρωμάτων βιταμίνης D πιθανώς οδηγεί σε μικρή ή καθόλου αλλαγή στη θνησιμότητα από κάθε αιτία και στη συχνότητα πτώσης, ενώ μπορεί να οδηγήσει σε μικρή διαφορά στα κατάγματα σε άτομα με σύνδρομο ευθραυστότητας. Για πολυσυμπληρώματα, η βεβαιότητα των διαθέσιμων στοιχείων ήταν πολύ χαμηλή για τις κύριες και τις δευτερεύουσες εκβάσεις. Η μελλοντική έρευνα για το σύνδρομο ευθραυστότητας θα πρέπει να επικεντρωθεί σε εκβάσεις που σχετίζονται με τον ασθενή, όπως η αλλαγή στα επίπεδα αδυναμίας, η γνωστική λειτουργία και σε λειτουργικά μέτρα.

Abstract

Introduction: Frail individuals are at risk of high morbidity and mortality. Low levels of a number of vitamins and minerals has been associated with higher frailty incidence and severity. Oral supplementation of these micronutrients is often used in clinical practice without enough backing evidence.

Objectives: To determine the effect of micronutrient supplementation on measures of physical frailty, mortality and other patient-related outcomes.

Methods: A systematic search in PubMed and Embase was conducted on September 2022 and randomized controlled trials investigating the effect of micronutrient supplementation in frail individuals were included. The GRADE guidelines were used to assess the certainty of available evidence.

Results: We included 18 studies described in 30 published articles, reporting on the effect of either Vitamin-D supplementation or multicomponent supplementation, which consisted of a number of micronutrients. Only 7 of 18 studies used a an established frailty scale to assess participants. Regarding risk of bias, overall quality of studies was moderate. For Vitamin D supplementation, 7 studies (2600 participants) reported all-cause mortality (RR: 1.04, 95% CI: 0.83 to 1.31, I^2=35%) with moderate certainty of evidence, while only one study reported on change in frailty levels. For multicomponent supplementation, we found 4 studies (180 participants) on mortality and 2 studies on change in frailty levels (MD= -0.28, 95% CI: -0.71 to 0.16, I^2=0%) with very low certainty of evidence for both outcomes.

Conclusion: Vitamin D supplementation probably leads to little or no change in all-cause mortality and fall incidence, while it may lead to little difference in fractures in frail individuals. For multicomponent supplementation, the certainty of evidence was very low for our main and secondary outcomes. Future research in frailty should focus on patient-related outcomes, such as change in frailty levels, cognitive function and functional measures.

Main Text

Introduction

Description of the Condition

Frailty syndrome is defined as a clinically recognizable state in the elderly population, that is associated with increased vulnerability resulting from decreased physiologic reserves and multi-organ dysfunction, that leads to inability cope with everyday and acute stressors.

Frailty syndrome is a disease of the elderly and is an emerging threat in modern societies. The ever-growing, aging population demands for change in public health policies in order to appropriately address this issue. According to WHO, by 2030 one in six people will be aged 60 years or over and as a result will be in risk of frailty.

The incidence of frailty differs between countries and settings, but it is estimated that one in six community-dwelling older people may have frailty (Ofori-Asenso *et al*, 2019). Additionally, pre-frailty, a state associated with increased risk of morbidity and frailty development, prevails in the elderly. Prevalence of pre-frailty is reported to be as high as 40% in older adults in Germany (Hajek *et al*, 2022).

Diagnosis of frailty is made using clinical criteria. While there is no ‘’gold standard’’ in the diagnosis of frailty, the two main methods to diagnose frailty are Fried’s Frailty Phenotype and Rockwood’s Frailty Index. Fried’s Frailty Phenotype assess physical frailty by measuring five characteristics: slowness, weakness, exhaustion, low physical activity and weight loss. Rockwood’s Frailty Index considers frailty to be a continuum of accumulated deficits and assesses a number of criteria to define frailty, including comorbidities, polypharmacy, difficulty in activities of daily living, cognitive examination and physical measures such as gait speed and handgrip strength. While these two definitions of frailty fundamentally differ in their approach towards the syndrome, both can reliably predict adverse outcomes associated with frailty. While Fried’s Phenotype can be used to assess individuals in a short amount of time, Frailty Index is considered to be more sensitive to longitudinal changes in frailty.

Description of the Intervention

Vitamins and minerals are essential *nutrients* that are required in small amounts for the proper function of biological organisms. Humans get them from food, but it is common among older people to take oral supplements with or without medical prescription. Oral supplements may include a number of components such as proteins, carbohydrates, fat, vitamins and minerals. Some available supplements may only contain a single micronutrient, more commonly vitamin D. In 2018 NHS published a guidance for over the counter medication, stating that the annual spend for vitamins and minerals is circa £ 48,100,000 and that with the exception of vitamin d they should not be routinely prescribed due to insufficient evidence of effectiveness. According to the National Institute for Health and Care Excellence, oral multivitamin and mineral supplements should help individuals who are eating poorly to meet their vitamin and mineral requirement.

How the Intervention might work

Vitamin D effects multiple pathways in humans, as vitamin D receptors are found throughout the human body. If affects muscle function, bone structure and cardiovascular health, as well as

inflammation, cell growth and metabolism. Vitamin D is linked with frailty, as it controls protein anabolism in muscle tissue and has anti-inflammatory properties *(*Bruyère *et al,* 2017*)*.

In pre-clinical models evidence show that vitamin supplementation could be used to attenuate frailty (Bisset and Howlett, 2022). Vitamin D3 supplementation in rats led to significantly lower frailty index compared to age-matched controls. Another study in mice reported increased physical frailty in vitamin D3 deficient rats.

Vitamin and mineral deficiency is common among frail older individuals. Observational studies in humans suggest a strong association between vitamin D deficiency and measures of physical frailty (Kotlarczyk *et al,* 2017). However there evidence on the efficacy of vitamin-d supplementation in elderly are controversial (Reid and Bolland, 2019; Murphy, 2022).

Large population based studies report that this association exists for other vitamins as well. Low intake of B6, C, E and folate was associated with a higher risk for frailty in Spanish population study (Balboa-Castillo *et al,* 2018). Greater severity of frailty was found in older hospitalized patients with vitamin-C deficiency (Sharma *et al,* 2021).

Similarly to vitamins, other micronutrient deficiency could lead to frailty through modulation of inflammation, oxidative stress, muscle and bone metabolism (Semba *et al,* 2006). According to a recent systematic review of observational studies, higher dietary and plasma levels of carotenoid were associated with reduced odds of frailty (Zupo *et al*, 2022). With regard to minerals, low levels of selenium (which has pleiotropic effects including protection from oxidative stress and inflammation) were associated with mortality in frail elderly (Giovannini *et al,* 2018). Additionally, magnesium seems to impact frailty development, based on a study in community-dwelling older Japanese women (Kaimoto *et al,* 2021).

Why it is important to do this review

Nutritional supplements are commonly used by elderly and frail individuals, but it is usually suggested with limited backing evidence. The aim of this systematic review is to determine the effect of micronutrient supplementation on measures of physical frailty, mortality and relevant clinical outcomes related with aging in frail elder adults.

Methods

Eligibility criteria

Types of studies

We Included randomized controlled trials (RCTs), cluster-RCTs and quasi-RCTs. As we are interested in the effectiveness of an intervention, non-randomized studies were excluded from this review. Editorials, letters, commentaries, cross-sectional studies were also not eligible for inclusion.

Types of participants

We included frail and pre-frail individuals irrespective of their housing conditions (resident homes, community-dwelling etc.). As there is no single gold-standard in the diagnosis of frailty, we considered all studies that described their population as frail eligible for this review. Studies that investigated the role of supplements in acute conditions (e.g. SARS-CoV2 infection) were excluded.

Types of interventions (and co interventions)

In this review we expected to find two types of interventions, either supplementation with a single vitamin or micronutrient or with a formulation including numerous vitamins and micronutrients. These two types of interventions were analyzed and synthesized separately.

Additionally we expected that some studies would investigated the effect of supplementation along or versus exercise. If possible, we excluded "exercise" arms or combined "exercise-supplementation" arms, but we included studies in which all participants received exercise.

We included studies that use the following comparison groups:

- Placebo
- No intervention
- Different Dose

Type of Outcome Measures

Primary Outcomes

1) All-cause mortality
2) Frailty levels, as measured by validated frailty scales, such as Frailty Index, and Fried's Frailty Phenotype.

Secondary Outcomes

1) Falls measured as count of falls, prevalence of falls, time to first fall.
2) Fractures, including hip fractures, vertebral fractures etc.
3) Muscle strength as measured by handgrip strength
4) Gait speed
5) Body mass measures including lean mass, fat free mass, total mass, BMI.
6) Cognitive function as measured by validated scales such as Mini Mental State Exam
7) Inflammatory markers including but not limited to cytokines and CRP.
8) Physical performance and functionality

Information Sources and Search Strategy

Electronic Searches

In September 2022, we conducted a comprehensive search on PubMed/Medline and Embase using the following terms: (vitamin* OR mineral* OR lycopene OR ascorbic OR tocopherol OR retinol OR folate OR carotenoid* OR betacarotene] OR selenium OR pyridoxine OR iron) AND ("frail elderly"[MeSH Terms] OR "frail*" OR "frailty").

Selection and Data Collection Process

After the removal of duplicate articles, screening was performed and full text copies of all relevant manuscripts were retrieved. Data were extracted using pre-specified forms.

Study risk of bias assessment

The Cochrane Risk of Bias Tool was used to assess potential risk of bias.

Effect Measures

Statistical analysis was performed using RevMan Web

For dichotomous data, number of events and number of participants for each group were extracted to calculate summary risk ratios and confidence intervals. For continuous data, mean difference, mean change from baseline or postintervention mean for each groups and the respective standard deviations were extracted to calculate summary mean difference and confidence intervals. If this measures were not available, the calculator tool in RevMan Web was used to transform p values, t values, 95% CI or SEMs.

Data synthesis

Random-effects were used in quantitative meta-analysis, due to the expected clinical heterogeneity of the populations in frailty studies, the definition of frailty and different types of intervention.. We included change scores in the meta-analysis using the guidance from the Cochrane Handbook (Chapter 10.5). When appropriate, we combined intervention groups as per the guidance from the Cochrane Handbook (Chapter 6.5.2).

Subgroup analysis and investigation of heterogeneity

We intended to perform subgroup analysis, if substantial heterogeneity was identified, to investigate the following variables:

- Frailty severity
- Different dose/form of intervention

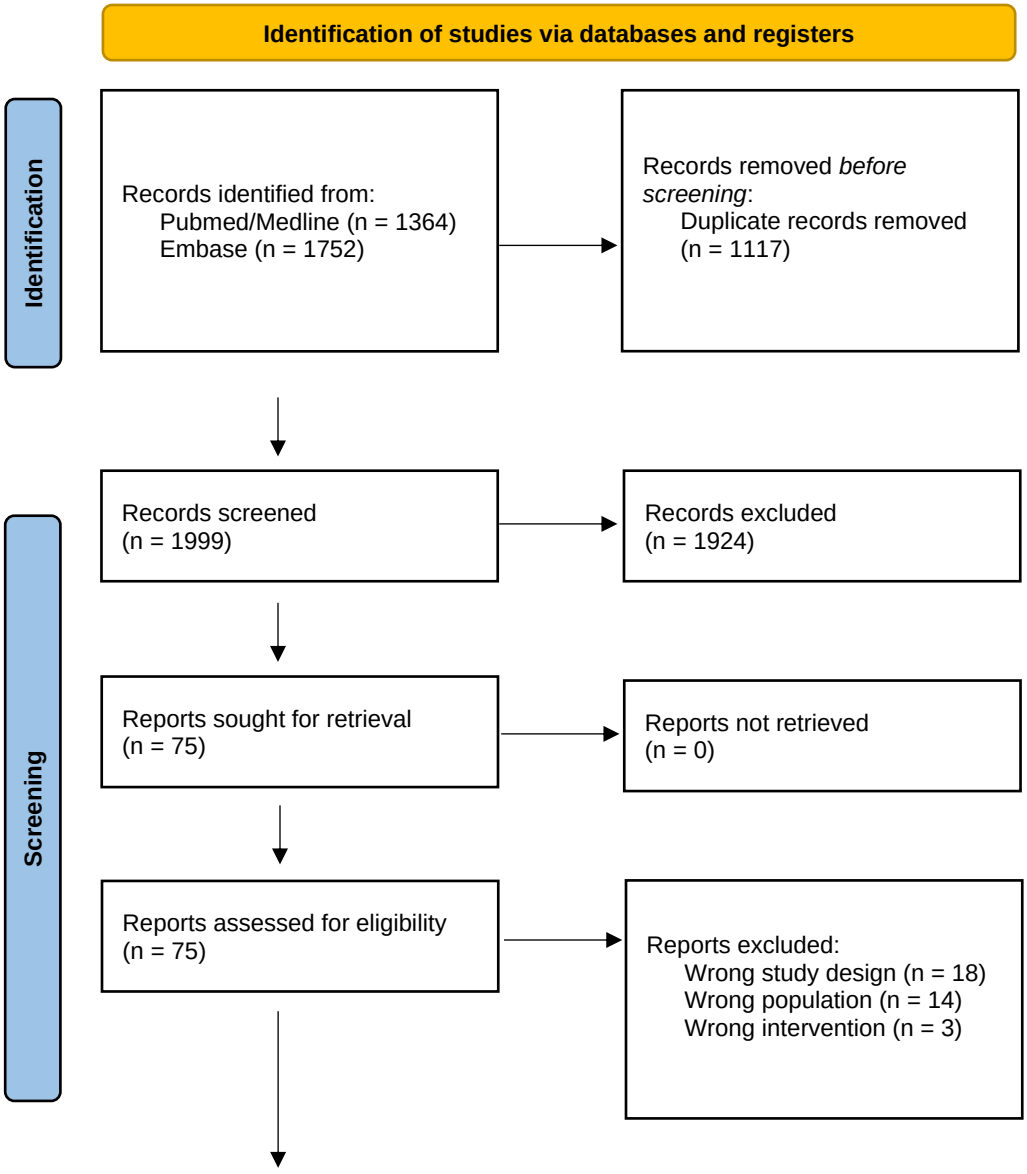
Sensitivity analysis

We planned to perform sensitivity analysis by excluding studies that did not use an established frailty scale, but enough data was not available

Summary of findings and assessment of the certainty of the evidence

The GRADE system was used to assess the certainty of the evidence. For reach outcome, we downgraded appropriately according to the trial's limitations.

Results



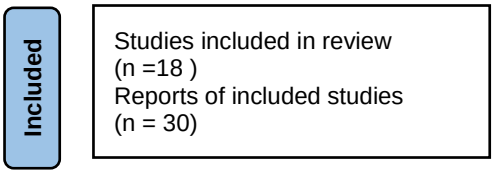


Figure 1: PRISMA Flowchart

Results of the search

Our search in Pub/Med and Embase resulted in 1364 and 1752 articles respectively. After duplicate removal, a total of 1999 articles were screened resulting in 75 potentially eligible reports. 18 reported were excluded due to wrong study design (non-randomized studies, study protocols), 14 due to wrong population and 3 due to wrong intervention. Finally, we included 18 studies described in 30 reports.

Included studies

We identified 9 studies (14 reports) investigating the effect of Vitamin D supplementation in frail elderly. 5 of these studies used an established frailty scale to assess participants; 3 used the Fried's Frailty Scale, 1 used the Winograd Criteria and 1 used the Frail Elderly Functional Assessment. The studies that did not used an established scale were relatively older with the most recent being published in 2011 (Neelemaat *et al,* 2011). The majority included studies administered daily doses of Vitamin D analogues (cholecalciferol, ergocalciferol, calcidiol, alphcalcidiol). Most of these studies used between 400 IU and 1000 IU per day, ranging from 200 IU to 4000 IU in the dose-finding phase STURDY trial (Appel *et al,* 2021). 2 older studies used very high doses of Vitamin D supplementation (Gloth *et al,* 1995: 400 IU per day to 100,000 IU every 3 months and Latham *et al,* 2003: single 300.000 IU dose).

Additionally, we found 9 studies (15 reports) on the effect of multicomponent supplementation in frail elderly. Only 2 of these studies used an established frailty scale, namely the Fried's Phenotype (Na *et al,*2021; Biesek *et al,* 2021). Different formulations and enriched products that contained a number of vitamins and minerals, but also protein carbohydrate and fat. Extensive description of the included studies can be found in the -*Table of Included Studies*-.

Risk of bias in included studies

The overall quality of studies was moderate as 12 out of 18 studies had at least one domain with high risk of bias and only 1 study had low risk of vias in all domains (Vaes *et al,* 2018).

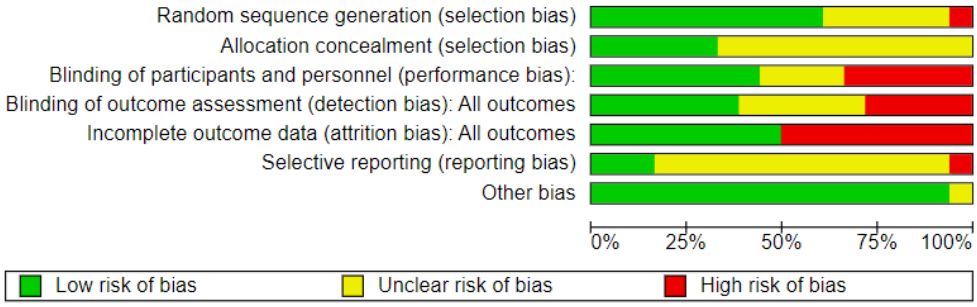


Figure 2: Risk of bias graph with overall bias in included studies

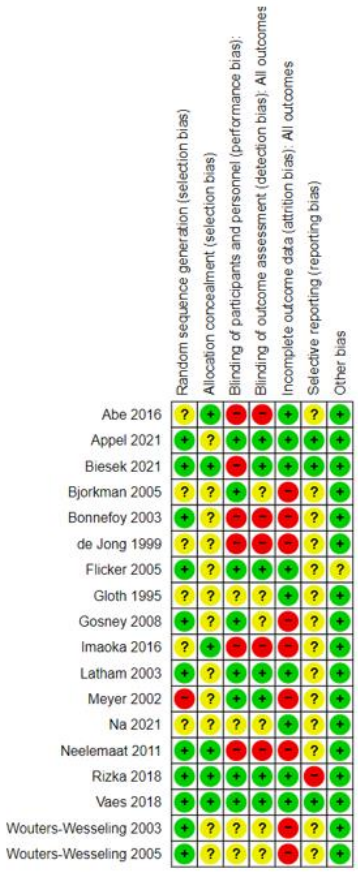


Figure 3: Risk of bias summary in included studies

Allocation

Out of 9 studies on Vitamin D supplementation, 6 had low, 2 unclear and 1 high risk of randomization bias. We considered the randomization method in the study by Meyer et al. to be insufficient, as they divided patients in groups based on date of birth. Additionally, allocation methods were not described adequately in 6 out of 9 studies (Meyer *et al*, 2002).

Regarding studies on multicomponent supplementation, 5 and 6 out of 9 studies did not provide enough details on randomization and allocation concealment respectively.

Blinding

Overall, blinding methods were judged to be adequate in studies on Vitamin D supplementation, with only 1 study having high risk of bias (participants, personnel and outcome assessors) (Neelemaat *et al,* 2011) as participants and researchers were not blinded to the intervention, 1 study had unclear risk of bias (blinding methods for participants personnel and outcome assessors were not described in text) (Gloth *et al,* 1995) and 1 had unclear risk only regarding blinding of outcome assessment (Bjorkman *et al,* 2008).

Contrariwise, studies investigating multicomponent supplementation had mostly high risk of detection and/or performance bias (5 out of 9 studies) and the remaining studies had unclear risk of bias in at least one of the two domains.

Incomplete outcome data

3 studies on Vitamin D supplementation and 5 studies on multicomponent supplementation had high risk of attrition bias, as less than 85% of randomized participants were analyzed in these studies.

Selective reporting

The study protocol was not available in 6 Vitamin D and 8 multicomponent supplementation studies, which were all judged to have unclear risk of bias in this domain. Additionally, we considered the study by Rizka to be in high risk of bias, as incidence of respiratory tract infection, which is the main outcome in the registered protocol, is not reported (Rizka *et al,* 2018).

Other potential sources of bias

Only 1 included study had unclear risk of other bias, as the supplement dose and frequency was changed during the conduction of the study. Reported conflicts of interest and funding for each can be found in the Table of Study Characteristics.

Effect of interventions

Intervention	**Outcome**	**Studies/ Participants**	**Summary Effect Measure**	**Heterogeneity**	**GRADE**
Vitamin D supplementation	All-cause mortality	7 studies/ 2600 participants	RR: 1.04, 95% CI:	I^2=35%	Moderate

vs placebo or control			0.83 to 1.31		
	Frailty levels	-			
	At least one fall	2 studies/ 847 participants	RR:0.99, 95% CI: 0.82 to 1.21	I^2=55%	Moderate
	Fracture	2 studies/ 1769 participants	RR:0.77, 95% CI: 0.59 to 1.01	I^2=0%	Low
	Muscle strength (kg)	3 studies/ 27 participants	MD:-0.62, 95% CI: -1.74 to 0.50	I^2=0%	Very low
	Gait speed	No quantitative synthesis			
	Weight Indices	No quantitative synthesis			
	Cognitive function	-			
	Inflammatory markers	No quantitative synthesis			
	Functional measures	No quantitative synthesis			
Multicomponent supplementation vs placebo or control	All-cause mortality	4 studies/ 180 participants (only 2 deaths were reported in total)			Very low
	Frailty levels (mean Fried's Frailty	2 studies/ 86 participants	MD: -0.28, 95% CI: -	I^2=0%	Very low

	Phenotype score)			0.71 to 0.16		
	At least one fall		No quantitative synthesis			
	Fracture		-			
	Muscle strength (kg)		4 studies/ 153 participants	MD:0.76, 95% CI: -1.35, 2.8	I^2=31%	Very low
	Gait speed		No quantitative synthesis			
	Weight Indices	BMI	2 studies/ 157 participants	MD: 0.69, 95% CI: -0.78 to 2.16	I^2=21%	Very low
		Body weight (kg)	4 studies/ 188 participants	MD: 1.23, 95% CI: -0.91 to 3.37	I^2=30%	Very low
	Cognitive function (MMSE mean score)		2 studies/ 89 participants	MD: 1.34, 95% CI: -1.45 to 4.14	I^2=0	Very low
	Inflammatory markers		No quantitative synthesis			
	Functional measures		No quantitative synthesis			

Table 1: Summary of findings table

Comparison 1: Vitamin D supplementation

Outcomes

1. All-cause mortality

Seven studies with a total of 2600 participants reported the effect of Vitamin D supplementation on mortality. The studies by Rizka and Vaes followed participants for 3 and 6 months respectively and did not report any deaths during the trial's period (Rizka *et al*, 2018; Vaes *et al, 2018)*. Compared to placebo or no intervention, Vitamin D supplementation probably results in little to no difference in mortality (RR: 1.04, 95% CI: 0.83 to 1.31, I^2=35%). The certainty of evidence was moderate, we downgraded one point for high risk of bias, mainly in the trial by Meyer (Meyer *et al*, 2002).

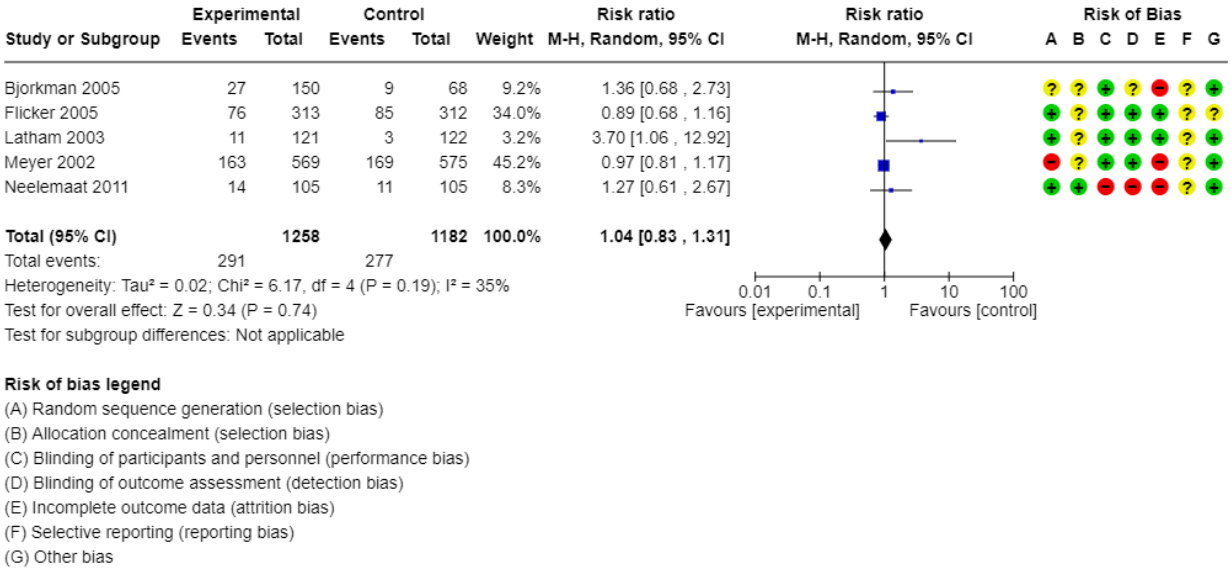


Figure 4: Meta-analysis of studies that reported the effect Vitamin-D supplementation on mortality in frail individuals

2. Frailty levels, as measured by validated frailty scales, such as Frailty Index, and Fried's Frailty Phenotype.

Two studies investigated the effect on Vitamin D supplementation on frailty status. In the trial by Appel, there was no difference between pooled higher doses (1000 IU/d, 2000 IU/d, 4000 IU/d) and control dose (200 IU/d) in risk of frailty incidence, frailty worsening or improving (Appel *et al*, 2021). Interestingly, during the dose-finding phase of the trial the 2000 IU/d group had higher risk of worsening frailty status (hazard ratio (HR) = 1.89, 95% CI: 1.13–3.16, $p = 0.015$), while the 4000 IU/d dose had lower risk for developing frailty (HR = 0.22, 95% CI: 0.05–0.97, $p = 0.045$). These differences might be spurious findings or might be attributed to baseline Vitamin D status.

Gloth et al. did not report the comparison between the two arms (Gloth *et al*, 1995).

3. Falls

We found three studies investigating the number of participants with at least one fall with each study considering a different dose of Vitamin D supplementation, ergocalciferol 1000 IU/d, single oral dose of 300.000 IU and high doses of cholecalciferol 1000-4000 IU/d (Flicker *et al*, 2005*;* Latham *et al*, 2003). The latter used a low dose of 200 IU/d in the control group. None of these studies found a sinificant effect on supplementation on the number of people with at least one fall. Vitamin D supplementation does not reduc e falls in frail individuals (RR=0.99, 95% CI: 0.82 to 1.21, I2=55%, Grade: Moderate, downgrade 1 point for inconsistency).

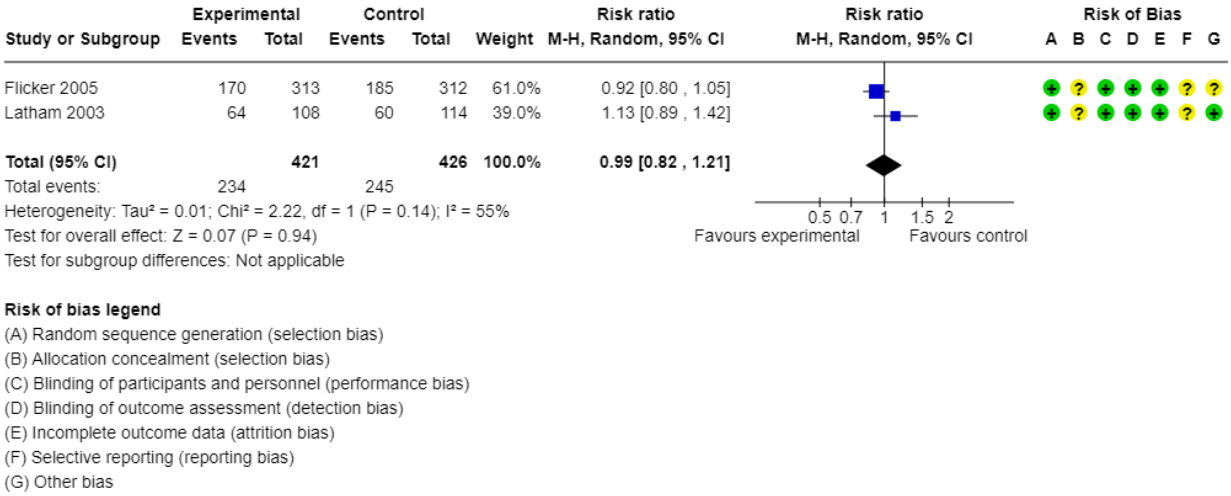
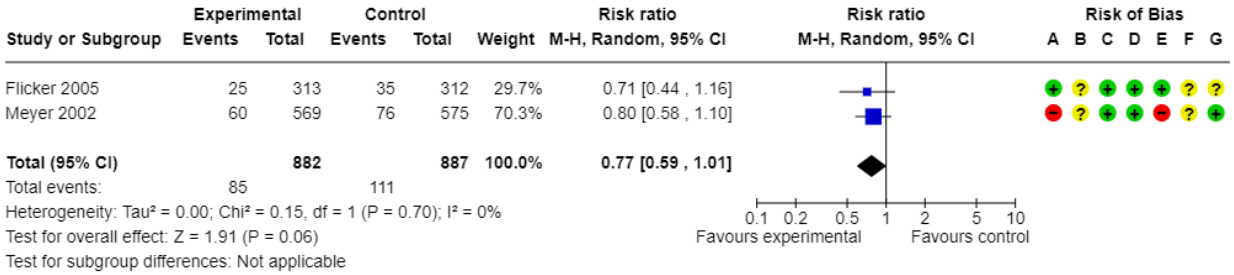


Figure 5: Meta-analysis of studies that reported the effect Vitamin-D supplementation on number of frail participants with at least one fall

4. Fractures, including hip fractures, vertebral fractures etc.

Fractures were reported by three studies. Compared to the other two studies Meyer et al. investigated a relatively lower dose of intervention of cholecalciferol 440 IU/d, but there was no significant differences between the intervention and the control group in any of the studies (Appel *et al*, 2021; Flicker *et al*, 2005*;* Meyer *et al*, 2002). Compared to placebo, Vitamin D supplementation may result to little or no difference on fractures (RR: 0.77, 95% CI: 0.59 to 1.01, I^2=0%, Grade: low, downgrade 1 point for wide CI, 1 point for ROB).

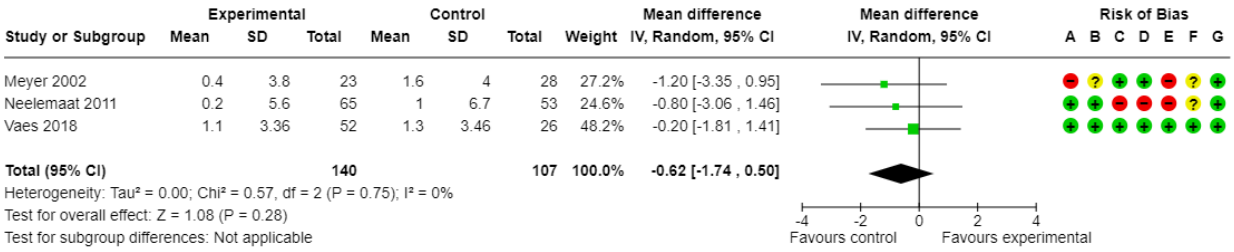


Risk of bias legend
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding of participants and personnel (performance bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

Figure 6: Meta-analysis of studies that reported the effect Vitamin-D supplementation on fractures in frail individuals

5. **Muscle strength as measured by handgrip strength**

Meyer, Neelemaat and Vaes investigated the change in handgrip strength after 12, 3 and 6 months of supplementation respectively (Meyer *et al*, 2002; Neelemaat *et al*, 2011; Vaes *et al*, 2018). All studies had negative findings. This outcome was available in a small number of participants of the Meyer trial (n=51) in a secondary report. In the quantitative synthesis, the two arms in the Vaes study (10 mcg/d of 25(OH)D3 and 20 mcg/d of cholecalciferol) were combined. The evidence is very uncertain about the effect of Vitamin D on handgrip strength (MD:-0.62, 95% CI: -1.74 to 0.50, I^2: 0%, Grade: Very low, downgrade 2 points for imprecision due to low number of participants and wide CI overlapping the line of no effect and 1 point for ROB).



Risk of bias legend
(A) Random sequence generation (selection bias)
(B) Allocation concealment (selection bias)
(C) Blinding of participants and personnel (performance bias)
(D) Blinding of outcome assessment (detection bias)
(E) Incomplete outcome data (attrition bias)
(F) Selective reporting (reporting bias)
(G) Other bias

Figure 7: Meta-analysis of studies that reported the effect Vitamin-D supplementation on handgrip strength in frail individuals

6. Gait speed

Regarding change in gait speed, Appel did not find significant differences between the higher pooled doses group vs the low dose group after 3 and 6 months of supplementation, but the higher doses provided a small but significant protective effect after 12 months of supplementation (MD=0.06 m/sec, 95% CI: 0.02-0.10) (Appel *et al,* 2021). Additionally, there was a significant reduction from baseline after 6 and 12 months of supplementation in both arms. Vaes followed patients up to 6 months and did not find any significant differences between the three arms (25OHD3, cholecalciferol and placebo) regarding gait speed (Vaes *et al,* 2018). Similarly to Appel, there was a within group decrease from baseline during the study period.

7. Body mass measures including lean mass, fat free mass, total mass, BMI.

Two studies reported changes in body mass indexes. Neelemaat did not find any significant differences in fat free mass after 3 months of supplementation, but in subgroup analysis there was a significant increase in participants with weight > 63.9 kg at baseline (mean difference 3.4, 95% CI: 0.2-6.6) (Neelemaat *et al,* 2011). Similarly, in the trial by Vaes supplementation with vitamin D for 6 months did not result in a change in total lean mass compared to placebo (Vaes *et al,* 2018).

8. Cognitive function as measured by validated scales such as Mini Mental State Exam

No studies reported this outcome.

9. Inflammatory markers including but not limited to cytokines and CRP.

Bjorkman et al. did not find any significant differences after different doses of cholecalciferol compared to placebo in either CRP (p=0.523) or fibrinogen (p=0.184). Extreme changes in CRP (e.g. -96.75 to 395.62 in the 1200IU/d arm) might be attributed to other factors, such as acute inflammatory procedures (Bjorkman *et al,* 2011).

In the trial by Rizka et al. supplementation with 0.5 mcg alphcalcidiol daily for 90 days result in significant changes in IL-10 levels, IL-6/IL-10 ratio, CD4/CD8 ratio and CD8+ CD28- percentage. This changes suggest a shift towards a more anti-inflammatory state (Rizka *et al,* 2018).

10. Functionality

Latham et al reported a number of different measures regarding functionality, including Barthel Index, Adelaide activities profile and Medical Outcomes Study 36-item short form questionnaire (SF-36), but did not find any significant differences 3 months after a single high dose vitamin D dose (Latham *et al*, 2003). Likewise, in the Neelemaat et al. study 3 month supplementation of 400 IU vitamin D3 did not lead in significant between group changes in functional limitation score, physical performance score or physical limitation score (Neelemaat *et al*, 2011).

Multicomponent Supplementation

1. All-cause mortality

Four studies with a total of 180 participants reported this outcome, but overall there were only 2 deaths (one in each group) in the study by Imaoka (Imaoka *et al*, 2016). Thus, the evidence is very uncertain about the effect of multicomponent supplementation on mortality in frail individuals.

2. Frailty levels, as measured by validated frailty scales, such as Frailty Index, and Fried's Frailty Phenotype.

Two studies with 86 participants evaluated the effect of multicomponent supplementation on frailty measured with the Fried's Frailty Phenotype (MD= -0.28, 95% CI: -0.71 to 0.16, I^2=0%) (Biesek *et al*, 2021; Na *et al*, 2021). The evidence is very uncertain about the effect of multicomponent supplementation on frailty levels (Grade: Very low, downgrade 2 for imprecision and 1 for risk of bias).

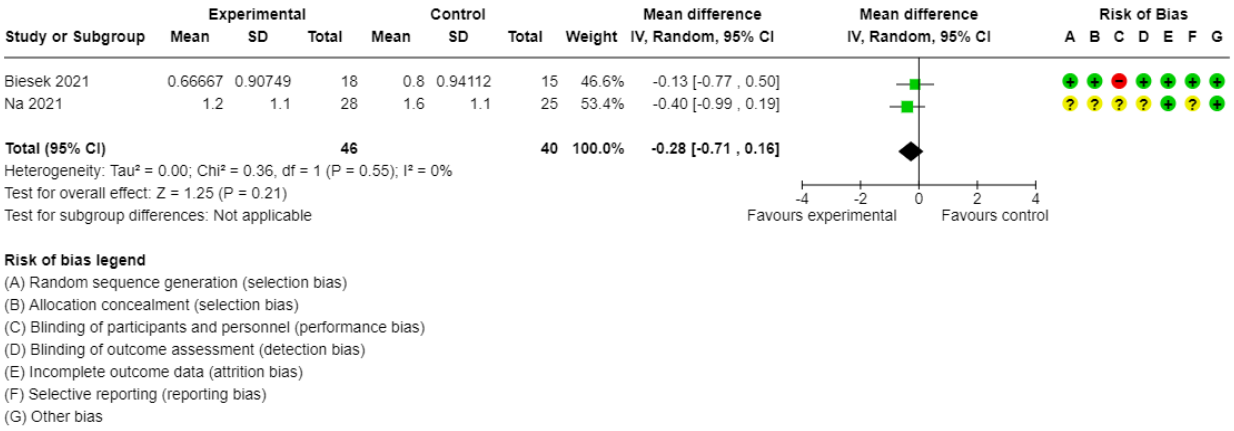


Figure 8: Meta-analysis of studies that reported the effect multicomponent supplementation on frailty levels in frail individuals

3. Falls

Only one study investigated the effect of multicomponent supplementation on falls ((Imaoka *et al*, 2016)). While they did not find significant differences between either the supplementation or the exercise group compared to the placebo, the group that received both interventions had a significantly lower hazard compared to placebo (adjusted for sex and age, HR= 0.276, 95% CI: 0.083-0.924, p=0.037).

4. Fractures, including hip fractures, vertebral fractures etc.

No studies reported this outcome.

5. Muscle strength as measured by handgrip strength

Four studies with a total of 153 participants were included in the meta-analysis (MD=0.76, 95% CI: -1.35, 2.87, I^2=31%) (Abe *et al*, 2016; Imaoka *et al*, 2016; Biesek *et al*, 2021; Na *et al*, 2021). All four studies had negative findings. Additionally de Jong, did not find significant differences between the supplementation and placebo group, but was not included in the meta-analysis as they reported median, 10th and 90th percentile values. The evidence is very uncertain about the effect of multicomponent supplementation on frailty levels (Grade: Very low, downgrade 2 for imprecision and 1 for ROB)

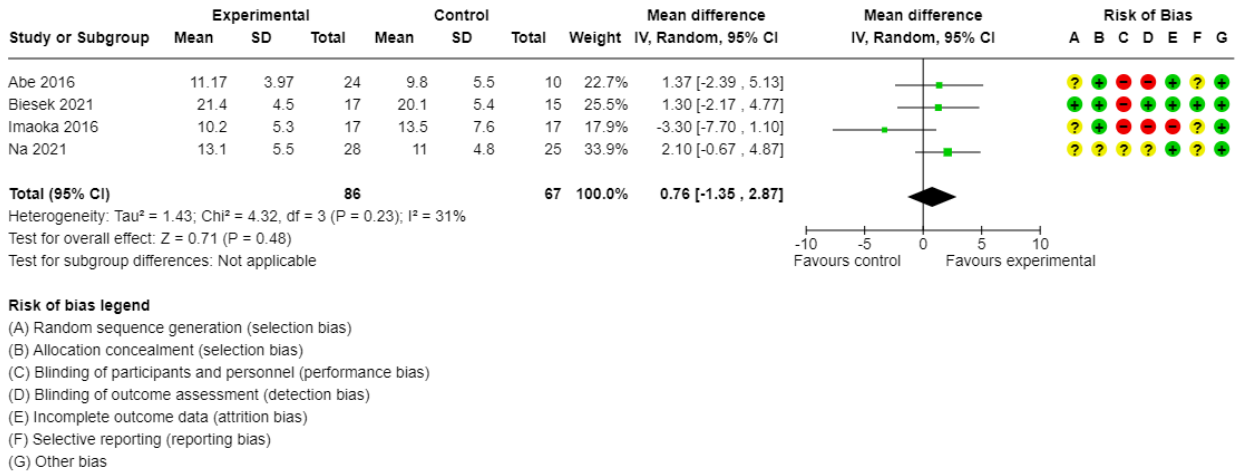


Figure 9: Meta-analysis of studies that reported the effect multicomponent supplementation on handgrip strength in frail individuals

6. Gait speed

Abe et al. analyzed changes after 3 months of supplementation and found a significant difference between the three arms, with the group receiving multicomponent supplementation that included medium-chain acids showing a slight increase compared to baseline (Abe *et al,* 2016). The other two groups (supplementation that included long-chain acids and control) showed a small decline from baseline. It must be noted that there was high attrition in this outcome (only 24 participants analyzed in total) and that the medium-chain acids group had numerically higher walking speed at baseline. Due to these differences, we did not pool the data from the two intervention groups. In the study by Bonnefoy, supplementation did not have a significant effect on 6 meter walk time at 3 and 9 months (Bonnefoy *et al*, 2003). Similarly in the trial by de Jong, changes from baseline were similar for the intervention and the control group (0.0±0.1 and 0.1±0.1 m/sec respectively).

7. Body mass measures including lean mass, fat free mass, total mass, BMI.

Five studies reported the effect of multicomponent supplementation on body mass indexes. We performed 2 separate meta-analyses for BMI (MD: 0.69, 95% CI: -0.78 to 2.16, I^2=21%) and body weight (MD: 1.23, 95% CI: -0.91 to 3.37, I^2=30%). The evidence is very uncertain about the effect of multicomponent supplementation on both indexes (Grade Very Low, downgrade 2 for imprecision and 1 for ROB). The findings of Bonnefoy et al. were not included in the analysis, as they only report % variation from baseline (Bonnefoy *et al* 2003). However they did find a significant increase in BMI compared to the control group after 3 and 9 months of supplementation using nutritional supplements consisting of proteins, carbohydrates, lipids, minerals and vitamins twice daily.

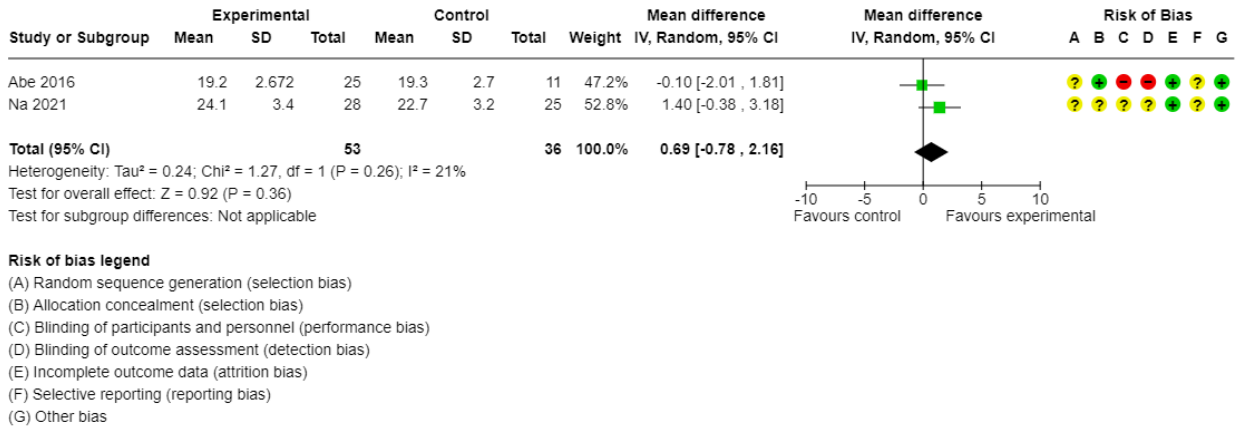


Figure 10: Meta-analysis of studies that reported the effect multicomponent supplementation on BMI in frail individuals

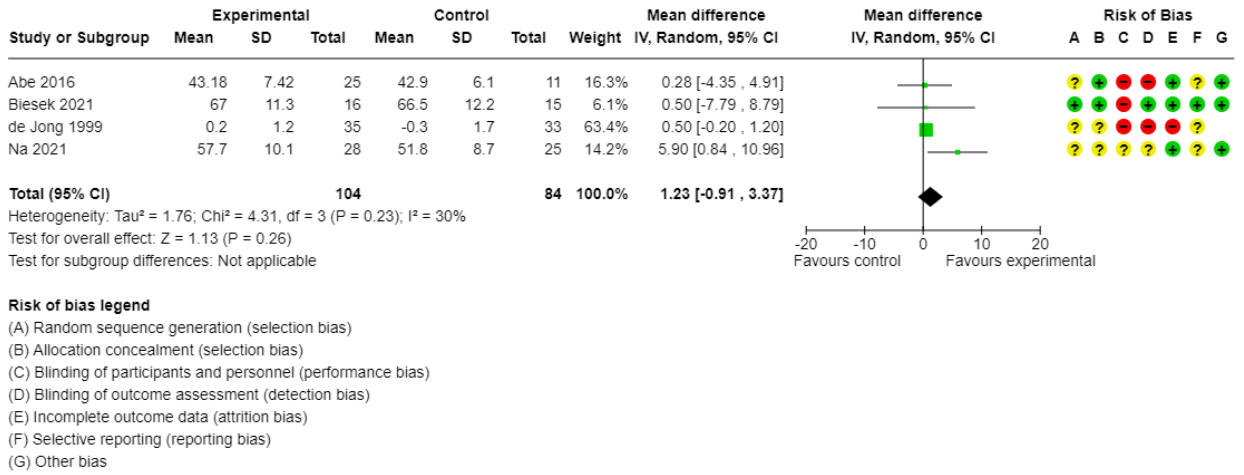


Figure 11: Meta-analysis of studies that reported the effect multicomponent supplementation on body weight in frail individuals

8. Cognitive function as measured by validated scales such as Mini Mental State Exam

Two studies with a total of 89 participants reported on the effect of multicomponent supplementation on MMSE score (MD: 1.34, 95% CI: -1.45 to 4.14, I^2=0%, Grade: Very low, downgrade 2 for imprecision due to low amount of events and participants and 1 for high ROB). The evidence is very uncertain about the effect of multicomponent supplementation MMSE score. Wouters-Wesseling et al. investigated a number of memory tests and found significant differences in word learning test and category fluency (professions) but not in delayed word learning test, recognition memory test for words or category fluency (animals) (Wouters-Wesseling *et al*, 2005). This study was in a high risk of bias due to attrition and these inconsistent findings require further validation.

Regarding dementia, there was a significant difference in Nishimura Geriatric Scale between the intervention groups (nutritional supplementation and different form of fatty acids) and the control group (MD: 9.10, 95% CI: 5.12 to 13.08) (Abe *et al*, 2016). Imaoka et al. did not find a significant effect on Hasegawa's Dementia Scale after 3 months of supplementation (Imaoka *et al*, 2016).

Gosney et al. evaluated the effect of multicomponent supplementation on mood scores (HAD anxiety and depression score and MADR depression score) (Gosney *et al*, 2008). They reported an trend towards positive effect in HAD depression score in the intervention group and contrariwise a trend towards positive effect in HAD anxiety score in the control group. This study was prone to significant attrition bias and enrolled a small number of participants (59 participants were analyzed).

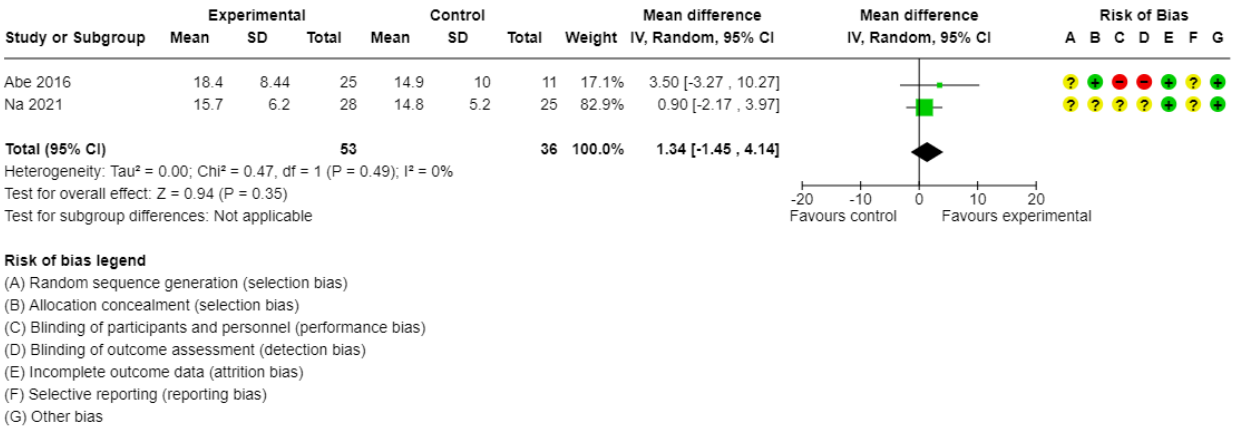


Figure 12: Meta-analysis of studies that reported the effect multicomponent supplementation on MMSE in frail individuals

9. Inflammatory markers including but not limited to cytokines and CRP.

Biesek et al. did not find a significant change in IL-6 levels neither between nor within groups after 3 months of supplementation (Biesek *et al*, 2021). It must be noted that only 9 and 11 participants were analyzed in the control and protein supplementation group respectively. Similar findings were reported by de Jong for CRP (MD: 0.3 CI:95%: −1.5 to 2.2) and ferritin (MD:−7, CI:95%: −18 to 5), but according to the authors there CRP was calculated only in 11 participants (de Jong *et al*, 1999). The exact number of participants that had a ferritin measurement is not reported by the authors.

10. Functionality

We identified three studies that reported a number of different measures regarding the effect of multicomponent supplementation on functionality. More specifically, Imaoka reported on functional independence measures after 6 months, de Jong on activities of daily living score, mobility score and self-care score after 17 weeks and Na on activities of daily living after 3 months (de Jong *et al*, 1999; Imaoka *et al*, 2016; Na *et al*, 2021). None of these between group comparison revealed a significant between group difference.

Discussion

We included 18 studies presented in a total of 30 reports, that compared either vitamin D or multicomponent supplementation to no treatment or placebo in frail elderly population. In outcomes with enough data to perform quantitative synthesis, we did not find significant differences between supplementation and control groups. Quality of evidence was moderate only in all-cause mortality and falls in the vitamin D analysis, while it was low or very low for all other outcomes. Meta-analysis of two studies (1769 participants) reporting on fractures showed a trend towards lower fracture incidence in participants receiving vitamin D supplementation. Limited positive findings were reported in the literature suggesting that vitamin D supplementation might lead towards a more prominent anti-inflammatory state.

According to our findings, high quality evidence on this subject are currently lacking. Misclassification of participants as frail due to not utilizing frailty scales might limit the generalizability of the findings in the included studies. We could not perform an appropriate a priori subgroup analysis and funnel plots to detect differential effects and publication bias respectively because of the paucity of the included studies. The overall quality of evidence of included studies was moderate to low, as 12 out of 18 studies had high risk of bias in at least one domain.

Similar to our study, a recent systematic review by Prokopidis et al. did not find a positive effect in sarcopenic older adults after vitamin d monotherapy (Prokopidis *et al,* 2022). Nevertheless, the inverse association between serum 25(OH)D concentration and frailty severity, make vitamin D analogues an attractive choice for further testing (Marcos-Pérez *et al,* 2020; Zhou *et al,* 2016). Considering the multi-faceted nature of frailty, multifactorial interventions might be more appropriate and supplementation monotherapy (Lee *et al,* 2020). On this notion, exercise has been shown to improve physical performance in frail individuals and the combination of these interventions might produce better results (Gielen *et* al, 2021; Huang *et al,* 2022; Kirwan *et al,* 2022; Weng *et al,* 2022).

Strengths and Limitations: We have performed a rigorous and strict search and screening strategy in order to locate all relevant studies on this subject. Manuscripts for all eligible studies were available. Inclusion of studies that do not utilize an established frailty scale to diagnose frailty syndrome, might limit the applicability of the results and introduce systematic bias.

Due to the low quality of available evidence, the results of this review cannot suggest for or against the use of micronutrient supplementation in the frail elderly in clinical practice. Prior to prescribing such formulations, medical practitioners should inform their patients on the lack of strong evidence on the respective clinical outcomes. Future research on this subject should perform proper geriatric assessment in all elderly participants to allow for more robust recommendations in this vulnerable population. Longer follow-up and consideration of frailty levels as a relevant outcome should be considered when designing such trials.

Conclusion

Vitamin D supplementation probably leads to little or no change in all-cause mortality and fall incidence, while it may lead to little difference in fractures in frail individuals. For multicomponent supplementation, the certainty of evidence was very low for our main and secondary outcomes. Future research in frailty should focus on patient-related outcomes, such as change in frailty levels, cognitive function and functional measures.

Conflicts of Interest: We have no conflicts of interest to disclose

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Tables

Study	Methods	Participants	Intervention	Outcomes	Notes
Vaes 2018 (incl Hangelbroek 2019	randomized, double-blind, placebo-controlled trial	≥65 y of age, had a serum 25(OH)D concentration between 20 and 50 nmol/L, a BMI (in kg/m2) between 18.5 and 35, and who were prefrail or frail based on the frailty criteria of Fried	Arms 1 (n=26), 2 (n=26) and 3(n=26) received a capsule of 10mcg 25(OH)D3 (calfecidiol), 20 mcg Vitamin D3 (cholecalciferol) or	Strength test and physical performance (maximal knee-extension and knee-flexion, hand grip strength, SPPB, timed up	

			placebo respectively. Study supplements were identical in appearance and taste	and go test, gait speed, chair rise-5 stands, 95% ellipse), serum 25(OH)D, plasma PTH, serum calcium, urine calcium-to-creatinine ratio, muscle fiber type and size, total lean mass, safety evaluation, transcriptomic analysis. Follow-up 6 months.	
Neelemaat 2011	randomized controlled trial	elderly patients (inclusion criteria: ≥60 years of age, expected length of hospital admission >2 days) newly admitted to the departments of general internal medicine, rheumatology, gastroenterology, dermatology, nephrology, orthopedics, traumatology, and vascular surgery. Participants described as frail	Intervention group (n=105) received 400 IU vitamin D3 and 500 mg calcium per day for 3 months. Additional oral	Functional limitations score, Physical performance score, Physical activity score, changes in body weight and	

		by study authors, but a frailty scale was not used.	nutritional supplement leading to an expected increase in intake of 2520 kJ/d and 24 g protein/d and telephone counseling by a dietitian. Control group (n=105) received usual care.	fat free mass, changes in handgrip strength.	
Flicker 2005	Randomized, placebo-controlled double-blind, trial	Older people resident in 60 hostels and 89 nursing homes. Participants described as frail by study authors, but a frailty scale was not used. Mean Age (83.3 ± 8.8 vs 83.6 ± 7.8). Subjects whose 25D level was less than 25 nmol/L or above 90 nmol/L were excluded.	All randomized subjects were prescribed 600 mg of elemental calcium in the form of calcium carbonate to be taken daily. Intervention group (n=313) received 10,000 IU ergocalciferol tablets once per	Falls, fractures, compliance with therapy.	

			week, which was changed to o 1000 IU ergocalciferol capsules given once daily due to discontinuation of the original product. Placebo arm received placebo identical to both supplements and in identical fashion.		
Appel 2021 (includes Cai 2022 and Wanigatunga 2021)	Two-stage, Bayesian, response-adaptive randomized trial	688 participants, community dwelling, aged ≥70 years, with elevated fall risk and serum 25-hydroxyvitamin D level of 25–72.5nmol/L. 61% of the participants were pre-frail and 13 % were frail at baseline (Fried's Frailty Phenotype). 91 more randomized at the confirmatory stage.	The control group dose, 200IU/day. The intervention group was assigned to 1000, 2000 or 4000 IU/day during the dose-finding part of the study. The 1000IU/day dose was	The primary outcome was time to first fall or death over two years, whichever occurred first. The secondary outcome was gait speed, although study documents	

			declared the best dose and participants previously randomized to 2000 and 4000IU/day were switched to 1000IU/day.	mentioned that other study outcomes were secondary. Gait speed was obtained from the timed 4-meter walk component of the Short Physical Performance Battery and was measured at baseline and 3, 12, and 24 months. Rates of SAEs, serious falls (fall resulting in a fracture or dislocation or a fall associated with a hospitalization or other SAE), falls with hospitalization, and	

				serious events potentially related to vitamin D were examined for safety. Frailty components (Fried's Frailty Phenotype) and additional information about falls (indoors, outdoors, fracture etc.) in separate publications.	
Latham 2003	Multicenter, randomized, controlled trial with a factorial design	Aged 65 and older, considered frail according to simple clinical measures of frailty as described by Winograd ((meets any one of the following criteria) Cerebrovascular accident Chronic and disabling illness Confusion Dependence in ADL's Depression Falls Impaired mobility Incontinence Malnutrition Polypharmacy Pressure sore Prolonged bedrest Restraints Sensory impairment	Factorial 2x2 design. Arm 1: The vitamin D intervention was given in a single oral dose. Patients received either six 1.25-mg calciferol (300,000 IU) or	Self-rated physical health (physical component of the SF-36 questionnaire) at 3 months and falls (number of falls, number of people who	Effects for each intervention are reported separately.

		Socioeconomic/family problems).	matching placebo tablets. Arm 2: The resistance exercise intervention consisted of a quadriceps exercise program using adjustable ankle cuff weights undertaken three times per week for 10 weeks. Arm 3: Combination. Arm 4: Control	fell) over the 6-month period.	
Gloth 1995	Randomized, controlled intervention study	Frail homebound community-dwelling older people. Patients who were less than 65 years old or who had been outside in the previous 6 months were ineligible for this study.	All subjects received either calcium or calcium and vitamin D (400 IU per day to 100,000 IU every 3 months of ergocalciferol).	Change in Frail Elderly Functional Assessment score.	Number of participants randomized in each group not reported.
Rizka 2018	Double blind	Elderly subjects aged >60 years old who visited a geriatric	IL-6, IL-10 and IFNγ		

	randomized controlled trial	clinic. 24.5% of the participants were pre-frail and 50.9% were frail.	and percentage of CD4, CD8, CD8+ CD28- T cell.		
Bjorkman 2005 (including Bjorkman 2009	Randomized double-blind controlled trial	Long-term inpatients aged over 65 years, chronically impaired mobility, stable general condition, and no known present disease (except osteoporosis) or medication (vitamin D supplements, glucocorticoids, antiepileptics, etc.) affecting calcium or bone metabolism.	Participants were randomized in three groups receiving cholecalciferol in Migliol oil in dose equivalent to 0 IU (n=68), 400 IU (n=77) or 1200 IU (n=73) respectively. Participants with insufficient consumption of dairy products received calcium supplementation during the intervention.	Mortality, 25(OH)Vit-d, PTH, Carboxy-terminal telopeptide of type I collagen, amino-terminal propeptide of type I procollagen, calcium, phosphorous, creatinine, GFR, albumin, CRP.	

Meyer 2002 (including Smedshaug 2007)	Double-blinded randomized controlled trial	Frail elderly nursing home residents, mean age at baseline was 84.7 years (SD ± 7.4 years).	The intervention group (n=569) received 5 ml of ordinary cod liver oil including 2.2 µg/ml vitamin D3 daily, whereas the placebo (n=575) was ordinary cod liver oil in which vitamin D had been removed (0.1-0.2 µg/ml vitamin D3). The difference between groups was 10 µg per 5-ml dose.	Hip fracture (defined as cervical or trochanteric fracture), all nonvertebral fractures, mortality, grip strength, 25-hydroxyvitamin D (calcidiol), osteocalcin, parathyroid hormone (PTH), and ionized calcium.	

Table 1: Characteristics of included studies on Vitamin D supplementation.

Study	Methods	Participants	Intervention	Outcomes	Notes
Imaoka 2016	randomized, non-blind, controlled clinical trial	Frail elderly who lived in the care facility and not received any regular supplementation of vitamin D during the previous 12 months. A	The control group (n = 23) was provided usual care. The low-exercise group (n = 22) did not perform group exercise, but were provided two sessions of individualized exercise each week. The nutrition group (n = 23) was given daily oral vitamin D (900 IU), via an Isocal jelly PCF (500 IU) and a supplement (400 IU). Supplementation included natrium, kalium, magnesium, phopsphorus, iron, zinc, copper, selenium, vitamin d, niacin, vitamin-b12 . The combined group (n = 23) performed the same exercise as the low-exercise group and received the same vitamin D supplementation as the nutrition group.	Mortality, hand grip strength, 25(OH)D, skeletal muscle index, hasegawa's dementia scale, functional independence measure, falls.	Only nutrition vs control groups were included in this review (n=46).
Bonnefoy 2003	factorial non blinded	Frail elderly living in	Factorial 2x2 design. Group 1:	Changes in quadriceps	Outcome measures are

	randomized controlled trial	retirement homes with mean age over 83 years, multiple diagnoses, several medications, and a length of stay of more than 3 years in retirement homes for the elderly.	Received nutritional supplements twice daily, consisting of proteins, carbohydrates, lipids, minerals (Ca, P, Mg, Fe) and vitamins (A, B1, B2, B5, B6, nicotinamide ,B12, C, E, D3, biotin, folic acid). Placebo for nutritional supplementation had an identical packaging. Group 2: Received moderate exercise three times weekly for 60 minutes. Weekly memory sessions served as placebo for exercise program. Group 3: Combination. Group 4: Placebo.	muscle power, changes in fat—free mass, 6m walk time, six-stair climb time, BMI, resting metabolic rate. Assessments were done at 3 and 9 months.	given for nutritional intervention (n=30) vs placebo (n=27) (effect of intervention) .
Biesek 2021	randomized controlled clinical trial	Pre-frail older women (according to Fried's Frailty Scale) with moderate" kidney functioning (i.e., a glomerular filtration rate (GFR) of 30–60	Participants were divided into five groups (18 participants in each group): control (CG); exergames training (ETG); protein supplementation (PSG); exergames combined with protein supplementation (ETPSG);	Frailty score after 12 weeks, bmi, lean mass, appendicular skeletal muscle mass, IL-6, 25(OH)D, handgrip strength, peak torque, food intake.	Only protein supplementation and control groups were included in this study.

		mL/min/1.73 m^2), estimated by the Chronic Kidney Disease Epidemiology Collaboration (CKD-EPI) equation; if presented, Type II diabetes had to be compensated (<8% glycated hemoglobin); and had adequate visual acuity assessed by the Snellen card (20/70 unilateral).	exergames combined with isoenergetic supplementation (ETISG). Protein supplementation group received a supplement once daily which included why protein isolate, carbohydrates, lipids, minerals (sodium, potassium, chloride, calcium, iron, phosphorus, magnesium, zinc, copper, manganese ,molybdenum, selenium, chromium, iodine) and vitamins (A, D, E, K, B1, B2, niacin, pantothenic acid, B6, folic acid, B12, biotin). Isoenergetic supplementation provided amount of kcal similar to the protein supplementation group. Exercise consisted of physical training twice a week for 12 weeks.		
Wouters-Wesseling 2003	Randomized, double-blind, placebo	Frail elderly people 65 years of age or older	The intervention group (n=28) received an enriched drink contained	Plasma antioxidant level after 6 months of	

	controlled trial	were selected based on a body mass index (BMI) of less than 25 kg/m2 and residency in a home for the elderly or sheltered housing. A frailty scale was not used.	energy (100 kcal/100 mL), protein, carbohydrate, fat and micronutrients in amounts of approximately 30% to 150% of US RDA, with higher levels of antioxidants, (sodium, potassium, chloride, calcium, phosphorus, magnesium, iron, zinc, copper, manganese, fluoride, molybdenum, selenium, chlorium, iodine, vitamins A, D, E, K, C, B1, B2, B6, B12, carotenoids, niacin, pantothenic acid, folate). The placebo (n=27) contained no energy or micronutrients.	supplementation. Vitamin E, Vitamin C, Trolox equivalent antioxidant capacity, uric acid, cysteine, total thiol, glutathione peroxidase.	
Wouters-Wesseling 2005	Randomized, double-blind, placebo-controlled trial	Frail white persons aged 65 years or older who had a BMI less than 25 kg/m2 and resided in a home for elderly persons or	The intervention group (n=34) received an enriched drink contained energy (100 kcal/100 mL), protein, carbohydrate, fat and micronutrients in amounts of approximately 30%	Neuropsychological tests, plasma homocysteine, plasma B12. Assessments were done after 6 months of supplementation.	

		sheltered housing residence.	to 150% of US RDA, with higher levels of antioxidants, (sodium, potassium, chloride, calcium, phosphorus, magnesium, iron, zinc, copper, manganese, fluoride, molybdenum, selenium, chlorium, iodine, vitamins A, D, E, K, C, B1, B2, B6, B12, carotenoids, niacin, pantothenic acid, folate). The placebo (n=33) contained no energy or micronutrients.		
Na 2021	Case-controlled, double-blind, and randomized controlled trial.	Elderly at community care facilities. 73% of the participants were pre-frail and 11% were frail at baseline. Mean age 80.8±7. The frailty status was determined using the Korean	Intervention group (n=31) received daily oral nutritional supplement including protein, carbohydrate, fat, minerals (sodium, calcium, phosphorus, potassium, magnesium, iron, zinc), vitamins (A, B1, B2, B6, B12, C, D, E, folic acid, niacin, pantothenic acid, biotin) for 90 days. Supplementation in	Change in weight, BMI, arm and calf circumference, body fat, lean mass, hand grip strength, appendicular skeletal muscle mass, activities of daily living, simplified nutritional appetite questionnaire, MMSE, frailty levels, changes in dietary	

		version of the fatigue, resistance, ambulation, illnesses, and loss of weight scale (K-FRAIL).	control group (n=31) was similar but had lower amounts of carbohydrate, fat, protein, calcium, phosphorus, zinc, vitamins A, C, D, E.	intake. Assessments were done after 3 months of supplementation.	
Gosney 2008	Double-blinded randomized controlled trial	Frail elderly residents from nursing and residential homes aged over 60 years. Median age was 82 years.	The micronutrient supplement and placebo (n=37) were identical in appearance. Participants took two tablets, twice a day for 8 weeks. The intervention group (n=33) received an active supplement including minerals (iron, zinc, copper, iodine, manganese, chromium, selenium, molybdenum, calcium, magnesium) and vitamins (A, D3, E, B1, B2, B6, B12, C nicotinamide, folic acid, biotin, calcium pantothenate).	HADS anxiety score, HADS depression score, MADRS score	
de Jong 1999	Randomized controlled trial	Free-living frail elderly Dutch people. The following	Factorial 2x2 design. Nutrition group (n=37) received of two enriched products	Dietary intake, blood vitamins levels, biochemical, hematological	

		criteria were used: requirement of health care, such as home care or meals-on-wheels service; age (≥ 70 y); no regular exercise; body mass index (BMI)3 belo w average (≤25 kg/m2 on the basis of self-reported weight and height) or recent weight loss; no use of multivitamin supplements ; and ability to understand the study procedures. A frailty scale was not used. 145 out of 217 randomized participants	daily , which delivered ~100% of the Dutch recommended daily allowance (RDA) of the following vitamins: D, E, thiamin, riboflavin, B-6, folic acid, B-12 and C and ~25–100% of the Dutch RDA of the following minerals: calcium (25%), magnesium (25%), zinc (50%), iron (50%) and iodine (100%). Subjects in the control (n=34) and exercise group received the natural amount of the regular products (amount of vitamins and minerals in regular products at the highest 15% of the concentration in enriched products. Exercise group (n=35) received twice daily moderate, gradually increasing intensity. Combinati on group (n=39) received both interventions.	and inflammatory markers serum levels, smell, taste and appetite changes, grip strength, walking speed, chair stands, fitness score, activities of daily living, mobility score, self-care score, lean body mass, performance score, neuropsycholo gical test, psychological wellbeing (SSWO score), subjective health, social involvement. Assessment after 17 weeks of intervention.	

		were analyzed.			
Abe 2016	Single-blind, randomized controlled trial	Frail elderly residing in nursing home and requiring special care from a helper. A frailty scale was not used.	The first group (*n* = 13) received an L-leucine (1.2 g) and cholecalciferol (20 μg)–enriched supplement with 6 g medium-chain TGs (LD + MCT). As an energy-matched control to the supplementation of MCTs, the second group (*n* = 13) received the same leucine and cholecalciferol–enriched supplement with 6 g long-chain TGs (LCTs) (LD + LCT). The control group (*n* = 12;) did not receive any supplements. The control group did not receive an energy-equivalent placebo. The L-leucine and cholecalciferol supplement also included carbohydrate, fat, sodium, thiamin, pyridoxine and cyanocobalamin.	Body weight, BMI, estimated muscle mass, hand-grip strength, walking speed, peak expiratory flow, leg open-close test, MMSE, Nishimura geriatric scale. Study duration was 3 months.	

Table 2: Characteristics of included studies on multicomponent supplementation.

Study	Random sequence Generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias
Vaes 2018 (incl Hangelbroek 2019	Low/ Randomization in permuted blocks and stratified by sex and BMI.	Low/ Investigators blinded to allocation treatment.	Low/ Double-Blinded and study supplements were identical in appearance and taste.	Low/ Investigators blinded to treatment.	Low/ 75 out of 78 participants completed the study.	Low/ Minimal deviations from published protocol.	Low/ Falls not reported, One author has a related patent, Sponsors not reported in manuscript.
Neelemaat 2011	Low/ A computerized random number generator was used to assign patients, in blocks of 10.	Low/ Consecutively numbered opaque envelope containing the patients' group assignment.	High/ Participants, research assistant, and researcher were not blinded.	High/ Research assistant, and researcher were not blinded. When performing the analyses, the primary investigator was not aware of the patients' group assignment.	High/ 30 out of 105 patients in each group were lost to follow up. Even higher attrition for some outcomes.	Unclear/ Netherlands registry is no longer available.	Low/ The Netherlands Organization for Health Research and Development (ZonMw) funded the trial, project number 94506203.

Flicker 2005	Low/ Subjects were randomized via computer-generated lists.	Unclear/ Not described in text.	Low/Residents, institutional staff, and study staff were blinded to treatment allocation.	Low/ Institutional staff, and study staff were blinded to treatment allocation.	Low/ 10% attrition after 1 year and 15% after 2 years of study.	Unclear/ Protocol not available.	Unclear / Study supplement changed during the trial. Funding for this study was provided by (Australian) National Health and Medical Research Council (NHMRC) Project Grants 964135 and 139124 and the Victorian Health Promotion Foundation (VHPF). The NHMRC and VHPF played no role in the study design or in the collection, analysis, or interpretation of data. Dr. Stein received financial support from the Wenkart Foundation and the Royal Australasian College of Physicians Vincent Fairfax Family Foundation Research Fellowship. Supplements and

							placebos were purchased commercially, and the suppliers played no role in the study design or in the collection, analysis, or interpretation of data.
Appel 2021 (includes Cai 2022 and Wanigatunga 2021)	Low/ Assignments were generated using a computer-generated random number and a web-based application.	Unclear/ Not described in text.	Low / Study personnel and participants were masked to randomized dose, occurrence of adaptations, and the transition from dose-finding to confirmatory stage.	Low/ Triple-blinded, study personnel and were masked to randomized dose, occurrence of adaptations, and the transition from dose-finding to confirmatory stage.	Low/ 779 patients randomized, data on 688 patients, 15% attrition during the 4-year duration of the trial.	Low/ All outcomes in study protocol have been reported.	Low/ The NIA encouraged several design features, including an adaptive trial to assess the efficacy and dose-response of vitamin D supplementation for fall prevention and a non-placebo control group. The NIA had no role in the collection, analysis, and interpretation of data; no role in the preparation, review, and approval of the manuscript; and no role in the decision to submit this manuscript for publication

Latham 2003	Low/ Computerized central randomization scheme.	Unclear/ Not described in text.	Low/ Participants and personnel blinded for Vitamin-d intervention.	Low/ "Research nurses who were blinded to the assigned treatments conducted follow-up visits at 3 and 6 months post randomization in the patients' place of residence."	Low/ 7 participants withdrawn.	Unclear/ Protocol not available.	Low/ Supported by grants from the Health Research Council of New Zealand, the Auckland University of Technology Research Fund, and a bequest from the Lenore Wilson Estate.
Gloth 1995	Unclear/ Not described in text.	Unclear/ Not described in text.	Unclear/ Not described in text.	Unclear/ Not described in text.	Low/ All subjects completed follow-up.	Unclear/ Protocol not available.	Low/ Funding not reported.
Rizka 2018	Low/ Computerized random sequence generation program.	Low/ Allocation concealment was performed.	Low/ Subjects and investigators were blinded.	Low/ Outcome assessors were blinded.	Low/ All randomized studies were analyzed.	High/ Incidence of respiratory tract infection (main	Low/ Sources of funding not reported.

						outcome in registered protocol) is not reported.	
Bjorkman 2005 (including Bjorkman 2009	Unclear/ Not described in text.	Unclear/ Not described in text.	Low/ Patients and ward nurses were blinded to the intervention.	Unclear/ Not described in text.	High/ 173 out of 218 participants analyzed (21% attrition).	Unclear/ Protocol not available.	Low/ The study was funded by a special governmental subsidy for health sciences research and training to Helsinki University Central Hospital.
Meyer 2002 (including Smedshaug 2007)	High/ Before the study started, the days of the month (1-31 days) were divided randomly into group A and group B, and based on the day of birth, a participant was placed	Unclear/ The nursing staff was not aware of the details in the allocation procedures	Low/ The participants, the nursing staff and the investigators were blinded.	Low/ The outcome assessor was not otherwise involved in the study and had no knowledge about the study participants	High/ 449 out of 1144 stopped treatment for other reasons except death.	Unclear/ Protocol not available.	Low/ Funding not reported.

	automatically in group A or group B when registered in the study database.						

Table 3: Risk of bias of included studies on Vitamin D supplementation.

Study	Random sequence Generation	Allocation concealment	Blinding of participants and personnel	Blinding of outcome assessment	Incomplete outcome data	Selective reporting	Other bias
Imaoka 2016	Unclear/ Not described in text.	Low/ Opaque envelopes were used	High/ Blinding was not performed.	High/ Blinding was not performed.	High/34 out of 46 participants were analyzed (26% attrition).	Unclear/ Protocol not available.	Low/ This study was funded by Nestle Health Science (Tokyo, Japan). The sponsor of the study had no role in the study

							design, conduct of the study, data collection, data interpretation or, preparation of the report.
Bonnefoy 2003	Low/ Centralized and stratified between the homes and occurred the same day before starting sessions for participants in each home, using a remote data-entry system	Unclear/ Not described in text.	High/ Blinding was not performed.	High/ Blinding was not performed.	High/42 out of 57 were analyzed (26% attrition).	Unclear/ Protocol not available.	Low/ Funding and conflicts of interest not reported.
Biesek 2021	Low/ Randomization into blocks was performed at randomization.com.	Low/ Described in study protocol.	High/ Personnel who carried out the interventions was blinded to the group, but participants in the control	Low/ In study protocol: "the researchers who carry out the evaluations and interventions	Low/ 3 participants lost to follow up in control group and 0 in the protein supplementation group.	Low/ Outcomes reported as described in protocol.	Low

			did not receive placebo.	will be blinded to the allocation of the groups and the block sizes''.			
Wouters-Wesseling 2003	Unclear/ Not described in text.	Unclear/ Not described in text.	Unclear/ Not described in text (only double-blind reported).	Unclear/ Not described in text (only double-blind reported).	Low/ No patients were lost to follow-up.	Unclear/ Protocol not available.	Low/The study was sponsored by Numico Research B.V.
Wouters-Wesseling 2005	Low/Randomly assigned, in groups of four matched for body mass index.	Unclear/ Not described in text.	Unclear/ Not described in text (only double-blind reported).	Unclear/ Not described in text (only double-blind reported).	High/ 67 out of 101 patients analyzed (33% attrition).	Unclear/ Protocol not available.	Low/ Sponsored by Numico Research B.V., Wageningen, The Netherlands
Na 2021	Unclear/ Randomization based on sex and frailty status via stratified cluster random sampling.	Unclear/ Not described in text.	Unclear/ Only characterized as double-blind with no further information.	Unclear/ only characterized as double-blind with no further information.	Low/ 53 out of 62 subjects analyzed (14% attrition).	Unclear/ Protocol not available.	Low/ NOS-NPO® and Placebo product were provided by Deasang Corporation, Korea.
Gosney 2008	Low/ Random numbers generated by the Hospital	Unclear/ Not described in text.	Low/ Participants and researchers	Unclear/ Not described in text.	High/ 59 out of 73 participants were analyzed (20% attrition).	Unclear/ Protocol not available.	Low/ The micronutrient supplement and placebo were

	Pharmacy Trials Unit.		blinded to the intervention.				supplied by Recip AB Sweden. Funding and potential conflicts of interest not reported.
de Jong 1999	Unclear/ Randomized through selection of sealed envelopes.	Unclear/ Randomized through selection of sealed envelopes.	High/ Participants and personnel were not blinded.	High/ Outcome assessors not blinded.	High/ 145 out of 217 randomized participants were analyzed (33% attrition).	Unclear/ Protocol not available.	Low/ Supported by funds from the Dutch Dairy Foundation on Nutrition and Health, Maarssen, The Netherlands, and the Health Research Council, The Netherlands.
Abe 2016	Unclear/ Through shuffling.	Low/ Allocation was conducted by a person who was not a member of this study. Sealed envelopes were used.	High/ Participants were only blinded between group 1 and group 2 and personnel	High/ Outcome assessor for walking speed was blinded, but this is not the	Low/36 out of 38 participants analyzed.	Unclear/ Protocol not available.	Low/ TGs were provided by the Nisshin OilliO Group Ltd. (Kanagawa, Japan). Potential conflicts of

			was not blinded.	case for other outcomes.			interest are not reported.

Table 4: Risk of bias of included studies on multicomponent supplementation.