Systematic review of health effects of dietary protein in older adults

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01 introduction

This background document belongs to the advisory report *Dietary reference values for protein* (in Dutch: *Voedingsnormen voor eiwitten – referentiewaarden voor de inname van eiwitten*), which has been prepared by the Committee on Nutrition of the Health Council of The Netherlands. In the advisory report, the Committee evaluated whether the dietary reference values (DRV) for protein for different age groups set by the European Food Safety Authority (EFSA) in 2012[1] could be adopted by The Netherlands.

EFSA, like other organisations that derived DRVs for protein,[2,3] decided to use the nitrogen (N) balance approach to determine protein requirement for adults. EFSA also considered several health outcomes that may be associated with protein intake. However, the available data on the effects of additional protein intake beyond the population reference intake (PRI) on muscle mass and function, on body weight control and obesity (risk) in children and adults, and on insulin sensitivity and glucose homeostasis did not provide evidence that could be considered as a criterion for determining DRVs for protein. Likewise, the available evidence did not permit the conclusion that an additional protein intake might affect bone mineral density and could be used as a criterion for the setting of DRVs for protein.

EFSA used the meta-analysis of N-balance studies in healthy adults by Rand et al.[4] and derived a population average requirement for protein of 0.66 g per kg of body weight (BW) per day, resulting in a PRI of 0.83 g/kg BW/d. Rand et al. observed a lower efficiency of N utilization in older adults (based on data of 14 older adults from one study) compared with younger adults. However, the estimated higher requirement of older adults was not significantly different from the requirement of younger adults. EFSA considered the protein requirement for (healthy) older adults (60 years and older) to be equal to that of (healthy) younger adults. EFSA also stated that the lower energy requirement of sedentary older adults may suggest that the protein-to-energy ratio of their requirement is higher than for younger age groups.

The Health Council's Committee on Nutrition agrees with the conclusions that EFSA drew based on the evidence available at that time. However, the Committee judged that, for older adults, there was a need to update the scientific literature because many publications on this topic emerged since the release of the EFSA report in 2012. Therefore, the Committee performed a systematic literature review with the aim of determining whether a protein intake higher than the from N-balance data derived DRV of 0.83 g/kg BW/d affects health outcomes in older adults.

This background document describes the methodology and the results of the systematic review on the effect of increased protein intake on various health outcomes in older adults.

02 methods

2.1 Literature search and study selection

The Committee initially aimed to base its evaluation on systematic reviews (SR), including meta-analyses (MA), of randomised controlled trials (RCT) and prospective cohort studies. Therefore, a systematic literature search was performed in PubMed to identify any potentially relevant SRs on the relationship between protein intake and health outcomes in older adults published up until 23 April 2020 (search string available in Annex A). An additional search for SRs in Scopus yielded no additional relevant SRs.

Studies in younger and older adults suggest that protein intake in combination with concomitant physical exercise has an additive or synergistic effect on muscle mass or strength compared to protein intake alone.[5] In addition, according to the current Dutch Physical Activity Guidelines (2017), adults – including older adults – are recommended to perform activities that strengthen the muscles and bones at least twice a week.[6] Therefore, both the effect of protein alone, as well as the effect of protein in the context of physical exercise (mainly muscle-strengthening activity) were considered relevant for this advisory report.

The Committee noticed that the majority of the SRs retrieved were based on RCTs (and not on cohort studies[2,7-9]). The SRs were limited with regard to the degree of detail of the individual studies included. For example, most of the SRs only provided information about the dose of protein that was provided, but not about the (daily) total protein intake (in g/kg body weight (BW)/d) of the participants. The latter is particularly relevant for deriving a population reference intake (PRI) or adequate intake (AI). Also, many SRs mixed various types of exposure (e.g. protein alone vs. protein in the context of physical exercise). The Committee, therefore, decided to base its evaluation on individual studies and not on SRs.

The relevant SRs that were retrieved through the original literature search were used to identify relevant individual studies. In addition, a second systematic literature search was carried out to identify the most recent individual studies (specifically RCTs) that had not yet been included in an SR. This search was limited to studies published in 2018, 2019 and 2020 (up until 23 April 2020) as this would cover the studies published after the inclusion date of the most recent SRs. This literature search was carried out in PubMed and Scopus (search string available in Annex A).

The Committee decided to only select RCTs, not prospective cohort studies, because: 1) many RCTs were available; 2) RCTs can provide evidence for a *causal* relationship, as opposed to prospective cohort studies; and 3) the majority of prospective cohort studies did not distinguish between multiple categories of total protein intake or did not report protein intake in g/kg BW/d. Moreover, they do often not include a protein category at the level of the current PRI. Because of this, it is very difficult to specify if any additional protein intake beyond the PRI of 0.83 g/kg

BW/d would elicit health benefits, and if so, what the *exact* optimal amount of protein would be.

All retrieved individual studies were further assessed for eligibility by using the pre-specified inclusion and exclusion criteria (Annex B). The Committee only included studies that included a control group and in which participants were randomly allocated to the intervention or control group (i.e. RCTs). Studies had to have a minimum duration of four weeks to be included. Older adults were defined as adults aged 60 and above, so the Committee has included studies of older adults with an *average* age of at least 65. As DRVs are intended for healthy persons, the Committee included studies of older adults who lived at home, in a care home, or in a nursing home, and excluded studies of older adults who had been admitted to hospital, studies conducted just before or after hospitalisation, and studies in which the study population consisted solely of individuals with a specific disease, such as diabetes or chronic lung disease. The Committee relied on studies in which the participants were exposed to protein or a mix of amino acids (numerous amino acids), such as protein supplements, amino acid supplements, and protein-rich or protein-enriched foods. These types of intervention best reflect the natural way of protein consumption through the diet. For this reason, the Committee has excluded studies with interventions involving a single or few specific individual amino acids. Because the Committee is specifically interested in the effect of *dietary* protein, it also excluded interventions with creatine and beta-hydroxy-beta-methylbutyrate (HMB). These substances are naturally produced by the body from amino acids (metabolites) and might elicit beneficial effects on muscle mass, but they occur only in very small quantities in food. The Committee also excluded studies in which the intervention groups and control groups differed intentionally in more ways than protein exposure alone. If the researchers intended to investigate the effect of a combination of extra protein and another substance (e.g. vitamin D), compared to a lower amount of both protein and the other substance, then that study was excluded. If the protein intervention was food-based and subjects were consequently given other nutrients along with the intended protein intervention, then that study was included. In the case of a food-based protein intervention (e.g. milk) or a high-protein diet versus a low-protein diet it is inevitable that other nutrients than protein are also to a certain extent involved. To limit the influence of energy balance on the outcome measures as much as possible, the Committee included isocaloric studies. Finally, any studies carried out in the context of a weight loss programme were excluded.

2.2 Study characteristics

Descriptive data (including information on study population, sample size, habitual protein intake, protein dose, and type of protein intervention) and results from the included individual studies were extracted and presented in tables.

2.2.1 Outcomes

The Committee evaluated the evidence for an effect of increased protein intake on health outcomes for each outcome separately. Nine health outcomes were selected (based on availability in the literature): lean body mass, muscle strength, physical function, bone health, blood pressure, glucose and insulin metabolism, serum lipid profile, kidney function, and cognition.

2.2.2 Protein intake with or without concomitant physical exercise

The Committee distinguished two exposure categories, according to whether the protein intervention took place in a study which also included a physical activity intervention:

1. Studies examining the effect of protein intake only;
2. Studies examining the effect of protein intake in the context of physical exercise.

In the first category, neither the intervention group nor the control group is exposed to a physical exercise intervention. In the second category, both the intervention group and the control group are exposed to a physical exercise intervention. So, studies were only included if protein intake was the only contrast between the intervention group and the control group.

2.2.3 Habitual protein intake and total protein intake

Because the underlying aim of this systematic literature review was to derive a DRV for protein (for older adults), the Committee was particularly interested in the *total* protein intake (preferentially expressed in g/kg BW/d) of the study population, rather than the prescribed or supplemented protein dose only. The total protein intake is the habitual protein intake plus the supplemented or prescribed protein dose (Text box 1). Habitual protein intake is the amount of protein that a person usually consumes on an average day outside the trial context. The studies in which the total protein intake of the control group was approximately 0.8 g/kg BW/d were of particular interest in terms of determining whether a protein intake higher than the from N-balance data estimated DRV (i.e. 0.83 g/kg BW/d) would yield health benefits in older adults. Therefore, the studies were grouped according to the total protein intake of the control group (which is mostly similar to the habitual protein intake). Four categories or ‘domains’ of total habitual protein intake were distinguished: ≥0.8 to <0.9 g/kg BW/d, ≥0.9 to <1.0 g/kg BW/d, ≥1.0 to <1.1 g/kg BW/d, and ≥1.1 g/kg BW/d. Studies in which the protein intake in the reference group was below 0.8 g/kg BW/d were not included, because this is below the PRI estimated from N-balance studies. The Committee evaluated all studies together (regardless of the domain) and additionally evaluated the evidence per domain of habitual protein intake, to better identify at what level of protein intake any potential health effects occurred.

2.2.4 Protein dose

The protein dose was defined as the difference in total protein intake between the intervention group and the control group during the intervention period (Text box 1). This difference was based on the achieved total protein intake (i.e. the reported habitual protein intake plus the consumed amount of supplemented or prescribed protein). The protein dose differs across studies and may account for differences in effects between studies. The Committee considered the protein dose in evaluating the scientific evidence and aimed to determine if there was a dose-response relationship. For this purpose, the Committee checked, within each domain of habitual protein intake, whether the protein dose in those studies that showed an effect was higher than the protein dose in those studies that showed no effect.

Text box 1. Definitions of habitual protein intake, total protein intake, and protein dose

Habitual protein intake is the amount of protein that a person usually consumes on an average day outside the trial context.

Total protein intake is the habitual protein intake plus the supplemented or prescribed protein dose. For the control group, the total protein intake is mostly similar to the habitual protein intake (since the control group is generally not provided or prescribed additional protein during the trial).

Protein dose is the amount of protein supplemented or prescribed during the trial. For the current advisory report, it is calculated as the difference in achieved total protein intake between the intervention group and the control group during follow-up.

2.2.5 Type of protein intervention

Across the selected studies, the participants were exposed to protein in a variety of ways. For example, in some studies, the entire diet was modified. In addition to modifying the level of protein intake, this will also have changed the intake of other nutrients that commonly occur in protein-rich foods, which may have affected the health outcome. In other studies, specific protein supplements (such as whey protein concentrate) were provided. When describing the results of the studies, the type of protein intervention involved was specified. The Committee has subdivided the types of protein intervention into the following three categories:

1. 'Pure' protein or amino acids
   These are often provided in the form of a powder or tablet, for example by means of whey hydrolysates, whey isolates, whey concentrates, or milk-protein concentrates, and dissolved in a (low-protein) drink;
2. One or a few food products with a high protein content
   These include protein-enriched products, such as commercially-available protein-enriched milk or 'ordinary' protein-rich foods, such as cow milk or soy milk. Consumption of these products is usually associated with consumption of more nutrients than protein alone;
3. High-protein diets
   The entire dietary pattern is modified to achieve a pre-specified total protein intake (e.g. 0.8 g/kg BW/d) in the control group and an increased total protein intake (e.g. 1.2 g/kg BW/d) in the intervention group.

2.2.6 Risk of bias

The internal validity of the included studies was assessed using the RoB 2 Cochrane collaboration tool for risk of bias assessment.[10] The RoB 2 tool addresses five domains of bias: 1) bias arising from the randomisation process, 2) bias due to deviations from intended interventions, 3) bias due to missing outcome data, 4) bias in measurement of the outcome and 5) bias in selection of the reported result. The risk of bias in each domain was scored as 'low risk of bias', 'some concerns', or 'high risk of bias'. These five judgements together resulted in an overall judgment of the risk of bias, also in terms of 'low risk of bias', 'some concerns' or 'high risk of bias'. A detailed description of the RoB 2 tool is provided in Annex C.

2.2.7 Statistical power

In its evaluation of the literature, the Committee considered the statistical power of the studies. If studies show that an increased protein intake has no effect on a given outcome, that may be due to insufficient statistical power for that specific outcome. This could result in the over-representation of studies with neutral findings (i.e. no effect). For this reason, as a sensitivity analysis, the Committee restricted itself to those studies where the power analysis was based on that outcome measure. Unless indicated otherwise in the individual studies, the Committee has made two assumptions in this regard. Firstly, the Committee assumed that a calculated sample size is the same for all outcome measures within the domain of a given outcome. In other words, if the power analysis is based

on handgrip strength (an outcome measure within the 'muscle strength' domain), for example, it is likely that the requisite sample size derived in this way will also apply to other outcome measures within the 'muscle strength' domain (such as knee extensor strength or leg press). Secondly, the Committee assumed (based on expert judgement) that for demonstrating a statistically significant effect, lean body mass generally requires a smaller sample size than muscle strength, and that muscle strength requires a smaller sample size than physical function. This amounts to the assumption that a study in which the power analysis is based on muscle strength will also have sufficient statistical power for lean body mass. Similarly, a study in which the power analysis is based on physical function will also have sufficient statistical power for lean body mass and muscle strength. No assumptions have been made for the other outcomes. Of note, for various reasons (higher drop-out rate, higher variation, or smaller effect than anticipated) the sample size derived from the power analysis may still have been too small to provide sufficient statistical power. Conversely, a significant effect might even be found for outcomes that, prior to the study, were expected to have insufficient statistical power. The Committee used the available information on this topic, as derived from the studies. It did not perform its own power analyses (post-intervention) to determine whether or not the sample size was (in retrospect) sufficient for a particular outcome measure.

2.3 Evaluation of the evidence

2.3.1 Drawing conclusions

In order to derive a DRV for protein, the Committee evaluated the available scientific evidence and drew conclusions regarding the effect of increased protein intake on health outcomes in older adults. The Committee distinguished six categories of conclusions: 'a convincing (beneficial/unfavourable) effect', 'a likely (beneficial/unfavourable) effect', 'a possible (beneficial/unfavourable) effect', 'ambiguous evidence', 'likely no effect', or 'too few studies'. The Committee based its conclusions primarily on the total number of studies, the percentage of studies showing an effect, and the direction of the effect. Conclusions were drawn based on predefined rules of thumb (Text box 2).

The Committee drew conclusions for each of the nine health outcomes. The selected studies often used multiple measures for such a health outcome (hereinafter referred to as 'specific outcome measure'), for example handgrip strength, knee extensor strength, and leg press to assess muscle strength. So, for one health outcome, a study may have explored multiple specific outcome measures, which are defined as 'contrasts'. This implies that one study might find multiple similar effects, due to correlated specific outcome measures, or differential effects for the same health outcome. The Committee decided to consider the studies that showed a beneficial effect for at least one of the specific outcome measures (or *contrasts*) as a study with a beneficial effect. By way of

illustration, the Committee considered a study in which five specific outcome measures (or *contrasts*) were assessed for the health outcome 'lean mass' and in which a statistically significant effect was found for one outcome measure (but not for the remaining four) to be 'a study with an effect'. Accordingly, the Committee based its approach on the percentage of studies that showed an effect. There are, however, substantial differences between studies in terms of the number of contrasts tested for a given health outcome. Studies that test large numbers of contrasts are more likely to demonstrate a statistically significant effect, but multiple testing also increases the likelihood of chance findings. These could be reasons for basing the approach on the percentage of contrasts (instead of studies) that showed an effect. One drawback of that approach is that it can produce an overestimate of the number of beneficial effects found, due to the correlation between specific outcome measures within a given health outcome. Thus, studies with numerous contrasts would have a larger share in the overall weighting of the evidence. The Committee has decided to apply the rules of thumb primarily at the level of studies and secondarily at the level of contrasts. Thus, if the percentage of contrasts with an effect differs substantially from the percentage of studies with an effect, this was explicitly mentioned and could lead to a modification of the conclusion.

Text box 2. Set of possible conclusions for the effect of increased protein intake on health outcomes[a,b,c]

- There is **a convincing beneficial effect** if a total of ≥3 studies are available, ≥75% of which involve a beneficial effect and none of which show an unfavourable effect.
- There is **a likely beneficial effect** if a total of ≥3 studies are available, ≥50 to 74% of which involve a beneficial effect and none of which show an unfavourable effect.
- There is **a possible beneficial effect** if a total of ≥3 studies are available, ≥25 to 49% of which involve a beneficial effect and none of which show an unfavourable effect.
- The available research is **ambiguous** in situations where studies show opposite results. This involves a combination of both beneficial effects and unfavourable effects, without the overall effect clearly pointing in one direction.
- There is **likely no effect** if a total of ≥3 studies are available, <25% of which involve a beneficial effect and none of which show an unfavourable effect.
- There are **too few studies** to draw conclusions in situations where a total of <3 studies are available or where <3 studies with sufficient statistical power are available.

[a] When speaking of beneficial effects or unfavourable effects, these are statistically significant beneficial or statistically significant unfavourable effects, respectively.
[b] For all categories, there may be neutral studies involved, i.e. studies in which no statistically significant effect was found.
[c] The above rules also apply to an unfavourable effect. Unfavourable effects were not expected based on a first judgment of the literature, so in the interest of readability, these rules are not specified here.

2.3.2 Presentation of results and conclusions

The Committee evaluated the scientific evidence for each outcome separately. For each outcome, the evaluation is structured as follows:

1. The evaluation starts with a summary of the results concerning the overall effect of increased protein intake. This includes the number of selected studies concerning the outcome measure in question, the number of participants in those studies, the protein dose, the observed effects, and the risk of bias.
2. The summary of the results is followed by the preliminary conclusion concerning the overall effect of increased protein intake on the health outcome in question (drawn up using the pre-specified rules of thumb; Text box 2).
3. Where sufficient studies have been found, the Committee has used a further subdivision, by domain of habitual protein intake. In this case, the Committee also described the results for each domain of habitual protein intake, as ancillary evidence.
4. The Committee checked whether there were any indications of a dose-response relationship within the domain of habitual protein intake, based on the information about the protein dose. If the overall conclusion was that there is *likely no effect* or that there are *too few studies*, then no further subdivision was made by domain of habitual protein intake. After all, that would not provide any new insights.
5. Lastly, the Committee formulated a final conclusion for the outcome measure in question. This was based on the conclusion regarding the overall effect of protein, as well as on any evaluations of the subgroups.
6. The evaluation concludes with a summary table outlining the main characteristics and results of the selected studies concerning the relevant outcome measure. Within the summary table, the studies are grouped by habitual protein intake domain and ordered by protein dose.

03 results

3.1 Number of included studies

The literature search for systematic reviews (SR) (including meta-analyses and individual patient data analyses) in PubMed yielded 609 publications. After excluding publications based on title/abstract screening and full text assessment, 27 publications were selected for the evaluation. Checking reference lists yielded one additional SR. In total, 28 SRs[2,9,11-36] were used for identifying individual studies. From these SRs, 207 individual studies were retrieved.

Table 1. Health outcomes evaluated and examples of specific outcome measures.

Outcome	Examples of outcome measures
Lean body mass	Total LBM, appendicular LBM, trunk LBM, muscle CSA (calf, thigh), muscle volume (calf, thigh), fat-free mass, appendicular LBM relative to squared height, muscle fibre area
Muscle strength	Knee extensor strength, leg press peak power, chest press strength, hip extensor strength, muscle quality index, handgrip strength, early relaxation time, arm curl test, preacher curl test
Physical function	Gait speed, TUG, stair climb power, SPPB, chair rise time, standing balance, perceived physical function
Bone health	BMC, BMD (lumbar spine, femoral neck, total hip), bone CSA, serum P1NP, serum CTX
Blood pressure	Systolic blood pressure, diastolic blood pressure
Glucose and insulin metabolism	Fasting blood glucose, fasting insulin, HOMA-IR
Serum lipid profile	Total cholesterol, HDL cholesterol, LDL cholesterol, triglycerides
Kidney function	eGFR, serum creatinine, albumin/creatinine ratio
Cognition	MMSE

Abbreviations: BMC: bone mineral content, BMD: bone mineral density, CSA: cross-sectional area, CTX: C-terminal telopeptide of type 1 collagen, eGFR: estimated glomerular filtration rate, HDL: high-density lipoprotein, HOMA-IR: homeostatic model assessment of insulin resistance, LBM: lean body mass, LDL: low-density lipoprotein, MMSE: Mini-Mental State Examination, P1NP: N-terminal propeptides of type 1 procollagen, SPPB: short physical performance battery, TUG: Timed Up and Go.

The additional search for recent individual RCTs published in 2018, 2019, or 2020 in PubMed (n=649) and Scopus (n=559) yielded 1208 publications. After removal of duplicates (n=166), 1042 unique publications remained for title/abstract screening. Based on title/abstract screening 974 publications were excluded, leaving 68 publications for full-text assessment.

After removal of nine publications that were found via both search strategies, 266 unique publications remained for full-text assessment. Of these, 242 publications were excluded, leaving 24 publications for final inclusion (flow diagram available in Annex D). These 24 publications reported on 18 unique RCTs. The characteristics and results of the included studies are presented (per outcome) in Annex E.

3.2 Selected outcomes

A total of 18 unique RCTs (21 publications) reported on the effects of increased protein intake on lean body mass, 15 RCTs on muscle strength, 12 RCTs on physical function, four RCTs on bone health, four RCTs on blood pressure, six RCTs on glucose and insulin metabolism, seven RCTs on serum lipid profile, six RCTs on kidney function and one RCT on cognition. Specific measures of the outcomes are presented in Table 1. RCTs with and without concomitant physical exercise were available for all outcomes except cognition.

3.3 Characteristics of included studies

3.3.1 Protein intake

In most RCTs (n=15), the mean habitual protein intake was between 0.8 and 1.1 g/kg body weight (BW)/d. In five of these studies, the mean habitual protein intake was ≥0.8 to <0.9 g/kg BW/d, in another five it was ≥0.9 to <1.0 g/kg BW/d, and in the remaining five studies it was ≥1.0 to <1.1 g/kg BW/d. In one study, the mean habitual protein intake was ≥1.1 g/kg BW/d. In the other two studies, the habitual intake was unclear.[37,38] Nine of the 18 RCTs were performed in the context of a concomitant physical exercise intervention. In most cases, this involved resistance exercise training. The type of protein intervention was 'pure' protein in 11 studies, protein-rich foods in four studies, high-protein diets in two studies, and a combination of 'pure' protein and protein-rich foods in one study.

3.3.2 Risk of bias

The results of the risk of bias assessment are presented in Annex F. None of the studies had a low risk of bias, for 50% of the studies there were some concerns and 50% of the studies had a high risk of bias. The most prevalent limitations leading to the judgement of either 'some concerns regarding the risk of bias' or 'high risk of bias' are:

- In about half of the studies, it was unclear whether the allocation sequence had been randomised and/or blinded for all of the staff and participants involved in the study. This led to an increased risk of selection bias;
- In many cases (n=7), it was unclear whether the outcome assessors had been blinded. For some outcome measures (such as muscle strength and physical function), this may lead to an increased risk of information bias. In studies where this was not reported, this domain was scored as 'some concerns';
- More than half of the studies involved missing outcome data. In most studies, no analyses were performed to demonstrate that the result had not been influenced by these missing data. This may lead to an increased risk of attrition bias.

3.3.3 Statistical power

Annex G specifies the outcome (or outcomes), per study, on which the power analysis was based. In most studies, the power analysis was based on lean body mass, followed by muscle strength, physical function, and bone health. In the case of nine RCTs (12 publications), this information was either not reported or was unclear.[37-48]

3.4 Results and conclusions for the effect of increased protein intake on health outcomes

In this section, the scientific evidence for the effect of increased protein intake on health outcomes is evaluated and conclusions are drawn (according to the approach described in section 2.3). The Committee evaluated the scientific evidence for each outcome separately. Results regarding subgroups according to domain of habitual protein intake were described as ancillary evidence, but no conclusion was drawn since there were too few studies within a domain to apply the rules of thumb.

3.4.1 Lean body mass

An overview of the main characteristics and results of the evaluated RCTs examining the effect of increased protein intake on parameters of lean body mass in older adults is provided in Table 2. A detailed description of the characteristics and results of these RCTs is provided in Annex E (Table E1).

Overall

The Committee evaluated 18 RCTs (21 publications) on the effect of increased protein intake on lean body mass in older adults, with a total of 62 statistically tested contrasts. Those studies involved a total of approximately 1284 participants[a] (intervention group (IG)/control group (CG): 706/578). The smallest study involved 12 participants (IG/CG: 6/6), while the largest study had 207 participants (IG/CG: 105/102). Most studies (n=13) had an intervention period of 12 weeks (range: 10 weeks to 2 years). The *habitual* protein intake[b] ranged from 0.8 to 1.1 g/kg BW/d and the protein dose[c] ranged from 0.17 to 0.82 g/kg BW/d. The *total* protein intake[d] in the intervention groups ranged from 1.06 g/kg BW/d (compared to an habitual intake of 0.89 g/kg BW/d) to 1.7 g/kg BW/d (compared to 0.9 g/kg BW/d). The risk of bias in those studies was scored as 'some concerns' (n=9) or 'high' (n=9).

In 7 of the 18 studies (39%), involving a total of 461 participants (IG/CG: 242/219), a beneficial effect of increased protein intake on lean body mass was found for at least one of the statistically tested contrasts (21 of 62 contrasts, 34%). The duration of those studies ranged from 10 weeks to 18 months. In those studies that found an effect on lean body mass, the habitual protein intake ranged from 0.8 to 1.05 g/kg BW/d and the protein dose from 0.24 to 0.80 g/kg BW/d. The total protein intake in the intervention groups of those studies ranged from 1.24 g/kg BW/d (compared to an habitual intake of 1.0 g/kg BW/d) to 1.7 g/kg BW/d

[a] This number represents the participants included in the analyses for this outcome. The sample size may vary slightly, depending on the specific outcome measure used.

[b] Habitual protein intake is the amount of protein that a participant usually consumes on an average day outside the trial context. The habitual protein intake is mostly similar to the total protein of the control group (that is generally not provided or prescribed additional protein during the trial).

[c] Protein dose is the difference in achieved total protein intake between the intervention group and the control group during follow-up

[d] Total protein intake is the habitual protein intake plus the supplemented or prescribed protein intake. For the control group, the total protein intake is mostly similar to the habitual protein intake (since the control group is generally not provided or prescribed additional protein during the trial).

(compared to 0.9 g/kg BW/d). The risk of bias in those seven studies was scored as 'some concerns' (n=4) or 'high' (n=3). Increased protein intake was not found to have any unfavourable effects on lean body mass.
Four of the 9 studies (10 of 35 contrasts) on the effect of increased protein intake alone found a beneficial effect on lean body mass, as did 3 of the 9 studies (11 of 27 contrasts) on the effect of increased protein intake in the context of physical exercise.

The changes in lean body mass did not involve any change in body weight. Annex H provides the results of a total of seven RCTs on the effect of increased protein intake (isocaloric replacement for carbohydrates) on body weight. In none of these studies a difference in body weight was observed between intervention groups and control groups.

Preliminary conclusion: Based on the 18 evaluated RCTs, the Committee concluded that increased protein intake has a possible beneficial effect on lean body mass in older adults, which does not involve any change in body weight.

Habitual protein intake (reference): ≥0.8 to <0.9 g/kg BW/d
Against a background (habitual) intake of 0.8 to 0.9 g protein/kg BW/d, the Committee evaluated five RCTs on the effect of increased protein intake on lean body mass, with a total of 18 statistically tested contrasts. Those studies involved a total of approximately 287 participants (IG/CG: 150/137). The smallest study involved 12 participants (IG/CG: 6/6), while the largest study had 141 participants (IG/CG: 75/66). The protein dose ranged from 0.17 to 0.82 g/kg BW/d. The risk of bias in those studies was scored as 'some concerns' (n=2) or 'high' (n=3).

In 2 of the 5 studies (40%), a beneficial effect of increased protein intake was found for at least one of the statistically tested contrasts (4 of 18 contrasts, 22%). In those studies that found an effect, the protein dose ranged from 0.53 to 0.6 g/kg BW/d. Increased protein intake, within this domain of habitual protein intake, was not found to have any unfavourable effects on lean body mass.

Habitual protein intake (reference): ≥0.9 to <1.0 g/kg BW/d
Against a background intake of 0.9 to 1.0 g protein/kg BW/d, the Committee evaluated five RCTs on the effect of increased protein intake on lean body mass, with a total of 18 statistically tested contrasts. Those studies involved a total of approximately 379 participants (IG/CG: 211/168). The smallest study involved 29 participants (IG/CG: 14/15), while the largest study had 114 participants (IG/CG: 58/56). The protein dose ranged from 0.28 to 0.8 g/kg BW/d. The risk of bias in those studies was scored as 'some concerns' (n=3) or 'high' (n=2).

In 2 of the 5 studies (40%), a beneficial effect of increased protein intake was found for at least one of the statistically tested contrasts (7 of 18

contrasts, 39%). In those studies that found an effect, the protein dose ranged from 0.47 to 0.8 g/kg BW/d. Increased protein intake, within this domain of habitual protein intake, was not found to have any unfavourable effects on lean body mass.

Habitual protein intake (reference): ≥1.0 to <1.1 g/kg BW/d
Against a background intake of 1.0 to 1.1 g protein/kg BW/d, the Committee evaluated five RCTs on the effect of increased protein intake on lean body mass, with a total of 19 statistically tested contrasts. Those studies involved a total of approximately 407 participants (IG/CG: 237/170). The smallest study involved 17 participants (IG/CG: 8/9), while the largest study had 207 participants (IG/CG: 105/102). The protein dose ranged from 0.21 to 0.49 g/kg BW/d. The risk of bias in those studies was scored as 'some concerns' (n=3) or 'high' (n=2).

In 3 of the 5 studies (60%), a beneficial effect of increased protein intake was found for at least one of the statistically tested contrasts (10 of 19 contrasts, 53%). In those studies that found an effect, the protein dose ranged from 0.24 to 0.49 g/kg BW/d. Increased protein intake, within this domain of habitual protein intake, was not found to have any unfavourable effects on lean body mass.

Habitual protein intake (reference): ≥1.1 g/kg BW/d
Against a background intake of at least 1.1 g protein/kg BW/d, the Committee evaluated one RCT on the effect of increased protein intake on lean body mass, with a total of five statistically tested contrasts. This study involved a total of 181 participants (IG/CG: 93/88). The protein dose was 0.3 g/kg BW/d. This study, with some concerns regarding the risk of bias, found no effects of increased protein intake on lean body mass.

In two RCTs, the habitual protein intake was unclear. The Committee has included those studies in the evaluation of the overall effect of protein intake, but not in the subdivision by domain of habitual protein intake.

Final conclusion regarding lean body mass:

Based on the 18 evaluated RCTs, the Committee concluded that increased protein intake has a **possible beneficial effect** on lean body mass in older adults, which does not involve any change in body weight.

Furthermore, the Committee concluded that the results do not suggest that the effect on lean body mass of increased protein intake alone (not in the context of physical exercise) differs from the effect of increased protein intake in the context of concomitant physical exercise. Beneficial effects of increased protein intake on lean body mass were observed in participants with an habitual protein intake up to and including 1.05 g/kg BW/d and for a total protein intake up to and including 1.7 g/kg BW/d. The Committee found no indications of a dose-response relationship.

Table 2. Overview of the results of the 18 evaluated randomised controlled trials on the effect of increased protein intake on lean body mass in older adults, categorised according to habitual protein intake and ordered by protein dose

Study	Analytic *n* IG/CG	Total protein intake (g/kg BW/d) during intervention[a]	Protein dose[b] (g/kg BW/d)	Protein type[c]	With/without physical exercise	Risk of bias[d]	Outcome	Result[e]				Comments
								+	NS	-	?	
Habitual protein intake (reference): ≥0.8 to <0.9 g/kg BW/d												
Arnarson et al. 2013[49] Same study as [50]	75/66	IG: 1.06 ± 0.23; CG 0.89 ± 0.23	0.17	A	Ex	H	Total LBM§		✔			
							aSMM§		✔			
Bhasin et al. 2018[51]	42/39	IG: 1.17 ± 0.13; CG: 0.81 ± 0.10	0.36	A,B	NoEx	SC	Total LBM§		✔ *			* P=0.04 for relative total LBM (% of BW), which will be mainly due to a greater decrease in total fat mass (kg) in IG than in CG (P=0.02)
							Trunk LBM§		✔			
							aLBM§		✔			
Sugihara Junior et al. 2018,[46] Fernandes et al. 2018[40]	15/16	IG: 1.4 ± 0.1; CG: 0.87 ± 0.1	0.53	A	Ex	H	Upper limb LST		✔			
							Lower limb LST		✔			
							SMM	✔				
							Total LST	✔				
Wright et al. 2018[47]	12/10	IG: 1.4; CG: 0.8 (prescribed)[f,g]	0.6[g]	C	NoEx	H	Total LBM	✔ *				* No significant change in total fat mass (P>0.05)
							Trunk LBM	✔				
							aLBM		✔			
							Muscle CSA, thigh		✔			
							Muscle volume, thigh		✔			
							Muscle CSA, calf		✔			
							Muscle volume, calf		✔			
Campbell et al. 1995[39]	6/6	IG: 1.62 ± 0.02; CG: 0.80 ± 0.02	0.82	B	Ex	SC	Fat-free mass		✔			
							Muscle CSA, thigh		✔			
Subtotal (contrasts)								*4*	*14*	*0*	*0*	*Beneficial effect observed for 4 of 18 contrasts*
Subtotal (studies)[h]								*2*	*5*	*0*	*0*	*(2 of 5 studies)*

Study	Analytic *n* IG/CG	Total protein intake (g/kg BW/d) during intervention[a]	Protein dose[b] (g/kg BW/d)	Protein type[c]	With/without physical exercise	Risk of bias[d]	Outcome	Result[e]				Comments
								+	NS	-	?	
Habitual protein intake (reference): ≥0.9 to <1.0 g/kg BW/d												
Park et al. 2018[52]	40/40	IG1: 1.18 ± 0.23; CG: 0.90 ± 0.38	0.28	A	NoEx	SC	aSMM[§]		✔			
							aSMM relative to BW[§]		✔			
							aSMM relative to squared height[§]		✔			
							aSMM relative to BMI[§]		✔			
	40/40	IG2: 1.37 ± 0.26; CG: 0.90 ± 0.38	0.47				aSMM[§]	✔				
							aSMM relative to BW[§]	✔				
							aSMM relative to squared height[§]	✔				
							aSMM relative to BMI[§]	✔				
Ten Haaf et al. 2019[53]	58/56	IG: 0.92 ± 0.27 (without protein supplementation of 31 g/d); CG: 0.97 ± 0.23	0.36[g]	A	Ex	SC	Total LBM[§]		✔ *			* P=0.046 for relative total LBM (% of BW), which will be mainly due to a greater decrease in total fat mass (kg) in IG than in CG (P=0.013)
Chalé et al. 2013[54]	42/38	NR (baseline: 0.98)	0.38[i]	A	Ex	SC	Total LBM[§]		✔			
							Muscle CSA, thigh[§]		✔			
Ottestad et al. 2017[55]	17/19	IG: 1.4 ± 0.5; CG: 0.9 ± 0.4	0.5	B	NoEx	H	Total LBM[§]		✔			
							Trunk LBM[§]		✔			
							aLBM[§]		✔			
Mitchell et al. 2017[48]	14/15	IG: 1.7 ± 0.1; CG: 0.9 ± 0.1	0.8	C	NoEx	H	Total LBM	✔ *				* No significant change in body weight (P=0.174), but greater decrease in total and % fat mass in IG than in CG (both P<0.01)
							Trunk LBM	✔				
							aLBM	✔				
							Muscle CSA, thigh		✔			
Subtotal (contrasts)								*7*	*11*	*0*	*0*	*Beneficial effect observed for 7 of 18 contrasts*
Subtotal (studies)[h]								*2*	*5*	*0*	*0*	*(2 of 5 studies)*

Study	Analytic *n* IG/CG	Total protein intake (g/kg BW/d) during intervention[a]	Protein dose[b] (g/kg BW/d)	Protein type[c]	With/without physical exercise	Risk of bias[d]	Outcome	Result[e]				Comments
								+	NS	-	?	
Habitual protein intake (reference): ≥1.0 to <1.1 g/kg BW/d												
Ispoglou et al. 2016[41]	8/9	1.02-1.08 (without protein supplementation of ~0.21 g/kg BW/d in IG1)	0.21	A	NoEx	H	Total LTM		✔			
	8/9	1.02-1.08 (without protein supplementation of ~0.21 g/kg BW/d in IG2)	0.21				Total LTM		✔			
Nabuco et al. 2019c[42]	13/13	IG: 1.0 ± 0.23 (without ~35 g whey protein supplementation on 3 d/wk); CG: 1.0 ± 0.19	0.24[g]	A	Ex	SC	Total LST	✔				
							Lower LST	✔				
							aLST	✔				
Kerstetter et al. 2015[56]	105/102	IG: 1.30 ± 0.05; CG: 1.05 ± 0.04	0.25	A	NoEx	SC	Total LBM		✔ *			* P=0.069 (total LBM tended to decrease less in IG than in CG)
							Trunk LBM	✔ *				* No significant change in total fat mass (P>0.05)
Thomson et al. 2016[57]	34/23	IG1: 1.42 ± 0.14; CG: 1.08 ± 0.05	0.34	B	Ex	H	Total LBM[§]		✔			
	26/23	IG2: 1.45 ± 0.14; CG: 1.08 ± 0.05	0.37				Total LBM[§]		✔			
Nabuco et al. 2018,[43] Nabuco et al. 2019a,[44] Nabuco et al. 2019b[45]	22/23	IG1: 1.38 ± 0.26; CG: 1.0 ± 0.25	0.38	A	Ex	SC	Upper limb LST		✔			
							Lower limb LST	✔				
							SMM	✔				
							aLST		✔			
							Total LST	✔				
	21/23	IG2: 1.49 ± 0.46; CG: 1.0 ± 0.25	0.49				Upper limb LST		✔			
							Lower limb LST	✔				
							SMM	✔				
							aLST		✔			
							Total LST	✔				
Subtotal (contrasts)								*10*	*9*	*0*	*0*	*Beneficial effect observed for 10 of 19 contrasts (3 of 5 studies)*
Subtotal (studies)[h]								*3*	*4*	*0*	*0*	

Study	Analytic *n* IG/CG	Total protein intake (g/kg BW/d) during intervention[a]	Protein dose[b] (g/kg BW/d)	Protein type[c]	With/without physical exercise	Risk of bias[d]	Outcome	Result[e]				Comments
								+	NS	-	?	
Habitual protein intake (reference): ≥1.1 g/kg BW/d												
Zhu et al. 2015[58] Same study as [59,60]	93/88 (2-y follow-up)	IG: 1.4 ± 0.4; CG: 1.1 ± 0.4	0.3	A	NoEx	SC	Arm LBM§		✔			
							Leg LBM§		✔			
							aLBM§		✔			
							aLBM relative to squared height§		✔			
							Muscle CSA, calf§		✔			
Subtotal (contrasts) *Subtotal (studies)*[h]								*0* *0*	*5* *1*	*0* *0*	*0* *0*	*No effect observed for any of 5 contrasts (1 study)*
Habitual protein intake (reference): Unclear												
Dillon et al. 2009[37]	7/7	NR	0.20	A	NoEx	H	Total LBM		✔ *			* Results for time*group interaction (ANOVA) not reported, which suggests that protein has no effect
Mitchell et al. 2015[38]	16 (total)	NR	NR (15 g/d)	B	Ex	H	Muscle fibre area		✔			
Subtotal (contrasts) *Subtotal (studies)*[h]								*0* *0*	*2* *2*	*0* *0*	*0* *0*	*No effect observed for any of 2 contrasts (2 studies)*
Total (contrasts) Total (studies)[h]								21 7	41 17	0 0	0 0	Beneficial effect observed for 21 of 62 contrasts (7 of 18 studies)

Abbreviations: aLBM: appendicular lean body mass, aLST: appendicular lean soft tissue, aSMM: appendicular skeletal muscle mass, BW: body weight, CG: control group, CSA: cross-sectional area, Ex: with concomitant physical exercise intervention, H: high risk of bias, IG: intervention group, L: low risk of bias, LBM: lean body mass, LST: lean soft tissue, LTM: lean tissue mass, NoEx: without concomitant physical exercise intervention, NR: not reported, NS: not significant, SC: some concerns (regarding risk of bias), SMM: skeletal muscle mass.

Footnotes:

§ Sufficient statistical power to detect an effect is to be expected, based on the sample size calculation.

[a] Total protein intake during follow-up. If protein intake was assessed at multiple time points, the intake assessed at the final time point was considered.

[b] 'Protein dose' indicates the difference in achieved total protein intake between the intervention and control groups during follow-up (which is not necessarily equal to supplemented/prescribed amount of protein).

[c] 'Protein type' indicates the way in which a higher protein intake was achieved and is categorised into 'pure' protein or amino acids (or essential amino acids) (A), specific food(s) with a high protein content (B), or high-protein diets (C).

[d] Risk of bias assessment: risk of bias was assessed using the RoB 2 tool and scored as 'low' (L), 'some concerns' (SC), or 'high' (H).

[e] The results of the studies are indicated as follows: +, statistically significant beneficial effect (P<0.05); -, statistically significant unfavourable effect (P<0.05); NS, no statistically significant effect (P≥0.05); ?, result unclear. In cases where results were reported for multiple time points, only the result for the final time point is reported.

[f] Actual protein intake may have been different from the prescribed protein intake, due to non-compliance (compliance was 91% on average).

[g] Protein intake in g/kg BW/d was calculated by using protein intake in g/d and mean body weight (and compliance, if available).

[h] Some studies assessed multiple specific outcomes (i.e. multiple contrasts) for the health outcome 'lean body mass', so one study can show both a significant and a non-significant effect.

[i] (Achieved) protein dose was estimated using prescribed protein dose, compliance rate (72%), and mean body weight.

3.4.2 Muscle strength

An overview of the main characteristics and results of the evaluated RCTs examining the effect of increased protein intake on muscle strength in older adults is provided in Table 3. A detailed description of the characteristics and results of these RCTs is provided in Annex E (Table E2).

Overall

The Committee evaluated 15 RCTs on the effect of increased protein intake on muscle strength in older adults, with a total of 83 statistically tested contrasts. Those studies involved a total of approximately 1023[a] participants (IG/CG: 570/453). The smallest study involved 14 participants (IG/CG: 7/7), while the largest study had 141 participants (IG/CG: 75/66). Most studies (n=11) had an intervention period of 12 weeks (range: 10 weeks to 2 years). The habitual protein intake ranged from 0.81 to 1.1 g/kg BW/d and the protein dose ranged from 0.17 to 0.8 g/kg BW/d. The total protein intake in the intervention group ranged from 1.06 g/kg BW/d (compared to an habitual intake of 0.89 g/kg BW/d) to 1.7 g/kg BW/d (compared to 0.9 g/kg BW/d). The risk of bias in those studies was scored as 'some concerns' (n=7) or 'high' (n=8).

In 4 of the 15 studies (27%), involving a total of 206 participants (IG/CG: 114/92), a beneficial effect of increased protein intake on muscle strength was found for at least one of the statistically tested contrasts (14 of 83 contrasts, 17%). The duration of those studies ranged from 10 weeks to 6 months. In those studies that found a beneficial effect on muscle strength, the habitual protein intake ranged from 0.87 to1.0 g/kg BW/d and the protein dose ranged from 0.38 to 0.8 g/kg BW/d. The total protein intake in the intervention groups of those studies ranged from 1.36 g/kg BW/d (compared to an habitual intake of 0.98 g/kg BW/d) to 1.7 g/kg BW/d (compared to 0.9 g/kg BW/d). The risk of bias in those studies was scored as 'some concerns' (n=4) or 'high' (n=4). Studies on the effect of increased protein intake in the context of physical exercise (usually resistance exercise training) more often showed a beneficial effect on muscle strength (3 of 8 studies (38%); 13 of 55 contrasts (24%)) than studies on the effect of increased protein intake alone (1 of 7 studies (14%); 1 of 28 contrasts (4%)).

An unfavourable effect on muscle strength was observed in one study, which was performed in the context of physical exercise (1 of 8 studies (13%); 2 of 55 contrasts (4%)). This unfavourable effect was observed for two specific outcome measurements (i.e. leg press and total 8 RM; no effect was observed for other specific outcome measures of muscle strength) and only for the group that received soy protein (IG2) but not for the group that received a comparable amount of dairy protein (IG1). Both intervention groups and the control group gained muscle strength, but the increase was less in the soy protein group compared to the dairy protein

[a] This number represents the participants included in the analyses for this outcome. The sample size may vary slightly, depending on the specific outcome measure used.

group and the control group. The authors suggested that this finding can be explained by the isoflavones in soy foods that might attenuate the anabolic muscle response through reducing testosterone concentrations. As the unfavourable effect is likely due to the type of protein and not to the amount of protein, the Committee has given less weight to this result when drawing its conclusion.

Preliminary conclusion: Based on the 15 evaluated RCTs, the Committee concluded that the effect of protein may depend on whether or not the protein intervention was in the context of a concomitant physical exercise intervention. It has, therefore, formulated two (preliminary) conclusions: 1) Increased protein intake not in the context of physical exercise has likely no effect on muscle strength in older adults; 2) There is a possible beneficial effect of increased protein intake in combination with physical exercise compared to physical exercise alone on muscle strength in older adults.

Because increased protein intake alone (not in the context of physical exercise) has likely no effect on muscle strength in older adults, the Committee did not further subdivide those studies according to the domain of habitual protein intake. Those studies in which protein intake is combined with physical exercise (n=8) are further evaluated based on subgroups of habitual protein intake in the following paragraphs (see Annex I for the summary table for those eight RCTs only).

Habitual protein intake (reference): ≥0.8 to <0.9 g/kg BW/d
Against a background (habitual) intake of 0.8 to 0.9 g protein/kg BW/d, the Committee evaluated two RCTs on the effect of increased protein intake (in the context of physical exercise) on muscle strength, with a total of eight statistically tested contrasts. Those studies involved a total of 172 participants (IG/CG: 90/82). The smallest study involved 31 participants (IG/CG: 15/16), while the largest study had 141 participants (IG/CG: 75/66). The protein dose ranged from 0.17 to 0.53 g/kg BW/d. The risk of bias in both studies was scored as ‘high’.

In 1 of the 2 studies (50%), a beneficial effect of increased protein intake was found for at least one of the statistically tested contrasts (3 of 8 contrasts, 38%). In those studies that found an effect, the protein dose was 0.53 g/kg BW/d. Increased protein intake, within this domain of habitual protein intake, was not found to have any unfavourable effects on muscle strength.

Habitual protein intake (reference): ≥0.9 to <1.0 g/kg BW/d
Against a background intake of 0.9 to 1.0 g protein/kg BW/d, the Committee evaluated two RCTs on the effect of increased protein intake (in the context of physical exercise) on muscle strength, with a total of 15 statistically tested contrasts. Those studies involved a total of 175 participants (IG/CG: 116/59). The smallest study involved 80 participants (IG/CG: 42/38),

while the largest study had 57 participants (IG/CG: 34/23). The risk of bias in both studies was scored as ‘some concerns’.

The protein dose ranged from 0.36 to 0.38 g/kg BW/d. In 1 of the 2 studies (50%), a beneficial effect of increased protein intake was found for at least one of the statistically tested contrasts (4 of 15 contrasts, 27%). In this study that found an effect, the protein dose was 0.38 g/kg BW/d. Increased protein intake, within this domain of habitual protein intake, was not found to have any unfavourable effects on muscle strength.

Habitual protein intake (reference): ≥1.0 to <1.1 g/kg BW/d
Against a background intake of 1.0 to 1.1 g protein/kg BW/d, the Committee evaluated three RCTs on the effect of increased protein intake (in the context of physical exercise) on muscle strength, with a total of 28 statistically tested contrasts. Those studies involved a total of 194 participants (IG/CG: 100/94). The smallest study involved 26 participants (IG/CG: 13/13), while the largest study had 114 participants (IG/CG: 58/56). The protein dose ranged from 0.24 to 0.49 g/kg BW/d. The risk of bias in those studies was scored as ‘some concerns’ (n=2) or ‘high’ (n=1).

In 1 of the 3 studies (33%), a beneficial effect of increased protein intake was found for at least one of the statistically tested contrasts (6 of 28 contrasts, 21%). In this study that found a beneficial effect, the protein dose ranged from 0.38 to 0.49 g/kg BW/d and the risk of bias was scored as ‘some concerns’. One study (33%) found an unfavourable effect on muscle strength (2 of 28 contrasts, 7%). In this study that found an unfavourable effect, the protein dose was 0.37 g/kg BW/d and the risk of bias was scored as ‘high’.

Habitual protein intake (reference): ≥1.1 g/kg BW/d
The Committee found no RCTs within this domain of habitual protein intake.

In one RCT, the habitual protein intake was unclear. The Committee has included this study in the evaluation of the overall effect of protein intake, but not in the subdivision by domain of habitual protein intake.

Final conclusion regarding muscle strength:

Based on the 15 evaluated RCTs, the Committee concluded that increased protein intake alone (not in the context of physical exercise) has **likely no effect** on muscle strength in older adults. In contrast, the Committee concluded that increased protein intake with concomitant physical exercise (predominantly resistance exercise training) does have a **possible beneficial effect** on muscle strength in older adults, compared to physical exercise alone.

Beneficial effects of increased protein intake in the context of physical exercise on muscle strength were observed in participants with an habitual protein intake up to and including 1.0 g/kg BW/d and for a total protein intake up to and including 1.49 g/kg BW/d. The Committee found no indications of a dose-response relationship.

Table 3. Overview of the results of the 15 evaluated randomised controlled trials on the effect of increased protein intake on muscle strength in older adults, categorised according to habitual protein intake and ordered by protein dose

Study	Analytic *n* IG/CG	Total protein intake (g/kg BW/d) during intervention[a]	Protein dose[b] (g/kg BW/d)	Protein type[c]	With/without physical exercise	Risk of bias[d]	Outcome	Result[e]				Comments
								+	NS	-	?	
Habitual protein intake (reference): ≥0.8 to <0.9 kg BW/d												
Arnarson et al. 2013[49] Same study as [50]	75/66	IG: 1.06 ± 0.23; CG 0.89 ± 0.23	0.17	A	Ex	H	Quadriceps strength		✔			
Bhasin et al. 2018[51]	29-31†/ 32-34†	IG: 1.17 ± 0.13; CG: 0.81 ± 0.10	0.36	A,B	NoEx	SC	Leg press strength		✔			
							Chest press strength		✔			
							Leg press peak power		✔			
Sugihara Junior et al. 2018[46] Same study as [40]	15/16	IG: 1.4 ± 0.1; CG: 0.87 ± 0.1	0.53	A	Ex	H	Chest press strength	✔				
							Knee extension strength	✔				
							Preacher curl strength		✔*			* P=0.07 (strength tended to increase more in IG than in CG)
							Total strength[f]	✔				
							Lower limb muscle quality index[g]		✔			
							Upper limb muscle quality index[h]		✔			
							Total muscle quality index[i]		✔			
Subtotal (contrasts) *Subtotal (studies)[j]*								*3* *1*	*8* *3*	*0* *0*	*0* *0*	*Beneficial effect observed for 3 of 11 contrasts (1 of 3 studies)*
Habitual protein intake (reference): ≥0.9 to <1.0 kg BW/d												
Park et al. 2018[52]	40/40	IG1: 1.18 ± 0.23; CG: 0.90 ± 0.38	0.28	A	NoEx	SC	Handgrip strength (IG1 vs CG)		✔			
	40/40	IG2: 1.37 ± 0.26; CG: 0.90 ± 0.38	0.47				Handgrip strength (IG2 vs CG)		✔			
Ten Haaf et al. 2019[53]	58/56 for handgrip strength; 22-56† (total) for other outcome measures	IG: 0.92 ± 0.27 (without protein supplementation of 31 g/d); CG: 0.97 ± 0.23	0.36[k]	A	Ex	SC	Handgrip strength[§]		✔			
							Quadriceps MVC[§]		✔			
							Maximal rate of force rise, quadriceps[§]		✔			
							Early relaxation time, quadriceps[§]		✔			
							Half relaxation time, quadriceps[§]		✔			
							Fatigue[§]		✔			

Study	Analytic *n* IG/CG	Total protein intake (g/kg BW/d) during intervention[a]	Protein dose[b] (g/kg BW/d)	Protein type[c]	With/without physical exercise	Risk of bias[d]	Outcome	Result[e]				Comments
								+	NS	-	?	
Chalé et al. 2013[54]	42/38	NR (baseline: 0.98)	0.38[l]	A	Ex	SC	Double leg press strength, 1 RM[§]		✔			
							Knee extension, 1RM, right[§]		✔			
							Knee extension, 1RM, left[§]		✔			
							Double leg press peak power, 40% 1RM[§]		✔			
							Knee extension peak power, 40% 1RM, right[§]	✔				
							Knee extension peak power, 40% 1RM, left[§]	✔				
							Double leg press peak power, 70% 1RM[§]		✔			
							Knee extension peak power, 70% 1RM, right[§]	✔				
							Knee extension peak power, 70% 1RM, left[§]	✔				
Ottestad et al. 2017[55]	16-17[†]/ 18-19[†]	IG: 1.4 ± 0.5; CG: 0.9 ± 0.4	0.5	B	NoEx	H	Leg press strength		✔			
							Chest press strength		✔			
							Handgrip strength, dominant		✔			
							Handgrip strength, non-dominant		✔			
Mitchell et al. 2017[48]	14/15	IG: 1.7 ± 0.1; CG: 0.9 ± 0.1	0.8	C	NoEx	H	Hand grip strength		✔			
							Knee extension MVC		✔			
							Knee extension peak power	✔				
Subtotal (contrasts)								*5*	*19*	*0*	*0*	*Beneficial effect observed for 5 of 24 contrasts (2 of 5 studies)*
Subtotal (studies)[i]								*2*	*5*	*0*	*0*	
Habitual protein intake (reference): ≥1.0 to <1.1 kg BW/d												
Ispoglou et al. 2016[41]	8/9	1.02-1.08 (without protein supplementation of ~0.21 g/kg BW/d in IG1)	0.21	A	NoEx	H	Handgrip strength		✔			
							30-s arm-curl test		✔			
	8/9	1.02-1.08 (without protein supplementation of ~0.21 g/kg BW/d in IG2)	0.21				Handgrip strength		✔			
							30-s arm-curl test		✔			

Study	Analytic *n* IG/CG	Total protein intake (g/kg BW/d) during intervention[a]	Protein dose[b] (g/kg BW/d)	Protein type[c]	With/without physical exercise	Risk of bias[d]	Outcome	Result[e]				Comments
								+	NS	-	?	
Nabuco et al. 2019c[42]	13/13	IG: 1.0 ± 0.23 (without ~35 g whey protein supplementation on 3 d/wk); CG: 1.0 ± 0.19	0.24[k]	A	Ex	SC	Knee extension		✔			
							Chest press		✔			
							Preacher curl		✔			
							Total strength[f]		✔			
Thomson et al. 2016[57]	34/23	IG1: 1.42 ± 0.14; CG: 1.08 ± 0.05	0.34	B	Ex	H	Knee extensor strength[§]		✔			
							Handgrip strength[§]		✔			
							Leg press[§]		✔			
							Chest press[§]		✔			
							Knee extension strength[§]		✔			
							Lat pull down[§]				✔*	* Smaller % (but not absolute) increase in IG1 than in CG
							Leg curl[§]		✔			
							Total 8RM[§]		✔			
	26/23	IG2: 1.45 ± 0.14; CG: 1.08 ± 0.05	0.37				Knee extensor strength[§]		✔			* P=0.08 (strength tended to increase less in IG2 than in CG)
							Handgrip strength[§]		✔			
							Leg press[§]			✔		
							Chest press[§]		✔			
							Knee extension strength[§]		✔			
							Lat pull down[§]		✔			
							Leg curl[§]		✔			
							Total 8RM[§]			✔		

Study	Analytic *n* IG/CG	Total protein intake (g/kg BW/d) during intervention[a]	Protein dose[b] (g/kg BW/d)	Protein type[c]	With/without physical exercise	Risk of bias[d]	Outcome	Result[e]				Comments
								+	NS	-	?	
Nabuco et al. 2018[43] Same study [44,45]	22/23	IG1: 1.38 ± 0.26; CG: 1.0 ± 0.25	0.38	A	Ex	SC	Chest press	✔				
							Knee extension	✔				
							Preacher curl		✔			
							Total strength[f]	✔				
	21/23	IG2: 1.49 ± 0.46; CG: 1.0 ± 0.25	0.49				Chest press	✔				
							Knee extension	✔				
							Preacher curl		✔			
							Total strength[f]	✔				
Subtotal (contrasts) *Subtotal (studies)*[i]								*6* *1*	*23* *4*	*2* *1*	*1* *1*	*Beneficial effect observed for 6 of 32 contrasts (1 of 4 studies)* *Unfavourable effect observed for 2 of 32 contrasts (1 of 4 studies)*
Habitual protein intake (reference): ≥1.1 kg BW/d												
Zhu et al. 2015[58] Same study as [59,60]	93/88 (2-y follow-up)	IG: 1.4 ± 0.4; CG: 1.1 ± 0.4	0.3	A	NoEx	SC	Handgrip strength		✔			
							Ankle dorsiflexion strength		✔			
							Knee flexor strength		✔			
							Knee extensor strength		✔			
							Hip extensor strength		✔			
							Hip abductor strength		✔			
							Hip flexor strength		✔			
							Hip adductor strength		✔			
Subtotal (contrasts) *Subtotal (studies)*[i]								*0* *0*	*8* *1*	*0* *0*	*0* *0*	*No effect observed for any of 8 contrasts (1 study)*
Habitual protein intake (reference): Unclear												
Dillon et al. 2009[37]	7/7	NR	0.20	A	NoEx	H	Biceps curl		✔ *			* Results for time*group interaction (ANOVA) not reported, which suggests that protein has no effect (for all outcomes)
							Triceps extension		✔ *			
							Leg extension		✔ *			
							Leg curl		✔ *			

Study	Analytic *n* IG/CG	Total protein intake (g/kg BW/d) during intervention[a]	Protein dose[b] (g/kg BW/d)	Protein type[c]	With/without physical exercise	Risk of bias[d]	Outcome	Result[e]				Comments
								+	NS	-	?	
Mitchell et al. 2015[38]	16 (total)	NR	NR (15 g/d)	B	Ex	H	Knee extension isometric MVC		✔			
							Leg press		✔			
							Leg extension		✔			
							Chest press		✔			
Subtotal (contrasts) *Subtotal (studies)*[j]								*0* *0*	*8* *2*	*0* *0*	*0* *0*	*No effect observed for any of 8 contrasts (2 studies)*
Total (contrasts) Total (studies)[k]								14 4	66 15	2 1	1 1	Beneficial effect observed for 14 of 83 contrasts (4 of 15 studies) Unfavourable effect observed for 2 of 83 contrasts (1 of 15 studies)

Abbreviations: BW: body weight, CG: control group, Ex: with concomitant physical exercise intervention, H: high risk of bias, IG: intervention group, L: low risk of bias, MVC: maximal voluntary contraction, NoEx: without concomitant physical exercise intervention, NR: not reported, NS: not significant, RM: repetition maximum, SC: some concerns (regarding risk of bias).

Footnotes:

† Depending on specific outcome measure.

§ Sufficient statistical power to detect an effect is to be expected, based on the sample size calculation.

[a] Total protein intake during follow-up. If protein intake was assessed at multiple time points, the intake assessed at the final time point was considered.

[b] 'Protein dose' indicates the difference in achieved total protein intake between the intervention and control groups during follow-up (which is not necessarily equal to the supplemented/prescribed amount of protein).

[c] 'Protein type' indicates the way in which a higher protein intake was achieved and is categorised into 'pure' protein or amino acids (or essential amino acids) (A), specific food(s) with a high protein content (B), or high-protein diets (C).

[d] Risk of bias assessment: risk of bias was assessed using the RoB 2 tool and scored as 'low' (L), 'some concerns' (SC), or 'high' (H).

[e] The results of the studies are indicated as follows: +, statistically significant beneficial effect ($P<0.05$); -, statistically significant unfavourable effect ($P<0.05$); NS, no statistically significant effect ($P\geq0.05$); ?, result unclear. In cases where results were reported for multiple time points, only the result for the final time point is reported.

[f] Total strength was calculated as the sum of chest press, knee extension and preacher curl strength (kg).

[g] Lower limb muscle quality index was calculated as knee extension strength divided by lower limb lean soft tissue.

[h] Upper limb muscle quality index was calculated as preacher curl strength divided by upper limb lean soft tissue.

[i] Total muscle quality index was calculated as total strength divided by skeletal muscle mass.

[j] Some studies assessed multiple specific outcomes (i.e. multiple contrasts) for the health outcome 'muscle strength', so one study can show both a significant and a non-significant effect.

[k] Protein intake in g/kg BW/d was calculated by using protein intake in g/d and mean body weight (and compliance, if available).

[l] (Achieved) protein dose was estimated using prescribed protein dose, compliance rate (72%), and mean body weight.

3.4.3 Physical function

An overview of the main characteristics and results of the evaluated RCTs examining the effect of increased protein intake on physical function in older adults is provided in Table 4. A detailed description of the characteristics and results of these RCTs is provided in Annex E (Table E3).

Overall

The Committee evaluated 12 RCTs on the effect of increased protein intake on physical function in older adults, with a total of 44 statistically tested contrasts. Almost all studies used an objective measure of physical function (i.e. a physical performance test administered by a member of the research team, such as gait speed). Those studies involved a total of approximately 961[a] participants (IG/CG: 543/418). The smallest study involved 17 participants (IG/CG: 8/9), while the largest study had 181 participants (IG/CG: 93/88). Most studies (n=8) had an intervention period of 12 weeks (range: 10 weeks to 2 years). The habitual protein intake ranged from 0.81 to 1.1 g/kg BW/d and the protein dose from 0.17 to 0.8 g/kg BW/d. The total protein intake in the intervention groups ranged from 1.06 g/kg BW/d (compared to an habitual intake of 0.89 g/kg BWd/) to 1.7 g/kg BW/d (compared to 0.9 g/kg BW/d). The risk of bias in those studies was scored as 'some concerns' (n=7) or 'high' (n=5).

In 2 of the 12 studies (17%), involving a total of 146 participants (IG/CG: 83/63), a beneficial effect of increased protein intake on physical function was found for at least one of the statistically tested contrasts (3 of 44 contrasts, 7%). The duration of those studies was 12 weeks. In those studies that found a beneficial effect on physical function, the habitual protein intake ranged from 0.9 to 1.0 g/kg BW/d and the protein dose from 0.38 to 0.49 g/kg BW/d. The total protein intake in the intervention groups of those studies ranged from 1.37 g/kg BW/d (compared to an habitual intake of 0.9 g/kg BW/d) to 1.49 g/kg BW/d (compared to 1.0 g/kg BW/d). Increased protein intake was not found to have any unfavourable effects on physical function. There was little difference between the results obtained by studies on the effect of increased protein intake alone (beneficial effect in 1 of 6 studies (1 of 25 contrasts)) and those obtained by studies on the effect of increased protein intake in the context of physical exercise (beneficial effect in 1 of 6 studies (2 of 19 contrasts)).

Preliminary conclusion: Based on the 12 evaluated RCTs, the Committee concluded that increased protein intake has likely no effect on physical function in older adults.

[a] This number represents the participants included in the analyses for this outcome. The sample size may vary slightly, depending on the specific outcome measure used.

Habitual protein intake (reference): ≥0.8 to <0.9 g/kg BW/d
Against a background (habitual) intake of 0.8 to 0.9 g protein/kg BW/d, the Committee evaluated two RCTs on the effect of increased protein intake on physical function, with a total of seven statistically tested contrasts. Those studies involved a total of approximately 206 participants (IG/CG: 108/98). The smallest study involved 65 participants (IG/CG: 33/32), while the largest study had 141 participants (IG/CG: 75/66). The protein dose ranged from 0.17 to 0.36 g/kg BW/d. The risk of bias in those studies was scored as 'some concerns' (n=1) or 'high' (n=1). Those studies found no effects of increased protein intake on physical function.

Habitual protein intake (reference): ≥0.9 to <1.0 g/kg BW/d
Against a background intake of 0.9 to 1.0 g protein/kg BW/d, the Committee evaluated five RCTs on the effect of increased protein intake on physical function, with a total of 24 statistically tested contrasts. Those studies involved a total of approximately 374 participants (IG/CG: 210/164). The smallest study involved 29 participants (IG/CG: 14/15), while the largest study had 114 participants (IG/CG: 58/56). The protein dose ranged from 0.25 to 0.8 g/kg BW/d. The risk of bias in those studies was scored as 'some concerns' (n=3) or 'high' (n=2).

In 1 of the 5 studies (20%), a beneficial effect of increased protein intake was found for at least one of the statistically tested contrasts (1 of 24 contrasts, 4%). In this study that found an effect, the protein dose was 0.47 g/kg BW/d. Increased protein intake, within this domain of habitual protein intake, was not found to have any unfavourable effects on physical function.

Habitual protein intake (reference): ≥1.0 to <1.1 g/kg BW/d
Against a background intake of 1.0 to 1.1 g protein/kg BW/d, the Committee evaluated four RCTs on the effect of increased protein intake on physical function, with a total of 12 statistically tested contrasts. Those studies involved a total of approximately 200 participants (IG/CG: 132/68). The smallest study involved 17 participants (IG/CG: 8/9), while the largest study had 57 participants (IG/CG: 34/23). The protein dose ranged from 0.21 to 0.49 g/kg BW/d. The risk of bias in those studies was scored as 'some concerns' (n=2) or 'high' (n=2).

In 1 of the 4 studies (25%), a beneficial effect of increased protein intake was found for at least one of the statistically tested contrasts (2 of 12 contrasts, 17%). In this study that found an effect, the protein dose ranged from 0.38 to 0.49 g/kg BW/d. Increased protein intake, within this domain of habitual protein intake, was not found to have any unfavourable effects on physical function.

Habitual protein intake (reference): ≥1.1 g/kg BW/d
Against a background intake of at least 1.1 g protein/kg BW/d, the Committee evaluated one RCT on the effect of increased protein intake on physical function, with a total of one statistically tested contrast. This study

involved a total of 181 participants (IG/CG: 93/88). The protein dose was 0.3 g/kg BW/d. The risk of bias in this study was scored as ‘some concerns’. This study found no effect of increased protein intake on physical function.

Final conclusion regarding physical function:

Based on the 12 evaluated RCTs, the Committee concluded that increased protein intake has **likely no effect** on physical function in older adults.

Furthermore, the Committee concluded that the results do not suggest that the effect on physical function of increased protein intake alone (not in the context of physical exercise) differs from the effect of increased protein intake in the context of concomitant physical exercise. Beneficial effects of increased protein intake on physical function were observed in participants with an habitual protein intake up to and including 1.0 g/kg BW/d and for a total protein intake up to and including 1.49 g/kg BW/d. The Committee found no indications of a dose-response relationship.

Table 4. Overview of the results of the 12 evaluated randomised controlled trials on the effect of increased protein intake on physical function in older adults, categorised according to habitual protein intake and ordered by protein dose

Study	Analytic *n* IG/CG	Total protein intake (g/kg BW/d) during intervention[a]	Protein dose[b] (g/kg BW/d)	Protein type[c]	With/ without physical exercise	Risk of Bias[d]	Outcome	Result[e]				Comments
								+	NS	-	?	
Habitual protein intake (reference): ≥0.8 to <0.9 kg BW/d												
Arnarson et al. 2013[49] Same study as [50]	75/66	IG: 1.06 ± 0.23; CG 0.89 ± 0.23	0.17	A	Ex	H	Gait speed, 6-min		✔			
							TUG		✔			
Bhasin et al. 2018[51]	33-42†/ 32-40†	IG: 1.17 ± 0.13; CG: 0.81 ± 0.10	0.36	A,B	NoEx	SC	Gait speed, 6-min		✔			
							Gait speed, 50-m		✔			
							Stair climb power, unloaded		✔ *			* P=0.08 (power tended to increase less in IG than in CG)
							Stair climb power, loaded		✔			
							Perceived physical function		✔			
Subtotal (contrasts)								*0*	*7*	*0*	*0*	*No effect observed for any of*
Subtotal (studies)[f]								*0*	*2*	*0*	*0*	*7 contrasts (2 studies)*
Habitual protein intake (reference): ≥0.9 to <1.0 kg BW/d												
Park et al. 2018[52]	40/40	IG1: 1.18 ± 0.23; CG: 0.90 ± 0.38	0.28	A	NoEx	SC	SPPB		✔			
							Gait speed, 4-m		✔			
							Standing balance		✔			
							Chair rise time		✔			
							TUG		✔			
	40/40	IG2: 1.37 ± 0.26; CG: 0.90 ± 0.38	0.47				SPPB		✔			
							Gait speed, 4-m	✔				
							Standing balance		✔			
							Chair rise time		✔			
							TUG		✔			
Ten Haaf et al. 2019[53]	58/56 (except chair rise time: total n=111)	IG: 0.92 ± 0.27 (without protein supplementation of 31 g/d); CG: 0.97 ± 0.23	0.36[g]	A	Ex	SC	SPPB[§]		✔			
							Standing balance[§]		✔			
							Gait speed, 4-m[§]		✔			
							Chair rise time[§]		✔			
							TUG[§]		✔			

Study	Analytic *n* IG/CG	Total protein intake (g/kg BW/d) during intervention[a]	Protein dose[b] (g/kg BW/d)	Protein type[c]	With/ without physical exercise	Risk of Bias[d]	Outcome	Result[e]				Comments
								+	NS	-	?	
Chalé et al. 2013[54]	42/38	NR (baseline: 0.98)	0.38[h]	A	Ex	SC	Gait speed, 400-m[§]		✔			
							Stair climb time[§]		✔			
							Chair rise time[§]		✔			
							SPPB[§]		✔			
Ottestad et al. 2017[55]	16/15-17[†]	IG: 1.4 ± 0.5; CG: 0.9 ± 0.4	0.5	B	NoEx	H	Chair rise time		✔			
							Stair climb time, unloaded		✔			
							Stair climb time, loaded		✔			
Mitchell et al. 2017[48]	14/15	IG: 1.7 ± 0.1; CG: 0.9 ± 0.1	0.8	C	NoEx	H	SPPB		✔			
							TUG		✔			
Subtotal (contrasts) *Subtotal (studies)[f]*								*1* *1*	*23* *5*	*0* *0*	*0* *0*	*Beneficial effect observed for 1 of 24 contrasts (1 of 5 studies)*
Habitual protein intake (reference): ≥1.0 to <1.1 kg BW/d												
Ispoglou et al. 2016[41]	8/9	1.02-1.08 (without protein supplementation of ~0.21 g/kg BW/d in IG1)	0.21	A	NoEx	H	Gait speed, 6-min		✔			
							30-s chair-stand test		✔			
	8/9	1.02-1.08 (without protein supplementation of ~0.21 g/kg BW/d in IG2)	0.21				Gait speed, 6-min		✔			
							30-s chair-stand test		✔			
Nabuco et al. 2019c[42]	13/13	IG: 1.0 ± 0.23 (without ~35 g whey protein supplementation on 3 d/wk); CG: 1.0 ± 0.19	0.24[g]	A	Ex	SC	Gait speed, 10-m		✔			
							Chair rise time		✔			
Thomson et al. 2016[57]	34/23	IG1: 1.42 ± 0.14; CG: 1.08 ± 0.05	0.34	B	Ex	H	Gait speed, 6-min		✔			
	26/23	IG2: 1.45 ± 0.14; CG: 1.08 ± 0.05	0.37				Gait speed, 6-min		✔			
Nabuco et al. 2018[43] Same study [44,45]	22/23	IG1: 1.38 ± 0.26; CG: 1.0 ± 0.25	0.38	A	Ex	SC	Gait speed, 10-m at fast pace	✔				
							Chair rise time		✔			
	21/23	IG2: 1.49 ± 0.46; CG: 1.0 ± 0.25	0.49				Gait speed, 10-m at fast pace	✔				
							Chair rise time		✔			
Subtotal (contrasts) *Subtotal (studies)[f]*								*2* *1*	*10* *4*	*0* *0*	*0* *0*	*Beneficial effect observed for 2 of 12 contrasts (1 of 4 studies)*

Study	Analytic *n* IG/CG	Total protein intake (g/kg BW/d) during intervention[a]	Protein dose[b] (g/kg BW/d)	Protein type[c]	With/ without physical exercise	Risk of Bias[d]	Outcome	Result[e]				Comments
								+	NS	-	?	
Habitual protein intake (reference): ≥1.1 kg BW/d												
Zhu et al. 2015[58] Same study as [59,60]	93/88 (2-y follow-up)	IG: 1.4 ± 0.4; CG: 1.1 ± 0.4	0.3	A	NoEx	SC	TUG		✔			
Subtotal (contrasts) *Subtotal (studies)*[f]								*0* *0*	*1* *1*	*0* *0*	*0* *0*	*No effect observed for the 1 contrast (1 study)*
Total (contrasts) Total (studies)[f]								3 2	41 12	0 0	0 0	Beneficial effect observed for 3 of 44 contrasts (2 of 12 studies)

Abbreviations: ADL: activities of daily living, BW: body weight, CG: control group, Ex: with concomitant physical exercise intervention, H: high risk of bias, IG: intervention group, L: low risk of bias, NoEx: without concomitant physical exercise intervention, NR: not reported, NS: not significant, SC: some concerns (regarding risk of bias), SPPB: short physical performance battery, TUG: Timed Up and Go.

Footnotes:

† Depending on specific outcome measure.

§ Sufficient statistical power to detect an effect is to be expected, based on the sample size calculation.

[a] Total protein intake during follow-up. If protein intake was assessed at multiple time points, the intake assessed at the final time point was considered.

[b] 'Protein dose' indicates the difference in achieved total protein intake between the intervention and control groups during follow-up (which is not necessarily equal to supplemented/prescribed amount of protein).

[c] 'Protein type' indicates the way in which a higher protein intake was achieved and is categorised into 'pure' protein or amino acids (or essential amino acids) (A), specific food(s) with a high protein content (B), or high-protein diets (C).

[d] Risk of bias assessment: risk of bias was assessed using the RoB 2 tool and scored as 'low' (L), 'some concerns' (SC), or 'high' (H).

[e] The results of the studies are indicated as follows: +, statistically significant beneficial effect (P<0.05); -, statistically significant unfavourable effect (P<0.05); NS, no statistically significant effect (P≥0.05); ?, result unclear. In cases where results were reported for multiple time points, only the result for the final time point is reported.

[f] Some studies assessed multiple specific outcomes (i.e. multiple contrasts) for the health outcome 'physical function', so one study can show both a significant and a non-significant effect.

[g] Protein intake in g/kg BW/d was calculated by using protein intake in g/d and mean body weight (and compliance, if available).

[h] (Achieved) protein dose was estimated using prescribed protein dose, compliance rate (72%), and mean body weight.

3.4.4 Bone health

An overview of the main characteristics and results of the evaluated RCTs examining the effect of increased protein intake on bone health in older adults is provided in Table 5. A detailed description of the characteristics and results of these RCTs is provided in Annex E (Table E4).

Overall

The Committee evaluated four RCTs on the effect of increased protein intake on bone health, with a total of 23 statistically tested contrasts. In the majority of the studies, the specific outcome measure was bone mineral density (BMD).The total number of participants varied from 279[a] (IG/CG: 144/135) to 448 (IG/CG: 233/215), depending on the specific outcome measure. The smallest study involved 17 participants (IG/CG: 8/9), while the largest study had 208 participants (IG/CG: 106/102). The intervention period ranged from 12 weeks to 2 years. The habitual protein intake ranged from 0.87 to 1.1 g/kg BW/d and the protein dose ranged from 0.21 to 0.53 g/kg BW/d. The total protein intake in the intervention groups ranged from 1.23 g/kg BW/d (compared to an habitual intake of 1.02 g/kg BW/d) to 1.4 g/kg BW/d (compared to 0.87 g/kg BW/d). The risk of bias in those studies was scored as ‘some concerns’ (n=2) or ‘high’ (n=2).

In 1 of the 4 studies (25%), involving a total of 121 participants (IG/CG: 61/60), a beneficial effect of increased protein intake on bone health was found for at least one of the statistically tested contrasts (1 of 23 contrasts, 4%). This concerned the P1NP serum biomarker[b]. The duration of this study was 18 months. In the study that found this beneficial effect, the habitual protein intake was 1.05 g/kg BW/d and the protein dose was 0.25 g/kg BW/d. The risk of bias in this study was scored as ‘some concerns’. Increased protein intake was not found to have any unfavourable effects on bone health. For bone health, only one study examined the effect of increased protein intake in the context of physical exercise (in which no effect was found). This precluded any comparison of the results of studies with and without a concomitant physical exercise intervention.

The Committee noted that the percentage of beneficial effects based on contrasts (4%) is much lower than the percentage of beneficial effects based on studies (25%). The only beneficial effect observed was for a surrogate outcome (serum marker). Furthermore, the effect estimates for the majority of tested contrast are close to zero (Table E4), suggesting no effect of increased protein intake on parameters of bone health. The Committee judged that the evidence is too weak to conclude that there is possibly a beneficial effect. As a result, it has moderated its conclusion.

[a] This number represents the participants included in the analyses for this outcome. The sample size may vary slightly, depending on the specific outcome measure used.

[b] P1NP is a bone-turnover parameter (for bone formation).

Preliminary conclusion: Based on the four evaluated RCTs, the Committee concluded that increased protein intake has likely no effect on bone health in older adults.

Because an effect of increased protein intake was found for only one contrast, the results were not further subdivided by domain of habitual protein intake.

Final conclusion regarding bone health:

Based on the four evaluated RCTs, the Committee concluded that increased protein intake has **likely no effect** on bone health in older adults.

Table 5. Overview of the results of the four evaluated randomised controlled trials on the effect of increased protein intake on bone health in older adults, categorised according to habitual protein intake and ordered by protein dose

Study	Analytic *n* IG/CG	Total protein intake (g/kg BW/d) during intervention[a]	Protein dose[b] (g/kg BW/d)	Protein type[c]	With/without physical exercise	Risk of Bias[d]	Outcome	Result[e]				Comments
								+	NS	-	?	
Habitual protein intake (reference): ≥0.8 to <0.9 kg BW/d												
Fernandes et al. 2018[40] Same study as [46]	16/16	IG: 1.4 ± 0.1; CG: 0.87 ± 0.1	0.53	A	Ex	H	Total BMC		✔			
Subtotal (contrasts)								*0*	*1*	*0*	*0*	*No effect observed for the*
Subtotal (studies)[f]								*0*	*1*	*0*	*0*	*1 contrast (1 study)*
Habitual protein intake (reference): ≥1.0 to <1.1 kg BW/d												
Ispoglou et al. 2016[41]	8/9	1.02-1.08 (without protein supplementation of ~0.21 g/kg BW/d in IG1)	0.21	A	NoEx	H	Total BMC		✔			
							Total BMD		✔			
	8/9	1.02-1.08 (without protein supplementation of ~0.21 g/kg BW/d in IG2)	0.21				Total BMC		✔			
							Total BMD		✔			
Kerstetter et al. 2015[56]	105-106[†]/ 102 for DXA measurements; 45/44 for QCT measurements; 61/60 for serum markers (all 18-mo follow-up)	IG: 1.30 ± 0.05; CG: 1.05 ± 0.04	0.25	A	NoEx	SC	BMD lumbar spine (DXA)[§]		✔			
							BMD total hip (DXA)[§]		✔			
							BMD femoral neck (DXA)[§]		✔			
							BMD lumbar spine (QCT)[§]		✔			
							BMD femoral neck, cortical (QCT)[§]		✔			
							BMD femoral neck, trabecular (QCT)[§]		✔			
							BMD femoral total, cortical (QCT)[§]		✔			
							BMD femoral total, trabecular (QCT)[§]		✔			
							Serum P1NP[§]	✔				
							Serum CTX[§]		✔ *			* No difference at 18 months, but at 9 months serum CTX increased more in IG than in CG (P=0.0007)
							Serum OC[§]		✔			
Subtotal (contrasts)								*1*	*14*	*0*	*0*	*Beneficial effect observed for*
Subtotal (studies)[f]								*1*	*2*	*0*	*0*	*1 of 15 contrasts (1 of 2 studies)*

Study	Analytic *n* IG/CG	Total protein intake (g/kg BW/d) during intervention[a]	Protein dose[b] (g/kg BW/d)	Protein type[c]	With/without physical exercise	Risk of Bias[d]	Outcome	Result[e]				Comments
								+	NS	-	?	
Habitual protein intake (reference): ≥1.1 kg BW/d												
Zhu et al. 2011[60] Same study as [58,59]	95/88 for DXA measurements; 67/66 for QCT measurements (all 2-y follow-up)	IG: 1.4 ± 0.4; CG: 1.1 ± 0.4	0.3	A	NoEx	SC	Total hip aBMD (DXA)[§]		✔			
							Femoral neck aBMD (DXA)[§]		✔			
							Total hip volumetric BMD (QCT)[§]		✔			
							Femoral neck vBMD (QCT)[§]		✔			
							Femoral neck bone CSA (QCT)[§]		✔			
							Femoral neck buckling ratio (QCT)[§]		✔			
							Femoral neck polar CSMI (QCT)[§]		✔			
Subtotal (contrasts) *Subtotal (studies)*[f]								*0* *0*	*7* *1*	*0* *0*	*0* *0*	*No effect observed for any of 7 contrasts (1 study)*
Total (contrasts) Total (studies)[f]								1 1	22 4	0 0	0 0	Beneficial effect observed for 1 of 23 contrasts (1 of 4 studies)

Abbreviations: aBMD: areal bone mineral density, BMC: bone mineral content, BMD: bone mineral density, BW: body weight, CG: control group, CSMI: cross-sectional moment of inertia, . CTX: C-terminal telopeptide of type 1 collagen, DXA: dual-energy x-ray absorptiometry, Ex: with concomitant physical exercise intervention, H: high risk of bias, IG: intervention group, L: low risk of bias, NoEx: without concomitant physical exercise intervention, NR: not reported, NS: not significant, OC: osteocalcin, P1NP: N-terminal propeptides of type 1 procollagen, QCT: quantitative computed tomography, SC: some concerns (regarding risk of bias), vBMD: volumetric bone mineral density.

Footnotes:

† Depending on specific outcome measure.

§ Sufficient statistical power to detect an effect is to be expected, based on the sample size calculation.

[a] Total protein intake during follow-up. If protein intake was assessed at multiple time points, the intake assessed at the final time point was considered.

[b] 'Protein dose' indicates the difference in achieved total protein intake between the intervention and control groups during follow-up (which is not necessarily equal to supplemented/prescribed amount of protein).

[c] 'Protein type' indicates the way in which a higher protein intake was achieved and is categorised into 'pure' protein or amino acids (or essential amino acids) (A), specific food(s) with a high protein content (B), or high-protein diets (C).

[d] Risk of bias assessment: risk of bias was assessed using the RoB 2 tool and scored as 'low' (L), 'some concerns' (SC), or 'high' (H).

[e] The results of the studies are indicated as follows: +, statistically significant beneficial effect (P<0.05); -, statistically significant unfavourable effect (P<0.05); NS, no statistically significant effect (P≥0.05); ?, result unclear. In cases where results were reported for multiple time points, only the result for the final time point is reported.

[f] Some studies assessed multiple specific outcomes (i.e. multiple contrasts) for the health outcome 'bone health', so one study can show both a significant and a non-significant effect.

3.4.5 Blood pressure

An overview of the main characteristics and results of the evaluated RCTs examining the effect of increased protein intake on blood pressure in older adults is provided in Table 6. A detailed description of the characteristics and results of these RCTs is provided in Annex E (Table E5).

Overall

The Committee evaluated four RCTs on the effect of increased protein intake on blood pressure (systolic or diastolic), with a total of ten statistically tested contrasts. Those studies involved a total of 263 participants (IG/CG: 107/156) The smallest study involved 22 participants (IG/CG: 12/10), while the largest study had 219 participants (IG/CG: 109/110). Three studies had an intervention period of 12 weeks, while the other study had intervention periods of 1 and 2 years. The habitual protein intake ranged from 0.8 to 1.0 g/kg BW/d and the protein dose ranged from 0.24 to 0.6 g/kg BW/d. The total protein intake in the intervention groups ranged from 1.24 to 1.49 g/kg BW/d (both compared to an habitual intake of 1.0 g/kg BW/d). The risk of bias in those studies was scored as 'some concerns' (n=3) or 'high' (n=1).

None of those four studies found an effect of increased protein intake on blood pressure. There is thus also no indication for a difference in effect of increased protein intake alone (examined in 2 RCTs) compared to increased protein intake in the context of physical exercise (examined in 2 RCTs) on blood pressure.

The Committee noted that the sample size (n=219) of the only study in which the power analysis was based on blood pressure[59] is substantially higher than the sample size (n=22-45) in the other three studies where it is not known whether the power analysis was based on blood pressure. Also, the Committee noted the effect sizes in some of those studies were not close to 0. It presumed that those three studies may have insufficient statistical power to demonstrate an effect on blood pressure. The Committee, therefore, could not completely exclude the possibility that increased protein intake may have an effect on blood pressure. More studies with sufficient statistical power are needed to draw a conclusion.

Preliminary conclusion: Based on the four evaluated RCTs, the Committee concluded that there are too few studies (with sufficient statistical power) to draw any conclusions about the effect of increased protein intake on blood pressure in older adults.

Because no effects of increased protein intake on blood pressure were found in any of the studies, the results were not further subdivided by domain of habitual protein intake.

Final conclusion regarding blood pressure:

Based on the four evaluated RCTs, the Committee concluded that there are **too few studies (with sufficient statistical power)** to determine whether or not increased protein intake affects blood pressure in older adults.

Table 6. Overview of the results of the four evaluated randomised controlled trials on the effect of increased protein intake on blood pressure in older adults, categorised according to habitual protein intake and ordered by protein dose

Study	Analytic *n* IG/CG	Total protein intake (g/kg BW/d) during intervention[a]	Protein dose[b] (g/kg BW/d)	Protein type[c]	With/without physical exercise	Risk of Bias[d]	Outcome	Result[e]				Comments
								+	NS	-	?	
Habitual protein intake (reference): ≥0.8 to <0.9 kg BW/d												
Wright et al. 2018[47]	12/10	IG: 1.4; CG: 0.8 (prescribed)[f,g]	0.6[f]	C	NoEx	H	Systolic blood pressure		✔			
							Diastolic blood pressure		✔			
Subtotal (contrasts)								*0*	*2*	*0*	*0*	*No effect observed for any of*
Subtotal (studies)[h]								*0*	*1*	*0*	*0*	*2 contrasts (1 study)*
Habitual protein intake (reference): ≥1.0 to 1.1 kg BW/d												
Nabuco et al. 2019c[42]	13/13	IG: 1.0 ± 0.23 (without ~35 g whey protein supplementation on 3 d/wk); CG: 1.0 ± 0.19	0.24[f]	A	Ex	SC	Systolic blood pressure		✔			
							Diastolic blood pressure		✔			
Nabuco et al. 2019a[44] Same study as [43,45]	22/23	IG1: 1.38 ± 0.26; CG: 1.0 ± 0.25	0.38	A	Ex	SC	Systolic blood pressure		✔			
							Diastolic blood pressure		✔			
	21/23	IG2: 1.49 ± 0.46; CG: 1.0 ± 0.25	0.49				Systolic blood pressure		✔			
							Diastolic blood pressure		✔			
Subtotal (contrasts)								*0*	*6*	*0*	*0*	*No effect observed for any of*
Subtotal (studies)[h]								*0*	*2*	*0*	*0*	*6 contrasts (2 studies)*
Habitual protein intake (reference): ≥1.1 kg BW/d												
Hodgson et al. 2012[59] Same study as [58,60]	109/110	IG: 1.4 ± 0.4; CG: 1.1 ± 0.4	0.3	A	NoEx	SC	Systolic blood pressure[§]		✔			
							Diastolic blood pressure[§]		✔			
Subtotal (contrasts)								*0*	*2*	*0*	*0*	*No effect observed for any of*
Subtotal (studies)[h]								*0*	*1*	*0*	*0*	*2 contrasts (1 study)*
Total (contrasts)								0	10	0	0	No effect observed for any of
Total (studies)[h]								0	4	0	0	10 contrasts (4 studies)

Abbreviations: BW: body weight, CG: control group, Ex: with concomitant physical exercise intervention, H: high risk of bias, IG: intervention group, L: low risk of bias, NoEx: without concomitant physical exercise intervention, NR: not reported, NS: not significant, SC: some concerns (regarding risk of bias).

Footnotes:

§ Sufficient statistical power to detect an effect is to be expected, based on the sample size calculation.

[a] Total protein intake during follow-up. If protein intake was assessed at multiple time points, the intake assessed at the final time point was considered.

[b] 'Protein dose' indicates the difference in achieved total protein intake between the intervention and control groups during follow-up (which is not necessarily equal to supplemented/prescribed amount of protein).

[c] 'Protein type' indicates the way in which a higher protein intake was achieved and is categorised into 'pure' protein or amino acids (or essential amino acids) (A), specific food(s) with a high protein content (B), or high-protein diets (C).

[d] Risk of bias assessment: risk of bias was assessed using the RoB 2 tool and scored as 'low' (L), 'some concerns' (SC), or 'high' (H).

[e] The results of the studies are indicated as follows: +, statistically significant beneficial effect (P<0.05); -, statistically significant unfavourable effect (P<0.05); NS, no statistically significant effect (P≥0.05); ?, result unclear. In cases where results were reported for multiple time points, only the result for the final time point is reported.

[f] Protein intake in g/kg BW/d was calculated by using protein intake in g/d and mean body weight.

[g] Actual protein intake may have been different from the prescribed protein intake, due to non-compliance (compliance was 91% on average).

[h] Some studies assessed multiple specific outcomes (i.e. multiple contrasts) for the health outcome 'blood pressure', so one study can show both a significant and a non-significant effect.

3.4.6 Glucose and insulin metabolism

An overview of the main characteristics and results of the evaluated RCTs examining the effect of increased protein intake on glucose and insulin metabolism in older adults is provided in Table 7. A detailed description of the characteristics and results of these RCTs is provided in Annex E (Table E6).

Overall

The Committee evaluated six RCTs on the effect of increased protein intake on parameters of glucose and insulin metabolism, with a total of 16 statistically tested contrasts. Those studies involved a total of 301 participants (IG/CG: 181/120). The smallest study involved 22 participants (IG/CG: 12/10), while the largest study had 80 participants (IG/CG: 40/40). The duration of these studies was 12 weeks. The habitual protein intake ranged from 0.8 to 1.0 g/kg BW/d and the protein dose ranged from 0.24 to 0.6 g/kg BW/d. The total protein intake in the intervention groups ranged from 1.18 g/kg BW/d (compared to an habitual intake of 0.90 g/kg BW/d) to 1.49 g/kg BW/d (compared to 1.0 g/kg BW/d). The risk of bias in those studies was scored as ‘some concerns’ (n=3) or ‘high’ (n=3).

None of those six studies found an effect of increased protein intake on parameters of glucose and insulin metabolism. There is thus also no indication for a difference in effect of increased protein intake alone (examined in 3 RCTs) compared to increased protein intake in the context of physical exercise (examined in 3 RCTs) on glucose and insulin metabolism.

The Committee cited a lack of statistical power as a possible explanation for the fact that these RCTs showed no effects. In 2 of the 6 studies[52,55] the power analysis was not based on a parameter of glucose or insulin metabolism. With regard to the other four studies, there is no information on this point. The Committee presumed that the sample sizes were too small to demonstrate an effect on glucose and insulin metabolism. The Committee, therefore, could not completely exclude the possibility that increased protein intake may have an effect on glucose and insulin metabolism. More studies with sufficient statistical power are needed to draw a conclusion.

Preliminary conclusion: Based on the six evaluated RCTs, the Committee concluded that there are too few studies (with sufficient statistical power) to draw any conclusions about the effect of increased protein intake on glucose and insulin metabolism in older adults.

Because no effects of increased protein intake on glucose and insulin metabolism were found in any of the studies, the results were not further subdivided by domain of habitual protein intake.

Final conclusion regarding glucose and insulin metabolism:

Based on the six evaluated RCTs, the Committee concluded that there are **too few studies (with sufficient statistical power)** to determine whether or not increased protein intake affects glucose and insulin metabolism in older adults.

Table 7. Overview of the results of the six evaluated randomised controlled trials on the effect of increased protein intake on glucose and insulin metabolism in older adults, categorised according to habitual protein intake and ordered by protein dose

Study	Analytic *n* IG/CG	Total protein intake (g/kg BW/d) during intervention[a]	Protein dose[b] (g/kg BW/d)	Protein type[c]	With/without physical exercise	Risk of Bias[d]	Outcome	Result[e]				Comments
								+	NS	-	?	
Habitual protein intake (reference): ≥0.8 to <0.9 g/kg BW/d												
Fernandes et al. 2018[40] Same study as [46]	16/16	IG: 1.4 ± 0.1; CG: 0.87 ± 0.1	0.53	A	Ex	H	Fasting blood glucose		✔			
Wright et al. 2018[47]	12/10	IG: 1.4; CG: 0.8 (prescribed)[f,g]	0.6[f]	C	NoEx	H	Fasting blood glucose		✔			
							Fasting insulin		✔			
							HOMA-IR		✔			
Subtotal (contrasts) *Subtotal (studies)*[h]								*0* *0*	*4* *2*	*0* *0*	*0* *0*	*No effect observed for any of 4 contrasts (2 studies)*
Habitual protein intake (reference): ≥0.9 to <1.0 g/kg BW/d												
Park et al. 2018[52]	40/40	IG1: 1.18 ± 0.23; CG: 0.90 ± 0.38	0.28	A	NoEx	SC	Fasting blood glucose		✔			
	40/40	IG2: 1.37 ± 0.26; CG: 0.90 ± 0.38	0.47				Fasting blood glucose		✔			
Ottestad et al. 2017[55]	17/18	IG: 1.4 ± 0.5; CG: 0.9 ± 0.4	0.5	B	NoEx	H	Fasting blood glucose		✔			
Subtotal (contrasts) *Subtotal (studies)*[h]								*0* *0*	*3* *2*	*0* *0*	*0* *0*	*No effect observed for any of 3 contrasts (2 studies)*
Habitual protein intake (reference): ≥1.0 to <1.1 g/kg BW/d												
Nabuco et al. 2019c[42]	13/13	IG: 1.0 ± 0.23 (without ~35 g whey protein supplementation on 3 d/wk); CG: 1.0 ± 0.19	0.24[f]	A	Ex	SC	Fasting blood glucose		✔			
							Fasting insulin		✔			
							HOMA-IR		✔			
Nabuco et al. 2019a[44] Same study as [43,45]	22/23	IG1: 1.38 ± 0.26; CG: 1.0 ± 0.25	0.38	A	Ex	SC	Fasting blood glucose		✔			
							Fasting insulin		✔			
							HOMA-IR		✔			
	21/23	IG2: 1.49 ± 0.46; CG: 1.0 ± 0.25	0.49				Fasting blood glucose		✔			
							Fasting insulin		✔			
							HOMA-IR		✔			
Subtotal (contrasts) *Subtotal (studies)*[h]								*0* *0*	*9* *2*	*0* *0*	*0* *0*	*No effect observed for any of 9 contrasts (2 studies)*
Total (contrasts) Total (studies)[h]								0 0	16 6	0 0	0 0	No effect observed for any of 16 contrasts (6 studies)

Abbreviations: BW: body weight, CG: control group, Ex: with concomitant physical exercise intervention, H: high risk of bias, HOMA-IR: homeostatic model assessment of insulin resistance, IG: intervention group, L: low risk of bias, NoEx: without concomitant physical exercise intervention, NR: not reported, NS: not significant, SC: some concerns (regarding risk of bias).
Footnotes:
[§] Sufficient statistical power to detect an effect is to be expected, based on the sample size calculation.
[a] Total protein intake during follow-up. If protein intake was assessed at multiple time points, the intake assessed at the final time point was considered.
[b] 'Protein dose' indicates the difference in achieved total protein intake between the intervention and control groups during follow-up (which is not necessarily equal to supplemented/prescribed amount of protein).
[c] 'Protein type' indicates the way in which a higher protein intake was achieved and is categorised into 'pure' protein or amino acids (or essential amino acids) (A), specific food(s) with a high protein content (B), or high-protein diets (C).
[d] Risk of bias assessment: risk of bias was assessed using the RoB 2 tool and scored as 'low' (L), 'some concerns' (SC), or 'high' (H).
[e] The results of the studies are indicated as follows: +, statistically significant beneficial effect (P<0.05); -, statistically significant unfavourable effect (P<0.05); NS, no statistically significant effect (P≥0.05); ?, result unclear. In cases where results were reported for multiple time points, only the result for the final time point is reported.
[f] Protein intake in g/kg BW/d was calculated by using protein intake in g/d and mean body weight.
[g] Actual protein intake may have been different from the prescribed protein intake, due to non-compliance (compliance was 91% on average).
[h] Some studies assessed multiple specific outcomes (i.e. multiple contrasts) for the health outcome 'glucose and insulin metabolism', so one study can show both a significant and a non-significant effect.

3.4.7 Serum lipid profile

An overview of the main characteristics and results of the evaluated RCTs examining the effect of increased protein intake on serum lipid profile in older adults is provided in Table 8. A detailed description of the characteristics and results of these RCTs is provided in Annex E (Table E7).

Overall

The Committee evaluated seven RCTs on the effect of increased protein intake on serum lipid profiles, with a total of 43 statistically tested contrasts. Those studies involved a total of approximately 385[a] participants (IG/CG: 223/162). The smallest study involved 22 participants (IG/CG: 12/10), while the largest study had 80 participants (IG/CG: 40/40). Six studies had an intervention period of 12 weeks, while the other study had an intervention period of 6 months. The habitual protein intake ranged from 0.8 to 1.0 g/kg BW/d and the protein dose ranged from 0.24 to 0.6 g/kg BW/d. The total protein intake in the intervention groups ranged from 1.17 g/kg BW/d (compared to an habitual intake of 0.81 g/kg BW/d) to 1.49 g/kg BW/d (compared to 1.0 g/kg BW/d). The risk of bias in those studies was scored as 'some concerns' (n=4) or 'high' (n=3).

In 2 of the 7 studies (29%), involving a total of 66 participants (IG/CG: 32/34), a beneficial effect of increased protein intake on serum lipid profiles was found for at least one of the statistically tested contrasts (2 of 43 contrasts, 5%). The duration of those studies was 12 weeks. In both studies that found a beneficial effect on lipid profiles, the habitual protein intake was approximately 0.9 g/kg BW/d and the protein dose was 0.5 g/kg BW/d. An unfavourable effect on serum lipid profiles was observed in one study, specifically concerning LDL (low-density lipoprotein) cholesterol (1 of 43 contrasts, 2%). The habitual protein intake in this study was 0.8 g/kg BW/d and the protein dose was 0.6 g/kg BW/d. In the three studies showing an effect (either beneficial or unfavourable), the risk of bias was scored as 'high'. There was little difference between the results obtained by studies on the effect of increased protein intake alone (beneficial effect in 1 of 4 studies (1 of 21 contrasts); unfavourable effect in 1 of 4 studies (1 of 21 contrasts)) and those obtained by studies on the effect of increased protein intake in the context of physical exercise (beneficial effect in 1 of 3 studies (1 of 22 contrasts)).

Preliminary conclusion: Based on the seven evaluated RCTs, the Committee concluded that there is ambiguous evidence regarding the effect of increased protein intake on serum lipid profiles in older adults. The high degree of ambiguity is caused by the opposite directions of the effects found, the wide variety of lipid measures used, and the difference in the percentage of beneficial effects at the level of studies compared to the percentage of beneficial effects at the level of contrasts.

[a] This number represents the participants included in the analyses for this outcome. The sample size may vary slightly, depending on the specific outcome measure used.

Habitual protein intake (reference): ≥0.8 to <0.9 g/kg BW/d
Against a background (habitual) intake of 0.8 to 0.9 g protein/kg BW/d, the Committee evaluated three RCTs on the effect of increased protein intake on serum lipid profiles, with a total of 15 statistically tested contrasts. These studies involved a total of approximately 139 participants (IG/CG: 71/68). The smallest study involved 22 participants (IG/CG: 12/10), while the largest study had 85 participants (IG/CG: 43/42). The protein dose ranged from 0.36 to 0.6 g/kg BW/d. The risk of bias in those studies was scored as 'some concerns' (n=1) or 'high' (n=2).

In 1 of the 3 studies (33%), a beneficial effect of increased protein intake was found for at least one of the statistically tested contrasts (1 of 15 contrasts, 7%). This concerned the total-to-HDL (high-density lipoprotein) cholesterol ratio. In the study that found this beneficial effect, the protein dose was 0.53 g/kg BW/d. One other study found an unfavourable effect of increased protein intake on serum lipid profiles (1 of 15 contrasts, 7%), specifically concerning LDL cholesterol. This study used a protein dose of 0.6 g/kg BW/d.

Habitual protein intake (reference): ≥0.9 to <1.0 g/kg BW/d
Against a background intake of 0.9 to 1.0 g protein/kg BW/d, the Committee evaluated two RCTs on the effect of increased protein intake on serum lipid profiles, with a total of 12 statistically tested contrasts. Those studies involved a total of approximately 154 participants (IG/CG: 96/58). The smallest study involved 34 participants (IG/CG: 16/18), while the largest study had 80 participants (IG/CG: 40/40). The protein dose ranged from 0.28 to 0.5 g/kg BW/d. The risk of bias in those studies was scored as 'some concerns' (n=1) or 'high' (n=1).

In 1 of the 2 studies (50%), a beneficial effect was found for at least one of the statistically tested contrasts (1 of 12 contrasts, 8%), specifically concerning triglycerides. In the study that found this beneficial effect, the protein dose was 0.5 g/kg BW/d. Increased protein intake, within this domain of habitual protein intake, was not found to have any unfavourable effects on serum lipid profile.

Habitual protein intake (reference): ≥1.0 to <1.1 g/kg BW/d
Against a background intake of 1.0 to 1.1 g protein/kg BW/d, the Committee evaluated two RCTs on the effect of increased protein intake on serum lipid profiles, with a total of 16 statistically tested contrasts. These studies involved a total of approximately 92 participants (IG/CG: 56/36). The smallest study involved 26 participants (IG/CG: 13/13), while the largest study had 44 participants (IG/CG: 21/23). The protein dose ranged from 0.24 to 0.49 g/kg BW/d. The risk of bias in those studies was scored as 'some concerns'. Those studies found no effects of increased protein intake on serum lipid profile.

Final conclusion regarding serum lipid profile:

Based on the seven evaluated RCTs, the Committee concluded that there is **ambiguous evidence** regarding the effect of increased protein intake on serum lipid profiles in older adults. The ambiguity is caused by opposite directions of the observed effects of protein intake, the variety in lipid measures, and the substantial difference in the proportion of beneficial effects based on the number of studies and the proportion of beneficial effects based on the number of contrasts.

Furthermore, the Committee concluded that the results do not suggest that the effect on serum lipid profiles of increased protein intake alone (not in the context of physical exercise) differs from the effect of increased protein intake in the context of concomitant physical exercise. Beneficial effects of increased protein intake on serum lipid profiles were observed in participants with an habitual protein intake up to and including 0.9 g/kg BW/d and for a total protein intake up to and including 1.4 g/kg BW/d. The Committee found no indications of a dose-response relationship.

Table 8. Overview of the results of the seven evaluated randomised controlled trials on the effect of increased protein intake on serum lipid profile in older adults, categorised according to habitual protein intake and ordered by protein dose

Study	Analytic *n* IG/CG	Total protein intake (g/kg BW/d) during intervention[a]	Protein dose[b] (g/kg BW/d)	Protein type[c]	With/without physical exercise	Risk of Bias[d]	Outcome	Result[e]				Comments
								+	NS	-	?	
Habitual protein intake (reference): ≥0.8 to <0.9 g/kg BW/d												
Bhasin et al. 2018[51]	40-46[£]/ 38-46[£]	IG: 1.17 ± 0.13; CG: 0.81 ± 0.10	0.36	A,B	NoEx	SC	Total cholesterol		✔			
							LDL cholesterol		✔			
							HDL cholesterol		✔			
							Triglycerides		✔*			* P=0.055 (triglyceride levels tended to decrease more in IG than in CG)
Fernandes et al. 2018[40] Same study as [46]	16/16	IG: 1.4 ± 0.1; CG: 0.87 ± 0.1	0.53	A	Ex	H	Total cholesterol		✔			
							LDL cholesterol		✔			
							HDL cholesterol		✔			
							Triglycerides		✔			
							Total/HDL cholesterol ratio	✔				
							LDL/HDL cholesterol ratio		✔			
Wright et al. 2018[47]	12/10	IG: 1.4; CG: 0.8 (prescribed)[f,g]	0.6[f]	C	NoEx	H	Total cholesterol		✔			
							LDL cholesterol			✔		
							HDL cholesterol		✔			
							Triglycerides		✔			
							Total/HDL cholesterol ratio		✔			
Subtotal (contrasts)								*1*	*13*	*1*	*0*	*Beneficial effect observed for 1 of 15 contrasts (1 of 3 studies)*
Subtotal (studies)[h]								*1*	*3*	*1*	*0*	*Unfavourable effect observed for 1 of 15 contrasts (1 of 3 studies)*

Study	Analytic *n* IG/CG	Total protein intake (g/kg BW/d) during intervention[a]	Protein dose[b] (g/kg BW/d)	Protein type[c]	With/without physical exercise	Risk of Bias[d]	Outcome	Result[e]				Comments
								+	NS	-	?	
Habitual protein intake (reference): ≥0.9 to <1.0 g/kg BW/d												
Park et al. 2018[52]	40/40	IG1: 1.18 ± 0.23; CG: 0.90 ± 0.38	0.28	A	NoEx	SC	Total cholesterol		✔			
							LDL cholesterol		✔			
							HDL cholesterol		✔			
							Triglycerides		✔			
	40/40	IG2: 1.37 ± 0.26; CG: 0.90 ± 0.38	0.47				Total cholesterol		✔			
							LDL cholesterol		✔			
							HDL cholesterol		✔			
							Triglycerides		✔			
Ottestad et al. 2017[55]	16-17[†]/18	IG: 1.4 ± 0.5; CG: 0.9 ± 0.4	0.5	B	NoEx	H	Total cholesterol		✔ *			* P=0.06 (total cholesterol tended to decrease more in IG than in CG)
							LDL cholesterol		✔			
							HDL cholesterol		✔			
							Triglycerides	✔				
Subtotal (contrasts)								*1*	*11*	*0*	*0*	*Beneficial effect observed for 1 of 12 contrasts*
Subtotal (studies)[h]								*1*	*2*	*0*	*0*	*(1 of 2 studies)*
Habitual protein intake (reference): ≥1.0 to <1.1 g/kg BW/d												
Nabuco et al. 2019c[42]	13/13	IG: 1.0 ± 0.23 (without ~35 g whey protein supplementation on 3 d/wk); CG: 1.0 ± 0.19	0.24[f]	A	Ex	SC	Total cholesterol		✔			
							LDL cholesterol		✔			
							HDL cholesterol		✔			
							Triglycerides		✔			

Study	Analytic *n* IG/CG	Total protein intake (g/kg BW/d) during intervention[a]	Protein dose[b] (g/kg BW/d)	Protein type[c]	With/without physical exercise	Risk of Bias[d]	Outcome	Result[e]				Comments
								+	NS	-	?	
Nabuco et al. 2019a[44] Same study as [43,45]	22/23	IG1: 1.38 ± 0.26; CG: 1.0 ± 0.25	0.38	A	Ex	SC	Total cholesterol		✔			
							LDL cholesterol		✔			
							HDL cholesterol		✔			
							Triglycerides		✔			
							Total/HDL cholesterol ratio		✔			* P=0.081 (Total/HDL cholesterol ratio tended to increase more in IG1 than in CG)
							LDL/HDL cholesterol ratio		✔			
	21/23	IG2: 1.49 ± 0.46; CG: 1.0 ± 0.25	0.49				Total cholesterol		✔			
							LDL cholesterol		✔			
							HDL cholesterol		✔			
							Triglycerides		✔			
							Total/HDL cholesterol ratio		✔			
							LDL/HDL cholesterol ratio		✔			
Subtotal (contrasts) *Subtotal (studies)*[h]								*0* *0*	*16* *2*	*0* *0*	*0* *0*	*No effect observed for any of 16 contrasts (2 studies)*
Total (contrasts) Total (studies)[h]								2 2	40 7	1 1	0 0	Beneficial effect observed for 2 of 43 contrasts (2 of 7 studies) Unfavourable effect observed for 1 of 43 contrasts (1 of 7 studies)

Abbreviations: BW: body weight, CG: control group, Ex: with concomitant physical exercise intervention, H: high risk of bias, HDL: high-density lipoprotein, IG: intervention group, L: low risk of bias, LDL: low-density lipoprotein, NoEx: without concomitant physical exercise intervention, NR: not reported, NS: not significant, SC: some concerns (regarding risk of bias).

Footnotes:

£ The exact number of participants included in the analyses is not reported. The number must be between the number of participants who were randomised and the number of participants who completed the study.

† Depending on the specific outcome measure.

[a] Total protein intake during follow-up. If protein intake was assessed at multiple time points, the intake assessed at the final time point was considered.

[b] 'Protein dose' indicates the difference in achieved total protein intake between the intervention and control groups during follow-up (which is not necessarily equal to supplemented/prescribed amount of protein).

[c] 'Protein type' indicates the way in which a higher protein intake was achieved and is categorised into 'pure' protein or amino acids (or essential amino acids) (A), specific food(s) with a high protein content (B), or high-protein diets (C).

[d] Risk of bias assessment: risk of bias was assessed using the RoB 2 tool and scored as 'low' (L), 'some concerns' (SC), or 'high' (H).

[e] The results of the studies are indicated as follows: +, statistically significant beneficial effect (P<0.05); -, statistically significant unfavourable effect (P<0.05); NS, no statistically significant effect (P≥0.05); ?, result unclear. In cases where results were reported for multiple time points, only the result for the final time point is reported.

[f] Protein intake in g/kg BW/d was calculated by using protein intake in g/d and mean body weight.

[g] Actual protein intake may have been different from the prescribed protein intake, due to non-compliance (compliance was 91% on average).

[h] Some studies assessed multiple specific outcomes (i.e. multiple contrasts) for the health outcome 'serum lipid profile', so one study can show both a significant and a non-significant effect.

3.4.8 Kidney function

An overview of the main characteristics and results of the evaluated RCTs examining the effect of increased protein intake on kidney function in older adults is provided in Table 9. A detailed description of the characteristics and results of these RCTs is provided in Annex E (Table E8).

Overall

The Committee evaluated six RCTs on the effect of increased protein intake on kidney function, with a total of 12 statistically tested contrasts. These studies involved a total of approximately 710 participants[a].

The smallest study involved 35 participants, while the largest study had 237 participants. Most studies (n=4) had an intervention period of 12 weeks (range: 12 weeks to 18 months). The habitual protein intake ranged from 0.81 to 1.05 g/kg BW/d and the protein dose ranged from 0.17 to 0.5 g/kg BW/d. The total protein intake in the intervention groups ranged from 1.06 g/kg BW/d (compared to an habitual intake of 0.89 g/kg BW/d) to 1.4 g/kg BW/d (compared to 0.9 g/kg BW/d). The risk of bias in these studies was scored as 'some concerns' (n=4) or 'high' (n=2).

In 1 of the 6 studies (17%), involving a total of 35 participants (IG/CG: 17/18), a beneficial effect of increased protein intake on kidney function was found for at least one of the statistically tested contrasts (1 of 12 contrasts, 8%). The duration of this study was 12 weeks. In the study that found this beneficial effect, the habitual protein intake was 0.9 g/kg BW/d and the protein dose was 0.5 g/kg BW/d. The risk of bias in this study was scored as 'high'. Increased protein intake was not found to have any unfavourable effects on kidney function. There was little difference between the results obtained by studies on the effect of increased protein intake alone (beneficial effect in 1 of 4 studies (1 of 8 contrasts)) and those obtained by studies on the effect of increased protein intake in the context of physical exercise (no effect in 2 studies).

The Committee noted that high-risk groups for deteriorating kidney function (e.g. those with diabetes, hypertension, heart problems or pre-existing impaired kidney function) are often excluded from studies. Protein may have a different effect in older adults with poor kidney function or with pre-existing risk factors for kidney problems compared to those with good kidney function, but this could not be evaluated based on the existing literature.

The Committee also noted important limitations when using serum creatinine or estimated glomerular filtration rate (eGFR) based on serum creatinine as indicator of kidney function in the context of protein intervention studies. First, an increase in protein intake is accompanied by an increase in serum creatinine levels, even without the true GFR changing. The eGFR, however, will decrease as it is based on the (higher)

[a] This number represents the participants included in the analyses for this outcome. The sample size may vary slightly, depending on the specific outcome measure used.

serum creatinine level.[61] This would falsely suggest an unfavorable effect of increased protein intake on kidney function. Second, it is presumed that long-term high protein intake might lead to renal hyperfiltration, which, in certain circumstances, might have unfavorable effects on the long-term.[62] However, the presence of renal hyperfiltration would probably not be demonstrated by serum creatinine or the creatinine-based eGFR as the first-mentioned mechanism may neutralize this effect. Thus, based on the current data, an effect on renal hyperfiltration cannot be ruled out. Lastly, low protein intake may result in a reduction in lean body (muscle) mass in older adults, and low muscle mass may lead to lower serum creatinine levels,[63] translating into a higher eGFR (again even without the true GFR changing). Altogether, the Committee judged that serum creatinine and the creatinine-based eGFR are inappropriate measures to determine the effect of protein intake. Therefore, data are insufficient to exclude an adverse effect of a long-term increase of protein intake on GFR in older adults.

Preliminary conclusion: Based on the six evaluated RCTs, the Committee concluded that there are too few studies (with appropriate outcome measures) to determine whether or not increased protein intake affects kidney function in older adults.

Because an effect of increased protein intake was found for only one contrast, the results were not further subdivided by domain of habitual protein intake.

Final conclusion regarding kidney function:

Based on the six evaluated RCTs, the Committee concluded that there are **too few studies (with appropriate outcome measures)** to determine whether or not increased protein intake affects kidney function in older adults.

Table 9. Overview of the results of the six evaluated randomised controlled trials on the effect of increased protein intake on kidney function in older adults, categorised according to habitual protein intake and ordered by protein dose

Study	Analytic *n* IG/CG	Total protein intake (g/kg BW/d) during intervention[a]	Protein dose[b] (g/kg BW/d)	Protein type[c]	With/without physical exercise	Risk of Bias[d]	Outcome	Result[e]				Comments
								+	NS	-	?	
Habitual protein intake (reference): ≥0.8 to <0.9 g/kg BW/d												
Ramel et al. 2013[50] Same study as [49]	237 (total)	IG: 1.06 ± 0.23; CG 0.89 ± 0.23	0.17	A	Ex	H	eGFR		✔			
Bhasin et al. 2018[51]	40-46[£]/ 38-46[£]	IG: 1.17 ± 0.13; CG: 0.81 ± 0.10	0.36	A,B	NoEx	SC	Serum creatinine		✔			
Subtotal (contrasts)								*0*	*2*	*0*	*0*	*No effect observed for any of 2 contrasts*
Subtotal (studies)[f]								*0*	*2*	*0*	*0*	*(2 studies)*
Habitual protein intake (reference): ≥0.9 to <1.0 g/kg BW/d												
Ten Haaf et al. 2019[53]	109-114[†] (total)	IG: 0.92 ± 0.27 (without protein supplementation of 31 g/d); CG: 0.97 ± 0.23	0.36[g]	A	Ex	SC	Serum creatinine		✔			
							eGFR		✔			
							Albumin/ creatinine ratio		✔			
Park et al. 2018[52]	40/40	IG1: 1.18 ± 0.23; CG: 0.90 ± 0.38	0.28	A	NoEx	SC	Serum creatinine		✔			
							eGFR		✔			
	40/40	IG2: 1.37 ± 0.26; CG: 0.90 ± 0.38	0.47				Serum creatinine		✔			
							eGFR		✔			
Ottestad et al. 2017[55]	17/18	IG: 1.4 ± 0.5; CG: 0.9 ± 0.4	0.5	B	NoEx	H	Serum creatinine	✔				
							eGFR		✔ *			* P=0.09 (eGFR tended to decrease less in IG than in CG)
Subtotal (contrasts)								*1*	*8*	*0*	*0*	*Beneficial effect observed for 1 of 9 contrasts*
Subtotal (studies)[f]								*1*	*3*	*0*	*0*	*(1 of 3 studies)*
Habitual protein intake (reference): ≥1.0 to <1.1 g/kg BW/d												
Kerstetter et al. 2015[56]	61/60 (18-mo follow-up)	IG: 1.30 ± 0.05; CG: 1.05 ± 0.04	0.25	A	NoEx	SC	eGFR		✔ *			* No difference at 18 months, but at 9 months eGFR increased more in IG than in CG (P=0.006)
Subtotal (contrasts)								*0*	*1*	*0*	*0*	*No effect observed for the 1 contrast (1 study)*
Subtotal (studies)[f]								*0*	*1*	*0*	*0*	
Total (contrasts)								1	11	0	0	Beneficial effect observed for 1 of 12 contrasts
Total (studies)[f]								1	6	0	0	(1 of 6 studies)

Abbreviations: BW: body weight, CG: control group, eGFR: estimated glomerular filtration rate, Ex: with concomitant physical exercise intervention, H: high risk of bias, IG: intervention group, L: low risk of bias, NoEx: without concomitant physical exercise intervention, NR: not reported, NS: not significant, SC: some concerns (regarding risk of bias).

Footnotes:

£ The exact number of participants included in the analyses is not reported. The number must be between the number of participants who were randomised and the number of participants who completed the study.

† Depending on the specific outcome measure.

§ Sufficient statistical power to detect an effect is to be expected, based on the sample size calculation.

[a] Total protein intake during follow-up. If protein intake was assessed at multiple time points, the intake assessed at the final time point was considered.

[b] 'Protein dose' indicates the difference in achieved total protein intake between the intervention and control groups during follow-up (which is not necessarily equal to supplemented/prescribed amount of protein).

[c] 'Protein type' indicates the way in which a higher protein intake was achieved and is categorised into 'pure' protein or amino acids (or essential amino acids) (A), specific food(s) with a high protein content (B), or high-protein diets (C).

[d] Risk of bias assessment: risk of bias was assessed using the RoB 2 tool and scored as 'low' (L), 'some concerns' (SC), or 'high' (H).

[e] The results of the studies are indicated as follows: +, statistically significant beneficial effect (P<0.05); -, statistically significant unfavourable effect (P<0.05); NS, no statistically significant effect (P≥0.05); ?, result unclear. In cases where results were reported for multiple time points, only the result for the final time point is reported.

[f] Some studies assessed multiple specific outcomes (i.e. multiple contrasts) for the health outcome 'kidney function', so one study can show both a significant and a non-significant effect.

[g] Protein intake in g/kg BW/d was calculated by using protein intake in g/d, mean body weight, and compliance.

3.4.9 Cognition

An overview of the main characteristics and results of the evaluated RCT examining the effect of increased protein intake on cognition in older adults is provided in Table 10. A detailed description of the characteristics and results of this RCT is provided in Annex E (Table E9).

Final conclusion regarding cognition:

Based on one evaluated RCT, the Committee concluded that there are **too few studies** to determine whether or not increased protein intake affects cognition in older adults.

Overall

The Committee evaluated one RCT on the effect of increased protein intake on cognition (MMSE), with a total of 2 statistically tested contrasts. This study involved a total of 120 participants (IG1/IG2/CG: 40/40/40). The intervention period was 12 weeks. The habitual protein intake was 0.9 g/kg BW/d and the protein dose ranged from 0.28 (IG1) to 0.47 (IG2) g/kg BW/d. The risk of bias in this study was scored as ‘some concerns’.

This study found no effect of increased protein intake on cognition. This health outcome only involved one study, which precluded any comparison of the results of studies with and without a concomitant physical exercise intervention.

Preliminary conclusion: There are too few studies to draw any conclusions about the effect of increased protein intake on cognition in older adults.

Because there is only one study available for this health outcome, the results were not further subdivided by domain of habitual protein intake.

Table 10. Overview of the results of the evaluated randomised controlled trial on the effect of increased protein intake on cognition in older adults, categorised according to habitual protein intake and ordered by protein dose

Study	Analytic *n* IG/CG	Total protein intake (g/kg BW/d) during intervention[a]	Protein dose[b] (g/kg BW/d)	Protein type[c]	With/without physical exercise	Risk of Bias[d]	Outcome	Result[e]				Comments
								+	NS	-	?	
Habitual protein intake (reference): ≥0.9 to <1.0 g/kg BW/d												
Park et al. 2018[52]	40/40	IG1: 1.18 ± 0.23; CG: 0.90 ± 0.38	0.28	A	NoEx	SC	Korean MMSE		✔			
	40/40	IG2: 1.37 ± 0.26; CG: 0.90 ± 0.38	0.47				Korean MMSE		✔			
Total (contrasts)								0	2	0	0	No effect observed for any of 2 contrasts (1 study)
Total (studies)[f]								0	1	0	0	

Abbreviations: BW: body weight, CG: control group, IG: intervention group, MMSE: Mini-Mental State Examination, NoEx: without concomitant physical exercise intervention, NS: not significant, SC: some concerns (regarding risk of bias).
Footnotes:

§ Sufficient statistical power to detect an effect is to be expected, based on the sample size calculation.

[a] Total protein intake during follow-up. If protein intake was assessed at multiple time points, the intake assessed at the final time point was considered.

[b] 'Protein dose' indicates the difference in achieved total protein intake between the intervention and control groups during follow-up (which is not necessarily equal to supplemented/prescribed amount of protein).

[c] 'Protein type' indicates the way in which a higher protein intake was achieved and is categorised into 'pure' protein or amino acids (or essential amino acids) (A), specific food(s) with a high protein content (B), or high-protein diets (C).

[d] Risk of bias assessment: risk of bias was assessed using the RoB 2 tool and scored as 'low' (L), 'some concerns' (SC), or 'high' (H).

[e] The results of the studies are indicated as follows: +, statistically significant beneficial effect (P<0.05); -, statistically significant unfavourable effect (P<0.05); NS, no statistically significant effect (P≥0.05); ?, result unclear. In cases where results were reported for multiple time points, only the result for the final time point is reported.

[f] Some studies assessed multiple specific outcomes (i.e. multiple contrasts) for the health outcome 'cognition', so one study can show both a significant and a non-significant effect.

3.4.10 Overall conclusion

The Committee included a total of 18 RCTs (from 24 publications) in its evaluation of the scientific evidence concerning the effect of increased protein intake on nine different health outcomes in older adults. The Committee considered it possible that increased protein intake (up until 1.0-1.1 g/kg BW/d), with isocaloric replacement (usually for carbohydrates), has a beneficial effect on lean body mass in older adults, which does not involve any change in body weight. The Committee also considered it possible that increased protein intake with concomitant physical exercise (resistance exercise training) has a beneficial effect on muscle strength compared to physical exercise alone. Increased protein intake alone (not in the context of physical exercise) has likely no effect on muscle strength. The Committee also considered it likely that increased protein intake has no effect on physical function and bone health. It is unclear whether increased protein intake has any effect on serum lipid profiles, and whether such an effect would be beneficial or unfavourable. Too few studies (with sufficient statistical power) are available to draw any conclusions about the effect of increased protein intake on blood pressure, glucose and insulin metabolism, and cognition in older adults. Also, too few studies (with appropriate outcome measures) are available to draw any conclusions about the effect of increased protein intake on kidney function. Overall, the results do not suggest that the effect of increased protein intake on the evaluated outcome measures (aside from muscle strength) would differ between older adults who engage in (concomitant) physical exercise and those who are not physically active. There is no evidence of a dose-response relationship.

The risk of bias was scored as ‘some concerns’ in one half of the studies and as ‘high’ in the other half. There seem to be no indications that studies with a high risk of bias show an effect more often (or less often) than other studies. Accordingly, the Committee saw no reason to believe that the degree of risk of bias affects the conclusions drawn. The lack of information concerning the power calculations in more than a third of the selected studies makes it difficult to determine the extent to which statistical power influenced the results obtained. The Committee had, therefore, adopted a prudent approach and has moderated some conclusions.

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annexes

A search strategy for literature search in PubMed and Scopus

Search for systematic reviews

Search strategy used by the Health Council's Committee on Nutrition to identify systematic reviews of randomised controlled trials and prospective cohort studies on the relationship between protein intake and health outcomes in older adults. The literature search was carried out in PubMed on 23 April 2020.

PubMed

1. Exposure
   (Dietary Proteins[MeSH] OR dietary protein*[tiab] OR protein intake[tiab] OR protein supplement*[tiab] OR nutritional protein*[tiab] OR dietary protein*[tiab] OR Amino Acids, Essential[MeSH] OR amino acid* OR casein[tiab] OR whey*[tiab] OR nutritional requirements[MeSH] OR nutritional requirement*[tiab] OR milk protein* OR (protein*[tiab] AND supplement*[tiab]))
2. Population
   (Aged[MeSH] OR aged[tiab] OR Middle Aged[MeSH] OR middle aged[tiab] OR elderly [tiab] OR old* people [tiab] OR old* person*[tiab] OR old* adult*[tiab] OR old* population*[tiab] OR healthy older adults[tiab])
3. Design
   a. (Systematic review [publication type] OR "Systematic Reviews as Topic"[Mesh] OR systematic review [tiab])
   b. (meta-analysis [publication type] OR "Meta-Analysis as Topic"[Mesh] OR meta-analysis [tiab])
   c. individual participant data [tiab]

Search string: 1 AND 2 AND (3a OR 3b OR 3c)
No filters applied.

Search for recent individual randomised controlled trials

Search strategy used by the Health Council's Committee on Nutrition to identify recent individual randomised controlled trials on the effect of increased protein intake on health outcomes in older adults. The literature search was carried out in PubMed and Scopus on 23 April 2020.

PubMed

1. Exposure
   (Dietary Proteins[MeSH] OR dietary protein*[tiab] OR protein intake[tiab] OR protein supplement*[tiab] OR nutritional protein*[tiab] OR dietary protein*[tiab] OR Amino Acids, Essential[MeSH] OR amino acid* OR casein[tiab] OR whey*[tiab] OR nutritional requirements[MeSH] OR nutritional requirement*[tiab] OR milk protein* OR (protein*[tiab] AND supplement*[tiab]))

2. Population
   (Aged[MeSH] OR aged[tiab] OR Middle Aged[MeSH] OR middle aged[tiab] OR elderly [tiab] OR old* people [tiab] OR old* person*[tiab] OR old* adult*[tiab] OR old* population*[tiab] OR healthy older adults[tiab])
3. Design
   (clinical trial [publication type] OR “Clinical Trials as Topic”[Mesh] OR clinical trial [tiab])

Search string: 1 AND 2 AND 3
Limit: 2018, 2019, 2020

Scopus

1. Exposure
   (TITLE-ABS-KEY(Dietary-Protein*) OR TITLE-ABS-KEY(protein-intake) OR TITLE-ABS-KEY(protein-supplement*) OR TITLE-ABS-KEY(protein-source*) OR TITLE-ABS-KEY(source-of-protein) OR TITLE-ABS-KEY(nutritional-protein*) OR TITLE-ABS-KEY(dietary-protein*) OR TITLE-ABS-KEY(Amino-Acids*) OR TITLE-ABS-KEY(casein) OR TITLE-ABS-KEY(whey*) OR TITLE-ABS-KEY(Milk proteins) OR TITLE-ABS-KEY(nutritional- requirement*) OR TITLE-ABS-KEY(Protein) AND TITLE-ABS-KEY(supplement))
2. Population
   (TITLE-ABS-KEY(aged) OR TITLE-ABS-KEY(elderly) OR TITLE-ABS-KEY(old*-people) OR TITLE-ABS-KEY(old*-person*) OR TITLE-ABS-KEY(old*-population*) OR TITLE-ABS-KEY (old*-adult*) OR TITLE-ABS-KEY(healthy-older-adults) OR TITLE-ABS-KEY(middle aged))
3. Design
   TITLE-ABS-KEY(clinical-trial)

Search string: 1 AND 2 AND 3
Limit: 2018, 2019, 2020

B inclusion and exclusion criteria for selection of studies

Inclusion criteria

1. Randomised controlled trials;
2. Minimal intervention duration of 4 weeks;
3. Sampling age of ≥50 and(/or) a mean age of ≥65;
4. Community-dwelling older adults or older adults living in a nursing home or care home.

Exclusion criteria

1. Study population comprising people with a specific (chronic) disease or condition, such as cancer, diabetes, chronic obstructive pulmonary disease, heart failure, polymyalgia rheumatic, or osteoporosis;
2. Hospitalised patients;
3. Immobilised patients;
4. Studies conducted directly prior or subsequent to surgery or hospitalisation (e.g. pre- or postoperative studies or studies after hospital discharge);
5. Exposure is individual amino acids (e.g. leucine), combinations of only two or three amino acids (e.g. branched-chain amino acids (BCAAs[a])), or dipeptides (e.g. beta-alanine or carnosine);
6. Exposure is not protein, amino acids or protein-(en)rich(ed) food products (e.g. creatine, dehydro-epiandrosteron (DHEA), beta-hydroxy-beta-methylbutyrate (HMB), and milk fat globule membrane (MFGM) are excluded);
7. Outcome is muscle protein synthesis;
8. No control arm;
9. No isocaloric control intervention;
10. The intervention comprises more than just protein (*intentionally*), i.e. the intervention group and control group differ in more ways than the amount of protein involved[b];
11. The intervention is part of a weight loss programme (e.g. energy-restricted diet).

[a] The branched-chain amino acids are three essential amino acids: leucine, isoleucine, and valine.

[b] The Committee distinguished between a ‘hard’ and a ‘soft’ contrast. An example of a hard contrast is when the intervention group is given a protein supplement as well as a vitamin D supplement, whereas the control group is given neither. The researchers’ *intention* was to investigate the effect of the combination of protein and vitamin D. An example of a soft contrast is when the intervention group is given a food-based protein supplement (e.g. milk) to investigate the effect of higher protein intake, as this also causes the group to consume (albeit unintentionally) other nutrients that are present in the supplement/provided food.

C the RoB 2 Cochrane collaboration tool to assess risk of bias

The RoB 2 Cochrane collaboration tool[10] was used to assess risk of bias. Table C1 summarises the bias domains and issues addressed in the RoB 2 tool. The Committee retrieved additional detailed information from the guidance document[64] and the handbook[65] for scoring the individual studies.

Table C1. Bias domains and issues addressed in the RoB 2 Cochrane collaboration tool[10,65]

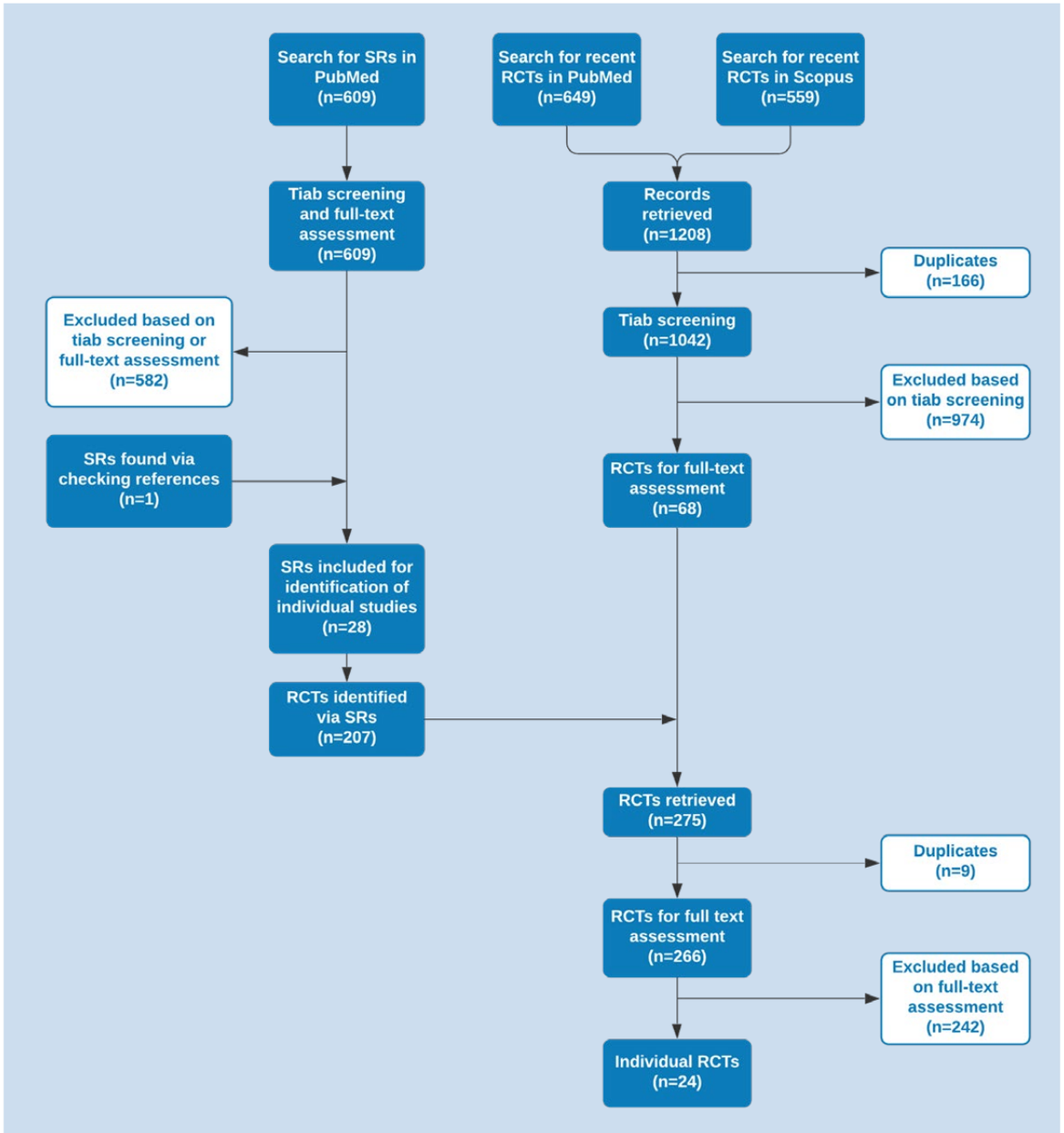
Bias domains		Issues addressed
1.	Bias arising from the randomisation process	Whether: • the allocation sequence was random; • the allocation sequence was adequately concealed; • baseline differences between intervention groups suggest a problem with the randomisation process.
2.	Bias due to deviations from intended interventions	Whether: • the participants were aware of their assigned intervention during the trial; • the carers and people delivering the interventions were aware of the participants' assigned intervention during the trial. *When the review authors' interest is in the effect of assignment to intervention:* • (if applicable) deviations from the intended intervention arose because of the experimental context (i.e. they do not reflect usual practice); and, if so, whether they were unbalanced between groups and likely to have affected the outcome; • an appropriate analysis was used to estimate the effect of assignment to intervention; and, if no, whether this could potentially have had a substantial impact on the result. *When the review authors' interest is in the effect of adhering to intervention:* • (if applicable) important non-protocol interventions were balanced across intervention groups; • (if applicable) failures in implementing the intervention could have affected the outcome; • (if applicable) study participants adhered to the assigned intervention regimen; • (if applicable) an appropriate analysis was used to estimate the effect of adhering to the intervention.
3.	Bias due to missing outcome data	Whether: • data for this outcome were available for all, or nearly all, of the participants randomised; • (if applicable) there was evidence that the result was not biased by missing outcome data; • (if applicable) missingness in the outcome was likely to depend on its true value (e.g. the proportions of missing outcome data, or the reasons for missing outcome data differ between intervention groups).
4.	Bias in measurement of the outcome	Whether: • the method of measuring the outcome was inappropriate; • measurement or ascertainment of the outcome could have differed between intervention groups; • outcome assessors were aware of the intervention received by study participants; • (if applicable) assessment of the outcome was likely to have been influenced by knowledge of the intervention received.
5.	Bias in selection of the reported result	Whether: • the trial was analysed in accordance with a pre-specified plan that was finalised before unblinded outcome data were available for analysis; • the numerical result being assessed is likely to have been selected, on the basis of the results, from multiple outcome measurements within the outcome domain; • the numerical result being assessed is likely to have been selected, on the basis of the results, from multiple analyses of the data.

Overall judgement

It is recommended that the following rules be used to reach an overall risk of bias judgement for a specific outcome:[10]

- Low risk (+). The study is judged to be at low risk of bias for all domains for this result.
- Some concerns (-). The study is judged to raise some concerns in at least one domain, for this result, but not to be at high risk of bias for any domain.
- High risk (x). The study is judged to be at high risk of bias in at least one domain for this result, OR The study is judged to have some concerns for multiple domains.

D flow diagram of study selection



RCT: randomised controlled trial, SR: systematic review, tiab: title and abstract

E overview of characteristics and results of included studies

Tables E1 to E9 provide the characteristics and results of RCTs examining the effect of increased protein intake on health outcomes in older adults. The studies are grouped according to whether or not the protein intervention was carried out in the context of a physical exercise intervention.

Table E1. Results from randomised controlled trials on the effect of increased protein intake on lean body mass in older adults, grouped according to whether or not the protein intervention was carried out in the context of (concomitant) physical exercise

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Not in the context of (concomitant) physical exercise										
Bhasin et al. 2018,[51] USA	Community-dwelling older men aged ≥65 with moderate physical function limitations and with habitual protein intake ≤0.83 g/kg BW/d; mean BMI: 30.3 ± 4.9 kg/m^2	Individualised diets providing 0.7 g protein/kg BW/d with additional discretionary foods (0.1 g protein/kg BW) and protein supplements (0.5 g/kg BW) to achieve a total of 1.3 g/kg BW/d, [A,B]	Individualised diets providing 0.7 g protein/kg BW/d with additional discretionary foods (0.1 g protein/kg BW) and placebo supplements (0.5 g CHO/kg BW) to achieve a total of 0.8 g/kg BW/d	Foods (4-6 mo): IG: 77.1 ± 13%, CG: 74.5 ± 23.2% Supplements (4-6 mo): IG: 91.2 ± 12.4%, CG: 92.6 ± 11.0%	Baseline: IG: 0.72 ± 0.11, CG: 0.69 ± 0.15 Follow-up (1-3 mo): IG: 1.18 ± 0.15, CG: 0.84 ± 0.07 Follow-up (4-6 mo): IG: 1.17 ± 0.13, CG: 0.81 ± 0.10	6 mo	Some concerns	Total LBM[§] (kg; DXA)	42/39 (mITT)	MD (95%-CI): +0.31 (-0.46 to +1.08) P=0.43 For relative total LBM (% of BW), MD (95%-CI): +0.81 (+0.05 to +1.58), P=0.04. Mainly a result of the significantly greater decrease in total fat mass (kg) in IG than in CG (P=0.02).
								Trunk LBM[§] (kg; DXA)	42/39 (mITT)	MD (95%-CI): +0.24 (-0.17 to +0.66) P=0.24
								aLBM[§] (kg; DXA)	42/39 (mITT)	MD (95%-CI): +0.04 (-0.48 to +0.55) P=0.89
Dillon et al. 2009,[37] USA	Community-dwelling older women; without vascular disease, hypertension or cardiac abnormality	EAA. 7.5 g EAA, ingested twice a day in between meals (total: 15 g EAA/d), [A]	CHO placebo. Isocaloric amount of lactose, ingested twice a day in between meals	NR	Baseline: NR Follow-up: NR	3 mo	High	Total LBM (kg; DXA)	7/7	Authors reported no results for time*group interaction (ANOVA), which suggests that protein has no effect
Ispoglou et al. 2016,[41] UK	Community-dwelling older men and women aged 65-75; good health, without major chronic diseases (e.g. diabetes, vascular disease, hypertension)	EAA. Standard EAA mixture with 20% leucine (IG1) and with 40% leucine (IG2), ingested at breakfast and dinner (total: 0.21 g/kg BW/d = ~11-21 g/d), [A]	CHO placebo. Isocaloric amount of lactose, ingested at breakfast and dinner	74-83%	Baseline: 0.95-1.10 Follow-up: 1.02-1.08 (without supplementation)	12 wk	High	Total LTM (kg; DXA)	8/8/9 (PP)	Mean % change ± SD: IG1: +0.2 ± 2.4 IG2: +1.1 ± 1.1 CG: +0.8 ± 1.3 Time*group interaction NS

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Kerstetter et al. 2015,[56] USA	Older men (aged >70) and women (aged >60) with BMI of 19-32 kg/m^2 and protein intake of 0.6-1.0 g/kg BW; without major chronic diseases (e.g. diabetes, renal disease, inflammatory bowel disease) or cancer within past 18 months	Whey protein. 45 g of whey protein isolate (~40 g of protein) + vitamin D (400 IU) + calcium (1200 mg), [A]	CHO placebo. Isocaloric amount of maltodextrin + vitamin D (400 IU) + calcium (1200 mg)	NR	Baseline: IG: 1.07 ± 0.03, CG: 1.06 ± 0.03 Follow-up: IG: 1.30 ± 0.05, CG: 1.05 ± 0.04	18 mo	Some concerns	Total LBM (kg; DXA)	105/102 (mITT)	LSMD (95%-CI): -0.52 (-1.08 to 0.04) P=0.069 (No significant change in total fat mass (P>0.05))
								Trunk LBM (kg; DXA)	105/102 (mITT)	LSMD (95%-CI): -0.33 (-0.660 to -0.003) **P=0.048** (trunk LBM decreased less in IG than in CG)
Mitchell 2017,[48] New Zealand	Community-dwelling older men aged >70; able to perform ADLs without mobility aids; without major chronic diseases (e.g. cancer, diabetes, thyroid diseases)	High-protein diet (2*RDA = 1.6 g/kg BW/d), omnivorous, 28-31 E% fat, [C]	Low-protein diet (1*RDA = 0.8 g/kg BW/d), omnivorous, 28-31 E% fat; difference made up of CHO	IG: 97.5%, CG: 98.9%	Baseline: IG: 1.1 ± 0.3, CG: 1.2 ± 0.4 Follow-up: IG: 1.7 ± 0.1, CG: 0.9 ± 0.1	10 wk	High	Total LBM[§] (kg; DXA)	14/15 (PP)	Mean change ± SD: IG: +1.49 ± 1.30 CG: -0.55 ± 1.49 **P for time*group interaction=0.001** (No significant change in body weight (P=0.174), but greater decrease in total and % fat mass in IG than in CG (both P<0.01))
								Trunk LBM[§] (kg; DXA)	14/15 (PP)	**P for time*group interaction<0.001**
								aLBM[§] (kg; DXA)	14/15 (PP)	**P for time*group interaction=0.022**
								Muscle CSA, thigh[§] (mm^2; QCT)	14/15 (PP)	Mean change ± SD: IG: -120 ± 292 CG: -539 ± 786 P for time*group interaction=0.112

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Ottestad et al. 2017,[55] Norway	Community-dwelling older adults aged ≥70; relatively healthy (no diabetes, CVD, cancer, COPD, CKD); not malnourished; with reduced muscle strength or performance	Protein-enriched milk. 400 ml drink containing 20 g protein, consumed twice a day (total: 40 g protein/d), [B]	CHO placebo. 400 ml drink containing an isocaloric amount of CHO, consumed twice a day	IG: 97.8 ± 3.8%, CG: 96.8 ± 5.7%	Baseline: IG: 1.0 ± 0.3, CG: 1.0 ± 0.3 Follow-up: IG: 1.4 ± 0.5, CG: 0.9 ± 0.4	12 wk	High	Total LBM§ (kg; DXA)	17/19 (mITT)	Mean change (95%-CI): IG: +0.4 (0.0 to +0.8) CG: +0.4 (0.0 to +0.9) P=0.85
								Trunk LBM§ (kg; DXA)	17/19 (mITT)	Mean change (95%-CI): IG: +0.2 (0.0 to +0.5) CG: 0.0 (-0.3 to +0.4) P=0.33
								aLBM§ (kg; DXA)	17/19 (mITT)	Mean change (95%-CI): IG: +0.1 (-0.1 to +0.4) CG: +0.2 (0.0 to +0.4) P=0.54
Park et al. 2018,[52] Korea	Community-dwelling (pre-)frail older adults aged 70-85 at risk of malnutrition (MNA ≤23.5); no kidney or liver failure; able to walk	Whey protein. Multiple 10-g packs of protein powder (9.3 g whey protein/pack), dissolved in 340 ml tea, were provided in addition to habitual protein intake up to 1.2 g/kg BW/d (IG1) and 1.5 g/kg BW/d (IG2), [A]	CHO placebo. Multiple 10-g packs of CHO powder (9.3 g maltodextrin per/pack), dissolved in 340 ml tea, were provided in addition to habitual protein intake up to 0.8 g/kg (CG) or 1.2 g/kg (IG1) (CG were given only CHO powder, IGs were given a combination of protein and CHO powder.)	IG1: 98%, IG2: 96%, CG: 97%	Baseline: IG1: 0.77 ± 0.24, IG2: 0.80 ± 0.21, CG: 0.84 ± 0.28 Follow-up: IG1: 1.18 ± 0.23, IG2: 1.37 ± 0.26, CG: 0.90 ± 0.38	12 wk	Some concerns	aSMM§ (kg; DXA)	40/40/40 (ITT)	**P for time*group interaction<0.05** (aSMM increased more in IG2, but not IG1, than in CG) (Result was similar for relative aSMM (% of BW): P for time*group interaction<0.05; % aSMM increased more in IG2, but not IG1, than in CG)
								ASMM relative to BW§ (%)	40/40/40 (ITT)	**P for time*group interaction<0.05** (aSMM/BW increased more in IG2, but not IG1, than in CG)
								ASMM relative to squared height§ (kg/m^2)	40/40/40 (ITT)	**P for time*group interaction<0.05** (aSMM/height^2 increased more in IG2, but not IG1, than in CG)
								ASMM relative to BMI§	40/40/40 (ITT)	**P for time*group interaction<0.05** (aSMM/BMI increased more in IG2, but not IG1, than in CG)

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Wright et al. 2018,[47] USA	Older adults aged 50-80 with overweight or obesity (BMI of 25-38 kg/m^2); without diabetes	High-protein diet: 1.4 g/kg BW/d (~27 E% protein, ~43 E% CHO, ~30 E% fat). Majority of additional protein (59%) came from eggs (3 eggs/d), [C]	Normal-protein diet: 0.8 g/kg BW/d (~15 E% protein, ~55 E% CHO, ~30 E% fat) (Normal-protein diet provided on average ~50 g/d less protein than high-protein diet.)	91% (overall)	Baseline: IG: 84 ± 15 g/d, CG: 79 ± 15 g/d (calculated by using mean BW: IG: 0.93 g/kg BW/d, CG: 0.88 g/kg BW/d Follow-up: NR	12 wk	High	Total LBM (kg; DXA)	12/10 (PP)	Mean change ± SD: IG: -0.28 ± 0.97 CG: -1.29 ± 0.97 **P for time*group interaction=0.05** (No significant change in total fat mass (P>0.05))
								Trunk LBM (kg; DXA)	12/10 (PP)	Mean change ± SD: IG: +0.12 ± 0.63 CG: -0.68 ± 0.72 **P for time*group interaction=0.015**
								aLBM (kg; DXA)	12/10 (PP)	Mean change ± SD: IG: -0.39 ± 0.71 CG: -0.58 ± 0.66 Time*group interaction NS
								Muscle CSA, thigh (mm^2; MRI)	18 (total; PP)	Mean change ± SD (*10^4): IG: +9.3 ± 114.0 CG: -29.2 ± 65.1 Time*group interaction NS
								Muscle volume, thigh (mm^3; MRI)	18 (total; PP)	Mean change ± SD (*10^4): IG: -16.2 ± 60.5 CG: -7.4 ± 28.3 Time*group interaction NS
								Muscle CSA, calf (mm^2; MRI)	15 (total; PP)	Mean change ± SD (*10^4): IG: -1.9 ± 4.8 CG: +2.0 ± 15.5 Time*group interaction NS
								Muscle volume, calf (mm^3; MRI)	15 (total; PP)	Mean change ± SD (*10^4): IG: -0.5 ± 3.0 CG: +1.2 ± 6.9 Time*group interaction NS

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Zhu et al. 2015,[58] Australia Same study as Hodgson et al. 2012[59] and Zhu et al. 2011[60]	Community-dwelling older women aged 70-80 with habitual protein intake <1.5 g/kg BW/d; without metabolic bone disease, osteoporotic fracture, diabetes, hepatic or renal insufficiency	Whey protein isolate. 250 ml skim milk-based high-protein supplement drink containing 30 g of whey protein + calcium, [A]	CHO placebo. 250 ml skim milk-based supplement drink containing 2.1 g of protein and isocaloric amount of maltodextrin + calcium	IG: 87.1%, CG: 80.8 % (P=0.03)	Baseline: IG: 1.2 ± 0.3, CG: 1.1 ± 0.3 Follow-up (1 y): IG: 1.4 ± 0.4, CG: 1.1 ± 0.3 Follow-up (2 y): IG: 1.4 ± 0.4, CG: 1.1 ± 0.4	1 y and 2 y	Some concerns	Arm LBM§ (kg; DXA)	1 y: 101/95 (mITT)	Mean change ± SEM: IG: +0.02 ± 0.02 CG: +0.09 ± 0.02 Time*group interaction NS
									2 y: 93/88 (mITT)	Mean change ± SEM: IG: 0 ± 0.02 CG: +0.03 ± 0.02 Time*group interaction NS
								Leg LBM§ (kg; DXA)	1 y: 101/95 (mITT)	Mean change ± SEM: IG: +0.18 ± 0.05 CG: +0.18 ± 0.06 Time*group interaction NS
									2 y: 93/88 (mITT)	Mean change ± SEM: IG: 0 ± 0.06 CG: +0.03 ± 0.02 Time*group interaction NS
								aLBM§ (kg; DXA)	1 y: 101/95 (mITT)	Mean change ± SEM: IG: +0.20 ± 0.06 CG: +0.27 ± 0.07 Time*group interaction NS
									2 y: 93/88 (mITT)	Mean change ± SEM: IG: -0.03 ± 0.07 CG: +0.03 ± 0.08 Time*group interaction NS
								aLBM relative to squared height§ (kg/m^2)	1 y: 101/95 (mITT)	Mean change ± SEM: IG: +0.09 ± 0.02 CG: +0.11 ± 0.02 Time*group interaction NS
									2 y: 93/88 (mITT)	Mean change ± SEM: IG: +0.02 ± 0.03 CG: +0.05 ± 0.03 Time*group interaction NS

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
								Muscle CSA, calf[§] (cm^2; QCT)	1 y: 101/95 (mITT)	Mean change ± SEM: IG: -0.01 ± 0.13 CG: +0.13 ± 0.15 Time*group interaction NS
									2 y: 93/88 (mITT)	Mean change ± SEM: IG: -0.71 ± 0.15 CG: -0.83 ± 0.17 Time*group interaction NS
In the context of (concomitant) physical exercise										
Arnarson et al. 2013,[49] Iceland Same study as Ramel et al. 2013[50]	Community-dwelling older men and women aged ≥65; without major orthopaedic disease or musculoskeletal disorders	Whey protein. Drink containing 20 g of whey protein isolate (+ 20 g of CHO), consumed after WBR (so only on training days), [A] WBR, 3 times/wk, 3 sets of 6-8 reps, 75-80% 1RM	CHO placebo. Drink containing 40 g of CHO, consumed after WBR (so only on training days) WBR, 3 times/wk, 3 sets of 6-8 reps, 75-80% 1RM	NR	Baseline: IG: 1.00 ± 0.26, CG: 0.92 ± 0.30 Follow-up: IG: 1.06 ± 0.23, CG 0.89 ± 0.23	12 wk	High	Total LBM[§] (kg; DXA)	75/66	Mean change ± SD: IG: +0.7 ± 1.1 CG: +0.9 ± 1.5 P=0.365
								aSMM[§] (kg; DXA)	75/66	Mean change ± SD: IG: +0.6 ± 1.2 CG: +0.5 ± 0.8 P=0.938
Campbell et al. 1995,[39] USA	Generally healthy older men and women aged 56-80	High-protein diet (2*RDA = 1.6 g/kg BW/d). Lacto-ovo-vegetarian diet providing 0.6 g protein/kg BW + milk-based beverages providing 1.0 g protein/kg BW + multivitamin-multimineral supplement, [B] WBR, 3 times/d, 2-3 sets of 8-12 reps, 80% 1RM	Low-protein diet (1*RDA = 0.8 g/kg BW/d). Lacto-ovo-vegetarian diet providing 0.6 g protein/kg BW + milk-based beverages providing 0.2 g protein/kg BW (non-protein intake was 55% CHO and 45% fat) + multivitamin-multimineral supplement WBR, 3 times/d, 2-3 sets of 8-12 reps, 80% 1RM	NR	Baseline (after a 2-wk run-in period): IG: 1.62 ± 0.02, CG: 0.80 ± 0.02 Follow-up: Similar to baseline values	12 wk	Some concerns	Fat-free mass (kg, hydrostatic weighing)[d]	6/6 (ITT)	Time*group interaction NS
								Muscle CSA, thigh (cm^2; CT)	6/6 (ITT)	Authors reported no results for time*group interaction (repeated measures ANOVA), which suggests that protein has no effect

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Chalé et al. 2013,[54] USA	Community-dwelling, mobility-limited, sedentary older women aged 70-85	Whey protein concentrate: 20 g consumed after breakfast and 20 g consumed after evening meal each day (total: 40 g whey protein/d). On training days, one serving was consumed immediately after WBR, [A] WBR, 3 times/wk, 10 reps, 80% 1RM	CHO placebo. Isocaloric amount consumed after breakfast and after evening meal each day. On training days, one serving was consumed immediately after WBR. WBR, 3 times/wk, 10 reps, 80% 1RM	IG: 72.1 ± 29.3%, CG: 82.3 ± 21.9%	Baseline: IG: 0.97, CG: 0.98 Follow-up: NR	6 mo	Some concerns	Total LBM[§] (kg; DXA)	42/38 (ITT)	MD (95%-CI): +0.26 (-0.43 to +0.95) NS
								Muscle CSA, thigh[§] (cm^2; CT)	42/38 (ITT)	MD (95%-CI): +1 (-1 to +4) NS
Fernandes et al. 2018,[40] Brazil Same study as Sugihara Junior et al. 2018[46]	Older women aged ≥60; physically independent; free from cardiac or orthopaedic dysfunction; protein intake <1.2 g/kg BW	Whey protein. 35 g of hydrolysed whey protein containing 27.1 g of protein, dissolved in 200 ml sugar-free soft drink, ingested after WBR (so only on training days), [A] WBR, 3 times/wk, 3 sets of 8-12RM	CHO placebo. 35 g of maltodextrin, dissolved in 200 ml sugar-free soft drink, ingested after WBR (so only on training days) WBR, 3 times/wk, 3 sets of 8-12RM	NR	Baseline: IG: 0.85 ± 0.1, CG: 0.81 ± 0.1 Follow-up: IG: 1.4 ± 0.1, CG: 0.87 ± 0.1	12 wk	High	Total LST (kg; DXA)	16/16	Mean % change: IG: +3.8 CG: +2.0 **P for time*group interaction<0.05**
Mitchell et al. 2015,[38] Canada	Generally healthy older men; free of musculoskeletal or metabolic disorders; recreationally active; mean age: 74.4 ± 5.4 y	500-ml of chocolate milk containing 14 g of protein, consumed <15 min after WBR on training days and with breakfast on non-training days, [B] WBR, 3 times/wk, 3-4 sets, 75-85% 1RM	CHO placebo. 500-ml drink with isocaloric amount of CHO, consumed <15 min after WBR on training days and with breakfast on non-training days WBR, 3 times/wk, 3-4 sets, 75-85% 1RM	NR	Baseline: NR Follow-up: NR	12 wk	High	Muscle fibre area (biopsy)	16 (total)	Time*group interaction NS

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Nabuco et al. 2018,[43] Brazil Same study as Nabuco et al. 2019a[44] and Nabuco et al. 2019b[45]	Older women aged ≥60, physically independent, free from cardiac or orthopaedic dysfunction	Whey protein. 35 g of hydrolysed whey protein supplement containing 27.1 g protein (+ 5.2 g CHO), mixed with non-caloric drink. IG1: protein before and placebo after WBR; IG2: placebo before and protein after WBR (so only on training days), [A] WBR, 3 times/wk, 3 sets of 8-12 reps	CHO placebo containing 0.3 g protein and 33.3 g CHO, mixed with non-caloric drink; one before and one after WBR (so only on training days) WBR, 3 times/wk, 3 sets of 8-12 reps	NR	Baseline: IG1: 0.92 ± 0.20, IG2: 0.94 ± 0.36, CG: 0.95 ± 0.27 Follow-up: IG1: 1.38 ± 0.26, IG2: 1.49 ± 0.46, CG: 1.0 ± 0.25	12 wk	Some concerns	Upper limb LST (kg; DXA)	22/21/23 (mITT)	Mean % change ± SD: IG1: +3.4 ± 3.0 IG2: +5.9 ± 4.3 CG: +4.1 ± 3.5 P for time*group interaction=0.156
								Lower limb LST (kg; DXA)	22/21/23 (mITT)	Mean % change ± SD: IG1: +3.2 ± 2.9* IG2: +1.1 ± 2.2* CG: -4.3 ± 8.4 *** P<0.05 compared to CG**
								SMM (kg; DXA[f])	22/21/23 (mITT)	Mean % change ± SD: IG1: +3.4 ± 2.9* IG2: +4.2 ± 2.3* CG: +2.0 ± 2.1 *** P<0.05 compared to CG**
Nabuco et al. 2019a,[44] Brazil Same study as Nabuco et al. 2018[43] and Nabuco et al. 2019b[45]	Older women aged ≥60, physically independent, free from cardiac or orthopaedic dysfunction	Whey protein. 35 g of hydrolysed whey protein supplement containing 27.1 g protein (+ 5.2 g CHO), mixed with non-caloric drink. IG1: protein before and placebo after WBR; IG2: placebo before and protein after WBR (so only on training days), [A] WBR, 3 times/wk, 3 sets of 8-12 reps	CHO placebo containing 0.3 g protein and 33.3 g CHO, mixed with non-caloric drink; one before and one after WBR (so only on training days) WBR, 3 times/wk, 3 sets of 8-12 reps	NR	Baseline: IG1: 0.92 ± 0.20, IG2: 0.94 ± 0.36, CG: 0.95 ± 0.27 Follow-up: IG1: 1.38 ± 0.26, IG2: 1.49 ± 0.46, CG: 1.0 ± 0.25	12 wk	Some concerns	ALST (kg; DXA)	22/21/23 (mITT)	Mean % change: IG1: +3.1 IG2: +2.6 CG: +2.7 P for time*group interaction=0.600

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Nabuco et al. 2019b,[45] Brazil Same study as Nabuco et al. 2018[43] and Nabuco et al. 2019a[44]	Older women aged ≥60, physically independent, free from cardiac or orthopaedic dysfunction	Whey protein. 35 g of hydrolysed whey protein supplement containing 27.1 g protein (+ 5.2 g CHO), mixed with non-caloric drink. IG1: protein before and placebo after WBR; IG2: placebo before and protein after WBR (so only on training days), [A] WBR, 3 times/wk, 3 sets of 8-12 reps	CHO placebo containing 0.3 g protein and 33.3 g CHO, mixed with non-caloric drink; one before and one after WBR (so only on training days) WBR, 3 times/wk, 3 sets of 8-12 reps	NR	Baseline: IG1: 0.92 ± 0.20, IG2: 0.94 ± 0.36, CG: 0.95 ± 0.27 Follow-up: IG1: 1.38 ± 0.26, IG2: 1.49 ± 0.46, CG: 1.0 ± 0.25	12 wk	Some concerns	Total LST (kg; DXA)	22/21/23 (mITT)	Mean % change: IG1: +2.7* IG2: +3.7* CG: +1.5 *** P<0.05 compared to CG**
Nabuco et al. 2019c,[42] Brazil	Older women aged ≥60 with sarcopenic obesity; physically independent; free from cardiac or orthopaedic dysfunction	Whey protein. 35 g of hydrolysed whey protein supplement, mixed with non-caloric drink, ingested after WBR (so only on training days), [A] WBR, 3 times/wk, 3 sets of 8-12 reps	CHO placebo. Isocaloric amount of maltodextrin mixed with non-caloric drink, ingested after WBR (so only on training days) WBR, 3 times/wk, 3 sets of 8-12 reps	NR	Baseline IG: 0.93 ± 0.36, CG: 0.97 ± 0.28 Follow-up: IG: 1.0 ± 0.23, CG: 1.0 ± 0.19 (without supplementation)	12 wk	Some concerns	Total LST (kg; DXA)	13/13 (ITT)	Mean % change: IG: +3.8 CG: +1.0 **P for time*group interaction<0.001**
								Lower LST (kg, DXA)	13/13 (ITT)	Mean % change: IG: +4.8 CG: +1.3 **P for time*group interaction<0.001**
								ALST (kg, DXA)	13/13 (ITT)	Mean % change: IG: +6.1 CG: +2.4 **P for time*group interaction<0.001**

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Sugihara Junior et al. 2018,[46] Brazil Same study as Fernandes et al. 2018[40]	Older women aged ≥60; physically independent; free from cardiac or orthopaedic dysfunction; protein intake <1.2 g/kg BW	Whey protein. 35 g of hydrolysed whey protein containing 27.1 g of protein, dissolved in 200 ml sugar-free soft drink, ingested after WBR (so only on training days), [A] WBR, 3 times/wk, 3 sets of 8-12RM	CHO placebo. 35 g of maltodextrin, dissolved in 200 ml sugar-free soft drink, ingested after WBR (so only on training days) WBR, 3 times/wk, 3 sets of 8-12RM	NR	Baseline: IG: 0.85 ± 0.1, CG: 0.81 ± 0.1 Follow-up: IG: 1.4 ± 0.1, CG: 0.87 ± 0.1	12 wk	High	Upper limb LST (kg; DXA)	15/16	Mean % change: IG: +5.0 CG: +2.5 P for time*group interaction=0.68
								Lower limb LST (kg; DXA)	15/16	Mean % change: IG: +3.4 CG: +0.8 P for time*group interaction=0.11
								SMM (kg; DXA[g])	15/16	Mean % change: IG: +4.8 CG: +2.3 **P for time*group interaction=0.02**
Ten Haaf et al. 2019,[53] The Netherlands	Physically active older adults aged ≥65 with habitual protein intake <1.0 g/kg BW; without type 2 diabetes, cancer, renal insufficiency (eGFR <30) or COPD	Milk-protein concentrate. 250-ml protein drink containing 15.5 g protein (+ 1.1 g fat + 14.5 g lactose) consumed twice a day (total: 31 g protein/d), [A] Training (walking) for the Nijmegen Four Days Marches	CHO placebo. 250-ml isocaloric drink containing 1.1 g protein (+ 5.2 g fat + 36 g CHO) consumed twice daily Training (walking) for the Nijmegen Four Days Marches	IG: 96 ± 3%, CG: 95 ± 3%	Baseline: IG: 0.86 ± 0.23, CG: 0.92 ± 0.24 Follow-up: IG: 0.92 ± 0.27, CG: 0.97 ± 0.23 (without supplementation)	12 wk	Some concerns	Total LBM[§] (kg; DXA)	58/56 (PP)	Mean change ± SD: IG: +0.54 ± 1.13 CG: +0.31 ± 1.03 P for time*group interaction=0.27 For relative total LBM (% of BW), mean change ± SD: IG: +0.93 ± 1.22, CG: +0.44 ± 1.4, P for time*group interaction=0.046. Mainly a result of the significantly greater decrease in total fat mass (kg) in IG than in CG (P=0.013).
Thomson et al. 2016,[57] Australia	Older adults aged 50-79 and BMI of 20-35 kg/m^2 who are physically active (but not engaged in formal exercise); without major chronic diseases (e.g. diabetes, cancer, metabolic disease, cardiac abnormalities, musculoskeletal injury)	High-protein diet. Individualised diet providing 1.0 g/kg BW/d + ~27 g dairy-based protein drink each day (IG1); Individualised diet providing 1.0 g/kg BW/d + ~27 g soy protein drink each day (IG2), [B] WBR, 3 times/wk, 1-3 sets of 8-20 reps, 8RM	CHO placebo. Individualised diet providing 1.0 g/kg BW/d + ~27 g CHO drink each day WBR, 3 times/wk, 1-3 sets of 8-20 reps, 8RM	IG1: 97%, IG2: 98%, CG: 98%	Baseline: IG1: 1.06 ± 0.10, IG2: 1.08 ± 0.09, CG: 1.02 ± 0.05 Follow-up: IG1: 1.42 ± 0.14, IG2: 1.45 ± 0.14, CG: 1.08 ± 0.05	12 wk	High	Total LBM[§] (kg)	34/26/23 (PP[h])	Mean change ± SD: IG1: +1.0 ± 1.0 IG2: +1.4 ± 1.2 CG: +0.8 ± 1.1 Between-group difference NS (P≥0.1)

Statistically significant effects are shown in bold.
Abbreviations: ADP: air-displacement plethysmography, aLBM: appendicular lean body mass, ALST: appendicular lean soft tissue, aSMM: appendicular skeletal muscle mass, BMI: body mass index, BW: body weight, CSA: cross-sectional area, CI: confidence interval, CG: control group, CHO: carbohydrates, CKD: chronic kidney disease, COPD: chronic obstructive pulmonary disease, CVD: cardiovascular disease, DXA: dual-energy X-ray absorptiometry, E%: percentage of energy intake, EAA: essential amino acids, IG: intervention group, ITT: intention-to-treat analysis or modified intention-to-treat analysis (mITT, only those with missing outcome data were excluded from the analytic sample), IU: international units, LBM: lean body mass, LSMD: least square mean difference, LST: Lean soft tissue, LTM: lean tissue mass, MD: mean difference (i.e. difference in within-group change), mg: milligram, MNA: Mini Nutritional Assessment, mo: months, MRI: magnetic resonance imaging, *n*: number, NR: not reported, NS: not significant, PP: per-protocol analysis, QCT, quantitative computed tomography, RDA: recommended dietary allowance, reps: repetitions, RM: repetition maximum, RoB: risk of bias, SD: standard deviation, SEM: standard error of the mean, SMM: skeletal muscle mass, US: B-mode ultrasound, WBR: whole-body resistance training, wk: weeks, y: years.
Footnotes:

§ Sufficient statistical power to detect an effect is to be expected, based on the sample size calculation.

[a] 'Protein type' indicates the way in which a higher protein intake was achieved and is categorised into 'pure' protein or amino acids (or essential amino acids) (A), specific product with a high protein content (B), or high-protein diets (C).

[b] Risk of bias was assessed using the RoB 2 tool and scored as 'low' (L), 'some concerns' (SC), or 'high' (H). A more detailed explanation of the overall judgment can be found in Annex F.

[c] Two subjects completed both diet protocols, with an interval of ≥4 months between each study. Data analyses were performed with sample sizes of 12 and 10, with and without the two subjects who completed both diet protocols, respectively. The results were the same for both; thus, all 12 subjects were considered individually for this report.

[d] Estimated from body density and total body water using the three-compartment model of Siri.[66]

[e] Air-displacement plethysmography (ADP) is conducted using a Bod Pod.

[f] (Intermuscular adipose tissue-free) skeletal muscle mass (SMM) was calculated with a predictive equation obtained from Kim et al.[67]

[g] Skeletal muscle mass (SMM) was calculated with a predictive equation obtained from Kim et al.[68]

[h] Intention-to-treat analysis demonstrated a similar pattern to the per-protocol analysis (data not shown).

Table E2. Results from randomised controlled trials on the effect of increased protein intake on muscle strength in older adults, grouped according to whether or not the protein intervention was carried out in the context of (concomitant) physical exercise

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Not in the context of (concomitant) physical exercise										
Bhasin et al. 2018,[51] USA	Community-dwelling older men aged ≥65 with moderate physical function limitations and with habitual protein intake ≤0.83 g/kg BW/d; mean BMI: 30.3 ± 4.9 kg/m^2	Individualised diets providing 0.7 g protein/kg BW/d with additional discretionary foods (0.1 g protein/kg BW) and protein supplements (0.5 g/kg BW) to achieve a total of 1.3 g/kg BW/d, [A,B]	Individualised diets providing 0.7 g protein/kg BW/d with additional discretionary foods (0.1 g protein/kg BW) and placebo supplements (0.5 g CHO/kg BW) to achieve a total of 0.8 g/kg BW/d	Foods (4-6 mo): IG: 77.1 ± 13%, CG: 74.5 ± 23.2% Supplements (4-6 mo): IG: 91.2 ± 12.4%, CG: 92.6 ± 11.0%	Baseline: IG: 0.72 ± 0.11, CG: 0.69 ± 0.15 Follow-up (1-3 mo): IG: 1.18 ± 0.15, CG: 0.84 ± 0.07 Follow-up (4-6 mo): IG: 1.17 ± 0.13, CG: 0.81 ± 0.10	6 mo	Some concerns	Leg press strength, 1RM (N)	31/32 (mITT)	MD (95%-CI): +0.89 (-86.9 to +88.7) P=0.98
								Chest press strength, 1RM (N)	31/34 (mITT)	MD (95%-CI): -11.8 (-31.6 to +7.9) P=0.24
								Leg press peak power, 60% 1RM (W)	29/32 (mITT)	MD (95%-CI): +26.4 (-10.5 to +63.4) P=0.16
Dillon et al. 2009,[37] USA	Community-dwelling older women; without vascular disease, hypertension or cardiac abnormality	EAA. 7.5 g EAA, ingested twice a day in between meals (total: 15 g EAA/d), [A]	CHO placebo. Isocaloric amount of lactose, ingested twice a day in between meals	NR	Baseline: NR Follow-up: NR	3 mo	High	Bicep curl, 1RM	7/7	Authors reported no results for time*group interaction (ANOVA), which suggests that protein has no effect
								Triceps extension, 1RM	7/7	Authors reported no results for time*group interaction (ANOVA), which suggests that protein has no effect
								Leg extension, 1RM	7/7	Authors reported no results for time*group interaction (ANOVA), which suggests that protein has no effect
								Leg curl, 1RM	7/7	Authors reported no results for time*group interaction (ANOVA), which suggests that protein has no effect

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Ispoglou et al. 2016,[41] UK	Community-dwelling older men and women aged 65-75; good health, without major chronic diseases (e.g. diabetes, vascular disease, hypertension)	EAA. Standard EAA mixture with 20% leucine (IG1) and with 40% leucine (IG2), ingested at breakfast and dinner (total: 0.21 g/kg BW/d = ~11-21 g/d), [A]	CHO placebo. Isocaloric amount of lactose, ingested at breakfast and dinner	74-83%	Baseline: 0.95-1.10 g/kg BW/d Follow-up: 1.02-1.08 g/kg BW/d (without supplementation)	12 wk	High	Relative handgrip strength (N/kg BW)	8/8/9 (PP)	Mean % change ± SD: IG1: +11.5 ± 23.9 IG2: +4.8 ± 7.5 CG: -0.2 ± 8.9 P=0.300
								30-s arm-curl test (n)	8/8/9 (PP)	Mean % change ± SD: IG1: +15.0 ± 20.0 IG2: +8.3 ± 13.7 CG: +0.9 ± 11.2 P=0.193
Mitchell et al. 2017,[48] New Zealand	Community-dwelling older men aged >70; able to perform ADLs without mobility aids; without major chronic diseases (e.g. cancer, diabetes, thyroid diseases)	High-protein diet (2*RDA = 1.6 g/kg BW/d), omnivorous, 28-31 E% fat, [C]	Low-protein diet (1*RDA = 0.8 g/kg BW/d), omnivorous, 28-31 E% fat; difference made up of CHO	IG: 97.5%, CG: 98.9%	Baseline: IG: 1.1 ± 0.3, CG: 1.2 ± 0.4 Follow-up: IG: 1.7 ± 0.1, CG: 0.9 ± 0.1	10 wk	High	Handgrip strength (kg)	14/15 (PP)	P for time*group interaction=0.167
								Knee extension MVC (Nm)	14/15 (PP)	Mean change ± SD: IG: +7.5 ± 22.9 CG: -8.6 ± 24.2 P for time*group interaction=0.120
								Knee extension peak power (W)	14/15 (PP)	Mean change ± SD: IG: +26.6 ± 47.7 CG: -11.7 ± 31.0 **P for time*group interaction=0.012**
Ottestad et al. 2017[55] Norway	Community-dwelling older adults aged ≥70; relatively healthy (no diabetes, CVD, cancer, COPD, CKD); not malnourished; with reduced muscle strength or performance	Protein-enriched milk. 400 ml drink containing 20 g protein, consumed twice a day (total: 40 g protein/d), [B]	CHO placebo. 400 ml drink containing an isocaloric amount of CHO, consumed twice a day	IG: 97.8 ± 3.8%, CG: 96.8 ± 5.7%	Baseline: IG: 1.0 ± 0.3, CG: 1.0 ± 0.3 Follow-up: IG: 1.4 ± 0.5, CG: 0.9 ± 0.4	12 wk	High	Leg press strength, 1RM (kg)	16/17 (mITT)	Mean change (95%-CI): IG: +5.7 (-1.7 to +13.1) CG: +6.2 (-2.2 to +14.6) P=0.93
								Chest press strength, 1RM (kg)	17/18 (mITT)	Mean change (95%-CI): IG: +1.3 (+0.1 to +2.5) CG: +1.5 (0.0 to +3.0) P=0.85
								Handgrip strength, dominant side (kg)	17/18 (mITT)	Mean change (95%-CI): IG: +0.1 (-0.7 to +1.0) CG: -0.5 (-1.5 to +0.5) P=0.27
								Handgrip strength, non-dominant side (kg)	17/19 (mITT)	Mean change (95%-CI): IG: +0.5 (-0.5 to +1.5) CG: +0.5 (-0.4 to +1.4) P=0.99

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Park et al. 2018,[52] Korea	Community-dwelling (pre-)frail older adults aged 70-85 at risk of malnutrition (MNA ≤23.5); no kidney or liver failure; able to walk	Whey protein. Multiple 10-g packs of protein powder (9.3 g whey protein/ pack), dissolved in 340 ml tea, were provided in addition to habitual protein intake up to 1.2 g/kg BW/d (IG1) and 1.5 g/kg BW/d (IG2), [A]	CHO placebo. Multiple 10-g packs of CHO powder (9.3 g maltodextrin/ pack), dissolved in 340 ml tea, were provided in addition to habitual protein intake up to 0.8 g/kg (CG) or 1.2 g/kg (IG1) (CG were given only CHO powder, IGs were given a combination of protein and CHO powder.)	IG1: 98%, IG2: 96%, CG: 97%	Baseline: IG1: 0.77 ± 0.24, IG2: 0.80 ± 0.21, CG: 0.84 ± 0.28 Follow-up: IG1: 1.18 ± 0.23, IG2: 1.37 ± 0.26, CG: 0.90 ± 0.38	12 wk	Some concerns	Handgrip strength (kg)	40/40/40 (ITT)	P for time*group interaction=0.553
Zhu et al. 2015,[58] Australia Same study as Hodgson et al. 2012[59] and Zhu et al. 2011[60]	Community-dwelling older women aged 70-80 with habitual protein intake <1.5 g/kg BW/d; without metabolic bone disease, osteoporotic fracture, diabetes, hepatic or renal insufficiency	Whey protein isolate. 250 ml skim milk-based high-protein supplement drink containing 30 g of whey protein + calcium, [A]	CHO placebo. 250 ml skim milk-based supplement drink containing 2.1 g of protein and isocaloric amount of maltodextrin + calcium	IG: 87.1%, CG: 80.8 % (P=0.03)	Baseline: IG: 1.2 ± 0.3, CG: 1.1 ± 0.3 Follow-up (1 y): IG: 1.4 ± 0.4, CG: 1.1 ± 0.3 Follow-up (2 y): IG: 1.4 ± 0.4, CG: 1.1 ± 0.4	1 y and 2 y	Some concerns	Handgrip strength (kg)	1 y: 99/94 (mITT)	Mean change ± SEM: IG: -0.87 ± 0.40 CG: -1.11 ± 0.40 Time*group interaction NS
									2 y: 93/88 (mITT)	Mean change ± SEM: IG: -1.09 ± 0.41 CG: -1.53 ± 0.42 Time*group interaction NS
								Ankle dorsiflexion strength (kg)	1 y: 99/94 (mITT)	Mean change ± SEM: IG: +1.10 ± 0.38 CG: +1.44 ± 0.40 Time*group interaction NS
									2 y: 93/88 (mITT)	Mean change ± SEM: IG: +2.15 ± 0.48 CG: +2.71 ± 0.41 Time*group interaction NS

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
								Knee flexor strength (kg)	1 y: 99/94 (mITT)	Mean change ± SEM: IG: +1.81 ± 0.38 CG: +1.65 ± 0.41 Time*group interaction NS
									2 y: 93/88 (mITT)	Mean change ± SEM: IG: +3.18 ± 0.38 CG: +2.36 ± 0.49 Time*group interaction NS
								Knee extensor strength (kg)	1 y: 99/94 (mITT)	Mean change ± SEM: IG: +2.08 ± 0.54 CG: +2.83 ± 0.67 Time*group interaction NS
									2 y: 93/88 (mITT)	Mean change ± SEM: IG: +3.36 ± 0.68 CG: +3.17 ± 0.80 Time*group interaction NS
								Hip extensor strength (kg)	1 y: 99/94 (mITT)	Mean change ± SEM: IG: -0.62 ± 0.57 CG: +1.37 ± 0.66 Time*group interaction NS
									2 y: 93/88 (mITT)	Mean change ± SEM: IG: +0.12 ± 0.65 CG: +1.34 ± 0.64 Time*group interaction NS
								Hib abductor strength (kg)	1 y: 99/94 (mITT)	Mean change ± SEM: IG: +0.67 ± 0.41 CG: +0.85 ± 0.48 Time*group interaction NS
									2 y: 93/88 (mITT)	Mean change ± SEM: IG: +1.46 ± 0.41 CG: +1.28 ± 0.58 Time*group interaction NS

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
								Hip flexor strength (kg)	1 y: 99/94 (mITT)	Mean change ± SEM: IG: +1.51 ± 0.44 CG: +2.20 ± 0.49 Time*group interaction NS
									2 y: 93/88 (mITT)	Mean change ± SEM: IG: +3.55 ± 0.57 CG: +3.67 ± 0.55 Time*group interaction NS
								Hip adductor strength (kg)	1 y: 99/94 (mITT)	Mean change ± SEM: IG: -2.06 ± 0.53 CG: -1.59 ± 0.61 Time*group interaction NS
									2 y: 93/88 (mITT)	Mean change ± SEM: IG: -1.53 ± 0.53 CG: -2.05 ± 0.68 Time*group interaction NS
In the context of (concomitant) physical exercise										
Arnarson et al. 2013,[49] Iceland Same study as Ramel et al. 2013[50]	Community-dwelling older men and women aged ≥65; without major orthopaedic disease or musculoskeletal disorders	Whey protein. Drink containing 20 g of whey protein isolate (+ 20 g of CHO), consumed after WBR (so only on training days), [A] WBR, 3 times/wk, 3 sets of 6-8 reps, 75-80% 1RM	CHO placebo. Drink containing 40 g of CHO, consumed after WBR (so only on training days) WBR, 3 times/wk, 3 sets of 6-8 reps, 75-80% 1RM	NR	Baseline: IG: 1.00 ± 0.26, CG: 0.92 ± 0.30 Follow-up: IG: 1.06 ± 0.23, CG 0.89 ± 0.23	12 wk	High	Quadriceps strength (N)	75/66	Mean change ± SD: IG: +56.5 ± 59.4 CG: +53.8 ± 51.8 P=0.776

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Chalé et al. 2013,[54] USA	Community-dwelling, mobility-limited, sedentary older women aged 70-85	Whey protein concentrate: 20 g consumed after breakfast and 20 g consumed after evening meal each day (total: 40 g protein/d). On training days, one serving was consumed immediately after WBR, [A]	CHO placebo. Isocaloric amount consumed after breakfast and after evening meal each day. On training days, one serving was consumed immediately after WBR.	IG: 72.1 ± 29.3%, CG: 82.3 ± 21.9%	Baseline: IG: 0.97, CG: 0.98 Follow-up: NR	6 mo	Some concerns	Double leg press strength, 1RM§ (N)	42/38 (ITT)	MD (95%-CI): +58 (-87 to +202) NS
								Knee extension, 1RM, right§ (N)	42/38 (ITT)	MD (95%-CI): +10 (-31 to +52) NS
								Knee extension, 1RM, left§ (N)	42/38 (ITT)	MD (95%-CI): +37 (-37 to +111) NS
								Double leg press peak power, 40% 1RM§ (W)	42/38 (ITT)	MD (95%-CI): +53 (-33 to +140) NS
		WBR, 3 times/wk, 10 reps, 80% 1RM	WBR, 3 times/wk, 10 reps, 80% 1RM					Knee extension peak power, 40% 1RM, right§ (W)	42/38 (ITT)	MD (95%-CI): +15 (+1 to +29) **P <0.05**
								Knee extension peak power, 40% 1RM, left§ (W)	42/38 (ITT)	MD (95%-CI): +15 (+1 to +29) **P <0.05**
								Double leg press peak power, 70% 1RM§ (W)	42/38 (ITT)	MD (95%-CI): +61 (-61 to +137) NS
								Knee extension peak power, 70% 1RM, right§ (W)	42/38 (ITT)	MD (95%-CI): +27 (+9 to +45) **P <0.05**
								Knee extension peak power, 70% 1RM, left§ (W)	42/38 (ITT)	MD (95%-CI): +30 (+12 to +47) **P <0.05**

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Mitchell et al. 2015,[38] Canada	Generally healthy older men; free of musculoskeletal or metabolic disorders; recreationally active; mean age: 74.4 ± 5.4 y	Milk protein. 500-ml of chocolate milk containing 14 g of protein, consumed <15 min after WBR on training days and with breakfast on non-training days, [B] WBR, 3 times/wk, 3-4 sets, 75-85% 1RM	CHO placebo. 500-ml drink with isocaloric amount of CHO, consumed <15 min after WBR on training days and with breakfast on non-training days WBR, 3 times/wk, 3-4 sets, 75-85% 1RM	NR	Baseline: NR Follow-up: NR	12 wk	High	Knee extension isometric MVC (Nm)	16 (total)	Mean % change ± SD: IG: +29.5 ± 17.8 CG: +25.6 ± 49.7 Time*group interaction NS
								Leg press, 1RM (kg)	16 (total)	Mean % change ± SD: IG: +52.1 ± 43.1 CG: +35.9 ± 47.6 Time*group interaction NS
								Leg extension, 1RM (kg)	16 (total)	Mean % change ± SD: IG: +52.1 ± 43.1 CG: +35.9 ± 47.6 Time*group interaction NS
								Chest press, 1RM (kg)	16 (total)	Mean % change ± SD: IG: +9.8 ± 6.9 CG: +4.9 ± 10.7 Time*group interaction NS
Nabuco et al. 2018,[43] Brazil Same study as Nabuco et al. 2019a[44] and Nabuco et al. 2019b[45]	Older women aged ≥60, physically independent, free from cardiac or orthopaedic dysfunction	Whey protein. 35 g of hydrolysed whey protein supplement containing 27.1 g protein (+ 5.2 g CHO), mixed with non-caloric drink. IG1: protein before and placebo after WBR; IG2: placebo before and protein after WBR (so only on training days), [A] WBR, 3 times/wk, 3 sets of 8-12 reps	CHO placebo containing 0.3 g protein and 33.3 g CHO, mixed with non-caloric drink; one before and one after WBR (so only on training days) WBR, 3 times/wk, 3 sets of 8-12 reps	NR	Baseline: IG1: 0.92 ± 0.20, IG2: 0.94 ± 0.36, CG: 0.95 ± 0.27 Follow-up: IG1: 1.38 ± 0.26, IG2: 1.49 ± 0.46, CG: 1.0 ± 0.25	12 wk	Some concerns	Chest press, 1RM (kg)	22/21/23 (mITT)	Mean % change ± SD: IG1: +5.6 ± 1.7* IG2: +5.9 ± 1.6* CG: +4.5 ± 1.2 *** P<0.05 compared to CG**
								Knee extension, 1RM (kg)	22/21/23 (mITT)	Mean % change ± SD: IG1: +9.2 ± 2.5* IG2: +8.8 ± 2.2* CG: +7.5 ± 1.0 *** P<0.05 compared to CG**
								Preacher curl, 1RM (kg)	22/21/23 (mITT)	Mean % change ± SD: IG1: +11.3 ± 5.7 IG2: +12.4 ± 6.6 CG: +10.5 ± 5.3 P for time*group interaction=0.376
								Total strength[c] (kg)	22/21/23 (mITT)	Mean % change ± SD: IG1: +8.1 ± 1.6* IG2: +8.3 ± 2.3* CG: +7.0 ± 2.7 *** P<0.05 compared to CG**

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Nabuco et al. 2019c,[42] Brazil	Older women aged ≥60 with sarcopenic obesity; physically independent; free from cardiac or orthopaedic dysfunction	Whey protein. 35 g of hydrolysed whey protein supplement, mixed with non-caloric drink, ingested after WBR (so only on training days), [A] WBR, 3 times/wk, 3 sets of 8-12 reps	CHO placebo. Isocaloric amount of maltodextrin mixed with non-caloric drink, ingested after WBR (so only on training days) WBR, 3 times/wk, 3 sets of 8-12 reps	NR	Baseline IG: 0.93 ± 0.36, CG: 0.97 ± 0.28 Follow-up: IG: 1.0 ± 0.23, CG: 1.0 ± 0.19 (without supplementation)	12 wk	Some concerns	Knee extension, 1RM (kg)	13/13 (ITT)	Mean % change: IG: +6.3 CG: +4.8 P for time*group interaction=0.347
								Chest press, 1RM (kg)	13/13 (ITT)	Mean % change: IG: +4.7 CG: +4.5 P for time*group interaction=0.696
								Preacher curl, 1RM (kg)	13/13 (ITT)	Mean % change: IG: +12.4 CG: +10.2 P for time*group interaction=0.247
								Total strength[c] (kg)	13/13 (ITT)	Mean % change: IG: +6.8 CG: +5.7 P for time*group interaction=0.248

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Sugihara Junior et al. 2018,[46] Brazil Same study as Fernandes et al. 2018[40]	Older women aged ≥60; physically independent; free from cardiac or orthopaedic dysfunction; protein intake <1.2 g/kg BW	Whey protein. 35 g of hydrolysed whey protein containing 27.1 g of protein, dissolved in 200 ml sugar-free soft drink, ingested after WBR (so only on training days), [A] WBR, 3 times/wk, 3 sets of 8-12RM	CHO placebo. 35 g of maltodextrin, dissolved in 200 ml sugar-free soft drink, ingested after WBR (so only on training days) WBR, 3 times/wk, 3 sets of 8-12RM	NR	Baseline: IG: 0.85 ± 0.1, CG: 0.81 ± 0.1 Follow-up: IG: 1.4 ± 0.1, CG: 0.87 ± 0.1	12 wk	High	Chest press strength, 1RM (kg)	15/16	Mean % change: IG: +6.3 CG: +2.7 **P for time*group interaction<0.01**
								Knee extension strength, 1RM (kg)	15/16	Mean % change: IG: +8.5 CG: +4.4 **P for time*group interaction=0.01**
								Preacher curl strength, 1RM (kg)	15/16	Mean % change: IG: +14.0 CG: +10.5 P for time*group interaction=0.07
								Total strength, 1RM (kg)	15/16	Mean % change: IG: +8.7 CG: +4.9 **P for time*group interaction<0.01**
								Lower limb muscle quality index[d]	15/16	Mean % change: IG: +4.4 CG: +4.5 P for time*group interaction=0.68
								Upper limb muscle quality index[e]	15/16	Mean % change: IG: +11.5 CG: +7.4 P for time*group interaction=0.16
								Total muscle quality index[f]	15/16	Mean % change: IG: +2.9 CG: +1.5 P for time*group interaction=0.58

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Ten Haaf et al. 2019,[53] The Netherlands	Physically active older adults aged ≥65 with habitual protein intake <1.0 g/kg BW; without type 2 diabetes, cancer, renal insufficiency (eGFR <30) or COPD	Milk-protein concentrate. 250-ml protein drink containing 15.5 g protein (+ 1.1 g fat + 14.5 g lactose) consumed twice a day (total: 31 g protein/d), [A] Training (walking) for the Nijmegen Four Days Marches	CHO placebo. 250-ml isocaloric drink containing 1.1 g protein (+ 5.2 g fat + 36 g CHO) consumed twice daily Training (walking) for the Nijmegen Four Days Marches	IG: 96 ± 3%, CG: 95 ± 3%	Baseline: IG: 0.86 ± 0.23, CG: 0.92 ± 0.24 Follow-up: IG: 0.92 ± 0.27, CG: 0.97 ± 0.23 (without supplementation)	12 wk	Some concerns	Handgrip strength§ (kg)	58/56 (PP)	Mean change ± SD: IG: 0 ± 4 CG: +1 ± 4 P for time*group interaction=0.24
								Quadriceps MVC§ (N)	56 (total; PP)	Mean change ± SD: IG: +7.2 ± 71.6 CG: -8.7 ± 63.1 P for time*group interaction=0.38
								Maximal rate of force rise, quadriceps§ (%/ms)	44 (total; PP)	Mean change ± SD: IG: -0.06 ± 0.09 CG: -0.03 ± 0.11 P for time*group interaction=0.38
								Early relaxation time, quadriceps§ (ms)	33 (total; PP)	Mean change ± SD: IG: -0.27 ± 3.05 CG: +0.29 ± 2.7 P for time*group interaction=0.58
								Half relaxation time, quadriceps§ (ms)	22 (total; PP)	Mean change ± SD: IG: +1.1 ± 8.5 CG: +1.5 ± 4.5 P for time*group interaction=0.87
								Fatigue§ (%)	30 (total; PP)	Mean change ± SD: IG: -0.6 ± 8.5 CG: +1.1 ± 7.4 P for time*group interaction=0.57

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Thomson et al. 2016,[57] Australia	Older adults aged 50-79 and BMI of 20-35 kg/m^2 who are physically active (but not engaged in formal exercise); without major chronic diseases (e.g. diabetes, cancer, metabolic disease, cardiac abnormalities, musculoskeletal injury)	High-protein diet. Individualised diet providing 1.0 g/kg BW/d + ~27 g dairy-based protein drink each day (IG1); Individualised diet providing 1.0 g/kg BW/d + ~27 g soy protein drink each day (IG2), [B] WBR, 3 times/wk, 1-3 sets of 8-20 reps, 8RM	CHO placebo. Individualised diet providing 1.0 g/kg BW/d + ~27 g CHO drink each day WBR, 3 times/wk, 1-3 sets of 8-20 reps, 8RM	IG1: 97%, IG2: 98%, CG: 98%	Baseline: IG1: 1.06 ± 0.10, IG2: 1.08 ± 0.09, CG: 1.02 ± 0.05 Follow-up: IG1: 1.42 ± 0.14, IG2: 1.45 ± 0.14, CG: 1.08 ± 0.05	12 wk	High	Knee extensor strength[§] (Nm)	34/26/23 (PP[g])	Mean change ± SD: IG1: +25.5 ± 22.1 IG2: +18.4 ± 18.6 CG: +30.5 ± 24.8 Between-group difference NS; P=0.08 for change in IG2 compared to CG
								Handgrip strength[§] (kg)	34/26/23 (PP[g])	Mean change ± SD: IG1: +1.0 ± 3.1 IG2: +1.6 ± 3.1 CG: +2.0 ± 3.9 Between-group difference NS
								Leg press, 8RM[§] (kg)	34/26/23 (PP[g])	Mean (%) change ± SD: IG1: +65.2 ± 30.3 (+136.8 ± 88.2%) IG2: +47.4 ± 34.1 (+64.8 ± 35.2%) CG: +66.3 ± 25.4 (+135.0 ± 62.0%) **Significant difference between IG2 and CG**, but not between IG1 and CG
								Chest press, 8RM[§] (kg)	34/26/23 (PP[g])	Mean change ± SD: IG1: +17.4 ± 6.7 IG2: +17.4 ± 11.1 CG: +13.3 ± 4.5 Between-group difference NS
								Knee extension strength, 8RM[§] (kg)	34/26/23 (PP[g])	Mean change ± SD: IG1: +29.4 ± 14.1 IG2: +23.0 ± 11.7 CG: +25.6 ± 10.5 Between-group difference NS
								Lat pull down, 8RM[§] (kg)	34/26/23 (PP[g])	Mean (%) change ± SD: IG1: +10.1 ± 5.3 (+24.6 ± 12.2%) IG2: +11.3 ± 6.4 (+28.2 ± 11.7%) CG: +12.5 ± 5.6 (+35.1 ± 17.0%) Between-group difference in absolute change NS; **significant difference in % change between IG1 and CG**, but not between IG2 and CG

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
								Leg curl, 8RM[§] (kg)	34/26/23 (PP[g])	Mean change ± SD: IG1: +13.9 ± 9.0 IG2: +12.0 ± 6.3 CG: +11.3 ± 5.5 Between-group difference NS (for both absolute and % change)
								Total 8RM[§] (kg)	34/26/23 (PP[g])	Mean (%) change ± SD: IG1: +131.3 ± 54.2 (+92.1 ± 40.8%) IG2: +102.1 ± 50.7 (+63.0 ± 23.8%) CG: +126.1 ± 41.3 (+92.3 ± 35.4%) **Significant difference between IG2 and CG**, but not between IG1 and CG

Statistically significant effects are shown in bold.

Abbreviations: BMI: body mass index, BW: body weight, CI: confidence interval, CG: control group, CHO: carbohydrates, CKD: chronic kidney disease, COPD: chronic obstructive pulmonary disease, CVD: cardiovascular disease, E%: percentage of energy intake, EAA: essential amino acids, eGFR: estimated glomerular filtration rate, IG: intervention group, ITT: intention-to-treat analysis or modified intention-to-treat analysis (mITT: only those with missing outcome data were excluded from the analytic sample), MD: mean difference (i.e. difference in within-group change), MNA: Mini Nutritional Assessment, mo: months, ms: millisecond, MVC: maximal voluntary contraction, *n*: number, N: Newton, Nm: Newton-metre, NR: not reported, NS: not significant, PP: per-protocol analysis, reps: repetitions, RM: repetition maximum, SD: standard deviation, SEM: standard error of the mean, WBR: whole-body resistance training, wk: weeks, y: years.

Footnotes:

§ Sufficient statistical power to detect an effect is to be expected, based on the sample size calculation.

a 'Protein type' indicates the way in which a higher protein intake was achieved and is categorised into 'pure' protein or amino acids (or essential amino acids) (A), specific product with a high protein content (B), or high-protein diets (C).

b Risk of bias was assessed using the RoB 2 tool and scored as 'low' (L), 'some concerns' (SC), or 'high' (H). A more detailed explanation of the overall judgment can be found in Annex F.

c Total strength was calculated as the sum of chest press, knee extension and preacher curl strength (kg).

d Lower limb muscle quality index was calculated as knee extension strength divided by lower limb lean soft tissue.

e Upper limb muscle quality index was calculated as preacher curl strength divided by upper limb lean soft tissue.

f Total muscle quality index was calculated as total strength divided by skeletal muscle mass.

g Intention-to-treat analysis demonstrated a similar pattern to the per-protocol analysis (data not shown).

Table E3. Results from randomised controlled trials on the effect of increased protein intake on physical function in older adults, grouped according to whether or not the protein intervention was carried out in the context of (concomitant) physical exercise

Study reference, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Not in the context of (concomitant) physical exercise										
Bhasin et al. 2018,[51] USA	Community-dwelling older men aged ≥65 with moderate physical function limitations and with habitual protein intake ≤0.83 g/kg BW/d; mean BMI: 30.3 ± 4.9 kg/m^2	Individualised diets providing 0.7 g protein/kg BW/d with additional discretionary foods (0.1 g protein/kg BW) and protein supplements (0.5 g/kg BW) to achieve a total of 1.3 g/kg BW/d, [A,B]	Individualised diets providing 0.7 g protein/kg BW/d with additional discretionary foods (0.1 g protein/kg BW) and placebo supplements (0.5 g CHO/kg BW) to achieve a total of 0.8 g/kg BW/d	Foods (4-6 mo): IG: 77.1 ± 13%, CG: 74.5 ± 23.2% Supplements (4-6 mo): IG: 91.2 ± 12.4%, CG: 92.6 ± 11.0%	Baseline: IG: 0.72 ± 0.11, CG: 0.69 ± 0.15 Follow-up (1-3 mo): IG: 1.18 ± 0.15, CG: 0.84 ± 0.07 Follow-up (4-6 mo): IG: 1.17 ± 0.13, CG: 0.81 ± 0.10	6 mo	Some concerns	Gait speed, 6-min at fast pace (m)	40/37 (mITT)	MD (95%-CI): -0.89 (-32.1 to +30.4) P=0.96
								Gait speed, 50-m at fast pace, loaded (m/s)	34/32 (mITT)	MD (95%-CI): -0.01 (-0.10 to +0.07) P=0.81
								Stair climb power, 12 steps, unloaded at fast pace (W)	36/33 (mITT)	MD (95%-CI): -28.3 (-59.8 to +3.2) P=0.08
								Stair climb power, 12 steps, loaded at fast pace (W)	33/33 (mITT)	MD (95%-CI): -11.5 (-46.5 to +23.5) P=0.52
								Perceived physical function (SF-36)	42/40 (mITT)	MD (95%-CI): -1.98 (-9.11 to +5.15) P=0.58
Ispoglou et al. 2016,[41] UK	Community-dwelling older men and women aged 65-75; good health, without major chronic diseases (e.g. diabetes, vascular disease, hypertension)	EAA. Standard EAA mixture with 20% leucine (IG1) and with 40% leucine (IG2), ingested at breakfast and dinner (total: 0.21 g/kg BW/d = ~11-21 g/d), [A]	CHO placebo. Isocaloric amount of lactose, ingested at breakfast and dinner	74-83%	Baseline: 0.95-1.10 g/kg BW/d Follow-up: 1.02-1.08 g/kg BW/d (without supplementation)	12 wk	High	Gait speed, 6-min (m)	8/8/9 (PP)	Mean % change ± SD: IG1: +8.8 ± 10.0 IG2: +5.8 ± 6.6 CG: +1.4 ± 4.5 P=0.132
								30-s chair-stand test (n)	8/8/9 (PP)	Mean % change ± SD: IG1: +11.0 ± 11.5 IG2: +13.2 ± 16.0 CG: +4.7 ± 15.7 P=0.470

Study reference, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Mitchell et al. 2017,[48] New Zealand	Community-dwelling older men aged >70; able to perform ADLs without mobility aids; without major chronic diseases (e.g. cancer, diabetes, thyroid diseases)	High-protein diet (2*RDA = 1.6 g/kg BW/d), omnivorous, 28-31 E% fat, [C]	Low-protein diet (1*RDA = 0.8 g/kg BW/d), omnivorous, 28-31 E% fat; difference made up of CHO	IG: 97.5%, CG: 98.9%	Baseline: IG: 1.1 ± 0.3, CG: 1.2 ± 0.4 Follow-up: IG: 1.7 ± 0.1, CG: 0.9 ± 0.1	10 wk	High	SPPB (score)	14/15 (PP)	P for time*group interaction=0.185
								TUG (s)	14/15 (PP)	P for time*group interaction=0.313
Ottestad et al. 2017,[55] Norway	Community-dwelling older adults aged ≥70; relatively healthy (no diabetes, CVD, cancer, COPD, CKD); not malnourished; with reduced muscle strength or performance	Protein-enriched milk. 400 ml drink containing 20 g protein, consumed twice a day (total: 40 g protein/d), [B]	CHO placebo. 400 ml drink containing an isocaloric amount of CHO, consumed twice a day	IG: 97.8 ± 3.8%, CG: 96.8 ± 5.7%	Baseline: IG: 1.0 ± 0.3, CG: 1.0 ± 0.3 Follow-up: IG: 1.4 ± 0.5, CG: 0.9 ± 0.4	12 wk	High	Chair rise time, 5x (s)	16/17 (mITT)	Mean change (95%-CI): IG: -0.2 (-1.2 to +0.7) CG: -0.3 (-1.2 to +5.4) P=0.83
								Stair climb time, 20 steps, unloaded (s)	16/15 (mITT)	Mean change (95%-CI): IG: -0.4 (-0.8 to -0.1) CG: 0.0 (-0.7 to +0.7) P=0.22
								Stair climb time, 20 steps, 10-kg loaded (s)	16/15 (mITT)	Mean change (95%-CI): IG: -0.2 (-0.7 to +0.3) CG: -0.2 (-1.0 to +0.6) P=0.94
Park et al. 2018,[52] Korea	Community-dwelling (pre-)frail older adults aged 70-85 at risk of malnutrition (MNA ≤23.5); no kidney or liver failure; able to walk	Whey protein. Multiple 10-g packs of protein powder (9.3 g whey protein/pack), dissolved in 340 ml tea, were provided in addition to habitual protein intake up to 1.2 g/kg BW/d (IG1) and 1.5 g/kg BW/d (IG2), [A]	CHO placebo. Multiple 10-g packs of CHO powder (9.3 g maltodextrin/ pack), dissolved in 340 ml tea, were provided in addition to habitual protein intake up to 0.8 (CG) or 1.2 (IG1) g/ kg BW/d (CG were given only CHO powder, IGs were given a combination of protein and CHO powder.)	IG1: 98%, IG2: 96%, CG: 97%	Baseline: IG1: 0.77 ± 0.24, IG2: 0.80 ± 0.21, CG: 0.84 ± 0.28 Follow-up: IG1: 1.18 ± 0.23, IG2: 1.37 ± 0.26, CG: 0.90 ± 0.38	12 wk	Some concerns	SPPB (score)	40/40/40 (ITT)	P for time*group interaction=0.365
								Gait speed, 4-m (m/s)	40/40/40 (ITT)	**P for time*group interaction=0.007** (faster gait speed at 12 wk in IG2, but not IG1, than in CG)
								Standing balance	40/40/40 (ITT)	P for time*group interaction=0.319
								Chair rise time, 5x (s)	40/40/40 (ITT)	P for time*group interaction=0.881
								TUG (s)	40/40/40 (ITT)	P for time*group interaction=0.207

Study reference, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Zhu et al. 2015,[58] Australia Same study as Hodgson et al. 2012[59] and Zhu et al. 2011[60]	Community-dwelling older women aged 70-80 with habitual protein intake <1.5 g/kg BW/d; without metabolic bone disease, osteoporotic fracture, diabetes, hepatic or renal insufficiency	Whey protein isolate. 250 ml skim milk-based high-protein supplement drink containing 30 g of whey protein + calcium, [A]	CHO placebo. 250 ml skim milk-based supplement drink containing 2.1 g of protein and isocaloric amount of maltodextrin + calcium	IG: 87.1%, CG: 80.8 % (P=0.03)	Baseline: IG: 1.2 ± 0.3, CG: 1.1 ± 0.3 Follow-up (1 y): IG: 1.4 ± 0.4, CG: 1.1 ± 0.3 Follow-up (2 y): IG: 1.4 ± 0.4, CG: 1.1 ± 0.4	1 y and 2 y	Some concerns	TUG (s)	1 y: 99/94 (mITT) 2 y: 93/88 (mITT)	Mean change ± SEM: IG: -0.14 ± 0.13 CG: -0.17 ± 0.15 Time*group interaction NS Mean change ± SEM: IG: -0.46 ± 0.12 CG: -0.55 ± 0.12 Time*group interaction NS
In the context of (concomitant) physical exercise										
Arnarson et al. 2013,[49] Iceland Same study as Ramel et al. 2013[50]	Community-dwelling older men and women aged ≥65; without major orthopaedic disease or musculoskeletal disorders	Whey protein. Drink containing 20 g of whey protein isolate (+ 20 g of CHO), consumed after WBR (so only on training days) WBR, 3 times/wk, 3 sets of 6-8 reps, 75-80% 1RM	CHO placebo. Drink containing 40 g of CHO, consumed after WBR (so only on training days) WBR, 3 times/wk, 3 sets of 6-8 reps, 75-80% 1RM	NR	Baseline: IG: 1.00 ± 0.26, CG: 0.92 ± 0.30 Follow-up: IG: 1.06 ± 0.23, CG 0.89 ± 0.23	12 wk	High	Gait speed, 6-min (m) TUG (s)	75/66 75/66	Mean change ± SD: IG: +35.1 ± 38.0 CG: +39.9 ± 75.9 P=0.726 Mean change ± SD: IG: -0.6 ± 1.7 CG: -0.5 ± 0.8 P=0.151
Chalé et al. 2013,[54] USA	Community-dwelling, mobility-limited, sedentary older women aged 70-85	Whey protein concentrate: 20 g consumed after breakfast and 20 g consumed after evening meal each day (total: 40 g protein/d). On training days, one serving was consumed immediately after WBR, [A] WBR, 3 times/wk, 10 reps, 80% 1RM	CHO placebo. Isocaloric amount consumed after breakfast and after evening meal each day. On training days, one serving was consumed immediately after WBR. WBR, 3 times/wk, 10 reps, 80% 1RM	IG: 72.1 ± 29.3%, CG: 82.3 ± 21.9%	Baseline: IG: 0.97, CG: 0.98 Follow-up: NR	6 mo	Some concerns	Gait speed, 400-m[§] (m/s) Stair climb time[§] (s) Chair rise time, 10x[§] (s) SPPB[§] (score)	42/38 (ITT) 42/38 (ITT) 42/38 (ITT) 42/38 (ITT)	MD (95%-CI): +0.08 (-0.02 to +0.19) NS MD (95%-CI): +0.3 (-1.1 to +1.8) NS MD (95%-CI): -1.9 (-5.2 to +1.4) NS MD (95%-CI): +0.21 (-0.41 to +0.83) NS

Study reference, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Nabuco et al. 2018,[43] Brazil Same study as Nabuco et al. 2019a[44] and Nabuco et al. 2019b[45]	Older women aged ≥60, physically independent, free from cardiac or orthopaedic dysfunction	Whey protein. 35 g of hydrolysed whey protein supplement containing 27.1 g protein (+ 5.2 g CHO), mixed with non-caloric drink. IG1: whey protein before and placebo after WBR; IG2: placebo before and whey protein after WBR (so only on training days), [A] WBR, 3 times/wk, 3 sets of 8-12 reps	CHO placebo containing 0.3 g protein and 33.3 g CHO, mixed with non-caloric drink; one before and one after WBR (so only on training days) WBR, 3 times/wk, 3 sets of 8-12 reps	NR	Baseline: IG1: 0.92 ± 0.20, IG2: 0.94 ± 0.36, CG: 0.95 ± 0.27 Follow-up: IG1: 1.38 ± 0.26, IG2: 1.49 ± 0.46, CG: 1.0 ± 0.25	12 wk	Some concerns	Gait speed, 10-m at fast pace (s)	22/21/23 (mITT)	Mean % change ± SD: IG1: -10.8 ± 11.3* IG2: -11.8 ± 8.6* CG: -4.3 ± 8.4 *** P<0.05 compared to CG**
								Chair rise time, 5x (s)	22/21/23 (mITT)	Mean % change ± SD: IG1: -10.0 ± 12.4 IG2: -10.1 ± 5.4 CG: -5.7 ± 7.6 P for time*group interaction=0.176
Nabuco et al. 2019c,[42] Brazil	Older women aged ≥60 with sarcopenic obesity; physically independent; free from cardiac or orthopaedic dysfunction	Whey protein. 35 g of hydrolysed whey protein supplement, mixed with non-caloric drink, ingested after WBR (so only on training days), [A] WBR, 3 times/wk, 3 sets of 8-12 reps	CHO placebo. Isocaloric amount of maltodextrin mixed with non-caloric drink, ingested after WBR (so only on training days) WBR, 3 times/wk, 3 sets of 8-12 reps	NR	Baseline: IG: 0.93 ± 0.36, CG: 0.97 ± 0.28 Follow-up: IG: 1.0 ± 0.23, CG: 1.0 ± 0.19 (without supplementation)	12 wk	Some concerns	Gait speed, 10-m (s)	13/13 (ITT)	Mean % change: IG: -6.7 CG: -7.6 P for time*group interaction=0.792
								Chair rise time, 5x (s)	13/13 (ITT)	Mean % change: IG: -11.5 CG: -10.1 P for time*group interaction=0.694

Study reference, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Ten Haaf et al. 2019,[53] The Netherlands	Physically active older adults aged ≥65 with habitual protein intake <1.0 g/kg BW; without type 2 diabetes, cancer, renal insufficiency (eGFR <30) or COPD	Milk-protein concentrate. 250-ml protein drink containing 15.5 g protein (+ 1.1 g fat + 14.5 g lactose) consumed twice a day (total: 31 g protein/d), [A] Training (walking) for the Nijmegen Four Days Marches	CHO placebo. 250-ml isocaloric drink containing 1.1 g protein (+ 5.2 g fat + 36 g CHO) consumed twice daily Training (walking) for the Nijmegen Four Days Marches	IG: 96 ± 3%, CG: 95 ± 3%	Baseline: IG: 0.86 ± 0.23, CG: 0.92 ± 0.24 Follow-up: IG: 0.92 ± 0.27, CG: 0.97 ± 0.23 (without supplementation)	12 wk	Some concerns	SPPB[§] (score)	58/56 (PP)	Median change (IQR): IG: 0 (0 to +1) CG: 0 (0 to 0) P for time*group interaction=0.73
								Standing balance[§] (score)	58/56 (PP)	Median change (IQR): IG: 0 (0 to 0) CG: 0 (0 to 0) P for time*group interaction=1.00
								Gait speed, 4-m at usual pace[§] (s)	58/56 (PP)	Mean change ± SD: IG: -0.2 ± 0.5 CG: -0.2 ± 0.4 P for time*group interaction=0.95
								Chair rise time[§], 5x (s)	111 (total; PP)	Mean change ± SD: IG: -0.8 ± 2.2 CG: -0.7 ± 1.9 P for time*group interaction=0.86
								TUG[§] (s)	58/56 (PP)	Mean change ± SD: IG: -0.4 ± 0.9 CG: -0.5 ± 0.6 P for time*group interaction=0.50

Study reference, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Thomson et al. 2016,[57] Australia	Older adults aged 50-79 and BMI of 20-35 kg/m^2 who are physically active (but not engaged in formal exercise); without major chronic diseases (e.g. diabetes, cancer, metabolic disease, cardiac abnormalities, musculoskeletal injury)	High-protein diet. Individualised diet providing 1.0 g/kg BW/d + ~27 g dairy-based protein drink each day (IG1); Individualised diet providing 1.0 g/kg BW/d + ~27 g soy protein drink each day (IG2), [B] WBR, 3 times/wk, 1-3 sets of 8-20 reps, 8RM	CHO placebo. Individualised diet providing 1.0 g/kg BW/d + ~27 g CHO drink each day WBR, 3 times/wk, 1-3 sets of 8-20 reps, 8RM	IG1: 97%, IG2: 98%, CG: 98%	Baseline: IG1: 1.06 ± 0.10, IG2: 1.08 ± 0.09, CG: 1.02 ± 0.05 Follow-up: IG1: 1.42 ± 0.14, IG2: 1.45 ± 0.14, CG: 1.08 ± 0.05	12 wk	High	Gait speed, 6-min (m)	34/26/23 (PP[c])	Mean change ± SD: IG1: +36.5 ± 35.9 IG2: +25.1 ± 35.5 CG: +19.2 ± 54.1 Between-group difference NS

Statistically significant effects are shown in bold.

Abbreviations: ADL: activities of daily living, BMI: body mass index, BW: body weight, CI: confidence interval, CG: control group, CHO: carbohydrates, CKD: chronic kidney disease, COPD: chronic obstructive pulmonary disease, CVD: cardiovascular disease, E%: percentage of energy intake, EAA: essential amino acids, eGFR: estimated glomerular filtration rate, IG: intervention group, ITT: intention-to-treat analysis or modified intention-to-treat analysis (mITT, only those with missing outcome data were excluded from the analytic sample), m: metres, MD: mean difference (i.e. difference in within-group change), MNA: Mini Nutritional Assessment, mo: months, *n*: number, NR: not reported, NS: not significant, PEDro: Physiotherapy Evidence Database scale, PP: per-protocol analysis, reps: repetitions, RM: repetition maximum, s: seconds, SD: standard deviation, SEM: standard error of the mean, SF-36: 36-item Short Form Healthy Survey: physical functioning domain, SPPB: short physical performance battery, TUG: timed up-and-go, WBR: whole-body resistance training, wk: weeks, y: years.

Footnotes:

[a] 'Protein type' indicates the way in which a higher protein intake was achieved and is categorised into 'pure' protein or amino acids (or essential amino acids) (A), specific product with a high protein content (B), or high-protein diets (C).

[b] Risk of bias was assessed using the RoB 2 tool and scored as 'low' (L), 'some concerns' (SC), or 'high' (H). A more detailed explanation of the overall judgment can be found in Annex F.

[c] Intention-to-treat analysis demonstrated a similar pattern to the per-protocol analysis (data not shown).

Table E4. Results from randomised controlled trials on the effect of increased protein intake on bone health in older adults, grouped according to whether or not the protein intervention was carried out in the context of (concomitant) physical exercise

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Not in the context of (concomitant) physical exercise										
Ispoglou et al. 2016,[41] UK	Community-dwelling older men and women aged 65-75; good health, without major chronic diseases (e.g. diabetes, vascular disease, hypertension)	EAA. Standard EAA mixture with 20% leucine (IG1) and with 40% leucine (IG2), ingested at breakfast and dinner (total: 0.21 g/kg BW/d = ~11-21 g/d), [A]	CHO placebo. Isocaloric amount of lactose, ingested at breakfast and dinner	74-83%	Baseline: 0.95-1.10 g/kg BW/d Follow-up: 1.02-1.08 g/kg BW/d (without supplementation)	12 wk	High	Total BMC (kg; DXA)	8/8/9 (PP)	Mean % change ± SD: IG1: +0.1 ± 1.4 IG2: +0.0 ± 1.0 CG: +0.4 ± 1.0 NS
								Total BMD (g/cm^2; DXA)	8/8/9 (PP)	Mean % change ± SD: IG1: +0.2 ± 1.2 IG2: +0.3 ± 1.6 CG: -0.4 ± 1.1 NS
Kerstetter et al. 2015,[56] USA	Older men (aged >70) and women (aged >60) with BMI of 19-32 kg/m^2 and protein intake of 0.6-1.0 g/kg BW; without major chronic diseases (e.g. diabetes, renal disease, inflammatory bowel disease) or cancer within past 18 months	Whey protein. 45 g of whey protein isolate (~40 g of protein) + vitamin D (400 IU) + calcium (1200 mg), [A]	CHO placebo. Isocaloric amount of maltodextrin + vitamin D (400 IU) + calcium (1200 mg)	NR	Baseline: IG: 1.07 ± 0.03, CG: 1.06 ± 0.03 Follow-up: IG: 1.30 ± 0.05, CG: 1.05 ± 0.04	9 mo and 18 mo	Some concerns	BMD lumbar spine[§] (g/cm^2; DXA)	9 mo: 105/102 (mITT) 18 mo: 105/102 (mITT)	LSMD (95%-CI): -0.001 (-0.012 to +0.010) NS LSMD (95%-CI): +0.002 (-0.011 to +0.014) NS

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
								BMD total hip§ (g/cm^2; DXA)	9 mo: 106/102 (mITT)	LSMD (95%-CI): -0.001 (-0.007 to +0.005) NS
									18 mo: 106/102 (mITT)	LSMD (95%-CI): +0.001 (-0.007 to +0.009) NS
								BMD femoral neck§ (g/cm^2; DXA)	9 mo: 106/102 (mITT)	LSMD (95%-CI): +0.004 (-0.004 to +0.012) NS
									18 mo: 106/102 (mITT)	LSMD (95%-CI): +0.006 (-0.004 to +0.016) NS
								BMD lumbar spine§ (mg/cm^3; QCT)	18 mo: 45/44 (mITT)	LSMD (95%-CI): +4.151 (-0.169 to +8.470) NS
								BMD femoral neck, cortical§ (mg/cm^3; QCT)	18 mo: 45/44 (mITT)	LSMD (95%-CI): -0.863 (-50.756 to +49.029) NS
								BMD femoral neck, trabecular§ (mg/cm^3; QCT)	18 mo: 45/44 (mITT)	LSMD (95%-CI): -0.953 (-5.054 to +3.147) NS
								BMD femoral total, cortical§ (mg/cm^3; QCT)	18 mo: 45/44 (mITT)	LSMD (95%-CI): +1.926 (-29.754 to +33.606) NS

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
								BMD femoral total, trabecular[§] (mg/cm^3; QCT)	18 mo: 45/44 (mITT)	LSMD (95%-CI): -0.421 (-3.900 to +3.058) NS
								Serum P1NP (nmol/L)	9 mo: 61/60 (mITT)	**P=0.0007** (improvement in IG compared to CG)
									18 mo: 61/60 (mITT)	P=0.3952
								Serum CTX (ng/L)	9 mo: 61/60 (mITT)	P=0.0206 (improvement in IG compared to CG)
									18 mo: 61/60 (mITT)	**P=0.0414**
								Serum OC (nmol/L)	9 mo: 61/60 (mITT)	P=0.3332
									18 mo: 61/60 (mITT)	P=0.7747

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Zhu et al. 2011,[60] Australia Same study as Hodgson et al. 2012[59] and Zhu et al. 2015[58]	Community-dwelling older women aged 70-80 with habitual protein intake <1.5 g/kg BW/d; without metabolic bone disease, osteoporotic fracture, diabetes, hepatic or renal insufficiency	Whey protein isolate. 250 ml skim milk-based high-protein supplement drink containing 30 g of whey protein + calcium, [A]	CHO placebo. 250 ml skim milk-based supplement drink containing 2.1 g of protein and isocaloric amount of maltodextrin + calcium	IG: 87.1%, CG: 80.8 % (P=0.03)	Baseline: IG: 1.2 ± 0.3, CG: 1.1 ± 0.3 Follow-up: IG: 1.4 ± 0.4, CG: 1.1 ± 0.4	1 y and 2 y	Some concerns	Total hip aBMD§ (mg/cm²; DXA)	1 y: 101/91 (mITT) 2 y: 95/88 (mITT)	Time*group interaction NS Time*group interaction NS
								Femoral neck aBMD§ (mg/cm²; DXA)	1 y: 101/91 (mITT) 2 y: 95/88 (mITT)	Time*group interaction NS Time*group interaction NS
								Total hip volumetric BMD§ (mg/cm³; QCT)	67/66 (2 y; mITT)	Mean change ± SE: IG: -3.63 ± 1.10 CG: -3.82 ± 1.43 Time*group interaction NS
								Femoral neck vBMD§ (mg/cm²; QCT)	67/66 (2 y; mITT)	Mean change ± SE: IG: -2.39 ± 1.25 CG: -0.24 ± 1.19 Time*group interaction NS
								Femoral neck bone CSA§ (cm²; QCT)[c]	67/66 (2 y; mITT)	Mean change ± SE: IG: -0.04 ± 0.06 CG: -0.03 ± 0.06 Time*group interaction NS
								Femoral neck buckling ratio§ (QCT)[d]	67/66 (2 y; mITT)	Mean change ± SE: IG: +0.04 ± 0.09 CG: +0.07 ± 0.09 Time*group interaction NS
								Femoral neck polar CSMI§ (cm⁴; QCT)[e]	67/66 (2 y; mITT)	Mean change ± SE: IG: -0.06 ± 0.07 CG: -0.06 ± 0.08 Time*group interaction NS

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
In the context of (concomitant) physical exercise										
Fernandes et al. 2018,[40] Brazil Same study as Sugihara Junior et al. 2018[46]	Older women aged ≥60; physically independent; free from cardiac or orthopaedic dysfunction; protein intake <1.2 g/kg BW	Whey protein. 35 g of hydrolysed whey protein containing 27.1 g of protein, dissolved in 200 ml sugar-free soft drink, ingested after WBR (so only on training days), [A] WBR, 3 times/wk, 3 sets of 8-12RM	CHO placebo. 35 g of maltodextrin, dissolved in 200 ml sugar-free soft drink, ingested after WBR (so only on training days) WBR, 3 times/wk, 3 sets of 8-12RM	NR	Baseline: IG: 0.85 ± 0.1, CG: 0.81 ± 0.1 Follow-up: IG: 1.4 ± 0.1, CG: 0.87 ± 0.1	12 wk	High	Total BMC (kg; DXA)	16/16	Mean % change: IG: +1.3 CG: +0.8 P for time*group interaction=0.76

Statistically significant effects are shown in bold.

Abbreviations: aBMD: areal BMD, BMC: bone mineral content, BMD: bone mineral density, BMI: body mass index, BW: body weight, CI: confidence interval, CG: control group, CHO: carbohydrates, CSA: cross-sectional area, CSMI: cross-sectional moment of inertia, CTX: C-terminal telopeptide of type 1 collagen, DXA: dual-energy X-ray absorptiometry, EAA: essential amino acids, IG: intervention group, ITT: intention-to-treat analysis or modified intention-to-treat analysis (mITT, only those with missing outcome data were excluded from the analytic sample), IU: international units, LBM: lean body mass, LSMD: least square mean difference, MD: mean difference (i.e. difference in within-group change), mg: milligram, mo: months, ng: nanogram, nmol: nanomole, *n*: number, NR: not reported, NS: not significant, OC: osteocalcin, P1NP: N-terminal propeptides of type 1 procollagen, PP: per-protocol analysis, QCT: quantitative computed tomography, RM: repetition maximum, SD: standard deviation, SEM: standard error of the mean, vBMD: volumetric BMD, WBR: whole-body resistance training, wk: weeks, y: years.

Footnotes:

[a] 'Protein type' indicates the way in which a higher protein intake was achieved and is categorised into 'pure' protein or amino acids (or essential amino acids) (A), specific product with a high protein content (B), or high-protein diets (C).

[b] Risk of bias was assessed using the RoB 2 tool and scored as 'low' (L), 'some concerns' (SC), or 'high' (H). A more detailed explanation of the overall judgment can be found in Annex F.

[c] Neck bone cross-sectional area (CSA) relates to strength in compression.

[d] Buckling ratio relates to strength in buckling.

[e] Polar cross-sectional moment of inertia (CSMI) relates to strength in torsion.

Table E5. Results from randomised controlled trials on the effect of increased protein intake on blood pressure in older adults, grouped according to whether or not the protein intervention was carried out in the context of (concomitant) physical exercise

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Not in the context of (concomitant) physical exercise										
Hodgson et al. 2012,[59] Australia Same study as Zhu et al. 2011[60] and Zhu et al. 2015[58]	Community-dwelling older women aged 70-80 with habitual protein intake <1.5 g/kg BW/d; without metabolic bone disease, osteoporotic fracture, diabetes, hepatic or renal insufficiency	Whey protein isolate. 250 ml skim milk-based high-protein supplement drink containing 30 g of whey protein + calcium, [A]	CHO placebo. 250 ml skim milk-based supplement drink containing 2.1 g of protein and isocaloric amount of maltodextrin + calcium	1 y (n=196): IG: 78 ± 29%, CG: 72 ± 31% 2 y (n=181): IG: 88 ± 25%, CG: 81 ± 25%	Baseline: IG: 1.2 ± 0.3, CG: 1.1 ± 0.3 Follow-up (2 y): IG: 1.4 ± 0.4, CG: 1.1 ± 0.4	1 y and 2 y	Some concerns	Systolic BP[§] (mm Hg)	1 y: 109/110 (mITT[c]) 2 y: 109/110 (mITT[c])	MD (95%-CI): -2.3 (-5.3 to +0.7) P=0.14 MD (95%-CI): +1.6 (-1.5 to +4.7) P=0.30
								Diastolic BP[§] (mm Hg)	1 y: 109/110 (mITT[c]) 2 y: 109/110 (mITT[c])	MD (95%-CI): -1.5 (-3.6 to +0.6) P=0.15 MD (95%-CI): +0.3 (-1.9 to +2.4) P=0.82
Wright et al. 2018,[47] USA	Older adults aged 50-80 with overweight or obesity (BMI of 25-38 kg/m^2); without diabetes	High-protein diet: 1.4 g/kg BW/d (~27 E% protein, ~43 E% CHO, ~30 E% fat). Majority of additional protein (59%) came from eggs (3 eggs/d), [C]	Normal-protein diet: 0.8 g/kg BW/d (~15 E% protein, ~55 E% CHO, ~30 E% fat) (Normal-protein diet provided on average ~50 g/d less protein than high-protein diet.)	91% (overall)	Baseline: IG: 84 ± 15 g/d, CG: 79 ± 15 g/d (calculated by using mean BW: IG: 0.93 g/kg BW/d, CG: 0.88 g/kg BW/d Follow-up: NR	12 wk	High	Systolic BP (mm Hg)	12/10 (PP)	Mean change ± SD: IG: -7 ± 13 CG: -2 ± 12 Time*group interaction NS
								Diastolic BP (mm Hg)	12/10 (PP)	Mean change ± SD: IG: -5 ± 5 CG: -2 ± 6 Time*group interaction NS

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
In the context of (concomitant) physical exercise										
Nabuco et al. 2019a,[44] Brazil Same study as Nabuco et al. 2018[43] and Nabuco et al. 2019b[45]	Older women aged ≥60, physically independent, free from cardiac or orthopaedic dysfunction	Whey protein. 35 g of hydrolysed whey protein supplement containing 27.1 g protein (+ 5.2 g CHO), mixed with non-caloric drink. IG1: protein before and placebo after WBR; IG2: placebo before and protein after WBR (so only on training days), [A] WBR, 3 times/wk, 3 sets of 8-12 reps	CHO placebo containing 0.3 g protein and 33.3 g CHO, mixed with non-caloric drink; one before and one after WBR (so only on training days) WBR, 3 times/wk, 3 sets of 8-12 reps	NR	Baseline: IG1: 0.92 ± 0.20, IG2: 0.94 ± 0.36, CG: 0.95 ± 0.27 Follow-up: IG1: 1.38 ± 0.26, IG2: 1.49 ± 0.46, CG: 1.0 ± 0.25	12 wk	Some concerns	Systolic BP (mm Hg)	22/21/23 (mITT)	Mean % change: IG1: -2.1 IG2: -0.1 CG: -3.8 P for time*group interaction=0.304
								Diastolic BP (mm Hg)	22/21/23 (mITT)	Mean % change: IG1: -1.1 IG2: +0.5 CG: -3.7 P for time*group interaction=0.178
Nabuco et al. 2019c,[42] Brazil	Older women aged ≥60 with sarcopenic obesity; physically independent; free from cardiac or orthopaedic dysfunction	Whey protein. 35 g of hydrolysed whey protein supplement, mixed with non-caloric drink, ingested after WBR (so only on training days), [A] WBR, 3 times/wk, 3 sets of 8-12 reps	CHO placebo. Isocaloric amount of maltodextrin mixed with non-caloric drink, ingested after WBR (so only on training days) WBR, 3 times/wk, 3 sets of 8-12 reps	NR	Baseline IG: 0.93 ± 0.36, CG: 0.97 ± 0.28 Follow-up: IG: 1.0 ± 0.23, CG: 1.0 ± 0.19 (without supplementation)	12 wk	Some concerns	Systolic BP (mm Hg)	13/13 (ITT)	Mean % change: IG: -0.5 CG: +1.5 P for time*group interaction=0.451
								Diastolic BP (mm Hg)	13/13 (ITT)	Mean % change: IG: -1.9 CG: -3.0 P for time*group interaction=0.702

Statistically significant effects are shown in bold.
Abbreviations: BMI: body mass index, BW: body weight, CI: confidence interval, CG: control group, CHO: carbohydrates, E%: percentage of energy intake, EAA: essential amino acids, IG: intervention group, ITT: intention-to-treat analysis or modified intention-to-treat analysis (mITT, only those with missing outcome data were excluded from the analytic sample), MD: mean difference (i.e. difference in within-group change), *n*: number, NR: not reported, NS: not significant, PP: per-protocol analysis, reps: repetitions, SD: standard deviation, WBR: whole-body resistance training, wk: weeks, y: years.
Footnotes:
[a] 'Protein type' indicates the way in which a higher protein intake was achieved and is categorised into 'pure' protein or amino acids (or essential amino acids) (A), specific product with a high protein content (B)\, or high-protein diets (C).
[b] Risk of bias was assessed using the RoB 2 tool and scored as 'low' (L), 'some concerns' (SC), or 'high' (H). A more detailed explanation of the overall judgment can be found in Annex F.
[c] Per-protocol analysis demonstrated similar results to intention-to-treat analysis (data available).

Table E6. Results from randomised controlled trials on the effect of increased protein intake on glucose and insulin metabolism in older adults, grouped according to whether or not the protein intervention was carried out in the context of (concomitant) physical exercise

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Not in the context of (concomitant) physical exercise										
Ottestad et al. 2017,[55] Norway	Community-dwelling older adults aged ≥70; relatively healthy (no diabetes, CVD, cancer, COPD, CKD); not malnourished; with reduced muscle strength or performance	Protein-enriched milk. 400 ml drink containing 20 g protein, consumed twice a day (total: 40 g protein/d), [B]	CHO placebo. 400 ml drink containing an isocaloric amount of CHO, consumed twice a day	IG: 97.8 ± 3.8%, CG: 96.8 ± 5.7%	Baseline: IG: 1.0 ± 0.3, CG: 1.0 ± 0.3 Follow-up: IG: 1.4 ± 0.5, CG: 0.9 ± 0.4	12 wk	High	Fasting blood glucose (mmol/L)	17/18 (mITT)	Mean change (95%-CI): IG: -0.1 (-0.3 to +0.2) CG: +0.1 (-0.1 to +0.3) P=0.36
Park et al. 2018,[52] Korea	Community-dwelling (pre-)frail older adults aged 70-85 at risk of malnutrition (MNA ≤23.5); no kidney or liver failure; able to walk	Whey protein. Multiple 10-g packs of protein powder (9.3 g whey protein/pack), dissolved in 340 ml tea, were provided in addition to habitual protein intake up to 1.2 (IG1) or 1.5 (IG2) g/kg BW/d, [A]	CHO placebo. Multiple 10-g packs of CHO powder (9.3 g maltodextrin/pack), dissolved in 340 ml tea, were provided in addition to habitual protein intake up to 0.8 (CG) or 1.2 (IG1) g/kg BW/d. (CG were given only CHO powder, IGs were given a combination of protein and CHO powder.)	IG1: 98%, IG2: 96%, CG: 97%	Baseline: IG1: 0.77 ± 0.24, IG2: 0.80 ± 0.21, CG: 0.84 ± 0.28 Follow-up: IG1: 1.18 ± 0.23, IG2: 1.37 ± 0.26, CG: 0.90 ± 0.38	12 wk	Some concerns	Fasting blood glucose (mmol/L)	40/40/40 (ITT)	P for time*group interaction=0.315
Wright et al. 2018,[47] USA	Older adults aged 50-80 with overweight or obesity (BMI of 25-38 kg/m^2); without diabetes	High-protein diet: 1.4 g/kg BW/d (~27 E% protein, ~43 E% CHO, ~30 E% fat). Majority of additional protein (59%) came from eggs (3 eggs/d), [C]	Normal-protein diet: 0.8 g/kg BW/d (~15 E% protein, ~55 E% CHO, ~30 E% fat) (Normal-protein diet provided on average ~50 g/d less protein than high-protein diet.)	91% (overall)	Baseline: IG: 84 ± 15 g/d, CG: 79 ± 15 g/d (calculated by using mean BW: IG: 0.93 g/kg BW/d, CG: 0.88 g/kg BW/d Follow-up: NR	12 wk	High	Fasting blood glucose (mmol/L)	12/10 (PP)	Mean change ± SD: IG: -0.1 ± 0.4 CG: 0.0 ± 0.6 Time*group interaction NS
								Fasting insulin (mmol/L)	12/10 (PP)	Mean change ± SD: IG: -35.4 ± 34.7 CG: -8.3 ± 38.9 Time*group interaction NS
								HOMA-IR (score)	12/10 (PP)	Mean change ± SD: IG: -1.37 ± 1.51 CG: -0.35 ± 1.59 Time*group interaction NS

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
In the context of (concomitant) physical exercise										
Fernandes et al. 2018,[40] Brazil Same study as Sugihara Junior et al. 2018[46]	Older women aged ≥60; physically independent; free from cardiac or orthopaedic dysfunction; protein intake <1.2 g/kg BW	Whey protein. 35 g of hydrolysed whey protein containing 27.1 g of protein, dissolved in 200 ml sugar-free soft drink, ingested after WBR (so only on training days), [A] WBR, 3 times/wk, 3 sets of 8-12RM	CHO placebo. 35 g of maltodextrin, dissolved in 200 ml sugar-free soft drink, ingested after WBR (so only on training days) WBR, 3 times/wk, 3 sets of 8-12RM	NR	Baseline: IG: 0.85 ± 0.1, CG: 0.81 ± 0.1 Follow-up: IG: 1.4 ± 0.1, CG: 0.87 ± 0.1	12 wk	High	Fasting blood glucose (mg/dL)	16/16	Mean % change: IG: -3.3 CG: +4.0 P for time*group interaction=0.42
Nabuco et al. 2019a,[44] Brazil Same study as Nabuco et al. 2018[43] and Nabuco et al. 2019b[45]	Older women aged ≥60, physically independent, free from cardiac or orthopaedic dysfunction	Whey protein. 35 g of hydrolysed whey protein supplement containing 27.1 g protein (+ 5.2 g CHO), mixed with non-caloric drink. IG1: protein before and placebo after WBR; IG2: placebo before and protein after WBR (so only on training days), [A] WBR, 3 times/wk, 3 sets of 8-12 reps	CHO placebo containing 0.3 g protein and 33.3 g CHO, mixed with non-caloric drink; one before and one after WBR (so only on training days) WBR, 3 times/wk, 3 sets of 8-12 reps	NR	Baseline: IG1: 0.92 ± 0.20, IG2: 0.94 ± 0.36, CG: 0.95 ± 0.27 Follow-up: IG1: 1.38 ± 0.26, IG2: 1.49 ± 0.46, CG: 1.0 ± 0.25	12 wk	Some concerns	Fasting blood glucose (mg/dL)	22/21/23 (mITT)	Mean % change: IG1: -5.0 IG2: -0.2 CG: -0.2 P for time*group interaction=0.319
								Fasting insulin (µU/mL)	22/21/23 (mITT)	Mean % change: IG1: -4.1 IG2: +7.3 CG: +0.5 P for time*group interaction=0.125
								HOMA-IR (score)	22/21/23 (mITT)	Mean % change: IG1: -11.6 IG2: -18.8 CG: -8.1 P for time*group interaction=0.372

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Nabuco et al. 2019c,[42] Brazil	Older women aged ≥60 with sarcopenic obesity; physically independent; free from cardiac or orthopaedic dysfunction	Whey protein. 35 g of hydrolysed whey protein supplement, mixed with non-caloric drink, ingested after WBR (so only on training days), [A] WBR, 3 times/wk, 3 sets of 8-12 reps	CHO placebo. Isocaloric amount of maltodextrin mixed with non-caloric drink, ingested after WBR (so only on training days) WBR, 3 times/wk, 3 sets of 8-12 reps	NR	Baseline IG: 0.93 ± 0.36, CG: 0.97 ± 0.28 Follow-up: IG: 1.0 ± 0.23, CG: 1.0 ± 0.19 (without supplementation)	12 wk	Some concerns	Fasting blood glucose (mg/dL)	13/13 (ITT)	Mean % change: IG: -4.1 CG: -1.0 P for time*group interaction=0.251
								Fasting insulin (µU/mL)	13/13 (ITT)	Mean % change: IG: -4.9 CG: -1.9 P for time*group interaction=0.774
								HOMA-IR (score)	13/13 (ITT)	Mean % change: IG: -7.5 CG: -0.2 P for time*group interaction=0.511

Statistically significant effects are shown in bold.

Abbreviations: BMI: body mass index, BW: body weight, CG: control group, CHO: carbohydrates, CKD: chronic kidney disease, COPD: chronic obstructive pulmonary disease, CVD: cardiovascular disease, E%: percentage of energy intake, EAA: essential amino acids, HOMA-IR: homeostatic model assessment of insulin resistance, IG: intervention group, ITT: intention-to-treat analysis or modified intention-to-treat analysis (mITT, only those with missing outcome data were excluded from the analytic sample), MNA: Mini Nutritional Assessment, *n*: number, NR: not reported, NS: not significant, PP: per-protocol analysis, reps: repetitions, RM: repetition maximum, SD: standard deviation, WBR: whole-body resistance training, wk: weeks.

Footnotes:

[a] 'Protein type' indicates the way in which a higher protein intake was achieved and is categorised into 'pure' protein or amino acids (or essential amino acids) (A), specific product with a high protein content (B), or high-protein diets (C).

[b] Risk of bias was assessed using the RoB 2 tool and scored as 'low' (L), 'some concerns' (SC), or 'high' (H). A more detailed explanation of the overall judgment can be found in Annex F.

Table E7. Results from randomised controlled trials on the effect of increased protein intake on serum lipid profile in older adults, grouped according to whether or not the protein intervention was carried out in the context of (concomitant) physical exercise

Study reference, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Not in the context of (concomitant) physical exercise										
Bhasin et al. 2018,[51] USA	Community-dwelling older men aged ≥65 with moderate physical function limitations and with habitual protein intake ≤0.83 g/kg BW/d; mean BMI: 30.3 ± 4.9 kg/m^2	Individualised diets providing 0.7 g protein/kg BW/d with additional discretionary foods (0.1 g protein/kg BW) and protein supplements (0.5 g/kg BW) to achieve a total of 1.3 g/kg BW/d, [A,B]	Individualised diets providing 0.7 g protein/kg BW/d with additional discretionary foods (0.1 g protein/kg BW) and placebo supplements (0.5 g CHO/kg BW) to achieve a total of 0.8 g/kg BW/d	Foods (4-6 mo): IG: 77.1 ± 13%, CG: 74.5 ± 23.2% Supplements (4-6 mo): IG: 91.2 ± 12.4%, CG: 92.6 ± 11.0%	Baseline: IG: 0.72 ± 0.11, CG: 0.69 ± 0.15 Follow-up (1-3 mo): IG: 1.18 ± 0.15, CG: 0.84 ± 0.07 Follow-up (4-6 mo): IG: 1.17 ± 0.13, CG: 0.81 ± 0.10	6 mo	Some concerns	Total cholesterol (mg/dL)	40-46/ 38-46[c] (mITT)	MD (95%-CI): -4.45 (-13.10 to +4.19) P=0.308
								LDL cholesterol (mg/dL)	40-46/ 38-46[c] (mITT)	MD (95%-CI): -2.71 (-10.03 to +4.61) P=0.463
								HDL cholesterol (mg/dL)	40-46/ 38-46[c] (mITT)	MD (95%-CI): +1.95 (-0.67 to +4.56) P=0.142
								Triglycerides (mg/dL)	40-46/ 38-46[c] (mITT)	MD (95%-CI): -19.62 (-39.71 to +0.47) P=0.055
Ottestad et al. 2017,[55] Norway	Community-dwelling older adults aged ≥70; relatively healthy (no diabetes, CVD, cancer, COPD, CKD); not malnourished; with reduced muscle strength or performance	Protein-enriched milk. 400 ml drink containing 20 g protein, consumed twice a day (total: 40 g protein/d), [B]	CHO placebo. 400 ml drink containing an isocaloric amount of CHO, consumed twice a day	IG: 97.8 ± 3.8%, CG: 96.8 ± 5.7%	Baseline: IG: 1.0 ± 0.3, CG: 1.0 ± 0.3 Follow-up: IG: 1.4 ± 0.5, CG: 0.9 ± 0.4	12 wk	High	Total cholesterol (mmol/L)	16/18 (mITT)	Mean change (95%-CI): IG: -0.5 (-0.8 to -0.2) CG: -0.1 (-0.4 to +0.2) P=0.06
								LDL cholesterol (mmol/L)	16/18 (mITT)	Mean change (95%-CI): IG: -0.3 (-0.5 to -0.1) CG: -0.1 (-0.3 to +0.1) P=0.25
								HDL cholesterol (mmol/L)	17/18 (mITT)	Mean change (95%-CI): IG: 0.0 (-0.1 to +0.1) CG: 0.0 (-0.0 to +0.1) P=0.41
								Triglycerides (mmol/L)	16/18 (mITT)	Mean change (95%-CI): IG: -0.1 (-0.3 to 0.0) CG: +0.1 (-0.2 to +0.2) **P=0.05**

Study reference, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Park et al. 2018,[52] Korea	Community-dwelling (pre-)frail older adults aged 70-85 at risk of malnutrition (MNA ≤23.5); no kidney or liver failure; able to walk	Whey protein. Multiple 10-g packs of protein powder (9.3 g whey protein/ pack), dissolved in 340 ml tea, were provided in addition to habitual protein intake up to 1.2 (IG1) or 1.5 (IG2) g/kg BW/d, [A]	CHO placebo. Multiple 10-g packs of CHO powder (9.3 g maltodextrin/pack), dissolved in 340 ml tea, were provided in addition to habitual protein intake up to 0.8 (CG) or 1.2 (IG1) g/kg BW/d (CG were given only CHO powder, IGs were given a combination of protein and CHO powder.)	IG1: 98%, IG2: 96%, CG: 97%	Baseline: IG1: 0.77 ± 0.24, IG2: 0.80 ± 0.21, CG: 0.84 ± 0.28 Follow-up: IG1: 1.18 ± 0.23, IG2: 1.37 ± 0.26, CG: 0.90 ± 0.38	12 wk	Some concerns	Total cholesterol (mmol/L)	40/40/40 (ITT)	P for time*group interaction=0.478
								LDL cholesterol (mmol/L)	40/40/40 (ITT)	P for time*group interaction=0.887
								HDL cholesterol (mmol/L)	40/40/40 (ITT)	P for time*group interaction=0.363
								Triglycerides (mmol/L)	40/40/40 (ITT)	P for time*group interaction=0.837

Study reference, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Wright et al. 2018,[47] USA	Older adults aged 50-80 with overweight or obesity (BMI of 25-38 kg/m^2); without diabetes	High-protein diet: 1.4 g/kg BW/d (~27 E% protein, ~43 E% CHO, ~30 E% fat). Majority of additional protein (59%) came from eggs (3 eggs/d), [C]	Normal-protein diet: 0.8 g/kg BW/d (~15 E% protein, ~55 E% CHO, ~30 E% fat) (Normal-protein diet provided on average ~50 g/d less protein than high-protein diet.)	91% (overall)	Baseline: IG: 84 ± 15 g/d, CG: 79 ± 15 g/d (calculated by using mean BW: IG: 0.93 g/kg BW/d, CG: 0.88 g/kg BW/d Follow-up: NR	12 wk	High	Total cholesterol (mmol/L)	12/10 (PP)	Mean change ± SD: IG: -0.1 ± 0.6 CG: -0.2 ± 0.4 Time*group interaction NS
								LDL cholesterol (mmol/L)	12/10 (PP)	Mean change ± SD: IG: +0.1 ± 0.4 CG: -0.3 ± 0.3 **Time*group interaction<0.05**
								HDL cholesterol (mmol/L)	12/10 (PP)	Mean change ± SD: IG: -0.1 ± 0.2 CG: -0.1 ± 0.2 Time*group interaction NS
								Triglycerides (mmol/L)	12/10 (PP)	Mean change ± SD: IG: -0.3 ± 0.6 CG: +0.1 ± 0.4 Time*group interaction NS
								Total/HDL cholesterol ratio	12/10 (PP)	Mean change ± SD: IG: +0.14 ± 0.36 CG: -0.21 ± 0.56 Time*group interaction NS

Study reference, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
In the context of (concomitant) physical exercise										
Fernandes et al. 2018,[40] Brazil Same study as Sugihara Junior et al. 2018[46]	Older women aged ≥60; physically independent; free from cardiac or orthopaedic dysfunction; protein intake <1.2 g/kg BW	Whey protein. 35 g of hydrolysed whey protein containing 27.1 g of protein, dissolved in 200 ml sugar-free soft drink, ingested after WBR (so only on training days), [A] WBR, 3 times/wk, 3 sets of 8-12RM	CHO placebo. 35 g of maltodextrin, dissolved in 200 ml sugar-free soft drink, ingested after WBR (so only on training days) WBR, 3 times/wk, 3 sets of 8-12RM	NR	Baseline: IG: 0.85 ± 0.1, CG: 0.81 ± 0.1 Follow-up: IG: 1.4 ± 0.1, CG: 0.87 ± 0.1	12 wk	High	Total cholesterol (mg/dL)	16/16	Mean % change: IG: -2.8 CG: +0.5 P for time*group interaction=0.33
								LDL cholesterol (mg/dL)[d]	16/16	Mean % change: IG: -6.8 CG: +0.9 P for time*group interaction=0.14
								HDL cholesterol (mg/dL)	16/16	Mean % change: IG: +6.7 CG: +6.3 P for time*group interaction=0.78
								Triglycerides (mg/dL)	16/16	Mean % change: IG: -2.0 CG: -1.2 P for time*group interaction=0.93
								Total/HDL cholesterol ratio	16/16	Mean % change: IG: -11.8 CG: -7.3 **P for time*group interaction=0.04**
								LDL/HDL cholesterol ratio[c]	16/16	Mean % change: IG: -11.5 CG: -6.9 P for time*group interaction=0.42

Study reference, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Nabuco et al. 2019a,[44] Brazil Same study as Nabuco et al. 2018[43] and Nabuco et al. 2019b[45]	Older women aged ≥60, physically independent, free from cardiac or orthopaedic dysfunction	Whey protein. 35 g of hydrolysed whey protein supplement containing 27.1 g protein (+ 5.2 g CHO), mixed with non-caloric drink. IG1: protein before and placebo after WBR; IG2: placebo before and protein after WBR (so only on training days), [A] WBR, 3 times/wk, 3 sets of 8-12 reps	CHO placebo containing 0.3 g protein and 33.3 g CHO, mixed with non-caloric drink; one before and one after WBR (so only on training days) WBR, 3 times/wk, 3 sets of 8-12 reps	NR	Baseline: IG1: 0.92 ± 0.20, IG2: 0.94 ± 0.36, CG: 0.95 ± 0.27 Follow-up: IG1: 1.38 ± 0.26, IG2: 1.49 ± 0.46, CG: 1.0 ± 0.25	12 wk	Some concerns	Total cholesterol (mg/dL)	22/21/23 (mITT)	Mean % change: IG1: +4.9 IG2: +4.3 CG: +2.0 P for time*group interaction=0.357
								LDL cholesterol (mg/dL)[d]	22/21/23 (mITT)	Mean % change: IG1: -3.3 IG2: +1.0 CG: +0.3 P for time*group interaction=0.683
								HDL cholesterol (mg/dL)	22/21/23 (mITT)	Mean % change: IG1: -0.5 IG2: +3.3 CG: +3.7 P for time*group interaction=0.129
								Triglycerides (mg/dL)	22/21/23 (mITT)	Mean % change: IG1: +0.9 IG2: -3.5 CG: -6.3 P for time*group interaction=0.348
								Total/HDL cholesterol ratio	22/21/23 (mITT)	Mean % change: IG1: +5.2 IG2: 0 CG: -3.7 P for time*group interaction=0.081
								LDL/HDL cholesterol ratio[d]	22/21/23 (mITT)	Mean % change: IG1: -1.9 IG2: -4.5 CG: -2.9 P for time*group interaction=0.925

Study reference, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Nabuco et al. 2019c,[42] Brazil	Older women aged ≥60 with sarcopenic obesity; physically independent; free from cardiac or orthopaedic dysfunction	Whey protein. 35 g of hydrolysed whey protein supplement, mixed with non-caloric drink, ingested after WBR (so only on training days), [A] WBR, 3 times/wk, 3 sets of 8-12 reps	CHO placebo. Isocaloric amount of maltodextrin mixed with non-caloric drink, ingested after WBR (so only on training days) WBR, 3 times/wk, 3 sets of 8-12 reps	NR	Baseline IG: 0.93 ± 0.36, CG: 0.97 ± 0.28 Follow-up: IG: 1.0 ± 0.23, CG: 1.0 ± 0.19 (without supplementation)	12 wk	Some concerns	Total cholesterol (mg/dL)	13/13 (ITT)	Mean % change: IG: -4.7 CG: -3.8 P for time*group interaction=0.847
								LDL cholesterol (mg/dL)[d]	13/13 (ITT)	Mean % change: IG: -7.8 CG: -3.0 P for time*group interaction=0.542
								HDL cholesterol (mg/dL)	13/13 (ITT)	Mean % change: IG: +6.9 CG: +5.1 P for time*group interaction=0.689
								Triglycerides (mg/dL)	13/13 (ITT)	Mean % change: IG: -12.0 CG: -8.7 P for time*group interaction=0.782

Statistically significant effects are shown in bold.

Abbreviations: BMI: body mass index, BW: body weight, CI: confidence interval, CG: control group, CHO: carbohydrates, CKD: chronic kidney disease, COPD: chronic obstructive pulmonary disease, CVD: cardiovascular disease, E%: percentage of energy intake, EAA: essential amino acids, HDL: high-density lipoprotein, IG: intervention group, ITT: intention-to-treat analysis or modified intention-to-treat analysis (mITT, only those with missing outcome data were excluded from the analytic sample), LDL: low-density lipoprotein, MD: mean difference (i.e. difference in within-group change), MNA: Mini Nutritional Assessment, mo: months, *n*: number, NR: not reported, NS: not significant, PEDro: Physiotherapy Evidence Database scale, PP: per-protocol analysis, reps: repetitions, RM: repetition maximum, s: seconds, SD: standard deviation, SEM: standard error of the mean, SPPB: short physical performance battery, WBR: whole-body resistance training, wk: weeks.

Footnotes:

[a] 'Protein type' indicates the way in which a higher protein intake was achieved and is categorised into 'pure' protein or amino acids (or essential amino acids) (A), specific product with a high protein content (B), or high-protein diets (C).

[b] Risk of bias was assessed using the RoB 2 tool and scored as 'low' (L), 'some concerns' (SC), or 'high' (H). A more detailed explanation of the overall judgment can be found in Annex F.

[c] The exact number of participants included in the analyses is not reported. The number must be between the number of participants who were randomised and the number of participants who completed the study.

[d] LDL cholesterol was determined using the Friedewald equation: LDL cholesterol = total cholesterol - (HDL cholesterol + triglycerides / 5).

Table E8. Results from randomised controlled trials on the effect of increased protein intake on kidney function in older adults, grouped according to whether or not the protein intervention was carried out in the context of (concomitant) physical exercise

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Not in the context of (concomitant) physical exercise										
Bhasin et al. 2018,[51] USA	Community-dwelling older men aged ≥65 with moderate physical function limitations and with habitual protein intake ≤0.83 g/kg BW/d; mean BMI: 30.3 ± 4.9 kg/m^2	Individualised diets providing 0.7 g protein/kg BW/d with additional discretionary foods (0.1 g protein/kg BW) and protein supplements (0.5 g/kg BW) to achieve a total of 1.3 g/kg BW/d, [A,B]	Individualised diets providing 0.7 g protein/kg BW/d with additional discretionary foods (0.1 g protein/kg BW) and placebo supplements (0.5 g CHO/kg BW) to achieve a total of 0.8 g/kg BW/d	Foods (4-6 mo): IG: 77.1 ± 13%, CG: 74.5 ± 23.2% Supplements (4-6 mo): IG: 91.2 ± 12.4%, CG: 92.6 ± 11.0%	Baseline: IG: 0.72 ± 0.11, CG: 0.69 ± 0.15 Follow-up (1-3 mo): IG: 1.18 ± 0.15, CG: 0.84 ± 0.07 Follow-up (4-6 mo): IG: 1.17 ± 0.13, CG: 0.81 ± 0.10	6 mo	Some concerns	Serum creatinine (mg/dL)	40-46/ 38-46[c] (mITT)	MD (95%-CI): -0.01 (-0.05 to +0.03) P=0.540
Kerstetter et al. 2015,[56] USA	Older men (aged >70) and women (aged >60) with BMI of 19-32 kg/m^2 and protein intake of 0.6-1.0 g/kg BW; without major chronic diseases (e.g. diabetes, renal disease, inflammatory bowel disease) or cancer within past 18 months	Whey protein. 45 g of whey protein isolate (~40 g of protein) + vitamin D (400 IU) + calcium (1200 mg), [A]	CHO placebo. Isocaloric amount of maltodextrin + vitamin D (400 IU) + calcium (1200 mg)	NR	Baseline: IG: 1.07 ± 0.03, CG: 1.06 ± 0.03 Follow-up: IG: 1.30 ± 0.05, CG: 1.05 ± 0.04	9 mo and 18 mo	Some concerns	eGFR (mL/ min/1.73 m^2)	9 mo: 61/60 (mITT) 18 mo: 61/60 (mITT)	**P=0.006** (improvement in IG compared to CG) P=0.3394

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Ottestad et al. 2017[55] Norway	Community-dwelling older adults aged ≥70; relatively healthy (no diabetes, CVD, cancer, COPD, CKD); not malnourished; with reduced muscle strength or performance	Protein-enriched milk. 400 ml drink containing 20 g protein, consumed twice a day (total: 40 g protein/d), [B]	CHO placebo. 400 ml drink containing an isocaloric amount of CHO, consumed twice a day	IG: 97.8 ± 3.8%, CG: 96.8 ± 5.7%	Baseline: IG: 1.0 ± 0.3, CG: 1.0 ± 0.3 Follow-up: IG: 1.4 ± 0.5, CG: 0.9 ± 0.4	12 wk	High	Serum creatinine (µmol//L)	17/18 (mITT)	Mean change (95%-CI): IG: -0.1 (-2.8 to +2.7) CG: +6.0 (+1.0 to +11.0) **P=0.04**
								eGFR (mL/ min/1.73 m^2)	17/18 (mITT)	Mean change (95%-CI): IG: +0.29 (-3.1 to +2.5) CG: -4.4 (-8.6 to -0.3) P=0.09
Park et al. 2018,[52] Korea	Community-dwelling (pre-)frail older adults aged 70-85 at risk of malnutrition (MNA ≤23.5); no kidney or liver failure; able to walk	Whey protein. Multiple 10-g packs of protein powder (9.3 g whey protein/pack), dissolved in 340 ml tea, were provided in addition to habitual protein intake up to 1.2 (IG1) or 1.5 (IG2) g/kg BW/d, [A]	CHO placebo. Multiple 10-g packs of CHO powder (9.3 g maltodextrin/pack), dissolved in 340 ml tea, were provided in addition to habitual protein intake up to 0.8 (CG) or 1.2 (IG1) g/kg BW/d (CG were given only CHO powder, IGs were given a combination of protein and CHO powder.)	IG1: 98%, IG2: 96%, CG: 97%	Baseline: IG1: 0.77 ± 0.24, IG2: 0.80 ± 0.21, CG: 0.84 ± 0.28 Follow-up: IG1: 1.18 ± 0.23, IG2: 1.37 ± 0.26, CG: 0.90 ± 0.38	12 wk	Some concerns	Serum creatinine (µmol//L)	40/40/40 (ITT)	P for time*group interaction=0.265
								eGFR (mL/ min/1.73 m^2	40/40/40 (ITT)	P for time*group interaction=0.277
In the context of (concomitant) physical exercise										
Ramel et al. 2013,[50] Iceland Same study as Arnarson et al. 2013[49]	Community-dwelling older men and women aged ≥65; without major orthopaedic disease or musculoskeletal disorders	Whey or milk protein. Drink containing 20 g of whey protein isolate (+ 20 g of CHO; IG1); drink containing 20 g of milk protein isolate (+ 20 g of CHO; IG2), consumed after WBR (so on training days only), [A] WBR, 3 times/wk, 3 sets of 6-8 reps, 75-80% 1RM	CHO placebo. Drink containing 40 g of CHO, consumed after WBR (so on training days only) WBR, 3 times/wk, 3 sets of 6-8 reps, 75-80% 1RM	NR	Baseline: IG1: 1.00 ± 0.26, IG2: NR; CG: 0.92 ± 0.30 Follow-up: IG1: 1.06 ± 0.23, IG2: NR, CG 0.89 ± 0.23	12 wk	High	eGFR (mL/ min/1.73 m^2)	237 (total)	β (95%-CI) for IG1 vs. IG2: -0.948 (-6.121 to +4.224) P=0.718 β (95%-CI) for CG vs. IG2: -1.770 (-6.772 to +3.233) P=0.486

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Ten Haaf et al. 2019,[53] The Netherlands	Physically active older adults aged ≥65 with habitual protein intake <1.0 g/kg BW; without type 2 diabetes, cancer, renal insufficiency (eGFR <30) or COPD	Milk-protein concentrate. 250-ml protein drink containing 15.5 g protein (+ 1.1 g fat + 14.5 g lactose) consumed twice a day (total: 31 g protein/d), [A] Training (walking) for the Nijmegen Four Days Marches	CHO placebo. 250-ml isocaloric drink containing 1.1 g protein (+ 5.2 g fat + 36 g CHO) consumed twice daily Training (walking) for the Nijmegen Four Days Marches	IG: 96 ± 3%, CG: 95 ± 3%	Baseline: IG: 0.86 ± 0.23, CG: 0.92 ± 0.24 Follow-up: IG: 0.92 ± 0.27, CG: 0.97 ± 0.23 (without supplementation)	12 wk	Some concerns	Serum creatinine (µmol//L)	114 (total, PP)	Mean change ± SD: IG: +3.9 ± 11.0 CG: +4.9 ± 6.8 P for time*group interaction=0.56
								eGFR (mL/min/1.73 m^2)	109 (total, PP)	Mean change ± SD: IG: -1.9 ± 9.8 CG: -4.1 ± 7.0 P for time*group interaction=0.19
								Albumin/ creatinine ratio (mg/mmol; urine)	111 (total, PP)	Mean change ± SD: IG: -0.5 ± 3.3 CG: -0.4 ± 4.1 P for time*group interaction=0.86

Statistically significant effects are shown in bold.

Abbreviations: BMI: body mass index, BW: body weight, CI: confidence interval, CG: control group, CHO: carbohydrates, CKD: chronic kidney disease, COPD: chronic obstructive pulmonary disease, CVD: cardiovascular disease, EAA: essential amino acids, eGFR: estimated glomerular filtration rate, IG: intervention group, ITT: intention-to-treat analysis or modified intention-to-treat analysis (mITT, only those with missing outcome data were excluded from the analytic sample), IU: international units, MD: mean difference (i.e. difference in within-group change), mg: milligram, MNA: Mini Nutritional Assessment, mo: months, *n*: number, NR: not reported, NS: not significant, PP: per-protocol analysis, reps: repetitions, RM: repetition maximum, wk: weeks.

Footnotes:

[a] 'Protein type' indicates the way in which a higher protein intake was achieved and is categorised into 'pure' protein or amino acids (or essential amino acids) (A), specific product with a high protein content (B), or high-protein diets (C).

[b] Risk of bias was assessed using the RoB 2 tool and scored as 'low' (L), 'some concerns' (SC), or 'high' (H). A more detailed explanation of the overall judgment can be found in Annex F.

[c] The exact number of participants included in the analyses is not reported. The number must be between the number of participants who were randomised and the number of participants who completed the study.

Table E9. Results from randomised controlled trials on the effect of increased protein intake on cognition in older adults, grouped according to whether or not the protein intervention was carried out in the context of (concomitant) physical exercise

Study, country	Study population	Exposure (IG), [protein type[a]]	Comparison (CG)	Compliance	Total protein intake (g/kg BW/d)	Study duration	Risk of bias[b]	Outcome	Analytic *n* IG/CG	Results
Not in the context of (concomitant) physical exercise										
Park et al. 2018,[52] Korea	Community-dwelling (pre-)frail older adults aged 70-85 at risk of malnutrition (MNA ≤23.5); no kidney or liver failure; able to walk	Whey protein. Multiple 10-g packs of protein powder (9.3 g whey protein/pack), dissolved in 340 ml tea, were provided in addition to habitual protein intake up to 1.2 (IG1) or 1.5 (IG2) g/kg BW/d, [A]	CHO placebo. Multiple 10-g packs of CHO powder (9.3 g maltodextrin/ pack), dissolved in 340 ml tea, were provided in addition to habitual protein intake up to 0.8 (CG) or 1.2 (IG1) g/kg BW/d. (CG were given only CHO powder, IGs were given a combination of protein and CHO powder.)	IG1: 98%, IG2: 96%, CG: 97%	Baseline: IG1: 0.77 ± 0.24, IG2: 0.80 ± 0.21, CG: 0.84 ± 0.28 Follow-up: IG1: 1.18 ± 0.23, IG2: 1.37 ± 0.26, CG: 0.90 ± 0.38	12 wk	Some concerns	MMSE (score)	40/40/40 (ITT)	P for time*group interaction=0.702

Statistically significant effects are shown in bold.

Abbreviations: BW: body weight, CG: control group, CHO: carbohydrates, EAA: essential amino acids IG: intervention group, ITT: intention-to-treat analysis, MMSE: Mini-Mental State Examination, MNA: Mini Nutritional Assessment, *n*: number, NR: not reported, NS: not significant, wk: weeks.

Footnotes:

[a] 'Protein type' indicates the way in which a higher protein intake was achieved and is categorised into 'pure' protein or amino acids (or essential amino acids) (A), specific product with a high protein content (B), or high-protein diets (C).

[b] Risk of bias was assessed using the RoB 2 tool and scored as 'low' (L), 'some concerns' (SC), or 'high' (H). A more detailed explanation of the overall judgment can be found in Annex F.

F risk of bias assessment of included studies

The table below describes the risk of bias for each of the five domains of the RoB 2 Cochrane collaboration tool,[10] as well as the overall risk-of-bias judgement (in terms of ‘high risk of bias’, ‘some concerns’ or ‘low risk of bias’), for all of the selected studies. Where the judgement is ‘some concerns’ or ‘high risk of bias’, an explanation is provided. Funding and any conflicts of interest involving the authors are not part of the RoB 2 tool, however these are presented here as they might be an additional concern with regard to risk of bias.

Study	Risk of bias domain[a]					Overall RoB[b]	Comments	Funding and author's conflicts of interest[c]
	1	2[d]	3	4	5			
Arnarson et al. 2013[49]	+	+	x	+	+	x	Considerable proportion of missing outcome data (12%) and missingness in the outcome may depend on its true value.	Funding provided by: Icelandic Technology Development Fund, Research Fund of the University of Iceland, Landspitali University Hospital Research Fund, and Helga Jonsdottir and Sigurlidi Kristjansson Geriatric Research Fund. Authors declared no conflict of interest.
Bhasin et al. 2018[51]	+	+	-	+	+	-	Missingness in the outcome may depend on its true value.	Funding provided by: NIA (NIH grant), National Center for Advancing Translational Sciences (Boston University Clinical Translational Science Institute grant; NIH Award), Boston Nutrition Obesity Research Center, and Harvard University and affiliated health care centres. Dietary supplements provided by: Abbott Laboratories, Bariatrix Nutrition and the National Dairy Council. Testosterone provided by: Endo Pharmaceuticals. Funding sources had no role in study design, study conduct, manuscript preparation and publication. Some authors declared conflicts of interest related to food companies (e.g. receiving grants from Abbott Pharmaceuticals, acting as a consultant for Novartis or AbbVie, receiving fees from Novo Nordisk, receiving research support from The Beef Checkoff Program).
Campbell et al. 1995[51]	-	+	+	+	+	-	Unclear if allocation sequence was concealed.	Funding provided by: the US Department of Agricultural Research Service and NIH grant. Personal support by Kraft General Foods Predoctoral Fellowship (American Institute of Nutrition). Other authors' conflicts of interest NR.
Chalé et al. 2013[54]	+	-	+	-	+	-	Achieved protein intake is not reported for the entire analytic sample while ITT analyses were performed. For some outcomes, results of completer analyses and ITT analyses differed. Furthermore, it is unclear who assessed the outcomes and if the outcome assessors were blinded. It is unlikely that they were unblinded, but if they were, the outcome (e.g. muscle strength, performance) might have been influenced by the assessor's knowledge of the intervention received.	Funding provided by: Boston Claude D. Pepper Older Americans Independence Center (NIH/NIA), the Boston Nutrition/Obesity Research Center, a postdoctoral training grant and the US Department of Agriculture. Personal funding by Dairy Research Institute. Other authors' conflicts of interest NR.
Dillon et al. 2009[37]	-	x	x	+	x	x	Unclear if allocation sequence was concealed. Also, no information was provided on the number of participants that were recruited, how many were randomised and how many were lost to follow-up (and thus unclear if outcome data were missing and/or if the correct analyses were performed). Moreover, only the time effect was reported and not the time*group interaction (while ANOVA was applied).	Funding provided by: Boston Claude D. Pepper Older Americans Independence Center (NIH/NIA), United States Public Health Service and General Clinical Research Center. Authors declared no conflict of interest.
Fernandes et al. 2018[40]	-	-	x	+	x	x	No information was provided on allocation sequence. Furthermore, it is unclear if participants were lost to follow-up, and for what reason, and it is unclear what type of analysis (PP or (m)ITT or other) was applied. Also, possibility of selective reporting.	Funding provided by: Coordination for the Improvement of Higher Education Personnel, the Brazilian Ministry of Education and the Brazilian National Council for Scientific and Technological Development. Dietary supplements provided by: Arla Foods Ingredients and New Millen. Authors declared no conflict of interest.

Study	Risk of bias domain[a]					Overall RoB[b]	Comments	Funding and author's conflicts of interest[c]
	1	2[d]	3	4	5			
Hodgson et al. 2012[59]	+	+	-	+	+	-	Considerable proportion of missing outcome data (11%), but no strong evidence provided to show that the result was not biased by missing outcome data.	Funding provided by: Australian National Health Medical Research Council and University of Western Australia Research Grants Scheme. Dietary supplements provided by: Fonterra Brands Limited. Funding sources had no role in study design, study execution and manuscript preparation. Authors declared no conflict of interest.
Ispoglou et al. 2016[41]	-	-	x	-	+	x	Loss to follow-up (for reasons including dietary non-compliance) was 18%, but no strong evidence provided to show that results were not biased by missing outcome data (and it is unclear whether different groups had the same drop-out rate and the same reason for drop-out); unclear if allocation sequence was concealed; unclear who assessed the outcomes and if the outcome assessors were blinded. If unblinded, some outcomes (e.g. muscle strength, performance) might have been influenced by the assessor's knowledge of the intervention received.	Funding provided by: institutional grant. Authors declared no conflict of interest.
Kerstetter et a. 2015[56]	-	+	-	+	+	-	Unclear if allocation sequence was concealed. No strong evidence provided to show that result was not biased by missing outcome data.	Funding provided by: NIH National Institute of Arthritis and Musculoskeletal and Skin Diseases, Dairy Research Institute and the Yale Bone Center. Authors declared no conflict of interest.
Mitchell et al. 2015[38]	-	-	x	-	+	x	This was a brief communication. As a result, reporting was very limited and therefore risk of bias was difficult to judge. No information was provided on allocation sequence and baseline characteristics were not reported. Also, unclear how many participants were randomised, how many withdrew and how many were analysed, and information was lacking on the reasons for any drop-outs and/or missing outcome data. Furthermore, unclear who assessed the outcomes and if the outcome assessors were blinded. If unblinded, the outcome (e.g. muscle strength) might have been influenced by knowledge of the intervention received.	Funding provided by: Dairy Farmers of Canada. Authors' conflict of interest NR.
Mitchell et al. 2017[48]	x	+	+	-	+	x	Allocation sequence probably was not concealed. Furthermore, assessment of the outcome (muscle strength, physical function) might have been influenced by the outcome, since outcome assessors were probably not blinded.	Funding provided by: New Zealand Ministry of Business, Innovation and Employment International Relationships and the European Union, and AgResearch Limited. Two authors are employees of AgResearch Limited. Other authors declared no conflict of interest.
Nabuco et al. 2018[43]	-	+	+	-	+	-	Unclear if allocation sequence was concealed. Also, unclear who assessed the outcomes and if the outcome assessors were blinded[c]. It is unlikely that they were unblinded, but if they were, the outcome (e.g. muscle strength, performance) might have been influenced by knowledge of the intervention received.	Funding provided by: Coordination for the Improvement of Higher Education Personnel, National Council for Scientific and Technological Development, and Ministry of Education. Dietary supplements provided by: Arla Foods Ingredients Group and New Milen. Authors declared no conflict of interest.

Study	Risk of bias domain[a]					Overall RoB[b]	Comments	Funding and author's conflicts of interest[c]
	1	2[d]	3	4	5			
Nabuco et al. 2019a[44]	-	+	+	+	-	-	In a previous paper from the same RCT (Nabuco 2018[43]) other lean body mass parameters were addressed (upper limb LST, lower limb LST, skeletal muscle mass). In two of these a statistically significant effect was observed. It is unclear why appendicular LST was part of this separate paper (probably because the authors expected a significant effect on this outcome as well).	Funding provided by: Coordination for the Improvement of Higher Education Personnel, National Council for Scientific and Technological Development, and Ministry of Education. Dietary supplements provided by: Arla Foods Ingredients Group and New Milen. Authors declared no conflict of interest.
Nabuco et al. 2019b[45]	-	+	+	+	-	-	Unclear if allocation sequence was concealed. Furthermore, in two other papers (Nabuco 2018[43]; Nabuco 2019a[44]) other lean body mass parameters were addressed (upper limb LST, lower limb LST and skeletal muscle mass, and appendicular LST), for two of which a statistically significant effect was observed. It seems as if the authors expected a significant effect on total LST (as, indeed, there was) and, therefore, included the outcome in this next paper.	Funding provided by: Coordination for the Improvement of Higher Education Personnel, National Council for Scientific and Technological Development, and Ministry of Education. Dietary supplements provided by: Arla Foods Ingredients Group and New Milen. Authors declared no conflict of interest.
Nabuco et al. 2019c[42]	-	+	+	-	+	-	Unclear if allocation sequence was concealed. Also, unclear who assessed the outcomes and if the outcome assessors were blinded[c]. It is unlikely that they were unblinded but, if they were, the outcome (e.g. muscle strength, performance) might have been influenced by assessor's knowledge of the intervention received.	Funding provided by: Coordination for the Improvement of Higher Education Personnel, National Council for Scientific and Technological Development, and Ministry of Education. Dietary supplements provided by: Arla Foods Ingredients Group and New Milen. Authors declared no conflict of interest.
Ottestad et al. 2017[55]	+	+	x	+	+	x	The attrition rate was high (20%) and the missing outcome data might depend on its true value.	Funding provided by: Research Council of Norway. Dietary supplements provided by: TINE SA. Some authors declared conflicts of interest related to food or pharmacy companies (e.g. receiving grants or personal fees from Amgen, Mills DA, TINE DA or Olympic Seafood); none of which are related to the contents of this study. Other authors declared no conflict of interest.
Park et al. 2018[52]	+	+	-	-	+	-	No strong evidence provided to show that results were not biased by missing outcome data (18%). Also, unclear if bias might feature in the outcome measurement (because it is not known who performed the outcome measurements).	Funding provided by: Korea Health Industry Development Institute (funded by the Ministry of Health & Welfare). Authors declared no conflict of interest.
Ramel et al. 2013[50]	+	+	x	+	+	x	Missingness in the outcome may depend on the true value.	Funding provided by: Icelandic Technology Development Fund, Research Fund of the University of Iceland, Landspitali University Hospital Research Fund, and Helga Jonsdottir and Sigurlidi Kristjansson Geriatric Research Fund. Authors' conflict of interest NR.
Sugihara Junior et al. 2018[46]	-	-	x	+	+	x	No information was provided on allocation sequence. Also, unclear if participants were lost to follow-up (or that outcome data were missing; missing data could depend on its true value) and for what reasons; unclear what type of analysis (PP or (m)ITT or other) was applied.	Funding provided by: Coordination for the Improvement of Higher Education Personnel, the Brazilian Ministry of Education and the Brazilian National Council for Scientific and Technological Development. Dietary supplements provided by: Arla Foods Ingredients, New Millen and the Planeta Saúde Arapongas (supermarket). Authors declared no conflict of interest.

Study	Risk of bias domain[a]					Overall RoB[b]	Comments	Funding and author's conflicts of interest[c]
	1	2[d]	3	4	5			
Ten Haaf et al. 2019[53]	+	+	+	-	+	-	Unclear who performed the outcome assessments and if the outcome assessors were blinded. If unblinded, the outcome (e.g. muscle strength, physical function) might have been influenced by the assessor's knowledge of the intervention received.	Funding provided by: 'Topconsortia for Knowledge and Innovation (TKIs)' from the Ministry of Economic Affairs. One author is affiliated with FrieslandCampina. Authors declared no conflict of interest.
Thomson et al. 2016[57]	+	-	x	+	+	x	Attrition rate was high (36%) and the missing outcome data might depend on its true value. Also, reasons for attrition were not balanced across the groups, which raises some concerns regarding performance bias.	Funding provided by: Dairy Health and Nutrition Consortium (including food companies). The sponsor assisted with diet design but had no involvement in any other aspects of the study design, study execution, data analysis or manuscript preparation. Authors declared no conflict of interest.
Wright et al. 2018[47]	-	-	-	+	+	x	Unclear if allocation sequence was concealed. Also, PP analyses have been performed while 12% (n=3 of 26) were lost to follow-up because of dietary non-compliance and compliance was not reported separately for each group. Furthermore, no strong evidence provided to show that results were not biased by missing outcome data (because of dietary non-compliance).	Funding provided by: Egg Nutrition Center-American Egg Board and Purdue Ingestive Behavior Research Center. Funding sources had no role in study design, study execution, manuscript preparation or publication. Authors declared no conflict of interest.
Zhu et al. 2011[60]	+	+	-	+	+	-	Considerable proportion of missing outcome data (11%), but no strong evidence provided to show that the result was not biased by missing outcome data.	Funding provided by: Australian National Health Medical Research Council and University of Western Australia Research Grants Scheme. Funding sources had no role in study design, study execution or manuscript preparation. Dietary supplements provided by: Fonterra Brands Limited. Authors declared no conflict of interest.
Zhu et al. 2015[58]	+	+	-	+	+	-	Considerable proportion of missing outcome data (11%), but no strong evidence provided to show that the result was not biased by missing outcome data.	Funding provided by: Australian National Health Medical Research Council and University of Western Australia Research Grants Scheme. Funding sources had no role in study design, study execution or manuscript preparation. Dietary supplements provided by: Fonterra Brands Limited. Authors declared no conflict of interest.

Abbreviations: ITT: intention to treat, LST: lean soft tissue: mITT: modified ITT, NIA: National Institute on Aging, NIH: National Institutes of Health, NR: not reported, PP: per-protocol, RCT: randomised controlled trial.

Footnotes:

[a] Domains addressed: 1, bias arising from the randomisation process; 2, bias due to deviations from the intended interventions; 3, bias due to missing outcome data; 4, bias in measurement of the outcome; 5, bias in selection of the reported result. Judgements include: + low risk of bias; - some concerns; x high risk of bias.[10] The RoB 2 guidance document[64] was used to ensure the correct interpretation and judgment of each domain.

[b] The following rules were applied to reach an overall risk of bias judgement, based on:[10] the overall judgment is 'low risk of bias' (+) if the study is judged to be at low risk of bias for all domains; the overall judgment is 'some concerns' (-) if the study is judged to raise some concerns in at least one domain (but no more than two domains), but not to be at high risk of bias for any domain; the overall judgment is 'high risk of bias' (x) if the study is judged to be at high risk of bias in at least one domain for this result or if the study is judged to raise some concerns regarding multiple (at least three) domains.

[c] Funding and authors' conflict of interest are not part of the RoB 2 Cochrane collaboration tool, however these are presented here as they might be an additional concern in relation to risk of bias.

[d] For the second domain, the Committee slightly deviated from the original RoB 2 criteria. The Committee did not necessarily prefer intention-to-treat analyses over per-protocol analyses (or vice versa), the choice that should be made according to the RoB 2 tool. The Committee was interested in the difference in achieved protein intake (from the background (habitual) diet plus the intervention) between the intervention- and control group at follow-up, considered as the *protein dose* in this document. This protein dose incorporates any non-compliance to the dietary intervention. In addition to the criteria described in the RoB 2 tool, the Committee assessed whether or not achieved protein intake was measured/reported and what the level of compliance was. If achieved protein intake was not available, compliance was not reported and no per-protocol analyses was performed, this would lead to some concerns regarding the risk of bias. If measured actual protein intake at follow-up was reported and the Committee noted no other concerns, the risk of bias was judged as low.

G overview of outcome measures used for power analysis in the included studies

The table below presents for each of the evaluated RCTs on which outcome or outcomes the power analysis was based. For nine RCTs (12 publications) information on this point was either not reported or unclear.

Study	Outcome on which power analysis was based
Arnarson et al. 2013[49], Ramel et al. 2013[50]	Lean body mass
Bhasin et al. 2018[51]	Lean body mass
Campbell et al. 1995[39]	Unclear
Chalé et al. 2013[54]	Lean body mass, muscle strength, physical function
Dillon et al. 2009[37]	Unclear
Fernandes et al. 2018[40], Sugihara-Junior et al. 2018[46]	Unclear
Ispoglou et al. 2016[41]	Unclear
Kerstetter et al. 2015[56]	Bone health
Mitchell et al. 2015[38]	Unclear
Mitchell et al. 2017[48]	Unclear
Nabuco et al. 2018[43], Nabuco et al. 2019a[44], Nabuco et al. 2019b[45]	Unclear
Nabuco et al. 2019c[42]	Unclear
Ottestad et al. 2017[55]	Lean body mass
Park et al. 2018[52]	Lean body mass
Ten Haaf et al. 2019[53]	Muscle strength, physical function
Thomson et al. 2016[57]	Muscle strength
Wright et al. 2018[47]	Unclear
Zhu et al. 2011[60], Hodgson et al. 2012[59], Zhu et al. 2015[58]	Lean body mass, bone health

H studies on the effect of increased protein intake on body weight

Table H1. Overview of the results of randomised controlled trials on the effect of increased protein intake on body weight in older adults, grouped according to whether or not the protein intervention was carried out in the context of (concomitant) physical exercise

Study	Total protein intake (g/kg BW/d) during intervention[a]	Protein type[b]	Risk of Bias[c]	Outcome	Result[d]				Comments
					+	NS	-	?	
Not in the context of (concomitant) physical exercise									
Bhasin et al. 2018[51]	IG: 1.17 ± 0.13; CG: 0.81 ± 0.10	A,B	SC	Body weight		✔			
Mitchell et al. 2017[48]	IG: 1.7 ± 0.1; CG: 0.9 ± 0.1	C	H	Body weight		✔			
Ottestad et al. 2017[55]	IG: 1.4 ± 0.5; CG: 0.9 ± 0.4	B	H	Body weight		✔			
Zhu et al. 2015[58] Same study as [59,60]	IG: 1.4 ± 0.4; CG: 1.1 ± 0.4	A	SC	Body weight		✔			
Subtotal (comparisons) *Subtotal (studies)*[e]					*0* *0*	*4* *4*	*0* *0*	*0* *0*	*No effect observed for any of the 4 comparisons (4 studies)*
In the context of (concomitant) physical exercise									
Chalé et al. 2013[54]	NR	A	SC	Body weight		✔			
Ten Haaf et al. 2019[53]	IG: 0.92 ± 0.27 (without protein supplementation of 31g/d); CG: 0.97 ± 0.23	A	SC	Body weight		✔ *			* P=0.07 (body weight tended to decrease more in IG than in CG. Lean body mass did not change; see Table 2)
Thomson et al. 2016[57]	IG1: 1.42 ± 0.14; IG2: 1.45 ± 0.14; CG: 1.08 ± 0.05	B	H	Body weight		✔			
Subtotal (comparisons) *Subtotal (studies)*[e]					*0* *0*	*3* *3*	*0* *0*	*0* *0*	*No effect observed for any of the 3 comparisons (3 studies)*
Total (comparisons) Total (studies)[e]					0 0	7 7	0 0	0 0	No effect observed for any of the 7 comparisons (7 studies)

Abbreviations: BW: body weight, CG: control group, H: high risk of bias, IG: intervention group, L: low risk of bias, NR: not reported, NS: not significant, SC: some concerns (regarding risk of bias).

Footnotes:

[a] Total protein intake during follow-up. If protein intake was assessed at multiple time points, the intake assessed at the final time point was considered.

[b] 'Protein type' indicates the way in which a higher protein intake was achieved and is categorised into 'pure' protein or amino acids (or essential amino acids) (A), specific food(s) with a high protein content (B), or high-protein diets (C).

[c] Risk of bias assessment: risk of bias was assessed using the RoB 2 tool and scored as ‘low’ (L), ‘some concerns’ (SC), or ‘high’ (H).

[d] The results of the studies are indicated as follows: +, statistically significant beneficial effect (P<0.05); -, statistically significant unfavourable effect (P<0.05); NS, no statistically significant effect (P≥0.05); ?, result unclear. In cases where results were reported for multiple time points, only the result for the final time point is reported.

[e] Some studies assessed multiple specific outcomes (i.e. multiple contrasts) for the health outcome ‘body weight’, so one study can show both a significant and a non-significant effect.

I studies on the effect of increased protein intake in the context of physical exercise on muscle strength

Table I1. Overview of the results of randomised controlled trials on the effect of increased protein intake in the context of concomitant physical exercise on muscle strength in older adults, categorised according to habitual protein intake and ordered by protein dose

Study	Analytic *n* IG/CG	Total protein intake (g/kg BW/d) during intervention[a]	Protein dose[b] (g/kg BW/d)	Protein type[c]	With/without physical exercise	Risk of bias[d]	Outcome	Result[e]				Comments
								+	NS	-	?	
Habitual protein intake (reference): ≥0.8 to <0.9 kg BW/d												
Arnarson et al. 2013[49] Same study as [50]	75/66	IG: 1.06 ± 0.23; CG 0.89 ± 0.23	0.17	A	Ex	H	Quadriceps strength		✔			
Sugihara Junior et al. 2018[46] Same study as [40]	15/16	IG: 1.4 ± 0.1; CG: 0.87 ± 0.1	0.53	A	Ex	H	Chest press strength	✔				
							Knee extension strength	✔				
							Preacher curl strength		✔ *			* P=0.07 (strength tended to increase more in IG than in CG)
							Total strength[f]	✔				
							Lower limb muscle quality index[g]		✔			
							Upper limb muscle quality index[h]		✔			
							Total muscle quality index[i]		✔			
Subtotal (contrasts)								*3*	*5*	*0*	*0*	*Beneficial effect observed for 3 of 8 contrasts (1 of 2 studies)*
Subtotal (studies)[j]								*1*	*2*	*0*	*0*	

Study	Analytic *n* IG/CG	Total protein intake (g/kg BW/d) during intervention[a]	Protein dose[b] (g/kg BW/d)	Protein type[c]	With/without physical exercise	Risk of bias[d]	Outcome	Result[e]				Comments
								+	NS	-	?	
Habitual protein intake (reference): ≥0.9 to <1.0 kg BW/d												
Ten Haaf et al. 2019[53]	58/56 for handgrip strength; 22-56[†] (total) for other outcome measures	IG: 0.92 ± 0.27 (without protein supplementation of 31 g/d); CG: 0.97 ± 0.23	0.36[k]	A	Ex	SC	Handgrip strength[§]		✔			
							Quadriceps MVC[§]		✔			
							Maximal rate of force rise, quadriceps[§]		✔			
							Early relaxation time, quadriceps[§]		✔			
							Half relaxation time, quadriceps[§]		✔			
							Fatigue[§]		✔			
Chalé et al. 2013[54]	42/38	NR	0.38[l]	A	Ex	SC	Double leg press strength, 1RM[§]		✔			
							Knee extension, 1RM, right[§]		✔			
							Knee extension, 1RM, left[§]		✔			
							Double leg press peak power, 40% 1RM[§]		✔			
							Knee extension peak power, 40% 1RM, right[§]	✔				
							Knee extension peak power, 40% 1RM, left[§]	✔				
							Double leg press peak power, 70% 1RM[§]		✔			
							Knee extension peak power, 70% 1RM, right[§]	✔				
							Knee extension peak power, 70% 1RM, left[§]	✔				
Subtotal (contrasts)								*4*	*11*	*0*	*0*	*Beneficial effect observed for 4 of 15 contrasts (1 of 2 studies)*
Subtotal (studies)[i]								*1*	*2*	*0*	*0*	
Total habitual protein intake (reference): ≥1.0 to <1.1 kg BW/d												
Nabuco et al. 2019c[42]	13/13	IG: 1.0 ± 0.23 (without ~35 g whey protein supplementation on 3 d/wk); CG: 1.0 ± 0.19	0.24[k]	A	Ex	SC	Knee extension		✔			
							Chest press		✔			
							Preacher curl		✔			
							Total strength[f]		✔			

Study	Analytic *n* IG/CG	Total protein intake (g/kg BW/d) during intervention[a]	Protein dose[b] (g/kg BW/d)	Protein type[c]	With/without physical exercise	Risk of bias[d]	Outcome	Result[e]				Comments
								+	NS	-	?	
Thomson et al. 2016[57]	34/23	IG1: 1.42 ± 0.14; CG: 1.08 ± 0.05	0.34	B	Ex	H	Knee extensor strength§		✔			
							Handgrip strength§		✔			
							Leg press§		✔			
							Chest press§		✔			
							Knee extension strength§		✔			
							Lat pull down§				✔ *	* Smaller % (but not absolute) increase in IG1 than in CG
							Leg curl§		✔			
							Total 8RM§		✔			
	26/23	IG2: 1.45 ± 0.14; CG: 1.08 ± 0.05	0.37				Knee extensor strength§		✔			* P=0.08 (strength tended to increase less in IG2 than in CG)
							Handgrip strength§		✔			
							Leg press§			✔		
							Chest press§		✔			
							Knee extension strength§		✔			
							Lat pull down§		✔			
							Leg curl§		✔			
							Total 8RM§			✔		
Nabuco et al. 2018[43] Same study [44,45]	22/23	IG1: 1.38 ± 0.26; CG: 1.0 ± 0.25	0.38	A	Ex	SC	Chest press	✔				
							Knee extension	✔				
							Preacher curl		✔			
							Total strength[f]	✔				
	21/23	IG2: 1.49 ± 0.46; CG: 1.0 ± 0.25	0.49				Chest press	✔				
							Knee extension	✔				
							Preacher curl		✔			
							Total strength[f]	✔				

Study	Analytic *n* IG/CG	Total protein intake (g/kg BW/d) during intervention[a]	Protein dose[b] (g/kg BW/d)	Protein type[c]	With/without physical exercise	Risk of bias[d]	Outcome	Result[e]				Comments
								+	NS	-	?	
Subtotal (contrasts) *Subtotal (studies)*[j]								*6* *1*	*19* *3*	*2* *1*	*1* *1*	*Beneficial effect observed for 6 of 28 contrasts (1 of 3 studies)* *Unfavourable effect observed for 2 of 28 contrasts (1 of 3 studies)*
Total habitual protein intake (reference): Unclear												
Mitchell et al. 2015[38]	16 (total)	NR	NR (15 g/d)	B	Ex	H	Knee extension isometric MVC		✔			
							Leg press		✔			
							Leg extension		✔			
							Chest press		✔			
Subtotal (contrasts) *Subtotal (studies)*[j]								*0* *0*	*4* *1*	*0* *0*	*0* *0*	*No effect observed for any of 4 contrasts (1 study)*
Total (contrasts) Total (studies)[j]								13 3	39 8	2 1	1 1	Beneficial effect observed for 13 of 55 contrasts (3 of 8 studies) Unfavourable effect observed for 2 of 55 contrasts (1 of 15 studies)

Abbreviations: BW: body weight, CG: control group, Ex: with concomitant physical exercise intervention, H: high risk of bias, IG: intervention group, L: low risk of bias, MVC: maximal voluntary contraction, NoEx: without concomitant physical exercise intervention, NR: not reported, RM: repetition maximum, SC: some concerns (regarding risk of bias).

Footnotes:

† Depending on specific outcome measure.

§ Sufficient statistical power to detect an effect is to be expected, based on the sample size calculation.

[a] Total protein intake during follow-up. If protein intake was assessed at multiple time points, the intake assessed at the final time point was considered.

[b] 'Protein dose' indicates the difference in achieved total protein intake between the intervention and control groups during follow-up (which is not necessarily equal to supplemented/prescribed amount of protein).

[c] 'Protein type' indicates the way in which a higher protein intake was achieved and is categorised into 'pure' protein or amino acids (or essential amino acids) (A), specific food(s) with a high protein content (B), or high-protein diets (C).

[d] Risk of bias assessment: risk of bias was assessed using the RoB 2 tool and scored as 'low' (L), 'some concerns' (SC), or 'high' (H).

[e] The results of the studies are indicated as follows: +, statistically significant beneficial effect (P<0.05); -, statistically significant unfavourable effect (P<0.05); NS, no statistically significant effect (P≥0.05); ?, result unclear. In cases where results were reported for multiple time points, only the result for the final time point is reported.

[f] Total strength was calculated as the sum of chest press, knee extension and preacher curl strength (kg).

[g] Lower limb muscle quality index was calculated as knee extension strength divided by lower limb lean soft tissue.

[h] Upper limb muscle quality index was calculated as preacher curl strength divided by upper limb lean soft tissue.

[i] Total muscle quality index was calculated as total strength divided by skeletal muscle mass.

[j] Some studies assessed multiple specific outcomes (i.e. multiple contrasts) for the health outcome 'muscle strength', so one study can show both a significant and a non-significant effect.

[k] Protein intake in g/kg BW/d was calculated by using protein intake in g/d, mean body weight (and compliance, if available).

[l] (Achieved) protein dose was estimated using prescribed protein dose, compliance rate (72%), and mean body weight.

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