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Potential anabolic effects of protein supplementation in combination with resistance training

A systematic review and meta-analysis of intervention trials

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List of Abbreviations

Abbreviations	Explanations
1-RM	1-repetition maximum
6 MWD	6-min walk distance
BCAA	Branched-chain amino acids
BM	Body mass
BMC	Bone mineral content
BW	Body weight
BWP	Bio-enhanced whey protein
Cdk2	Cyclin-dependent kinase 2
CHO	Carbohydrates
CI	Confidence interval
CMJ	Countermovement jump
CSA	Cross-sectional area
d	Days
DCER	Dynamic constant external resistance
EAA	Essential amino acids
EMG	Electromyography
EWGSOP	Working Group on Sarcopenia in Older People
FAI	Frenchay Activities Index
FFM	Fat free mass
FM	Fat mass
FRT	Functional reach distance test
GDF-15	Growth/differentiation factor
HMB	Beta-hydroxy-beta-methylbutyrate
IGF-1	Insulin-like growth factor

LBM	Lean body mass
LL	Lower limb
LTM	Lean tissue mass
MD	Mean difference
MHC	Myosin heavy chain
MPS	muscle protein synthesis
mRNA	Messenger ribonucleic acid
mTORC1	mechanistic target of rapamycin complex-1
MVC	Maximum voluntary contraction
NA	did not report baseline protein intake
NS	Non-significant difference between groups
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
RDA	Recommended daily allowance
RE	Resistance exercise
RFD	Rate of force development
RPE	Ratings of perceived exertion
Reps	Repetitions
RT	Resistance training
SD	Standard deviation
SE	Standard error
SH	Standard beta-hydroxy beta-methylbutyric acid
SJ	Squat jump
SPPB	Short Physical Performance Battery
TBW	Total body water
TFM	Total fat mass

TRH	Time-release HMB
TTE	Treadmill time to exhaustion
TUG	Timed up and go test
TWL	Total weight lifted (weight x repetitions)
UL	Upper limb
VL	Vastus lateralis
WBG	Whey protein + L-glutamine + BCAA
WC	Whey protein + casein
Wk	Week
WMD	Weighted mean difference

1. Introduction

1.1. Background

The supplementation of proteins plays a central role in the world of sport, especially in the field of resistance training and particularly with respect to bodybuilding. The protein blends, shakes and powders promise a significant improvement in muscle hypertrophy and have become an indispensable part of the weight training scene. The use of supplements, as well as an adjustment of the protein requirements in exercising individuals and elderly has been controversially discussed for years [(1)].

The protein turnover in the human body is constant and both the synthesis of protein, as well as its breakdown is subject to anabolic and catabolic influences. The catabolic ones include aging and illness. By contrast, the uptake of dietary protein and physical activity are anabolic signals. Figure 1 illustrates the daily protein turnover of an adult, considering the nitrogen balance [(2)].

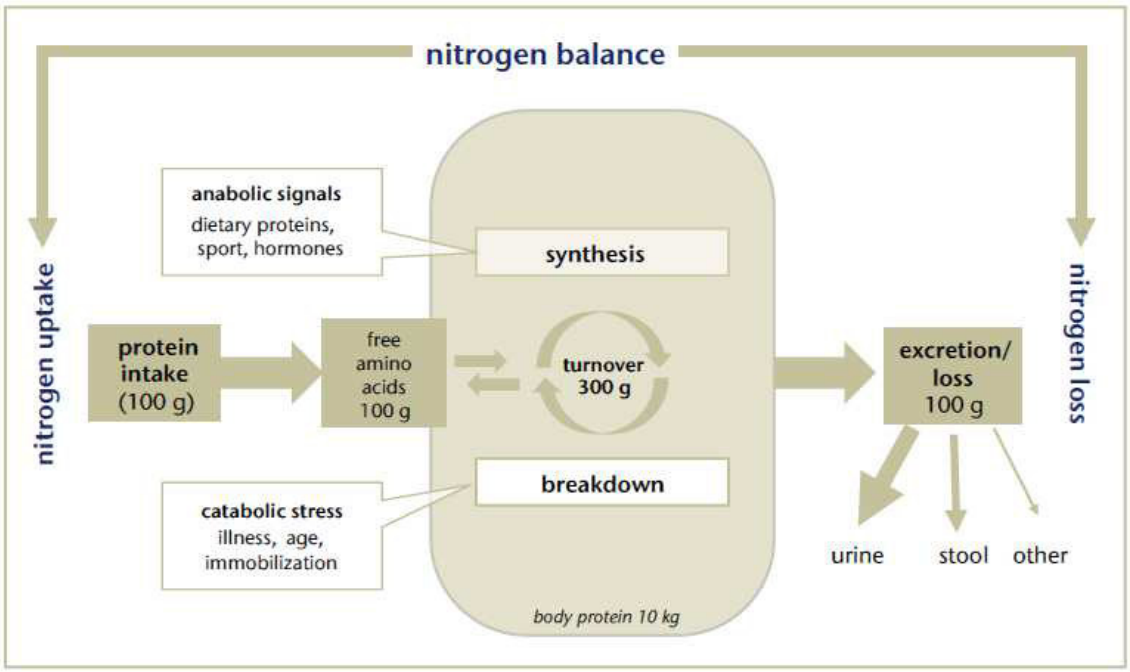

Figure 1: The daily protein turnover in the human body [(2)]

Recommendations for protein intake are derived from the nitrogen balance studies. Thus, an intake of 0.8 g/kg body weight per day (RDA) is recommended for adults aged 18 years and older. The general recommendations are aimed at maintaining existing muscle mass. This intake may not be sufficient for the elderly, as the aging process results in a loss of muscle mass. Accordingly, the recommended protein intake was increased to 1.0 g/kg body weight per day [(3)]. Individuals who practice weight training aim at an increase in muscle mass and maximum performance and therefore a higher intake of protein, 1.4 to 2.0 g protein/kg body weight per day, is recommended by the International Society of Sports Nutrition [(4)].

1.2. Protein supplements and resistance training

Systematic reviews already exist on this topic, e.g. Cermak et al. investigated the effect of protein supplements on the adaptive response of skeletal muscle after resistance training [(5)]. Amino acids are divided into non-essential and essential acids. Among the essential amino acids, three are also referred to as branched-chain amino acids (BCAAs), i.e. leucine, valine and isoleucine, which were supposed to have a special influence on muscle protein synthesis (MPS) [(4,6)].

The quality of the protein may play a role in influencing resistance exercise-induced hypertrophy, again with BCAAs such as leucine exerting special effects on muscle protein synthesis [(7)]. A recent study by Stokes et al. examined young adults and demonstrated that muscle protein synthesis was mainly stimulated by leucine. However, the authors stressed that an excessively high amount of proteins can probably also cause adverse effects and lead to possible disorders of the MPS (dose-response relationship) [(8)].

1.3. Definition of sarcopenia

Sarcopenia, the age-related loss of muscle mass, strength and functionality, is a common phenomenon in older people and is associated with frailty, a limitation of ability to walk and an increase of fall-related fractures [(9)].

The European Working Group on Sarcopenia in Older People (EWGSOP) defines sarcopenia based on the following criteria: low muscle mass, low muscle strength, and lower physical performance [10].

The possible effect of protein supplementation in combination with exercise training in the elderly has already been investigated. The systematic review and meta-analysis by Thomas et al. showed that protein supplements did not result in significant improvements considering muscle mass, muscle strength, anthropometric composition, and functional ability [11].

1.4. Protein paradox

The term "protein paradox" as coined by Klaus et al. [2] describes quite well the current study situation. On the one hand, some studies observed an increase in muscle mass [5], high-protein diets probably improve obesity, triglyceride and blood pressure [12], while on the other hand, other studies yielded favorable effects in the liver by reducing protein intake led to positive effects in mice [13]. Analyzing NHANES III data, Levine et al. reported a positive association between high protein intake and overall mortality as well as risk of type 2 diabetes and cancer in the population aged between 50 and 65 years. In contrast, in senior citizens aged higher than 65 years, there was an inverse association between protein intake and overall mortality and cancer risk, thereby adding to the “paradoxical” situation regarding protein [14].

1.5. Hypothesis

To gain further insight into the implications of protein supplementation for muscle synthesis, the aim of this systematic review and meta-analysis of intervention trials is to investigate the potential anabolic effects of protein supplementation in combination with resistance training. The meta-analyses focused on the impact on anthropometric parameters, muscle strength, muscle hypertrophy and velocity taking different subgroups into consideration (with respect to age, study length, and training condition).

Null hypothesis (H0): The use of protein supplements (whey protein, leucine, HMB, BCAA and EAA) in combination with resistance training, has no significant impact on anthropometric outcomes (BW, LBM, % FM and FM), as well as on parameters measuring muscle strength (leg press, bench press, knee extension, peak power, handgrip strength), muscle hypertrophy (muscle fiber-specific CSA) and velocity (gait speed, 6-min walk distance (MWD), timed up and go test (TUG), chair rise up time and stair climb time).

Alternative hypothesis (H1): The use of protein supplements (whey protein, leucine, HMB, BCAA and EAA) in combination with resistance training, has a significant impact on anthropometric outcomes (BW, LBM, % FM and FM), as well as on parameters measuring muscle strength (leg press, bench press, knee extension, peak power, handgrip strength), muscle hypertrophy (muscle fiber-specific CSA) and velocity (gait speed, 6-min walk distance (MWD), timed up and go test (TUG), chair rise up time and stair climb time).

2. Methods

The protocol of the systematic review and meta-analysis is registered in PROSPERO – International Prospective Register of Systematic Reviews. The preliminary registration number is 404418081013481167.

2.1. Data sources and literature searches

A systematic search of the literature was conducted until April 2018 by using the electronic databases the Cochrane Centre Register of Controlled Trials (CENTRAL), PubMed, and Web of Science. The following search terms were used in PubMed: (leucin*[tiab] OR isoleucin*[tiab] OR valin*[tiab] OR bcaa[tiab] OR branched chain amino acid* [tiab] OR branched-chain amino acid* [tiab] OR essential amino acid* [tiab] OR protein[tiab] OR amino acid*[tiab] OR eaa*[tiab] OR milk*[tiab] OR whey*[tiab] OR soy*[tiab] OR casein*[tiab]) AND ("exercise"[MeSH Terms] OR "exercise"[All Fields] OR "training"[All Fields]) AND ((Randomized Controlled Trial[ptyp] OR Clinical Trial[ptyp]) AND humans[MeSH Terms] AND adult[MeSH Terms]) NOT (Case-Control Studies[MeSH] OR Cohort Studies[MeSH] OR case-control[tiab] OR cohort[tiab] OR case-report[tiab] OR adolescents[All Fields] OR children[All Fields] OR gestational[tiab] OR pregnant[tiab] OR pregnancy[tiab]) NOT (rats[tiab] OR monkeys[tiab] OR primates[tiab] OR rabbits[tiab] OR cats[tiab] OR dogs[tiab] OR mice[tiab] OR pigs[tiab] OR cows[tiab]). The languages were limited to German and English, no restriction was made regarding the publication year.

2.2. Eligibility criteria

All randomized controlled trials or studies with a crossover design were included if they met the following criteria: 1) combination of resistance training plus protein supplements; 2) minimum intervention period six weeks with at least two exercise sessions per week; 3) humans only; 4) enrolling individuals 18 years or older; 5) supplementation with protein; 6) supplementation with leucine at least 2.0 g/d; 7) more than 1.2 g/kg body weight protein per day via diet.

The following anthropometric parameters were defined as primary outcome measures for the assessment of muscle mass and at least one of these parameters had to be available:

- Body weight
- Lean body mass
- Fat mass
- % Fat mass

In addition, secondary outcome measurements were determined, including parameters for the assessment of muscle strength:

- Leg press
- Bench press
- Knee extension
- Peak power
- Handgrip strength

Also included were parameters for the assessment of muscle fiber hypertrophy (muscle fiber type-specific cross-sectional area) and values that measured the velocity, for example gait speed, chair rise time, stair climb time, 6-min walk distance and the timed up and go test (TUG).

2.3. Exclusion criteria

A study was excluded if protein supplementation was given in combination with other supplements, for example creatinine, vitamin D, as well as studies with the intention to treat a clinical condition, such as the metabolic syndrome or obesity. Likewise, all studies on animals, participants under the age of 18, or interventions with a high-protein diet with simultaneous caloric restriction were removed.

2.4. Data extraction and risk of bias assessment

First, the titles and abstracts of the identified records were reviewed and then sorted out according to the inclusion and exclusion criteria. The individual steps of the selection process are shown in the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) flow diagram (Figure 2) [15]. The data from each study were extracted as follows: the first author's name and the year of publication, number of participants, gender, mean age, frail or non-frail (only by study populations with mean age > 70 years), study duration and frequency, type of resistance training, intensity, balance/functional training, type of protein and frequency, timing of intake, administered amounts, control treatment and the baseline protein intake. The study characteristics are shown in Table 1 for subjects with a mean age > 70 years and Table 2 for subjects with a mean age < 70 years. In addition, the outcome measurements of each study are presented in Table 3 and Table 4, respectively. The Cochrane Risk of Bias Tool for Randomized Controlled Trials was used to evaluate the quality of the studies [16]. The risk of bias assessment is presented using a graph (Figure 33) and a summary (Figure 38). Furthermore, the individual tables explain with a detailed depiction of the bias assessment of the respective domains for all studies included and the evaluations of the trials are attached in the appendix (Tables 21-43).

2.5. Statistical analysis

The statistical analyses were performed using the Review Manager Version 5.3 (Nordic Cochrane Center, Copenhagen) by the Cochrane Collaboration. The means ± standard deviations (SD) or the changes from baseline values ± standard deviations of both intervention and control groups were compared in a random-effects model. If the values were given as mean ± standard error (SE), the standard error was converted into standard deviation by using $SD = SE \times \sqrt{N}$. In a single study [17], the median, minimum and maximum were reported. These data were calculated using the formula according to the Method of Hozo et al. [18]. The outcomes are presented as forest plots and sensitivity analyses were performed according to the following subgroups.

- Study length (< 12 weeks vs. > 12 weeks)
- Age (mean age < 70 years vs. > 70 years)
- Training condition (trained vs. untrained)

The analysis regarding training condition considered only study participants with mean age < 70 years. In addition, the single study effect was examined on the overall result of each outcome measurement. Furthermore, funnel plots were evaluated by visual analysis of symmetry and were generated only for outcomes with more than eight trials. Funnel plots were used to identify possible publication bias via indicating a lack of data.

3. Results

The selection procedure starting with 4470 records in the databases and ending with 23 trials with a total of 1235 participants (baseline values), which were included in the systematic review, as well as 18 studies with a total of 635 subjects (completed the study) were included in the meta-analysis is given in Figure 2.

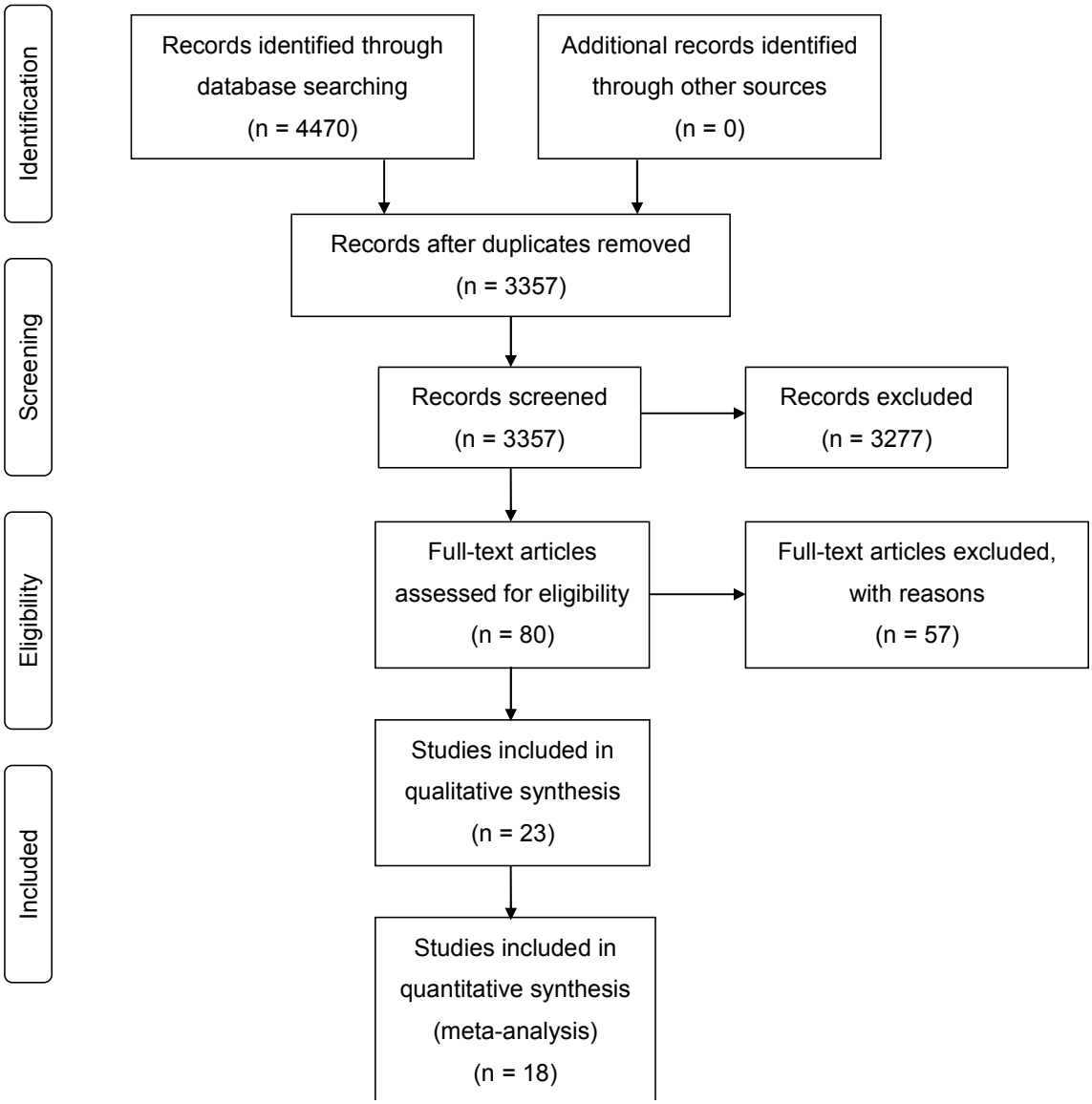

Figure 2: PRISMA flow diagram [15]

In addition, after screening and verification of eligibility 57 articles were excluded and the reasons are described as follows:

- Protein content or amount too low or unknown (8 studies)
- Hypo-caloric diet (4 studies)
- Study length too short (6 studies)
- Outcome not met (6 studies)
- Control group inadequate (7 studies)
- Supplement inappropriate (20 studies)
- Participants not comparable e.g. bodybuilder or the Olympic team (2 studies)
- Exercise training not suitable (2 studies)

The study by Kirn et al.[19] was excluded because only methods and design were described. Regarding the CALM Intervention study (Counteracting Age-related Loss of Skeletal Muscle Mass) by Bechshoft et al.[20], Lars Holm was contacted to find out if more data had already been published. However, it is an ongoing study and the article was therefore removed. Of 23 included studies, another five were excluded from quantitative synthesis (meta-analysis). Reasons for exclusion were: the results were only shown in figures by Kraemer et al.[21] or not available like the results of Ikeda et al.[22]; the standard deviations (Candow et al.[23]) or the values of the control group (Andersen et al.[24]) were missing. Furthermore, one study only reported the least square means ± standard errors by Trabal et al.[25]. The summary of the study characteristics was compiled on the basis of the division into two age categories. The category mean age > 70 years included participants from the following studies; Hofmann et al.[17], Ikeda et al.[22], Trabal et al.[25], Arnarson et al. [26], Chale et al.[27], Godard et al.[28], Karelis et al.[29], and Kim et al.[30].

For the category mean age < 70 years, included studies were: Kraemer et al.[21], Andersen et al. [24], Candow et al.[23], Antonio et al.[31], Coburn et al.[32], Farup et al.[33], Herda et al.[34], Hoffman et al.[35], Hulmi et al.[36], Ispoglou et al.[37], Joy et al.[38], Kerksick et al.[39], Slater et al.[40], Volek et al.[41], and Willoughby et al.[42]. The general characteristics of all studies enrolled in the present systematic review are shown in Table 1 and Table 2, respectively.

Table 1: Summary of study characteristics from subjects with a mean age > 70 years

Author, Year	Number of Participants	Gender	Intervention: Mean Age, Years	Control: Mean Age, Years	Frail/Non-frail	Study Duration and RT Frequency	Type of RT	Intensity	Balance/Functional Training	Type of Protein	Frequency	Timing of Intake	Amount	Control Treatment	Baseline Protein Intake ($g \cdot kg^{-1} \cdot day^{-1}$)
Arnarson et al., 2013 [26]	161	female + male	73.3	74.6	No	3d/wk for 12 weeks	LL+UL	"75%-80% 1-RM, 3 sets with 6-8 reps"	No	Whey concentrate	With training	After training	20 g	CHO	1.00
Chalé et al., 2013 [27]	80	female + male	78.0	77.3	Yes	3d/wk for 24 weeks	LL+UL	"80% 1-RM, 3 sets with 12 reps"	No	Whey	Daily	Breakfast: 20 g; Dinner: 20 g	40 g	CHO (maltodextrin)	0.97
Godard et al., 2002 [28]	17	male	70.8	72.1	No	3d/wk for 12 weeks	LL	80% 1-RM	No	"EAA (2.24 g L-Leucine, 1.20 g L-isoleucine, 1.86 g L-lysine, 1.40 g L-valine, 1.86 g L-phenylalanine, 1.30 g L-histidine, 1.76 g L-threonine, 0.38 g L-methionine)"	Daily	After training	12 g	Exercise	1.14
Hofmann et al., 2016 [17]	91	female	83.9	82.9	No	2d/wk for 24 weeks	LL+UL	light to heavy (yellow to black Thera-Band®)	No	"20.7 g whey protein isolate (3 g leucine, >10 g essential amino acids), 9.3 g carbohydrates, 3 g fat, vitamins (800 IU vitamin D, 2.9 mg vitamin B6, 3 µg vitamin B12) and minerals"	Daily	"every morning after breakfast […] after each training session"	20.7 g	Exercise	NA

Author, Year	Number of Participants	Gender	Intervention: Mean Age, Years	Control: Mean Age, Years	Frail/Non-frail	Study Duration and RT Frequency	Type of RT	Intensity	Balance/Functional Training	Type of Protein	Frequency	Timing of Intake	Amount	Control Treatment	Baseline Protein Intake ($g \cdot kg^{-1} \cdot day^{-1}$)
Ikeda et al., 2016 [22]	55	female + male	-	-	Yes	2d/wk for 28 weeks (1 weeks only RT without supplementation)	LL + UL	"3 sets of 20 repetitions, 30% of maximum voluntary contraction (MVC)"	Yes	"6-g tablet amino acid supplement (calories 25.5 kcal, Amino-Vital tablet, Ajinomoto Co., Inc., Tokyo, Japan). The supplement contained 500 mg of amino acids per 1 g: 260 mg of BCAA and 240 mg of conditionally essential amino acids (105 mg leucine, 85 mg isoleucine, 70 mg valine, 123 mg glutamate, and 117 mg arginine; the content percentage of leucine was 21%)"	With training	"10 minutes before the exercise"	6 g	CHO (maltodextrin)	NA
Karelis et al., 2015 [29]	99	female + male	69.9	71.0	No	3d/wk for 135 days	LL+UL	80% 1-RM	No	"20 g (2 x 10 g pouches) of the cysteine-rich whey protein isolate (Immunocal®)"	Daily	"one at breakfast and the second mid-morning or mid-afternoon"; on training days: "one at breakfast and the second within 1 hour after the end of each exercise session"	20 g	Casein	NA
Kim et al., 2012 [30]	77	female	79.5	79.0	Yes	2d/wk for 12 weeks	LL+UL	Moderate	Yes	"EAA (42% leucine, 14% lysine, 10.5% valine; 10.5% isoleucine, 10.5% threonine, 7% phenylalanine, 5.5% other)"	Daily	Twice daily	6 g (2.52 g leucine)	Exercise	NA
Trabal et al., 2015 [25]	30	female + male	85.0	84.0	No	3d/wk for 12 weeks	LL	65% 1-RM	Yes	Leucine	Daily	Lunch: 5 g, Dinner: 5 g	10 g	CHO	1.23

1-RM = 1-repetition maximum, BCAA = branched-chain amino acids, CHO = carbohydrates, EAA = essential amino acids, LL = lower limb, reps = repetitions, MVC = maximum voluntary contraction, NA = protein intake is not specified, UL = upper limb, wk = week

Table 2: Summary of study characteristics from subjects with a mean age < 70 years

Author, Year	Number of Participants	Gender	Intervention: Mean Age, Years	Control: Mean Age, Years	Fitness	Study Length and RT Frequency	Type of RT	RT Intensity	Balance/Functional Training	Type of Protein	Frequency	Timing of Ingestion	Amount (g)	Control Treatment	Baseline Protein Intake ($g \cdot kg^{-1} \cdot day^{-1}$)
Andersen et al., 2005 [24]	22	male	23.0	23.0	normal	3d/wk for 14 weeks	LL	4-15 1-RM	No	"16.6 g of whey protein; 2.8 g of casein; 2.5 g of egg white protein; 2.8 g of L-glutamine"	Daily	Before and after Training; on non-training days in the morning	Training days: 50 g; non-training days: 25 g	CHO	1.27
Antonio et al., 2000 [31]	21	female	26.9	27.4	untrained	3d/wk for 6 weeks	LL+UL+aerobic	6-12 1-RM	No	EAA 10 g provide 1. 964 g L-Leucine)	Daily	Before and after training; on non-workout days in the morning	Training days: 25.6 g; non-training days: 12.8 g	CHO	NA
Candow et al., 2006 [23]	31	female + male	24.0	-	untrained	3d/wk for 6 weeks	LL+UL	"60-90% of 1-RM"	No	Whey protein	Daily	Before and after training, "before going to bed"; on non-training days: 3 equal doses	"3 equal doses (i.e., 0.5 g/kg body mass supplement powder dissolved in water)"	CHO	1.60
Coburn et al., 2006 [32]	33	male	21.3	22.8	untrained	3d/wk for 8 weeks	LL	"80% of the DCER 1-RM"	No	Whey protein (20.0 g) + L-leucine (6.2 g)	Daily	Before and after training; on non-training days: before breakfast	Training days: 52.4 g; non-training days: 26.2 g	CHO	NA
Farup et al., 2014 [33]	22	male	23.7	24.1	untrained	3d/wk for 12 weeks	LL	moderate-high	No	Whey protein (Leucine 2.77 g, Histidine 0.41 g, Isoleucine 1.29 g, Lysine 1.50 g, Methionine 0.35 g, Phenylalanine 0.90 g, Threonine 1.46 g, Tryptophan 0.37 g, Valine 1.35 g)	With training	"half before and half after training"	39 g	CHO	NA
Herda et al, 2013 [34]	106	male	21.0	20.9	untrained	3d/wk for 8 weeks	LL+UL	80% 1-RM (6 repetitions)	No	"BWP (20 g Polyethylene glycosylated whey protein concentrate + 7 g leucine)"	Daily	"before and after each exercise session and one each on the nontraining day"	Training days: BWP 54 g; non-training days: BWP 27 g	CHO	1.39

Author, Year	Number of Participants	Gender	Intervention: Mean Age, Years	Control: Mean Age, Years	Fitness	Study Length and RT Frequency	Type of RT	RT Intensity	Balance/Functional Training	Type of Protein	Frequency	Timing of Ingestion	Amount (g)	Control Treatment	Baseline Protein Intake ($g \cdot kg^{-1} \cdot day^{-1}$)
Hoffman et al., 2009 [35]	33	male	19.9	20.7	trained	4d/wk for 10 weeks	LL+UL	4-10 RM for 2-4 sets	No	"42 g whey protein isolate + casein protein isolate; 3.6 g of leucine"	Daily	In the morning and evening or before and after training; also on non-training days	84 g	Exercise only	1.62
Hulmi et al., 2009 [36]	38	male	25.2	27.2	untrained	2d/wk for 21 weeks	LL+UL	"40-85% 1-RM"	No	"15 g whey isolate protein: histidine (0.2 g), isoleucine (1.0 g), leucine (1.7 g), lycine (1.4 g), methionine (0.4 g), phenylalanine (0.5 g), threonine (1.0 g), tryptophan (0.2 g) and valine (0.8 g)"	With training	"before and after each bout of RE"	30 g	Water	1.35
Ispoglou et al., 2011 [37]	40	male	28.5	28.2	untrained	2d/wk for 12 weeks	LL+UL	"8-12 RM"	No	L-Leucine	Daily	"immediately following exercise"; on non-training days "3 equal doses (morning, midday, evening)"	4 g (50 $mg \cdot kg^{-1} \cdot BW \cdot d^{-1}$)	CHO (lactose)	0.89
Joy et al., 2013 [38]	24	male	21.3	21.3	trained	3d/wk for 8 weeks	LL+UL	"2-12 RM for 3-5 sets"	No	Whey protein isolate (5.5 g leucine)	With training	"immediately following the workout"	48 g	Rice protein isolate (3,8 g leucine)	NS
Kerksick et al., 2006 [39]	44	female + male	31.0	31.0	trained	4d/wk for 10 weeks	LL+UL	80% 1-RM	No	WC: 40 g whey protein + 8 g casein (Leucine 5.37 mg) ; WBG: 40 g whey + 5 g L-glutamine + 3 g BCAA (Leucine 1504.64 mg)	Daily	After training; on non-training days in the morning	48 g	CHO	2.00
Kraemer et al., 2009 [21]	17	male	22.9	22.9	untrained	3d/wk for 12 weeks	LL+UL	"light: 12-14 RM, moderate: 8-10 RM, heavy: 3-5 RM; 3 sets"	No	"Muscle Armor™(1.5 g calcium HMB, 7 g arginine, 7 g glutamine, 3 g taurine, 5.824 g dextrose)"	Daily	"twice per day (once with breakfast, once with dinner)"	48.6 g	"Non-EAA (10 g glycine, 11.5 g alanine, 1.5 g glutamic acid, 1.5 g serine)" 2*24,5 g	NS

Author, Year	Number of Participants	Gender	Intervention: Mean Age, Years	Control: Mean Age, Years	Fitness	Study Length and RT Frequency	Type of RT	RT Intensity	Balance/Functional Training	Type of Protein	Frequency	Timing of Ingestion	Amount (g)	Control Treatment	Baseline Protein Intake ($g \cdot kg^{-1} \cdot day^{-1}$)
Slater et al., 2001 [40]	27	male	24.9	24.0	trained	2-3d/wk for 6 weeks	LL+UL	"4-6 for 3-5 sets, with a total of 24 to 32 sets per session"	No	HMB (one capsule: 250 mg)	Daily	To main meals three equivalent doses	3 g	Rice flour	NA
Volek et al., 2013 [41]	147	female + male	22.8	22.3	untrained	3d/wk for 9 months	LL+UL	light to heavy	No	Whey protein	Daily	Training days: after exercise; non-training days: after breakfast	20 g	CHO (dextrose)	1.23
Willoughby et al., 2007 [42]	20	male	19.0	19.0	untrained	4d/wk for 10 weeks	LL+UL	"85-90% 1-RM"	No	"28 g of protein (14 g whey protein concentrate, 6 g whey protein isolate, 4 g milk protein isolate, 4 g calcium caseinate), 12 g of free amino acids and 6 g leucine"	Daily	"1h before and immediately after exercise", on non-training days: in the morning	40 g	CHO (dextrose)	2.11

1-RM = 1-repetition maximum, BCAA = branched-chain amino acids, BWP = bio-enhanced whey protein, CHO = carbohydrates, DCER = dynamic constant external resistance, EAA = essential amino acids, LL = lower limb, reps = repetitions, NA = protein intake is not specified, RE = resistance exercise, UL = upper limb, WC = whey protein + casein, wk = week

The outcome measurements as well as the significant protein effects are summarized in Table 3 and Table 4, respectively.

Table 3: Outcome measurements from subjects with a mean age > 70 years

Author, Year	**Outcomes**		**Significant Protein Effect**
Arnarson et al., 2013 [26]	Body composition	LBM; appendicular skeletal muscle mass	NS
	Muscle strength	Knee extension, quadriceps strength (MVC)	
	Physical function	TUG; 6 MWD	
Chalé et al., 2013 [27]	Body composition	BM, LBM, FM	NS
	Muscle strength	Leg press; knee extensors (1-RM)	
	Muscle size	Total mid-thigh and total muscle CSA	
	Power	"peak power for both knee extensors and leg press"	
	Physical function	SPPB (stair-climb time and chair-rise time, 400 m walk)	
Godard et al., 2002 [28]	Muscle strength	Knee extensors MVC (1-RM)	NS
	Muscle size	Total mid-thigh muscle CSA (right)	
Hofmann et al., 2016 [17]	Muscle quality	Chair stand test (lower extremities)	NS
		Handgrip strength (right hand)	
	Biochemical parameters	Follistatin, IGF-1, Myostatin, Activin A, GDF-15	
Ikeda et al., 2016 [22]	Muscle strength	"upper and lower limb isometric strength (leg press, hip abduction, knee extension rowing)"	Leg press, knee extension P < 0,05
	Physical function	TUG, FRT, FAI measures physical activities	FRT P < 0.05
Karelis et al., 2015 [29]	Body composition	BM, LBM, FM	NS
	Muscle strength	Leg press; knee extensors (1-RM)	P < 0.05
Kim et al., 2012 [30]	Body composition	Muscle mass, appendicular skeletal muscle mass, leg muscle mass	NS
	Muscle strength	Knee extension	Knee extension strength P = 0.01
	Physical function	Self-paced and maximum gait velocity	NS
Trabal et al., 2015 [25]	Muscle size	"triceps skin fold, mid-upper arm muscle area (MUAMA), calf cicumference"	NS
	Muscle strength	Isometric leg strength	P = 0.056
	Physical function	SPPB: balance test, TUG, chair rise time, 4 m walk time	NS

1-RM = 1-repetition maximum, 6 MWD = 6-min walk distance, BM = body mass, CSA = cross-sectional area, FAI = Frenchay Activities Index, FM = fat mass, FRT = functional reach distance test, GDF-15 = growth/differentiation factor, IGF-1 = insulin growth factor, LBM = lean body mass, MVC = maximum voluntary contraction, NS = non-significant difference between groups, SPPB = Short Physical Performance Battery, TUG = timed up and go test

Table 4: Outcome measurements from subjects with a mean age < 70 years

Author, Year	Outcome		Significant Protein Effect
Andersen et al., 2005 [24]	Muscle size	fCSA analysis of m. vastus lateralis (right)	P < 0.01
	Muscle strength	Vertical jump performance (SJ, CMJ)	SJ P < 0.01
	Power	Peak torque (isokinetic and isometric, eccentric and concentric)	
Antonio et al., 2000 [31]	Body composition	BM, TBW, LBM, TFM, % Fat, BMC	NS
	Muscle strength	TWL	
	Aerobic endurance	TTE	TTE (min) P < 0.05
Candow et al., 2006 [23]	Body composition	LTM	P < 0.05
	Muscle strength	Squat and bench press strength (1-RM)	P < 0.05
	Biochemical parameters	3-methylhistidine (urinary analysis)	NS
Coburn et al., 2006 [32]	Body composition	BM, % Fat, FFM, FM	NS
	Muscle strength	1-RM DCER strength (trained + untrained limb)	Trained limb: P < 0.05
	Muscle size	M. quadriceps femoris CSA (trained + untrained limb)	Vastus lateralis at proximal level of untrained limb: 6.44%
Farup et al., 2014 [33]	Muscle size	CSA m. quadriceps femoris and patellar tendon	Quadriceps CSA: P < 0.001; patellar tendon CSA: P < 0.05
	Muscle strength	Isometric strength performance (MVC, RFD) + EMG	NS
Herda et al, 2013 [34]	Body composition	LBM, %FM, BM	NS
	Muscle size	muscle CSA (right thigh)	NS
	Muscle strength	1RM leg press and bench press	NS
	Biochemical parameters	Blood analysis	NS
Hoffman et al., 2009 [35]	Body composition	BM, % Fat, LBM, FM	NS
	Muscle strength	1-RM squat and bench press	Bench press P < 0.05
	Power	Bench press, squat	NS
	Biochemical parameters	Urinary nitrogen excretion	NS
Hulmi et al., 2009 [36]	Body composition	BM, % FM	NS
	Muscle size	Muscle CSA (right quadriceps femoris muscle, vastus lateralis)	Vastus lateralis P < 0.05
	Muscle strength	Dynamic 1-RM leg press, isometric leg press, knee extension, knee flexion and bench press	NS
	Gene expression	Muscle mRNA levels	cdk2 mRNA P = 0.08
Ispoglou et al., 2011 [37]	Body composition	FM, LBM	NS
	Muscle strength	"leg press, bench press, chest cross, pullover, overhead press, preacher curls, triceps press (All Nautilus, USA) and prone leg curl (Nautilus Nitro, USA)", 5-RM	Leg press (P = 0.010), bench press (P = 0.02), pullover (P = 0.03), preacher curls (P = 0.004), triceps press (P = 0.002), total strength (P < 0.001)
	Biochemical parameters	Blood analysis	NS
	Evaluation of the effort	RPE, 7-d Physical Activity Recall	NS

Author, Year	Outcome		Significant Protein Effect
Joy et al., 2013 [38]	Body composition	LBM, %FM	NS
	Muscle strength	Bench press, leg press (1-RM)	NS
	Muscle size	"muscle thickness of the biceps brachii, vastus lateralis and intermedius muscle"	NS
	Power	Max. cycling ergometry (10 sec) [wingate test]	NS
Kerksick et al., 2006 [39]	Body composition	BM, LBM, FM, %FM, FFM, BMC	NS
	Muscle strength	Leg press, bench press (1-RM)	NS
	Power	Anaerobic capacity, cycle ergometer (30 sec) [wingate test]	NS
	Biochemical parameters	Blood analysis	NS
Kraemer et al., 2009 [21]	Body composition	BM, LBM, %FM	$P \leq 0.05$
	Muscle strength	Squat and bench press (1-RM)	$P \leq 0.05$
	Muscle size	Circumference measurement, patella tendon thickness	Circumference: thigh $P \leq 0.05$
	Power	Verical jump performance (CMJ)	$P \leq 0.05$
	Biochemical parameters	Hormones, muscle damage markers	$P \leq 0.05$ (testosteron, creatine kinase)
Slater et al., 2001 [40]	Body composition	LBM, FM, BW	NS
	Muscle strength	Bench press, leg press, chins (3RM isoinertial strength test)	NS
	Biochemical parameters	Blood and urinary analyses	Plasma HMB: $P < 0.01$; urinary HMB excretion $P < 0.01$
Volek et al., 2013 [41]	Body composition	LBM, FM, BM, %FM	LBM $P < 0.05$
	Muscle strength	Squat, bench press (1-RM)	NS
	Biochemical parameters	Fasting plasma leucine versus LBM	$R^2 = 0.17$, $P < 0.005$
Willoughby et al., 2007 [42]	Body composition	BM, Body water, %FM, FM, FFM	BM, FFM $P \leq 0.05$
	Muscle strength	Bench press, leg press (1-RM)	$P \leq 0.05$
	Muscle size	Thigh mass	$P \leq 0.05$
	Biochemical parameters	Myofibrillar protein content, serum insulin, serum IGF-1 levels	Myofibrillar protein and IGF-1 $P \leq 0.05$
	Gene expression	Skeletal muscle IGF-1 mRNA, MHC isoform mRNA/protein composition	$P \leq 0.05$

1-RM = 1-repetition maximum, BM = body mass, BMC = bone mineral content, BW = body weight, cdk2 mRNA = cyclin-dependent kinase 2 messenger ribonucleic acid, CMJ = countermovement jump, CSA = cross-sectional area, DCER = dynamic constant external resistance, EMG = electromyography, FFM = fat free mass, FM = fat mass, HMB = beta-hydroxy beta-methylbutyric acid, IGF-1 = insulin-like growth factor, LBM = lean body mass, LTM = lean tissue mass, MHC = myosin heavy chain, MVC = maximum voluntary contraction, NS = non-significant difference between groups, RFD = rate of force development, RPE = ratings of perceived exertion, SJ = squat jump, TBW = total body water, TFM = total fat mass, TTE = treadmill time to exhaustion, TWL = total weight lifted (weight x repetitions)

3.1. Anthropometric measurements

3.1.1. Body weight

Figure 3 illustrates that the change in body weight in kilogram (kg) after protein supplementation was not significantly greater when compared to control treatments. Weighted mean difference (WMD) was 0.79 kg, 95% CI [-0.55, 2.13], P = 0.25. The WMD of the subgroup with a mean age < 70 years was 1.01 kg, 96% CI [-0.45, 2.47], P = 0.18 and for subjects with a mean age > 70 years, the corresponding data was -0.37 kg, 95% CI [-3.75, 3.02], P = 0.83.

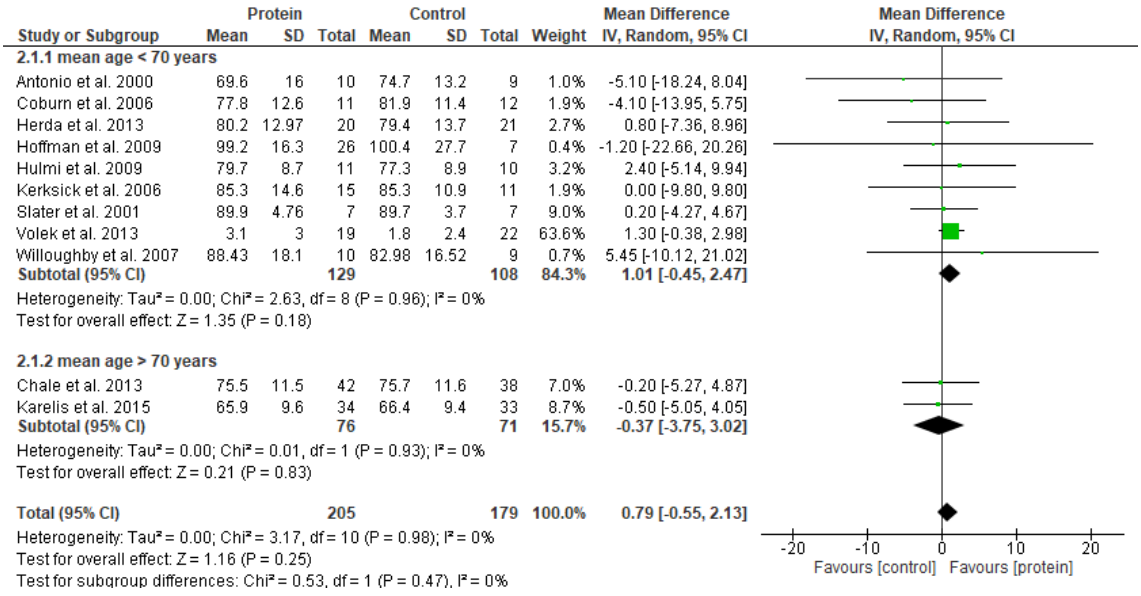

Figure 3: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for body weight in kilogram (kg) of 11 randomized controlled trials comparing the effect of protein supplementation versus control treatments. The data were separated into subgroups (mean age < 70 years vs. mean age > 70 years). The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond represents the mean difference with the 95% CI of the subgroups and the diamond at the bottom of the graph shows the pooled weighted mean differences including 95% CI.

Based on a further subgroup analysis, no significant difference between the intervention and the control treatment was found in trials with either a study length < 12 weeks or > 12 weeks. The weighted mean difference was -0.30 kg, 95% CI [-3.50, 2.89], P = 0.85 for a duration < 12 weeks while a study length > 12 weeks 1.03 kg, 95% CI [-0.45, 2.50], P = 0.17. The difference regarding protein supplementation versus control treatment was non-significant (Figure 4).

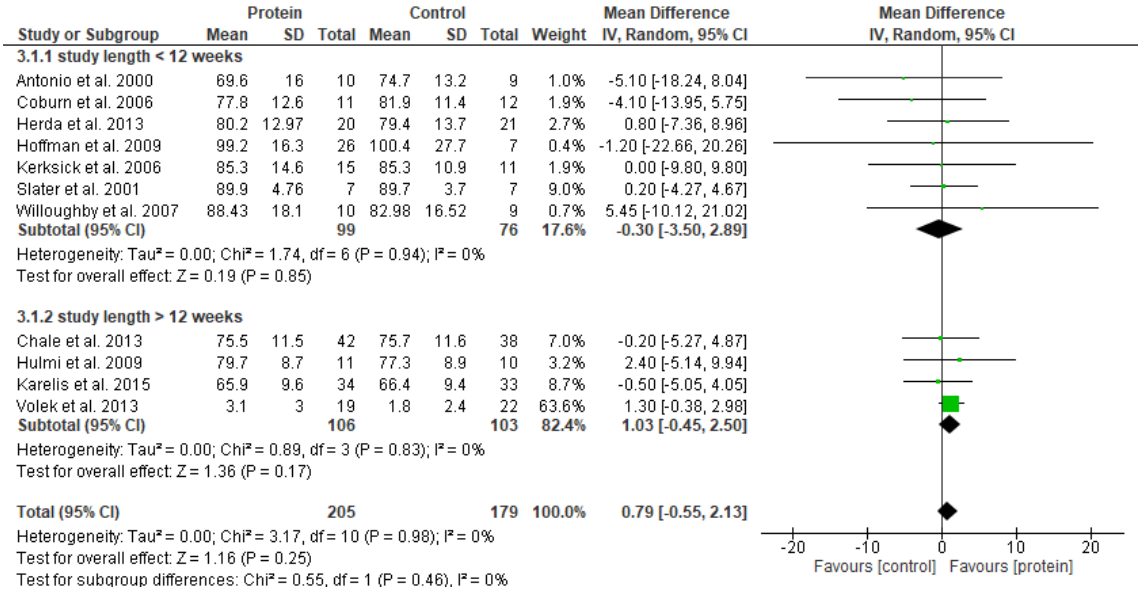

Figure 4: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for body weight in kilogram (kg) of 11 randomized controlled trials comparing the effect of protein supplementation versus control treatments. The data were separated into subgroups (< 12 weeks vs. > 12 weeks). The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond represents the mean difference with the 95% CI of the subgroups and the diamond at the bottom of the graph shows the pooled weighted mean differences including 95% CI.

Figure 5 shows the results for body weight for subgroups separated by training condition. WMD was 1.14 kg, 95% CI [-0.42, 2.71], P = 0.15 for untrained subjects, and 0.12 kg, 95% CI [-3.88, 4.11], P = 0.95 for trained subjects, respectively. This subgroup analysis considered only study participants with mean age < 70 years, the overall WMD was 1.01 kg, 95% CI [-0.45, 2.47], P = 0.18.

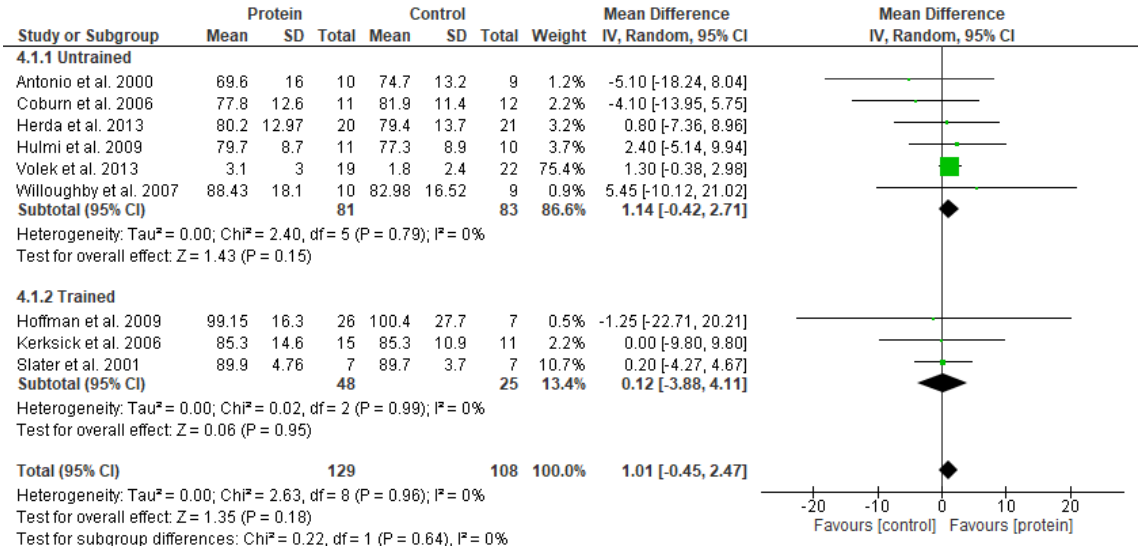

Figure 5: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for body weight in kilogram (kg) of 9 randomized controlled trials comparing the effect of protein supplementation versus control treatments. The data were separated into subgroups (untrained vs. trained). The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond represents the mean difference with the 95% CI of the subgroups and the diamond at the bottom of the graph shows the pooled weighted mean differences including 95% CI.

The sensitivity analysis of the single studies revealed no impact on the overall effect (Table 5).

Table 5: Results of the sensitivity analysis regarding the single study effect for the outcome body weight in kilogram (kg)

Without	Protein (n)	Control (n)	Mean Difference IV, Random, 95% CI	Test for overall effect Z (p)	I^2
Antonio et al. 2000	195	170	0.85 [-0.49, 2.20]	1.24 (0.21)	0%
Coburn et al. 2006	194	167	0.88 [-0.47, 2.24]	1.28 (0.20)	0%
Hoffman et al. 2009	179	172	0.80 [-0.54, 2.14]	1.17 (0.24)	0%
Karelis et al. 2015	171	146	0.91 [-0.49, 2.32]	1.28 (0.20)	0%
Chale et al. 2013	163	141	0.87 [-0.52, 2.26]	1.22 (0.22)	0%
Kerksick et al. 2006	190	168	0.81 [-0.55, 2.16]	1.17 (0.24)	0%
Slater et al. 2001	198	172	0.85 [-0.56, 2.26]	1.19 (0.24	0%
Herda et al. 2013	185	158	0.79 [-0.57, 2.15]	1.14 (0.25)	0%
Volek et al. 2013	186	157	-0.10 [-2.32, 2.12]	0.09 (0.93)	0%
Hulmi et al. 2009	194	169	0.74 [-0.62, 2.10]	1.06 (0.29)	0%
Willoughby et al. 2007	195	170	0.76 [-0.59, 2.10]	1.10 (0.27)	0%
Total	**205**	**179**	**0.79 [-0.55, 2.13]**	**1.16 (0.25)**	**0%**

CI = confidence interval, p = probability value, I^2 = heterogeneity, n = number of participants

3.1.2. Lean body mass

Figures 6 to 8 show the change in lean body mass in kilogram (kg) for each subgroup (age, study length and training condition) and the total weighted mean difference which was -0.00 kg, 95% CI [-0.39, 0.39], P = 1.00. When separating by age, no significant change in lean body mass could be observed either for study participants aged < 70 years (WMD 0.70 kg, 95% CI [-0.16, 1.55], P = 0.11) or for subjects aged > 70 years (WMD -0.18 kg, 95% CI [-0.61, 0.25], P = 0.42) (Figure 6).

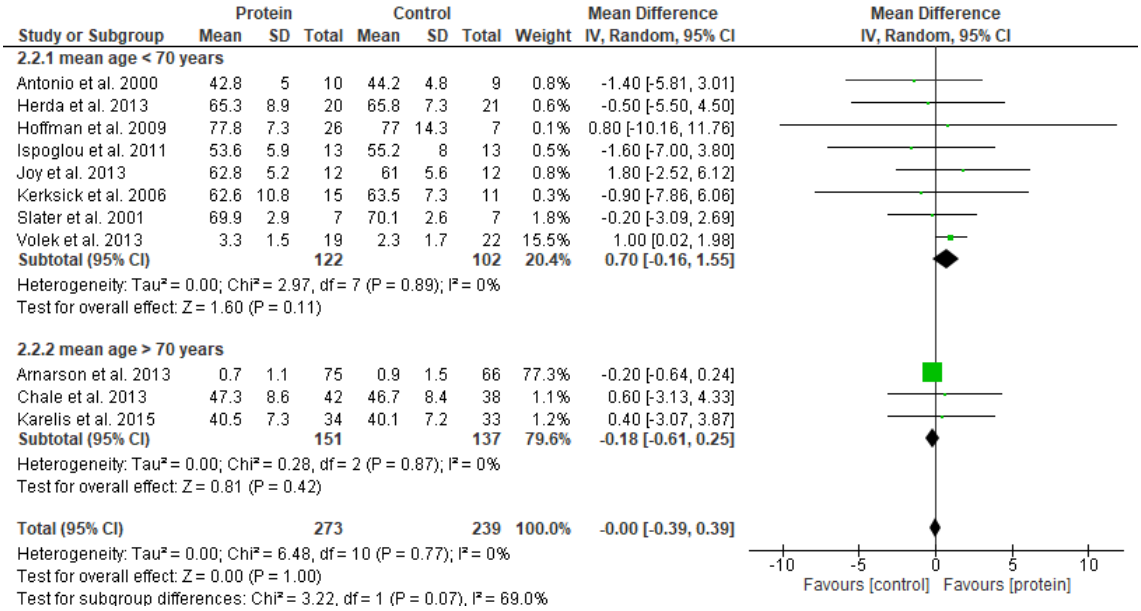

Figure 6: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for lean body mass in kilogram (kg) of 11 randomized controlled trials comparing the effect of protein supplementation versus control treatments. The data were separated into subgroups (mean age < 70 years vs. mean age > 70 years). The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond represents the mean difference with the 95% CI of the subgroup and the diamond at the bottom of the graph shows the pooled weighted mean differences including 95% CI.

The forest plot in Figure 7 presents the subgroup analysis for study length. The difference between protein supplements and control treatments was not significant, i.e. the WMD for subgroup with a study length < 12 weeks was -0.11 kg, 95% CI [-1.95, 1.74], P = 0.91, and 0.20 kg, 95% CI [-0.50, 0.90], P = 0.58 in intervention trials with a study duration > 12 weeks.

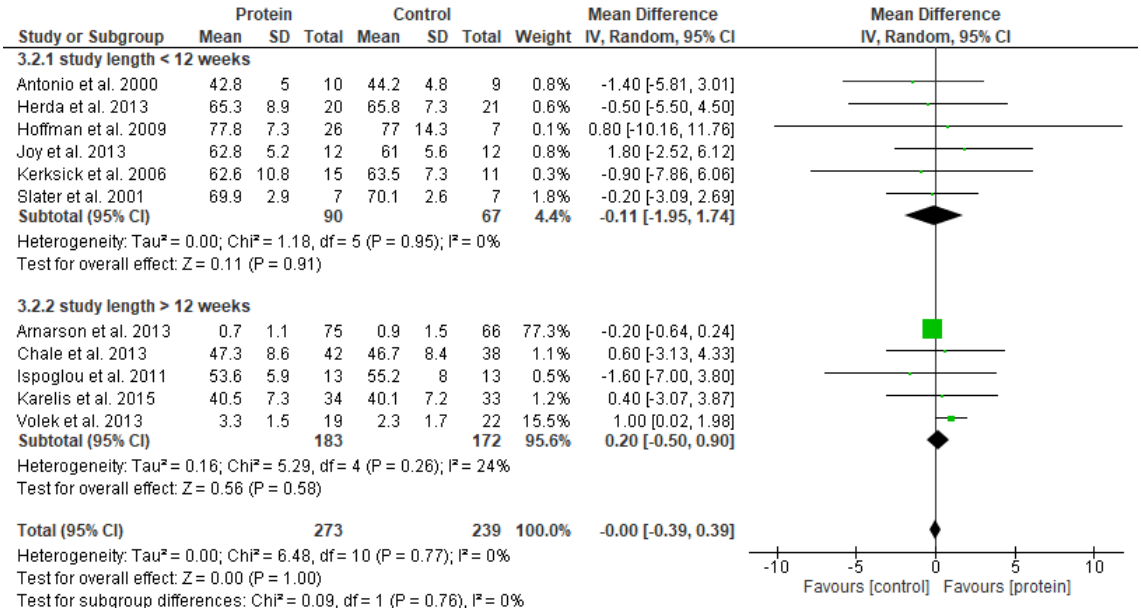

Figure 7: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for lean body mass in kilogram (kg) of 11 randomized controlled trials comparing the effect of protein supplementation versus control treatments. The data were separated into subgroups (< 12 weeks vs. > 12 weeks). The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond represents the mean difference with the 95% CI of the subgroup and the diamond at the bottom of the graph shows the pooled weighted mean differences including 95% CI.

Figure 8 demonstrates the mean difference of the subgroup analysis with respect to training condition (WMD 0.70 kg, 95% CI [-0.16, 1.55], P = 0.11). The mean difference of untrained subjects was 0.77 kg, 95% CI [-0.16, 1.69], P = 0.10, the corresponding values for trained volunteers was 0.30 kg, 95% CI [-1.92, 2.52], P = 0.79 of trained participants.

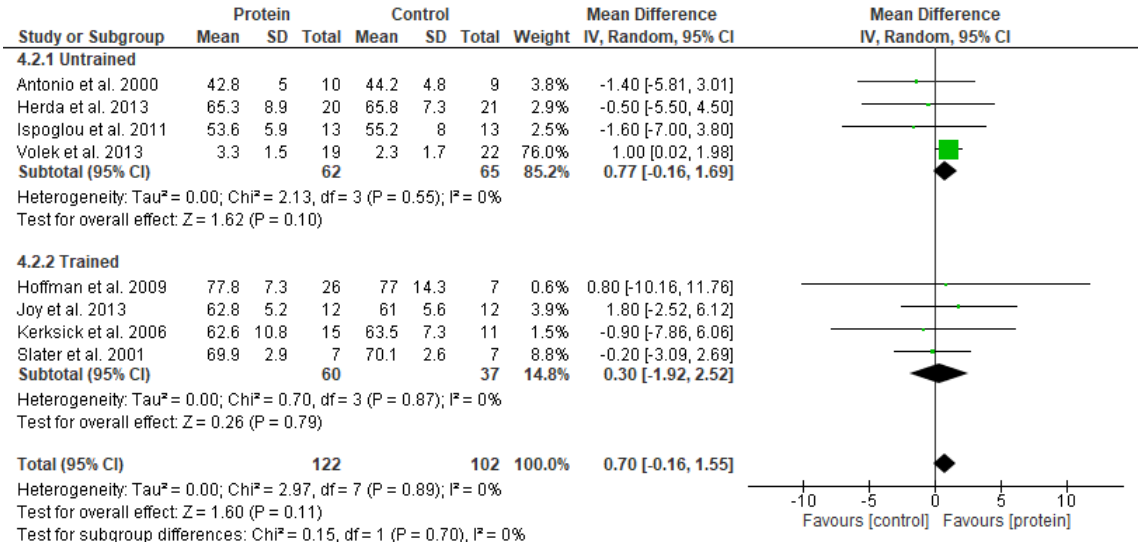

Figure 8: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for lean body mass in kilogram (kg) of 8 randomized controlled trials comparing the effect of protein supplementation versus control treatments. The data were separated into subgroups (untrained vs. trained). The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond represents the mean difference with the 95% CI of the subgroup and the diamond at the bottom of the graph shows the pooled weighted mean differences including 95% CI.

The sensitivity analysis of single study effects yielded no change in significance levels of the overall effect (Table 6).

Table 6: Results of the sensitivity analysis regarding the single study effect for the outcome lean body mass in kilogram (kg)

Without	Protein (n)	Control (n)	Mean Difference IV, Random, 95% CI	Test for overall effect Z (p)	I^2
Antonio et al. 2000	263	230	0.01 [-0.38, 0.40]	0.05 (0.96)	0%
Arnarson et al. 2013	198	173	0.68 [-0.13, 1.49]	1.64 (0.10)	0%
Chale et al. 2013	231	201	-0.01 [-0.40, 0.38]	0.04 (0.97)	0%
Herda et al. 2013	253	218	0.00 [-0.38, 0.39]	0.01 (0.99)	0%
Hoffman et al. 2009	247	232	-0.00 [-0.39, 0.38]	0.01 (0.99)	0%
Ispoglou et al. 2011	260	226	0.01 [-0.38, 0.39]	0.04 (0.97)	0%
Joy et al. 2013	261	227	-0.02 [-0.40, 0.37]	0.08 (0.94)	0%
Karelis et al. 2015	239	206	-0.01 [-0.39, 0.38]	0.03 (0.98)	0%
Kerksick et al. 2006	258	228	0.00 [-0.38, 0.39]	0.01 (0.99)	0%
Slater et al. 2001	266	232	0.00 [-0.39, 0.39]	0.01 (0.99)	0%
Volek et al. 2013	254	217	-0.18 [-0.60, 0.24]	0.86 (0.39)	0%
Total	**273**	**239**	**-0.00 [-0.39, 0.39]**	**0.00 (1.00)**	**0%**

CI = confidence interval, p = probability value, I^2 = heterogeneity, n = number of participants

3.1.3. Fat mass

The forest plot for the change in fat mass (kg) separated by age of participants is given in Figure 9. Overall WMD was -0.14 kg, 95% CI [-1.19, 0.92], P = 0.80. The mean difference of the subgroup with a mean age < 70 years was -0.05 kg, 96% CI [-1.28, 1.19], P = 0.94, and for subjects with a mean age > 70 years, WMD was -0.39 kg, 95% CI [-2.44, 1.66], P = 0.71. Taken together, the analysis showed a non-significant change in fat mass.

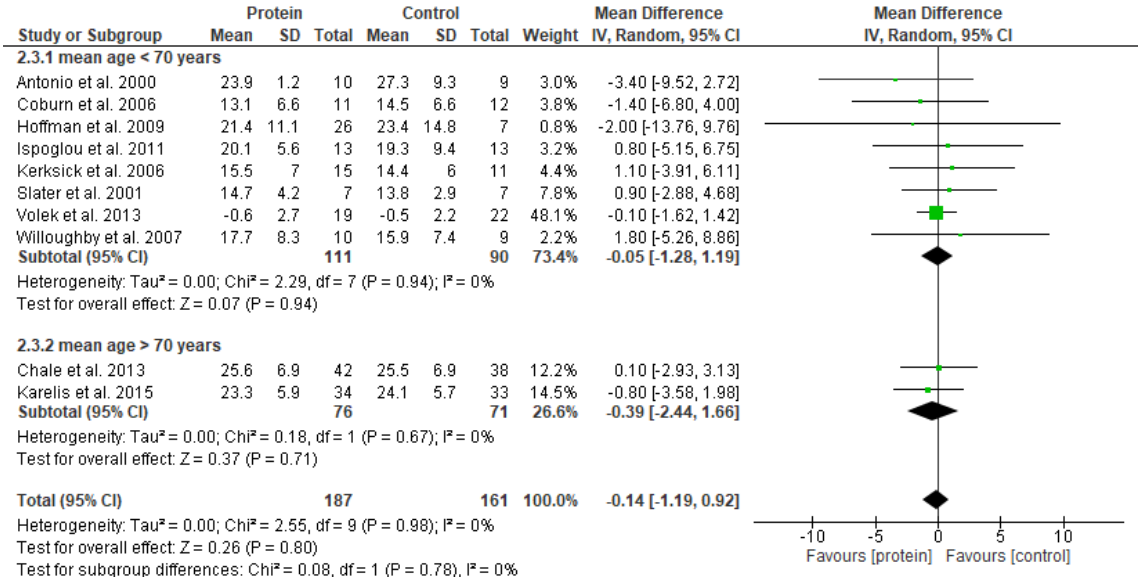

Figure 9: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for fat mass in kilogram (kg) of 10 randomized controlled trials comparing the effect of protein supplementation versus control treatments. The data were separated into subgroups (mean age < 70 years vs. mean age > 70 years). The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond represents the mean difference with the 95% CI of the subgroup and the diamond at the bottom of the graph shows the pooled weighted mean differences including 95% CI.

Figure 10 summarizes the outcome parameter fat mass following separation into subgroups according to study length. The WMD for the studies with a duration < 12 weeks was -0.05 kg, 95% CI [-2.30, 2.19], P = 0.96, and for studies with a duration > 12 weeks, WMD turned out to be -0.16 kg, 95% CI [-1.36, 1.03], P = 0.79.

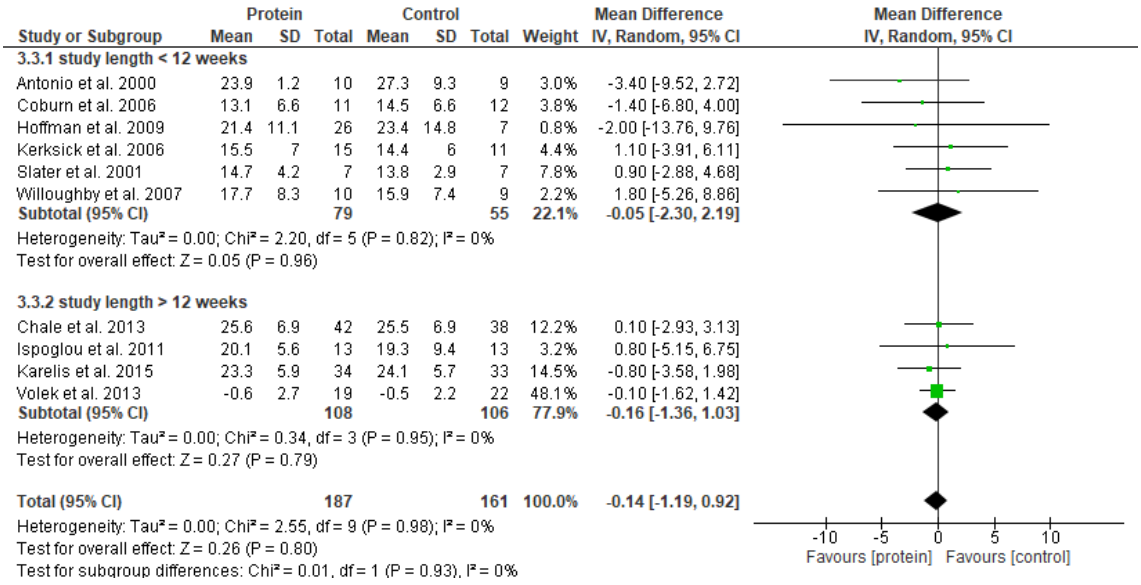

Figure 10: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for fat mass in kilogram (kg) of 10 randomized controlled trials comparing the effect of protein supplementation versus control treatments. The data were separated into subgroups (< 12 weeks vs. > 12 weeks). The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond represents the mean difference with the 95% CI of the subgroup and the diamond at the bottom of the graph shows the pooled weighted mean differences including 95% CI.

Based on a further subgroup analysis in terms of the training condition (Figure 11), there was no significant difference between the protein supplements and the control treatments (overall WMD -0.05 kg, 95% CI [-1.28, 1.19], P = 0.94). The weighted mean difference was -0.23 kg, 95% CI [-1.59, 1.13], P = 0.74 for untrained participants and 0.79 kg, 95% CI [-2.13, 3.71], P = 0.60 for trained participants, respectively.

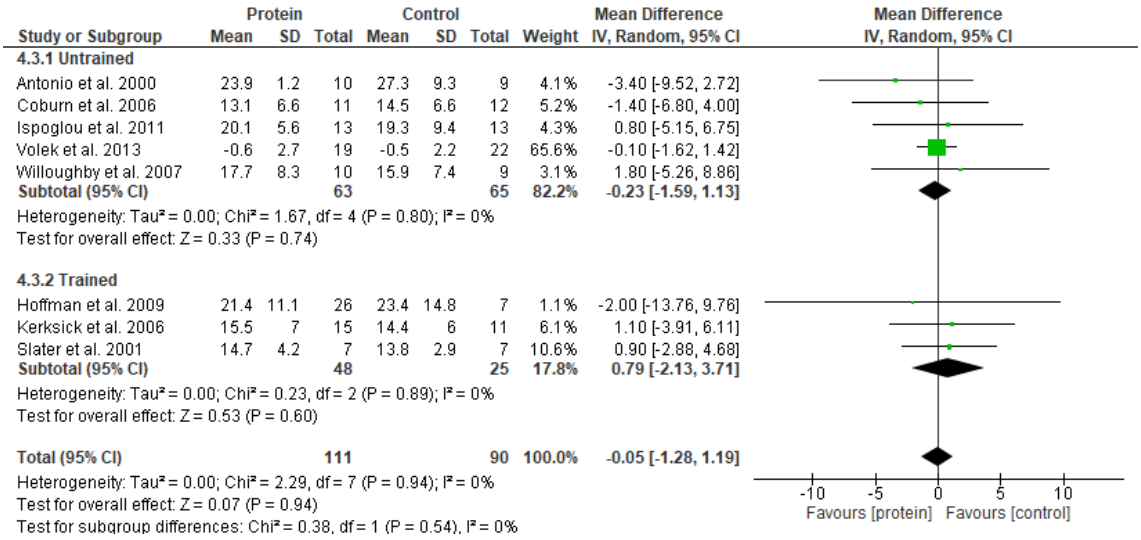

Figure 11: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for body weight in kilogram (kg) of 8 randomized controlled trials comparing the effect of protein supplementation versus control treatments. The data were separated into subgroups (untrained vs. trained). Study participants with a mean age > 70 years were not included in this subgroup analysis. The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond represents the mean difference with the 95% CI of the subgroup and the diamond at the bottom of the graph shows the pooled weighted mean differences including 95% CI.

Table 7 compiles the results of the sensitivity analyses regarding single study effects for fat mass showing no statistically significant changes in the initial probability values.

Table 7: Results of the sensitivity analysis regarding the single study effect for the outcome fat mass in kilogram (kg)

Without	Protein (n)	Control (n)	Mean Difference IV, Random, 95% CI	Test for overall effect Z (p)	I^2
Antonio et al. 2000	177	152	-0.04 [-1.11, 1.03]	0.07 (0.94)	0%
Chale et al. 2013	145	123	-0.17 [-1.30, 0.96]	0.30 (0.77)	0%
Coburn et al. 2006	176	149	-0.09 [-1.16, 0.99]	0.16 (0.87)	0%
Hoffman et al. 2009	161	154	-0.12 [-1.18, 0.94]	0.23 (0.82)	0%
Ispoglou et al. 2011	174	148	-0.17 [-1.24, 0.90]	0.31 (0.76)	0%
Karelis et al. 2015	153	128	-0.03 [-1.17, 1.12]	0.04 (0.96)	0%
Kerksick et al. 2006	172	150	-0.20 [-1.28, 0.89]	0.35 (0.72)	0%
Slater et al. 2001	180	154	-0.23 [-1.33, 0.87]	0.40 (0.69)	0%
Volek et al. 2013	168	139	-0.17 [-1.64, 1.29]	0.23 (0.82)	0%
Willoughby et al. 2007	177	152	-0.18 [-1.25, 0.89]	0.33 (0.74)	0%
Total	**187**	**161**	**-0.14 [-1.19, 0.92]**	**0.26 (0.80)**	**0%**

CI = confidence interval, p = probability value, I^2 = heterogeneity, n = number of participants

3.1.4. % Fat mass

The forest plot in Figure 12 presents the results of the meta-analysis of fat mass in %. No significant differences in overall WMD could be observed when comparing protein groups with the corresponding controls. The WMD in study participants aged < 70 years was -0.00, 95% CI [-1.16, 1.15], P = 1.00. For this parameter, there were no data available from intervention trials with participants aged > 70 years.

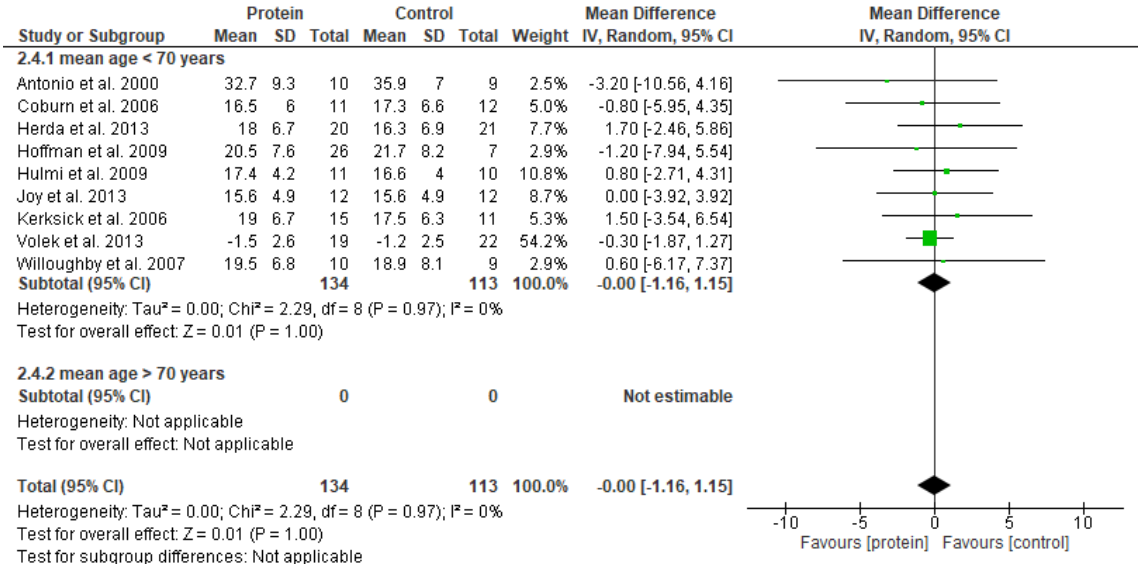

Figure 12: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for fat mass in percentage (%) of 9 randomized controlled trials comparing the effect of protein supplementation versus control treatments. The data were separated into subgroups (mean age < 70 years vs. mean age > 70 years). All study participants had a mean age < 70 years. The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond represents the mean difference with the 95% CI of the subgroup and the diamond at the bottom of the graph shows the pooled weighted mean differences including 95% CI.

Figure 13 shows the outcome parameter fat mass in % separating studies according to study length. WMD was 0.21 %, 95% CI [-1.74, 2.16], P = 0.83 for studies with a length < 12 weeks, and -0.12 %, 95% CI [-1.55, 1.31], P = 0.87 for studies with a length > 12 weeks, respectively.

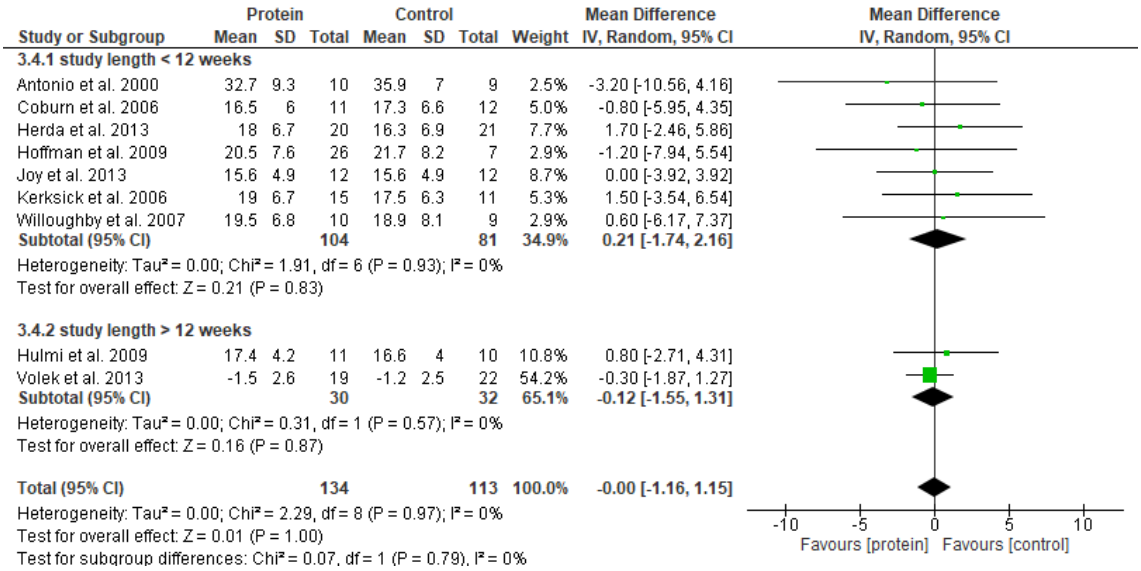

Figure 13: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for fat mass in percentage (%) of 9 randomized controlled trials comparing the effect of protein supplementation versus control treatments. The data were separated into subgroups (< 12 weeks vs. > 12 weeks). The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond represents the mean difference with the 95% CI of the subgroup and the diamond at the bottom of the graph shows the pooled weighted mean differences including 95% CI.

Subgroup analysis with respect to training conditions reveals no significant differences between protein supplementation and controls both for untrained participants (WMD -0.06 %, 95% CI [-1.32, 1.21], P = 0.93) as well as for trained participants (WMD 0.26 %, 95% CI [-2.55, 3.07], P = 0.86) (Figure 14).

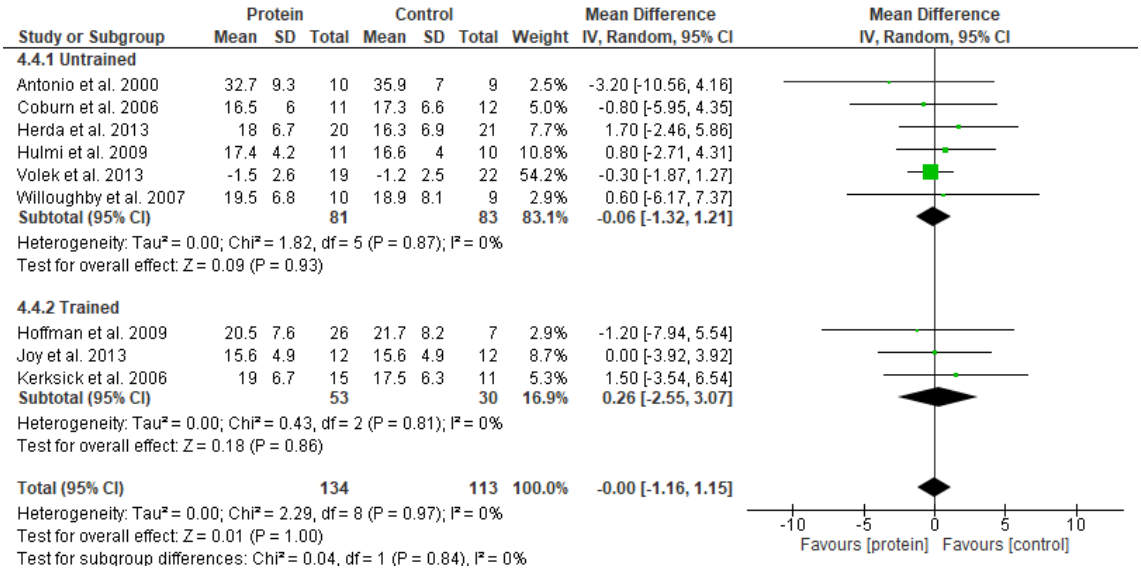

Figure 14: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for fat mass in percentage (%) of 9 randomized controlled trials comparing the effect of protein supplementation versus control treatments. The data were separated into subgroups (untrained vs. trained). The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond represents the mean difference with the 95% CI of the subgroup and the diamond at the bottom of the graph shows the pooled weighted mean differences including 95% CI.

The sensitivity analyses of single studies showed no impact on the overall effect (Table 8).

Table 8: Results of the sensitivity analysis regarding the single study effect for the outcome fat mass in percentage (%)

Without	Protein (n)	Control (n)	Mean Difference IV, Random, 95% CI	Test for overall effect Z (p)	I^2
Antonio et al. 2000	124	104	0.08 [-1.09, 1.25]	0.13 (0.90)	0%
Coburn et al. 2006	123	101	0.04 [-1.15, 1.22]	0.06 (0.95)	0%
Herda et al. 2013	114	92	-0.15 [-1.35, 1.06]	0.24 (0.81)	0%
Hoffman et al. 2009	108	106	0.03 [-1.14, 1.20]	0.06 (0.96)	0%
Hulmi et al. 2009	123	103	-0.10 [-1.32, 1.12]	0.16 (0.87)	0%
Joy et al. 2013	122	101	-0.00 [-1.21, 1.20]	0.01 (1.00)	0%
Kerksick et al. 2006	119	102	-0.09 [-1.27, 1.10]	0.14 (0.89)	0%
Volek et al. 2013	115	91	0.35 [-1.36, 2.05]	0.40 (0.69)	0%
Willoughby et al. 2007	124	104	-0.02 [-1.19, 1.15]	0.04 (0.97)	0%
Total	**134**	**113**	**-0.00 [-1.16, 1.15]**	**0.01 (1.00)**	**0%**

CI = confidence interval, p = probability value, I^2 = heterogeneity, n = number of participants

In summary, all analyses on anthropometric parameters showed no significant changes in the comparison of protein supplementation versus control treatments.

3.2. Muscle strength

The Figures and Tables below summarize the analyses of different muscle strength parameters.

3.2.1. Leg press

Figure 15 represents the forest plot for the outcome parameter leg press in kilogram (kg). Protein supplementation resulted in a significant increase in leg press muscle strength (WMD 0.73 kg, 95% CI [0.06, 1.39], P = 0.03) when compared to control treatments.

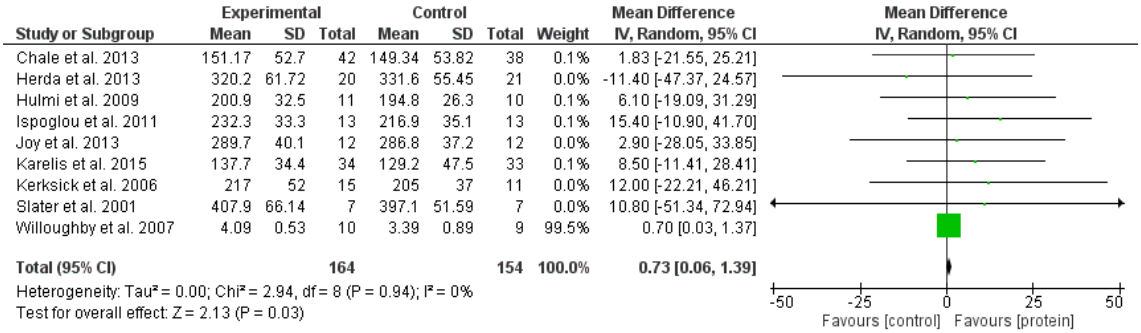

Figure 15: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for leg press of 9 randomized controlled trials comparing the effect of protein supplementation versus control treatments. The horizontal line shows the 95% CI of these effects. To improve the readability of the effect size, the arrow was used at the end of the CI. This study has a smaller sample size (n) and a larger standard deviation (SD).The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond at the bottom of the graph represents the pooled weighted mean differences including 95% CI.

However, this result could not be confirmed by any of the subgroup analyses according to mean age, study length, and training condition (Table 9).

Table 9: Results of the sensitivity analysis regarding the subgroups effect for the outcome leg press in kilogram (kg)

Subgroup	Protein (n)	Control (n)	Mean Difference IV, Random, 95% CI	Test for overall effect Z (p)	I^2
mean age < 70 years	88	83	0.72 [0.05, 1.38]	2.10 (0.04)	0%
mean age > 70 years	76	71	5.70 [-9.46, 20.86]	0.74 (0.46)	0%
study length < 12 weeks	64	60	0.70 [0.03, 1.37]	2.06 (0.04)	0%
study length > 12 weeks	100	94	7.69 [-3.96, 19.33]	1.29 (0.20)	0%
trained	34	30	7.45 [-14.08, 28.98]	0.68 (0.50)	0%
untrained	54	53	0.71 [0.04, 1.38]	2.08 (0.04)	0%
Total	**164**	**154**	**0.73 [0.06, 1.39]**	**2.13 (0.03)**	**0%**

CI = confidence interval, p = probability value, I^2 = heterogeneity, n = number of participants

Table 10 gives the results of the sensitivity analyses (single study effects) for the parameter leg press in kilogram (kg). Following removal of the study by Willoughby et al.[(42)], the overall result was no longer significant (WMD 6.21 kg, 95% CI [- 3.65, 16.06], P = 0.22).

Table 10: Results of the sensitivity analysis regarding the single study effect for the outcome leg press in kilogram (kg)

Without	Protein (n)	Control (n)	Mean Difference IV, Random, 95% CI	Test for overall effect Z (p)	I^2
Chale et al. 2013	122	116	0.72 [0.06, 1.39]	2.13 (0.03)	0%
Herda et al. 2013	144	133	0.73 [0.06, 1.40]	2.15 (0.03)	0%
Hulmi et al. 2009	153	144	0.72 [0.05, 1.39]	2.12 (0.03)	0%
Ispoglou et al. 2011	151	141	0.72 [0.05, 1.38]	2.10 (0.04)	0%
Joy et al. 2013	152	142	0.72 [0.06, 1.39]	2.13 (0.03)	0%
Karelis et al. 2015	130	121	0.72 [0.05, 1.38]	2.11 (0.04)	0%
Kerksick et al. 2006	149	143	0.72 [0.05, 1.39]	2.12 (0.03)	0%
Slater et al. 2001	157	147	0.72 [0.06, 1.39]	2.13 (0.03)	0%
Willoughby et al. 2007	154	145	6.21 [-3.65, 16.06]	1.23 (0.22)	0%
Total	**164**	**154**	**0.73 [0.06, 1.39]**	**2.13 (0.03)**	**0%**

CI = confidence interval, p = probability value, I^2 = heterogeneity, n = number of participants

3.2.2. Bench press

Likewise to leg press, improvements in muscle strength measured via bench presses were significantly more pronounced in the intervention group treated with protein supplements as compared to control treatments (Figure 16). The weighted mean difference was 0.31 kg, 95% CI [0.15, 0.47], P = 0.0001.

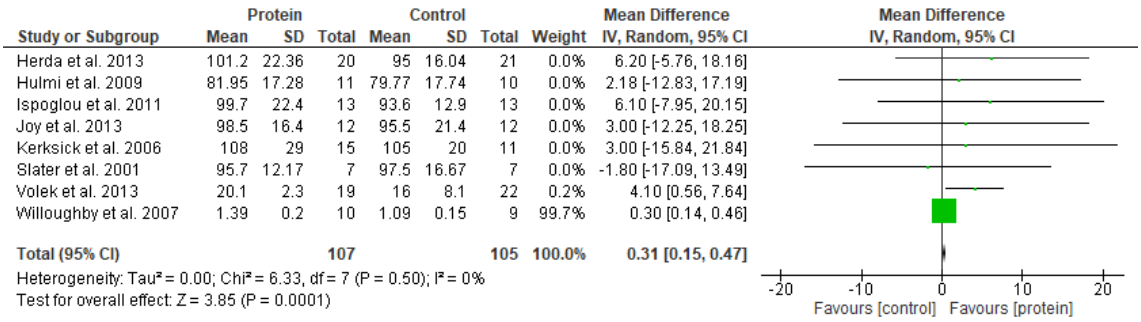

Figure 16: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for bench press in kilogram (kg) of 8 randomized controlled trials comparing the effect of protein supplementation versus control treatments. The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond at the bottom of the graph represents the pooled weighted mean differences including 95% CI.

Table 11 outlines the results of the subgroup analysis according to study length and training conditions (no data for mean age > 70 years were available). While separation via study length did not affect the significant outcome, the evaluation regarding training condition yielded only non-significant results.

Table 11: Results of the sensitivity analysis regarding the subgroups effect for the outcome bench press in kilogram (kg)

Subgroup	Protein (n)	Control (n)	Mean Difference IV, Random, 95% CI	Test for overall effect Z (p)	I^2
mean age < 70 years	107	105	0.31 [0.15, 0.47]	3.85 (0.0001)	0%
mean age > 70 years	-	-	-	-	-
study length < 12 weeks	64	60	0.30 [0.14, 0.46]	3.74 (0.0002)	0%
study length > 12 weeks	43	45	4.12 [0.77, 7.46]	2.41 (0.02)	0%
trained	34	30	1.20 [-8.17, 10.57]	0.25 (0.80)	0%
untrained	73	75	1.87 [-0.70, 4.44]	1.43 (0.15)	34%
Total	**107**	**105**	**0.31 [0.15, 0.47]**	**3.85 (0.0001)**	**0%**

CI = confidence interval, p = probability value, I^2 = heterogeneity, n = number of participants

The single study effects sensitivity analyses are summarized in Table 12. Overall result was no longer significant, following the removal of the studies by Hulmi et al.[36], Joy et al.[38], Kerksick et al.[39] and by Slater et al.[40], respectively.

Table 12: Results of the sensitivity analysis regarding the single study effect for the outcome bench press in kilogram (kg).

Without	Protein (n)	Control (n)	Mean Difference IV, Random, 95% CI	Test for overall effect Z (p)	I^2
Herda et al. 2013	87	84	0.31 [0.15, 0.47]	3.83 (0.0001)	0%
Hulmi et al. 2009	96	95	0.73 [-0.37, 1.82]	1.30 (0.19)	4%
Ispoglou et al. 2011	94	92	0.31 [0.15, 0.47]	3.84 (0.0001)	0%
Joy et al. 2013	95	93	0.65 [-0.34, 1.63]	1.29 (0.20)	3%
Kerksick et al. 2006	92	94	0.70 [-0.36, 1.75]	1.30 (0.19)	4%
Slater et al. 2001	100	98	0.73 [-0.34, 1.80]	1.33 (0.18)	4%
Volek et al. 2013	88	83	0.30 [0.14, 0.46]	3.75 (0.0002)	0%
Willoughby et al. 2007	97	96	3.94 [0.90, 6.99]	2.54 (0.01)	0%
Total	**107**	**105**	**0.31 [0.15, 0.47]**	**3.85 (0.0001)**	**0%**

CI = confidence interval, p = probability value, I^2 = heterogeneity, n = number of participants

3.2.3. Knee extension

WMD in knee extension in Newton (N) (6.06 N, 95% CI [-13.20, 25.33], P = 0.54) as an indicator of muscle strength was not significantly different when comparing protein supplementation versus control treatment (Figure 17).

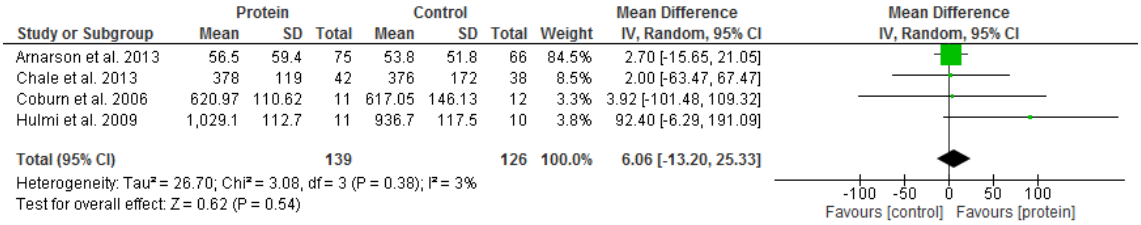

Figure 17: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for knee extension in Newton (N) of 4 randomized controlled trials comparing the effect of protein supplementation versus control treatments. The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond at the bottom of the graph represents the pooled weighted mean differences including 95% CI.

Subgroup analyses with respect to either mean age or study length (no data were available for trained subjects) demonstrated a statistically non-significant change in the probability value (Table 13).

Table 13: Results of the sensitivity analysis regarding the subgroups effect for the outcome knee extension in Newton (N)

Subgroup	Protein (n)	Control (n)	Mean Difference IV, Random, 95% CI	Test for overall effect Z (p)	I^2
mean age < 70 years	22	22	50.18 [-36.44, 136.79]	1.14 (0.26)	31%
mean age > 70 years	117	104	2.65 [-15.02, 20.32]	0.29 (0.77)	0%
study length < 12 weeks	11	12	3.92 [-101.48, 109.32]	0.07 (0.94)	not applicable
study length > 12 weeks	128	114	13.20 [-23.74, 50.13]	0.70 (0.48)	35%
trained	-	-	-	-	-
untrained	22	22	50.18 [-36.44, 136.79]	1.14 (0.26)	31%
Total	**139**	**126**	**6.06 [-13.20, 25.33]**	**0.62 (0.54)**	**3%**

CI = confidence interval, p = probability value, I^2 = heterogeneity, n = number of participants

Sensitivity analyses of single study effects did not result in significant changes of the initial overall effect (Table 14).

Table 14: Results of the sensitivity analysis regarding the single study effect for the outcome knee extension in Newton (N).

Without	Protein (n)	Control (n)	Mean Difference IV, Random, 95% CI	Test for overall effect Z (p)	I^2
Arnarson et al. 2013	64	60	26.16 [-28.48, 80.81]	0.94 (0.35)	17%
Chale et al. 2013	97	88	18.35 [-28.33, 65.04]	0.77 (0.44)	35%
Coburn et al. 2006	128	114	13.20 [-23.74, 50.13]	0.70 (0.48)	35%
Hulmi et al. 2009	128	116	2.68 [-14.75, 20.11]	0.30 (0.76)	0%
Total	**139**	**126**	**6.06 [-13.20, 25.33]**	**0.62 (0.54)**	**3%**

CI = confidence interval, p = probability value, I^2 = heterogeneity, n = number of participants

Figure 18 shows that the change in knee extension (measured as Nm) to determine muscle strength was not significantly more pronounced following protein supplementation as compared to placebos (WMD -4.08 Nm, 95% CI [-18.02, 9.87], P = 0.57).

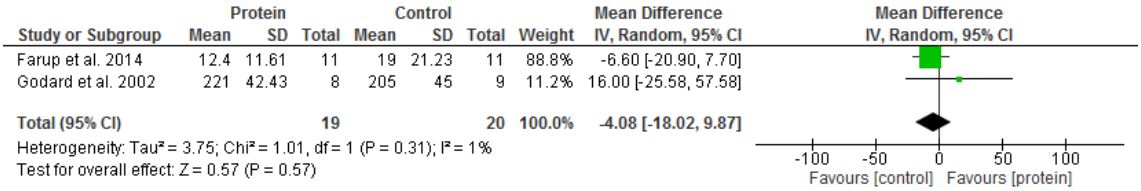

Figure 18: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for knee extension in Newton meter (Nm) of 2 randomized controlled trials comparing the effect of protein supplementation versus control treatments. The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond at the bottom of the graph represents the pooled weighted mean differences including 95% CI.

Overall WMD was not affected by a single study (Table 15).

Table 15: Results of the sensitivity analysis regarding the single study effect for the outcome knee extension in Newton meter (Nm).

Without	Protein (n)	Control (n)	Mean Difference IV, Random, 95% CI	Test for overall effect Z (p)	I^2
Farup et al. 2014	8	9	16.00 [-25.58, 57.58]	0.75 (0.45)	Not applicable
Godard et al. 2002	11	11	-6.60 [-20.90, 7.70]	0.90 (0.37)	Not applicable
Total	**19**	**20**	**-4.08 [-18.02, 9.87]**	**0.57 (0.57)**	**1%**

CI = confidence interval, p = probability value, I^2 = heterogeneity, n = number of participants

3.2.4. Peak power

Based on a further analysis, no significant difference between intervention groups and control groups was found, by measuring peak power in Watt (W). Weighted mean difference was 0.12 W, 95% CI [-31.99, 32.22], P = 0.99 (Figure 19).

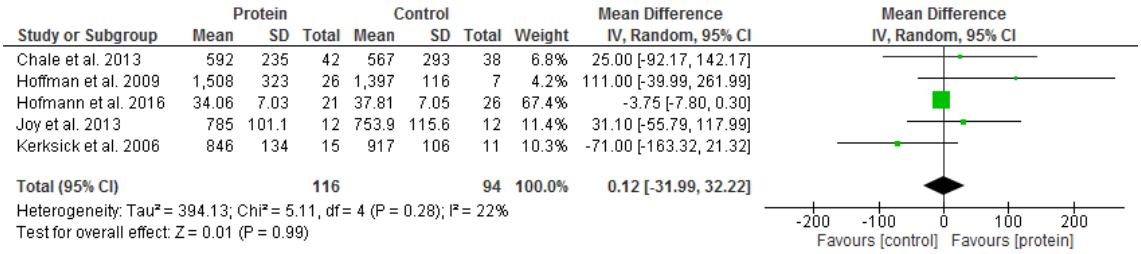

Figure 19: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for peak power in Watt (W) of 5 randomized controlled trials comparing the effect of protein supplementation versus control treatments. The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond at the bottom of the graph represents the pooled weighted mean differences including 95% CI.

Subgroup analysis via mean age revealed that peak power tended to be higher in populations > 70 years following protein supplementation. The subgroup analysis regarding study length came to the same conclusion with respect to long-term studies > 12 weeks, most likely due to the fact that these intervention trials predominantly enrolled subjects with a mean age > 70 years (WMD -3.72 W, 95% CI [-7.76, 0.33], P = 0.07) (Table 16).

Table 16: Results of the sensitivity analysis regarding the subgroups effect for the outcome peak power in Watt (W)

Subgroup	Protein (n)	Control (n)	Mean Difference IV, Random, 95% CI	Test for overall effect Z (p)	I^2
mean age < 70 years	53	30	11.70 [-83.54, 106.94]	0.24 (0.81)	59%
mean age > 70 years	63	64	-3.72 [-7.76, 0.33]	1.80 (0.07)	0%
study length < 12 weeks	53	30	11.70 [-83.54, 106.94]	0.24 (0.81)	59%
study length > 12 weeks	63	64	-3.72 [-7.76, 0.33]	1.80 (0.07)	0%
trained	53	30	11.70 [-83.54, 106.94]	0.24 (0.81)	59%
untrained	-	-	-	-	-
Total	**116**	**94**	**0.12 [-31.99, 32.22]**	**0.01 (0.99)**	**22%**

CI = confidence interval, p = probability value, I^2 = heterogeneity, n = number of participants

Table 17 shows the single study effects sensitivity analyses regarding peak power revealing no change in the overall effect.

Table 17: Results of the sensitivity analysis regarding the single study effect for the outcome peak power in Watt (W).

Without	Protein (n)	Control (n)	Mean Difference IV, Random, 95% CI	Test for overall effect Z (p)	I^2
Chale et al. 2013	74	56	-0.11 [-43.66, 43.43]	0.01 (1.00)	38%
Hoffman et al. 2009	90	87	-3.77 [-7.81, 0.27]	1.83 (0.07)	0%
Hofmann et al. 2016	95	68	11.83 [-57.28, 80.94]	0.34 (0.74)	39%
Joy et al. 2013	104	82	-3.00 [-47.94, 41.95]	0.13 (0.90)	33%
Kerksick et al. 2006	101	83	-2.22 [-13.68, 9.23]	0.38 (0.70)	2%
Total	**116**	**94**	**0.12 [-31.99, 32.22]**	**0.01 (0.99)**	**22%**

CI = confidence interval, p = probability value, I^2 = heterogeneity, n = number of participants

3.2.5. Handgrip strength

Figure 20 represents the analysis for handgrip strength in kilogram (kg) however, including only one study yielding non-significant results (WMD -0.09 kg, 95% CI [-0.24, 0.06], P = 0.24).

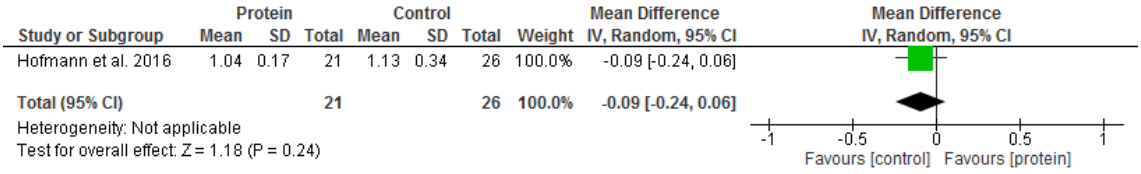

Figure 20: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for handgrip strength in kilogram (kg) of 1 randomized controlled trial comparing the effect of protein supplementation versus control treatments. The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond at the bottom of the graph represents the pooled weighted mean differences including 95% CI.

3.3. Muscle fiber hypertrophy

3.3.1. Muscle type-specific cross-sectional area

Figure 21 shows that the changes in muscle type-specific cross-sectional area in square centimeter (cm^2) to measure hypertrophy were significantly stronger in the intervention groups treated with protein supplements compared to control treatments (WMD 2.07 cm^2, 95% CI [0.43, 3.70], P = 0.01).

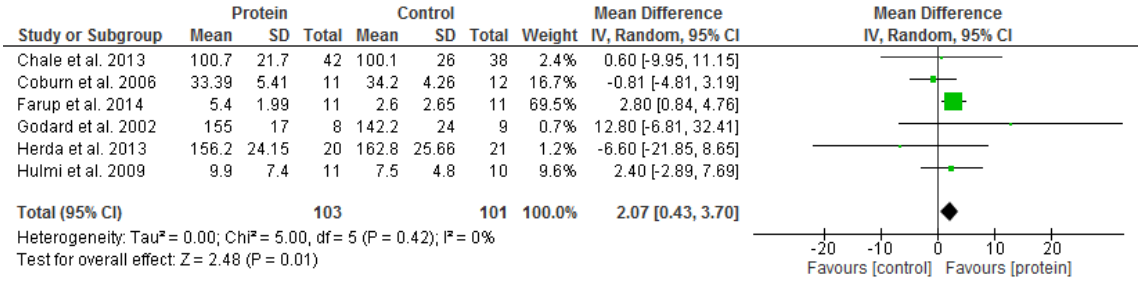

Figure 21: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for muscle type-specific cross-sectional area (CSA) in square centimeter (cm^2) of 6 randomized controlled trials comparing the effect of protein supplementation versus control treatments. The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond at the bottom of the graph represents the pooled weighted mean differences including 95% CI.

Table 18 presents the different subgroup analyses for the outcome parameter muscle type-specific CSA separated by mean age and study length (no data were available for trained subjects). The effect of protein supplements was not statistically significant when compared to placebos either considering the mean age or the study length subgroups.

Table 18: Results of the sensitivity analysis regarding the subgroups effect for the outcome muscle type-specific cross-sectional area (CSA) in square centimeter (cm^2)

Subgroup	Protein (n)	Control (n)	Mean Difference IV, Random, 95% CI	Test for overall effect Z (p)	I^2
mean age < 70 years	53	54	1.67 [-0.58, 3.91]	1.45 (0.15)	21%
mean age > 70 years	50	47	3.79 [-6.72, 14.29]	0.71 (0.48)	13%
study length < 12 weeks	31	33	-1.18 [-5.06, 2.69]	0.60 (0.55)	0%
study length > 12 weeks	72	68	2.77 [0.97, 4.58]	3.02 (0.003)	0%
trained	-	-	-	-	-
untrained	53	54	1.67 [-0.58, 3.91]	1.45 (0.15)	21%
Total	**103**	**101**	**2.07 [0.43, 3.70]**	**2.48 (0.01)**	**0%**

CI = confidence interval, p = probability value, I^2 = heterogeneity, n = number of participants

In the single study effects sensitivity analyses the probability value changed by removing the studies by Chale et al.[27], Farup et al.[33] and Hulmi et al.[36], i.e. the overall effect was no longer significantly different between the intervention group and the placebo group (Table 19).

Table 19: Results of the sensitivity analysis regarding the single study effect for the outcome muscle type-specific cross-sectional area (CSA) in square centimeter (cm^2)

Without	Protein (n)	Control (n)	Mean Difference IV, Random, 95% CI	Test for overall effect Z (p)	I^2
Chale et al. 2013	61	63	1.78 [-0.52, 4.08]	1.52 (0.13)	19%
Coburn et al. 2006	92	89	2.64 [0.86, 4.43]	2.90 (0.004)	0%
Farup et al. 2014	92	90	0.40 [-2.56, 3.36]	0.26 (0.79)	0%
Godard et al. 2002	95	92	1.99 [0.36, 3.63]	2.38 (0.02)	0%
Herda et al. 2013	83	80	2.17 [0.53, 3.81]	2.59 (0.010)	0%
Hulmi et al. 2009	92	91	1.52 [-1.13, 4.16]	1.12 (0.26)	20%
Total	**103**	**101**	**2.07 [0.43, 3.70]**	**2.48 (0.01)**	**0%**

CI = confidence interval, p = probability value, I^2 = heterogeneity, n = number of participants

3.4. Velocity measurements

3.4.1. Gait speed

Gait speed in meter per second (m/s) was significantly more increased following protein supplementation as compared to control when synthesizing data from two interventions. WMD was 0.18 m/s, 95% CI [0.00, 0.35], P = 0.05 (Figure 22). This was not affected by removing one of these studies (Table 20).

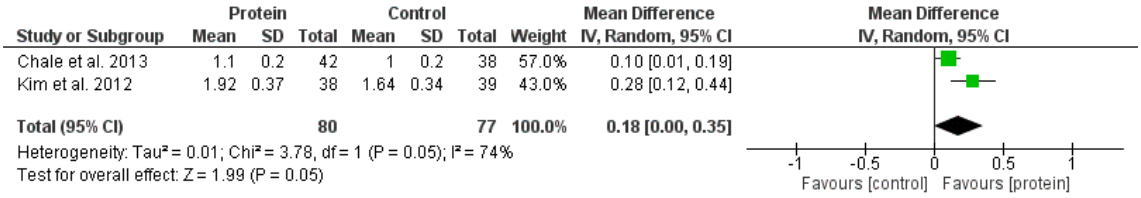

Figure 22: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for gait speed in meter per second (m/s) of 2 randomized controlled trials comparing the effect of protein supplementation versus control treatments. The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond at the bottom of the graph represents the pooled weighted mean differences including 95% CI.

Table 20: Results of the sensitivity analysis regarding the single study effect for the outcome gait speed in meter per second (m/s)

Without	Protein (n)	Control (n)	Mean Difference IV, Random, 95% CI	Test for overall effect Z (p)	I^2
Chale et al. 2013	38	39	0.28 [0.12, 0.44]	3.46 (0.0005)	Not applicable
Kim et al. 2012	42	38	0.10 [0.01, 0.19]	2.23 (0.03)	Not applicable
Total	**80**	**77**	**0.18 [0.00, 0.35]**	1.99 (0.05)	74%

CI = confidence interval, p = probability value, I^2 = heterogeneity, n = number of participants

3.4.2. Chair rise time

Figure 23 describes chair rise time in seconds (s). The overall effect was not significantly different between intervention group and control group (WMD -1.90 s, 95% CI [-4.71, 0.91], P = 0.18).

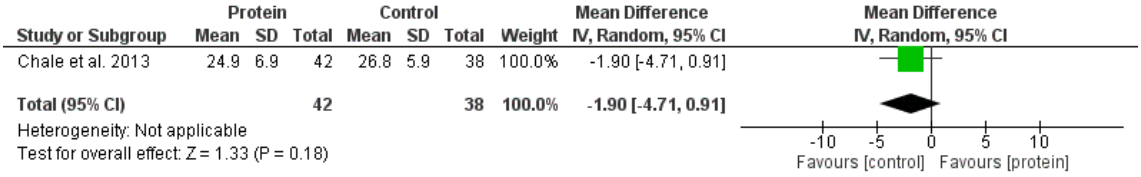

Figure 23: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for chair rise time in seconds (s) of 1 randomized controlled trial comparing the effect of protein supplementation versus control treatments. The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond at the bottom of the graph represents the pooled weighted mean differences including 95% CI.

3.4.3. Stair climb time

The change in stair climb time in seconds (s) did not differ significantly between the protein supplements group and the respective controls in the study by Chale et al. [27] The WMD was 0.10 s, 95% CI [-1.46, 1.66], P = 0.90 (Figure 24).

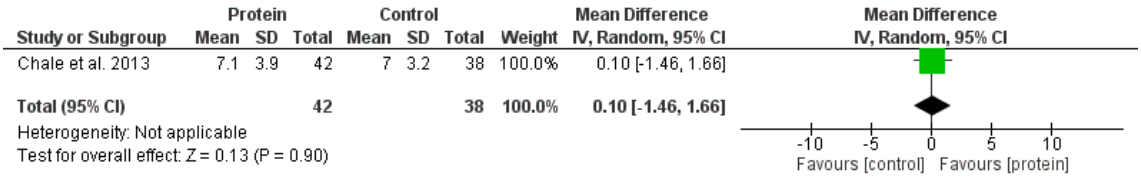

Figure 24: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for stair climb time in seconds (s) of 1 randomized controlled trial comparing the effect of protein supplementation versus control treatments. The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond at the bottom of the graph represents the pooled weighted mean differences including 95% CI.

3.4.4. Timed up and go test

The following Figure 25 presents the timed up and go test (TUG) in seconds (s). The forest plot showing that the change in this parameter was not significantly prominent, the weighted mean difference was -0.10 s, 95% CI [-0.53, 0.33], P = 0.65.

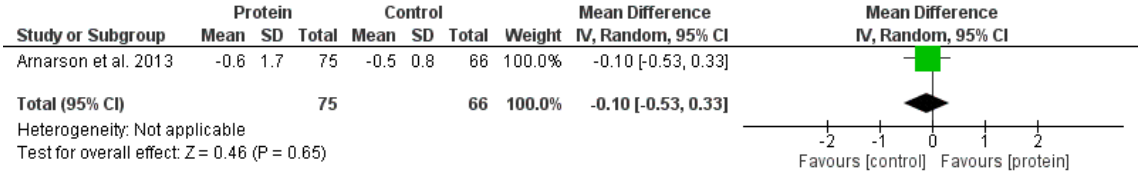

Figure 25: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for timed up and go test in seconds (s) of 1 randomized controlled trial comparing the effect of protein supplementation versus control treatments. The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond at the bottom of the graph represents the pooled weighted mean differences including 95% CI.

3.4.5. 6-min walk distance

Figure 26 shows that the improvement in 6-min walk distance was not significantly more pronounced following protein supplements compared to placebos (WMD -4.80 m, 95% CI [-25.03, 15.43], P = 0.64.

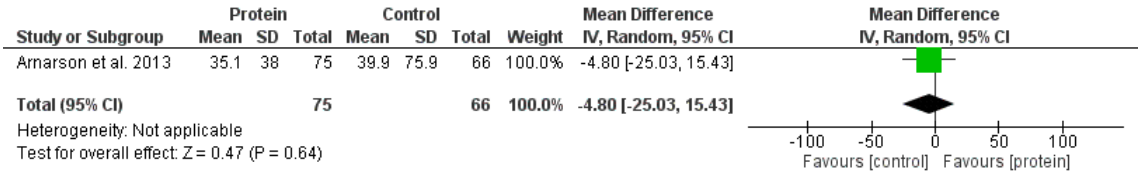

Figure 26: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for 6-minutes walk for distance in meter (m) of 1 randomized controlled trial comparing the effect of protein supplementation versus control treatments. The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond at the bottom of the graph represents the pooled weighted mean differences including 95% CI.

3.5. Heterogeneity

To determine the differences in measurement methods, study populations and interventions of the individual studies, the heterogeneity measure I^2 (%) was used. The measure indicates the proportion of total dispersion based on systematic differences between the studies. In accordance with recommendations by the Cochrane Society, the following categories were applied: I^2 = 0%, there is no heterogeneity; I^2 = 25%, there is little variability; I^2 = 50%, the studies have a medium heterogeneity and at I^2 = 75%, the differences are high [42]. The anthropometric parameters (body weight, lean body mass, fat mass and % fat mass) showed no heterogeneity, I^2 was 0% for all outcomes. Highest values for I^2 were found for body weight following separation into subgroups by mean age (I^2 = 69%). I^2 for secondary outcomes varied between 0% and 22% with the exception of gait speed (I^2 = 74%).

3.6. Publication bias

To identify the presence of a potential publication bias, funnel plots were created for the outcomes parameters body weight, lean body mass, fat mass, % fat mass, leg press and bench press. The funnel plots illustrating the SE of the corresponding parameter for each study against the respective mean difference are given in Figures 27 to 29, respectively. There is a symmetrical pattern, suggesting a low probability of an existing publication bias. In contrast, the funnel plots for the parameters fat mass in %, leg press and bench press (Figure 30-32) revealed an asymmetric scattering. This might be an indicator for a possible presence of a publication bias.

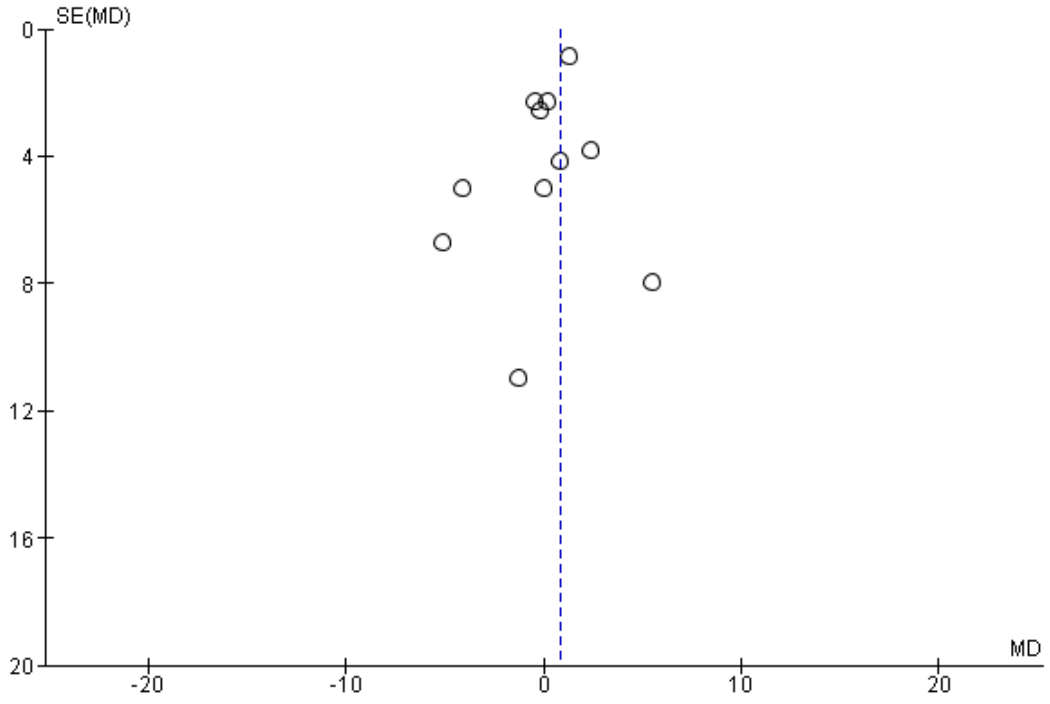

Figure 27: Funnel plot illustrating study precision against the mean difference (MD) with standard error (SE (MD)) for protein supplement interventions versus control and body weight (kg).

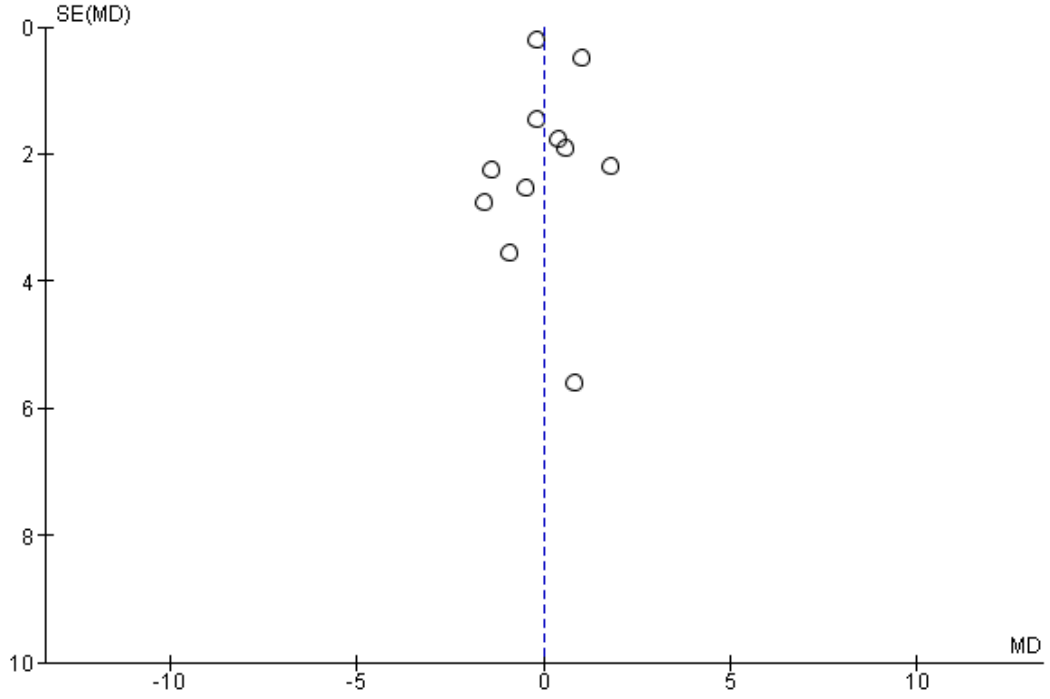

Figure 28: Funnel plot illustrating study precision against the mean difference (MD) with standard error (SE (MD)) for protein supplement interventions versus control and lean body mass (kg).

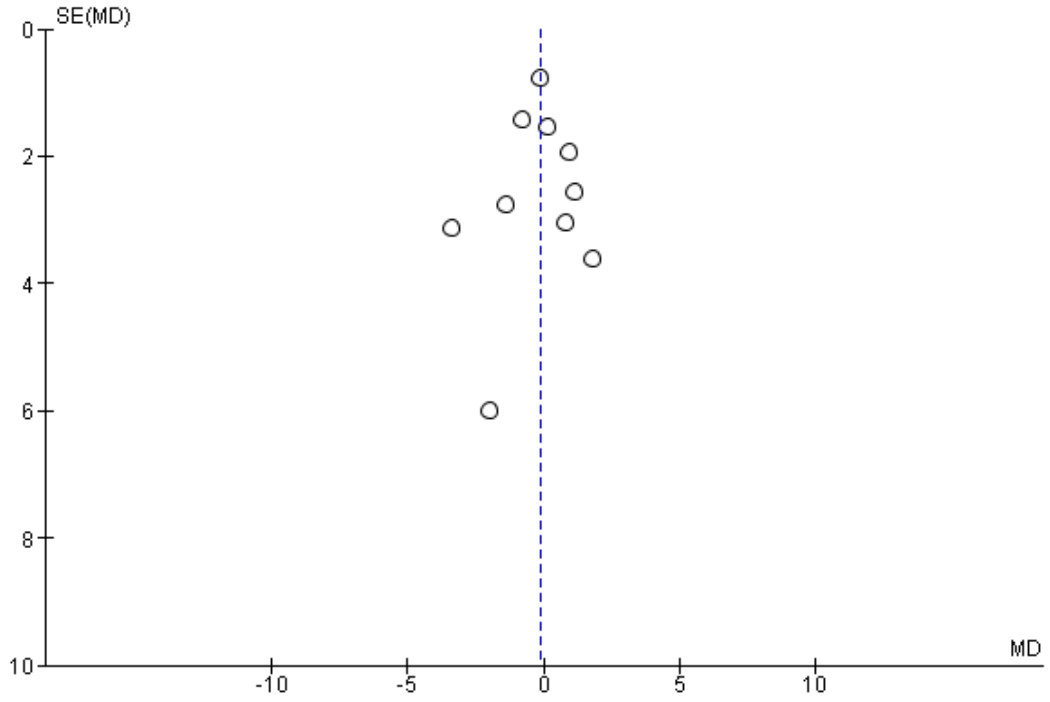

Figure 29: Funnel plot illustrating study precision against the mean difference (MD) with standard error (SE (MD)) for protein supplement interventions versus control and fat mass (kg).

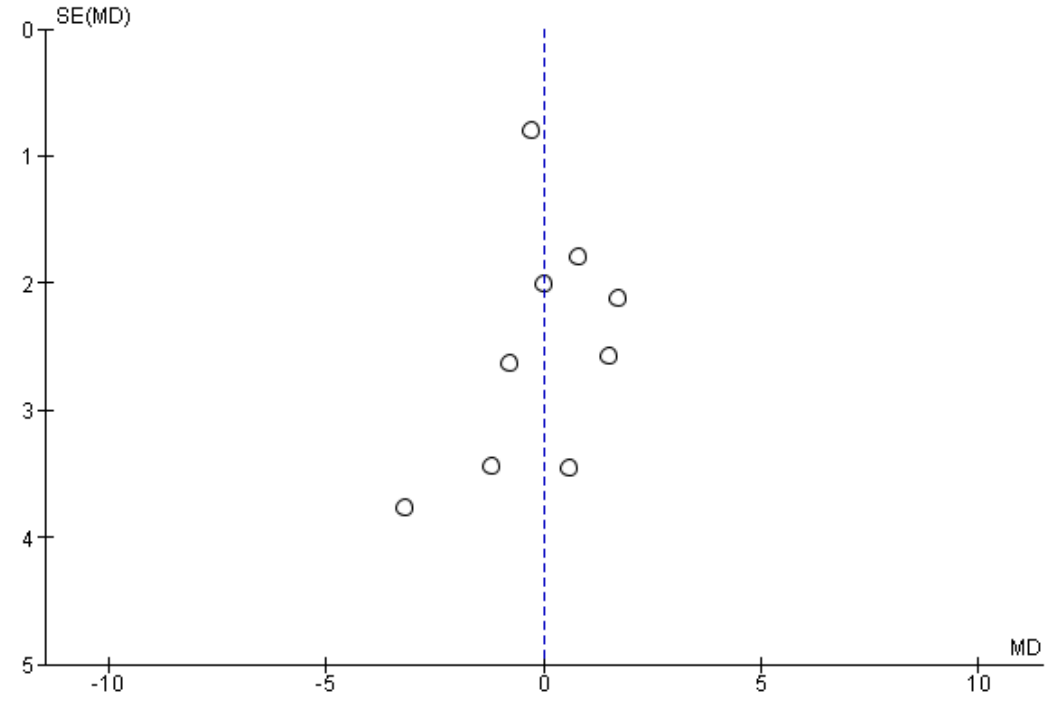

Figure 30: Funnel plot illustrating study precision against the mean difference (MD) with standard error (SE (MD)) for protein supplement interventions versus control and fat mass (%).

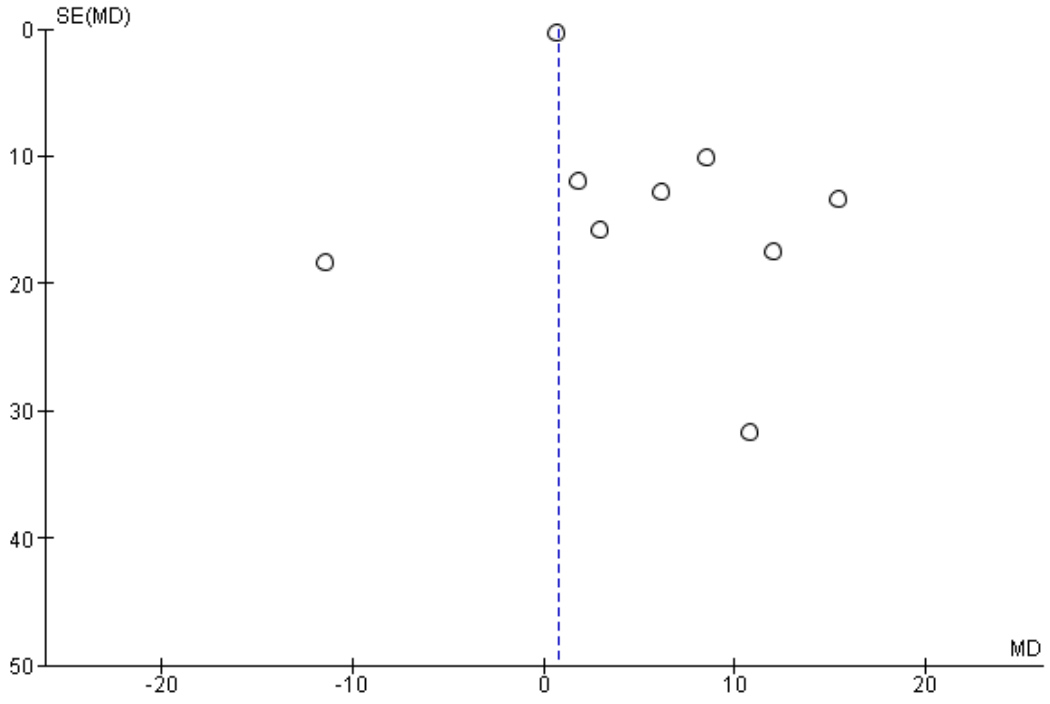

Figure 31: Funnel plot illustrating study precision against the mean difference (MD) with standard error (SE (MD)) for protein supplement interventions versus control and leg press (kg).

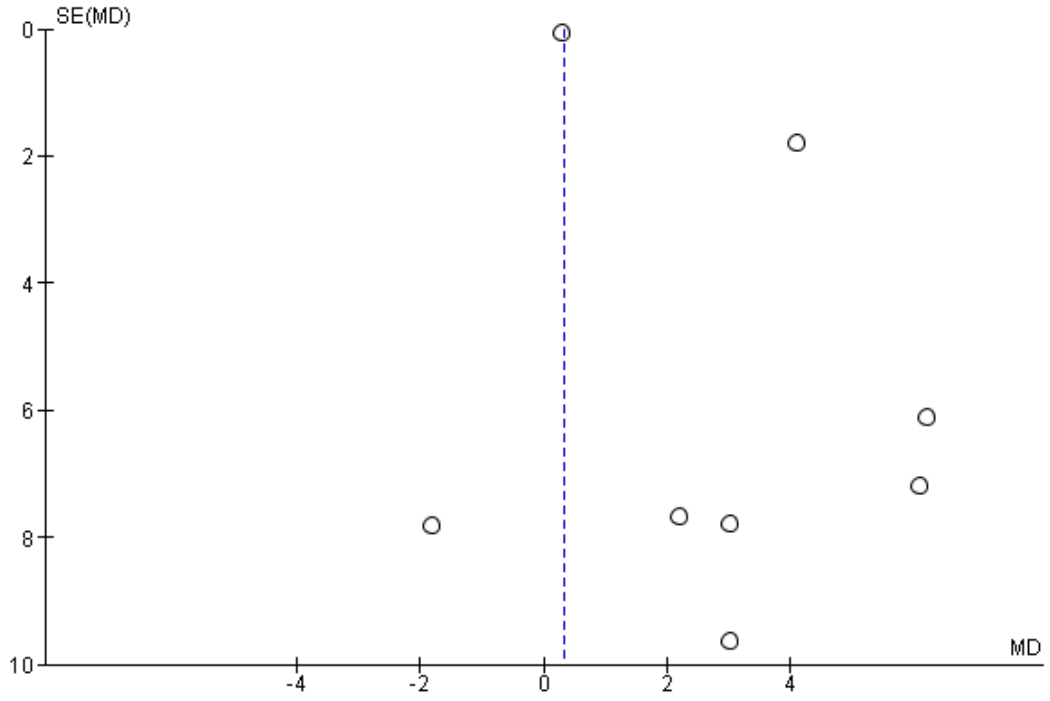

Figure 32: Funnel plot illustrating study precision against the mean difference (MD) with standard error (SE (MD)) for protein supplement interventions versus control and bench press (kg).

3.7. Risk of bias assessment

The graph below illustrates the entire bias potential of all included trials (Figure 33). The risk of bias is given for each domain in percentage and graded using the colors of a traffic light (green-yellow-red) according to the Cochrane Risk of Bias Tool for Randomized Controlled Trials [16].

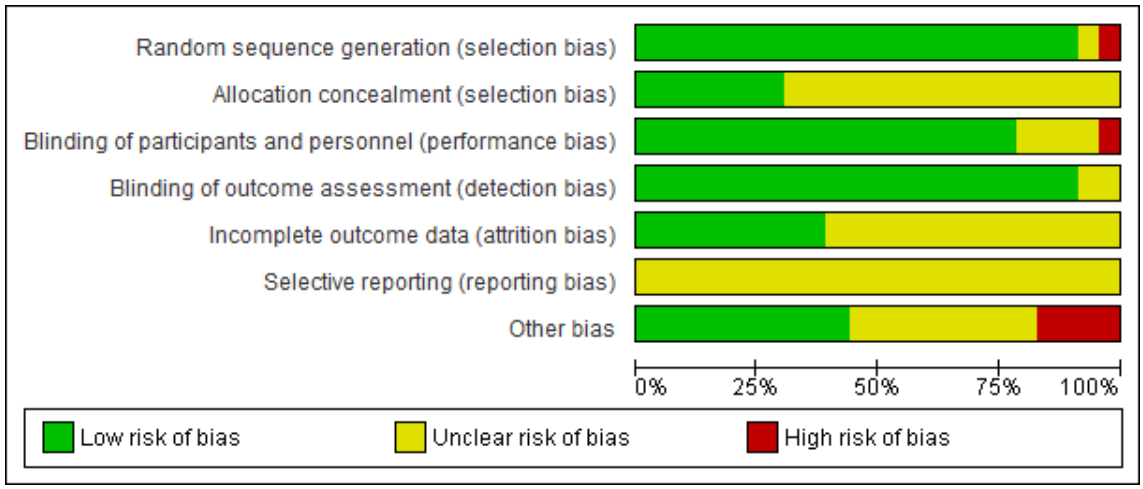

Figure 33: Cochrane risk of bias graph of included intervention trials

4. Discussion

This systematic review together with the subsequent meta-analysis investigated the potential anabolic effect of protein supplementation combined with resistance training. It could be shown that an additional protein/amino acid supplementation had no influence on the anthropometric parameters (body weight, lean body mass, fat mass and fat mass in %). However, synthesis of available data revealed a significant improvement in muscle strength parameters expressed as either bench press (WMD 0.31 kg, 95% CI [0.15, 0.47], P = 0.000) or leg press (WMD 0.73 kg, 95% CI [0.06, 1.39], P = 0.03) as well as a trend for enhancements in gait speed (WMD 0.18 m/s, 95% CI [0.00, 0.35], P = 0.05). Another parameter used to determine muscle strength, i.e. peak power, did not show a significant increase, however, based on a sensitivity analysis of subgroups, it was found that the long-term studies (> 12 weeks of intervention) resulted in a trend towards a positive effect (WMD -3.72 W, 95% CI [-7.76, 0.33], P = 0.07). Since these studies predominately enrolled participants with a mean age higher than 70 years, the subgroup analysis regarding age came to the same conclusion. In addition, the parameter for measuring muscle fiber hypertrophy was significantly more increased following protein supplementation as compared to control treatment (WMD 2.07 cm^2, 95% CI [0.43, 3.70], P = 0.01).

Some of these results are in sharp contrast to data from other systematic reviews or meta-analyses already published on this topic. Thus, Cermak et al. showed that supplementation with proteins during resistance training yielded significant increases in both muscle mass and strength [(5)]. The data of the present systematic review and meta-analysis cannot support this statement. There was no positive influence on the anthropometric parameters when comparing a protein supplementation versus control treatments. This seems to be more in line with a critical review by Reidy et al. stressing the high variability of the available data and concluding that the effect of protein/amino acid supplementation is very low and only applies to certain sub-groups [(44)].

Moreover, a systematic review by Thomas et al. investigated the possible effect of protein supplementation again in combination with exercise training in the elderly and postulated that protein supplements have no significant positive impact on muscle mass, muscle strength, anthropometric composition, and functional ability [11], which is in agreement to the present data.

Also, the subgroup analyses - by age (mean age < 70 years vs. mean age > 70 years), study length (< 12 weeks vs. > 12 weeks) and training condition (untrained vs. trained subjects) - of the four predefined anthropometric outcomes did not reveal any changes in the overall effect. In contrast, subgroup analyses of secondary outcomes yielded significant changes in the probability value. Thus, the evaluation of leg press measurements showed a significant benefit of protein supplementation compared to placebos. Further analyses demonstrated that this result applies only for studies which included untrained subjects with a mean age < 70 years and study duration > 12 weeks. The analysis regarding the outcome bench press showed a significant change of the overall effect on closer examination of the subgroup "training condition". Interestingly, trained and untrained subjects experienced a change in the probability value. In this case, the sensitivity analyses suggest that single studies contributed to the significant effect. In the analysis regarding peak power, it could be observed that although there were nearly significant changes in two subgroups, the single study effect analysis yielded a similar result when removing the study by Hoffman et al. [35] (WMD -3.77 W, 95% CI [-7.81, 0.27], P = 0.07). In addition, the results of subgroup analyses for the parameter muscle type-specific CSA remained significant only for studies with a length > 12 weeks.

In addition to the analysis of the parameters on the prescribed outcomes, which are represented in form of forest plots, sensitivity analyses were conducted. For the defined primary outcomes, the evaluation of the single study effect showed no significant change in the overall effect. Although only data from two studies were available for the outcome parameter gait speed, it was found that protein supplementation had a nearly significant impact compared to control treatment.

After removing a single study, the probability value changed and the significance of the overall effect was increased. Without the intervention trial by Chale et al. [27] the mean difference was 0.28 m/s, 95% CI [0.12, 0.44], P = 0.0005 and without the study by Kim et al.[30] MD was 0.10 m/s, 95% CI [0.01, 0.19], P = 0.03.

The question arises as to why the primary outcomes do not change when comparing the intervention groups to the control groups. For the parameter lean body mass, it can be assumed that the study by Arnarson et al. [26] has a clear influence on the overall results. This impact can probably be attributed to the high number of participants (intervention group n = 75, control group n = 66) and accordingly it gives the study a certain high amount of power. The effect might also be explained by the nature of the values, as the difference to baseline has been given.

Regarding the secondary outcome parameter leg press, the significant results may be due to the effect of the study by Willoughby et al. [42]. Here, the difference to the baseline was reported for the measurement of the 1-RM leg press and the data were given a weight of 99.5%. Consequently, following the removal of this study the effect of protein supplementation was no longer significantly different compared to control treatments. The significant positive impact of protein supplementation compared to placebos can only be explained by the high standard deviations of the studies by Kerksick et al. [39], Slater et al. [40], Hulmi et al. [36] and Joy et al. [38], indicating a wide dispersion of the measured values. The mean differences and confidence intervals of the four studies were in the same range, and after removing each study, the result also changed and the effect of the intervention group was no longer significantly different from the control group. The studies by Chale et al. [27], Hulmi et al. [36] and Farup et al. [33] significantly affected the data for the outcome parameter muscle type-specific CSA.

The study by Chale et al. [27] examined a significantly larger study population (intervention group n = 42, control group n= 38), and the studies by Hulmi et al. [36] and Farup et al. [33] reported the values in percent, therefore receiving a stronger weight.

The results of the secondary outcomes are explained by the heterogeneity of the data, because individual studies significantly influenced the overall result.

On the other hand, in the case of the primary results, the values are fairly homogeneous and can be compared well, but this meta-analysis indicated that the results of the intervention group did not differ significantly from the control group.

Measurements for assessing functional ability, like gait speed, timed up and go test or the chair rise time, were mainly from studies enrolling subjects with a mean age > 70 years. This meta-analysis showed that protein supplementation did not have a clearly positive effect compared to placebos, supporting the findings of the study by Thomas et al. [(11)]. The lack of effect might possibly be due to the inadequate protein intake at baseline. In the elderly, maintenance of muscle mass might be predominant and regular resistance training may counteract muscle catabolism, without exerting any anabolic effects.

Based on this work, it was not possible to issue a precise statement on the long-term or chronic effects of protein supplementation since there are no data available on defined “clinical” endpoints such as risk of morbidity or mortality.

So the exact mechanisms of proteins and amino acids in this regard are not yet sufficiently understood and still need to be investigated in further studies. Therefore it is not yet clear whether the ingestion or an increased supply of proteins over a very long period of time will have distinct side effects [(2)].

With respect to mechanisms of action, the amino acid leucine is one of the branched-chain amino acids (BCAAs) and was often given as a supplement in the included studies. Although the selected studies using > 2 g leucine per day did not result in any changes in anthropometric parameters suggesting only a limited potential anabolic effect, analyses of some of the secondary outcome parameters established beneficial effects of protein supplementation as compared to control treatments. Muscle protein synthesis is strongly stimulated by BCAAs, with leucine being a major trigger of anabolic effect on muscle mass [(6)]. This effect may be due to the mechanism of leucine

in the body because it activates the mechanistic target of rapamycin complex-1 (mTORC1) [7].

The study by Hamarsland et al. investigated the effect of native whey protein in comparison to whey protein concentrate and milk. Increased phosphorylation of p70S6K (specifically, a protein kinase) could only be observed when comparing native whey protein versus milk [45]. In addition to rapamycin and leucine, the activity of this complex is also influenced by HMB, insulin and growth factors. After leucine binds to a protein called sestrin2, it activates mTORC1. The activation of this complex leads to the phosphorylation (p70S6K) of the down-streaming proteins, thus the amino acid leucine stimulates muscle protein synthesis [7].

The present work has certain strengths as well as various limitations. The literature search was not limited according to publication date and the systematic review and meta-analysis include studies published between 2000 and 2017. However, the influence of unpublished studies cannot be excluded. The funnel plots for the parameters fat mass in %, leg press and bench press demonstrated an asymmetric pattern. This is an indication for a possible presence of a publication bias, i.e. unsuccessful attempts to publish negative or indifferent results. Due to occasional low number of studies, it was not possible to perform either subgroup or sensitivity analyses. Accordingly, no precise statement can be made for these outcome measurements. Heterogeneity was mostly modest, with considerable values being most likely due to variations in compositions, administered amounts and the frequency of protein supplements.

One of the strengths of the present systematic review is the qualitative evaluation applying the Cochrane Risk of Bias Tool for Randomized Controlled Trials [16] for all included studies. This tool assesses the study design, the methodology and the selection as well as the blinding of the subjects. In addition, the meta-analyses synthesized not only the usual anthropometric data, but were expanded to secondary outcomes measuring muscle strength and functional ability.

Due to thorough and restricted inclusion and exclusion criteria, it could be established that the supplementation, at least in these analyses, does not induce any improvement in the anthropometric parameters.

5. Conclusion

This systematic review and meta-analyses examined intervention trials with a study length of at least 6 weeks comparing the effects of resistance training together with protein supplementation against resistance training only. Major results were: no significant effect of protein administration on anthropometric parameters, but significant improvements following supplementation with respect to some of the parameters measuring muscle strength. This is in contrast to some of the data already published on this topic reporting a number of benefits of protein supplements on anthropometric outcomes such as muscle mass. Future studies should use consistent measurement techniques and a considerably higher number of participants in order to formulate a more accurate statement on this topic.

6. Summary

At present, supplementation with proteins and amino acids is a highly controversial issue and plays a major role in the field of resistance training. Here, the focus is primarily on the amino acid leucine, which is one of the BCAAs (branched-chain amino acids). The essential amino acid favors the stimulation of muscle protein synthesis, because the quality of added proteins has a beneficial effect on the buildup of muscle mass. In addition, the quantity is crucial, especially with regard to an adequate intake of older persons. Because the continuous loss of muscle mass (sarcopenia) in old age can lead to limitations of body functions and frailty. As part of this systematic review and meta-analysis of intervention trials, a possible anabolic effect of protein supplementation in combination with strength training was investigated. In the review, 23 studies with a minimum duration of six weeks were included and 18 studies included the quantitative analysis. The statistical analyses were performed using Review Manager 5.3, and a qualitative evaluation of the studies was also carried out. In addition, sensitivity analyses on the subgroups: age (subjects < 70 years vs. subjects > 70 years), study length (< 12 weeks vs. > 12 weeks) and training condition (untrained vs. trained) were evaluated. This systematic review and meta-analyses showed that protein supplementation in combination with resistance training has no anabolic effect on the anthropometric parameters (body weight, lean body mass, fat mass, and fat mass in %). However, there were significant improvements in the parameters for measuring muscle strength, muscle type-specific cross-sectional area, and functional performance. For example, parameters for measuring muscle strength achieved a significant change between protein supplements and control treatments (leg press WMD 0.73 kg, 95% CI [0.06, 1.39], P = 0.03 and bench press WMD 0.31 kg, 95% CI [0.15, 0.47], P = 0.0001).

7. Zusammenfassung

Derzeit wird die Supplementation mit Proteinen und Aminosäuren recht kontrovers diskutiert und spielt eine große Rolle im Bereich des Kraftsports. Hier steht vor allem die Aminosäure Leucin im Fokus, diese zählt zu den BCAAs (verzweigtkettige Aminosäuren). Die essentielle Aminosäure begünstigt die Stimulation der Muskelproteinsynthese, denn die Qualität zugeführter Proteine wirkt sich positiv auf den Aufbau der Muskelmasse aus. Darüber hinaus ist die Quantität entscheidend, vor allem im Hinblick auf eine adäquate Zufuhr älterer Personen. Denn der kontinuierliche Verlust von Muskelmasse (Sarkopenie) im Alter kann zu Einschränkungen der Körperfunktionen und Gebrechlichkeit führen. Im Rahmen dieser systematischen Übersichtarbeit mit zusätzlicher Metaanalyse wurde ein möglicher anaboler Effekt einer Proteinsupplementation in Kombination mit Krafttraining untersucht. In der Übersichtsarbeit wurden 23 Studien mit einer Mindestdauer von sechs Wochen berücksichtigt und 18 Studien schloss die quantitative Auswertung (Metaanalyse) ein. Die statistische Auswertung wurde mittels Review Manager 5.3 durchgeführt, ebenfalls erfolgte eine qualitative Bewertung der Studien. Außerdem wurden Sensitivitätsanalysen zu den Subgruppen Alter (Personen < 70 Jahre vs. Personen > 70 Jahre), Studienlänge (< 12 Wochen vs. > 12 Wochen) und Trainingszustand (untrainierte Personen vs. trainierte Personen) ausgewertet. Diese systematische Übersichtsarbeit und Metaanalyse ergab, dass eine Proteinsupplementation in Kombination mit Krafttraining keinen anabolen Effekt auf die anthropometrischen Parameter (Körpergewicht, magere Körpermasse, Fettmasse und Fettmasse in %) hat. Es gab jedoch signifikante Verbesserungen bei den Parametern zur Messung der Muskelstärke, der muskeltyp-spezifischen Querschnittfläche und der funktionellen Leistungsfähigkeit. Beispielsweise erzielten Parameter zu Erfassung der Muskelkraft eine signifikante Änderung bei der Betrachtung der Proteinsupplemente verglichen mit Placebos (leg press WMD 0.73 kg, 95% CI [0.06, 1.39], P = 0.03 und bench press WMD 0.31 kg, 95% CI [0.15, 0.47], P = 0.0001).

8. References

1. Phillips SM (2017) Current Concepts and Unresolved Questions in Dietary Protein Requirements and Supplements in Adults. *Front Nutr* **4,** 13.
2. Klaus S, Pfeiffer AFH, Boeing H, Laeger T, Grune T (2018) The protein paradox - how much dietary protein is good for health? *Ernahrungs Umschau* **65**(2), 42–47.
3. Deutsche Gesellschaft für Ernährung (DGE) (2018) D-A-CH Referenzwerte für die Nährstoffzufuhr: Protein. https://www.dge.de/wissenschaft/referenzwerte/protein/ (accessed October 2018).
4. Jäger R, Kerksick CM, Campbell BI *et al.* (2017) International Society of Sports Nutrition Position Stand: Protein and exercise. *J Int Soc Sports Nutr* **14,** 20.
5. Cermak NM, Res PT, Groot LC de *et al.* (2012) Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: A meta-analysis. *Am J Clin Nutr* **96,** 6, 1454–1464.
6. World Health Organization (2007) *Protein and Amino Acid Requirements in Human Nutrition*. Joint FAO/WHO/UNU expert consultation. WHO Technical Report Series no. 935. Geneva: WHO.
7. Phillips SM (2016) The impact of protein quality on the promotion of resistance exercise-induced changes in muscle mass. *Nutr Metab* **13,** 64.
8. Stokes T, Hector AJ, Morton RW *et al.* (2018) Recent Perspectives Regarding the Role of Dietary Protein for the Promotion of Muscle Hypertrophy with Resistance Exercise Training. *Nutrients* **10,** 180.
9. Rosenberg IH (1997) Sarcopenia: Origins and clinical relevance. *J Nutr* **127,** 5 Suppl, 990S-991S.
10. Cruz-Jentoft AJ, Baeyens JP, Bauer JM *et al.* (2010) Sarcopenia: European consensus on definition and diagnosis: Report of the European Working Group on Sarcopenia in Older People. *Age Ageing* **39,** 4, 412–423.
11. Thomas DK, Quinn MA, Saunders DH *et al.* (2016) Protein Supplementation Does Not Significantly Augment the Effects of Resistance Exercise Training in Older Adults: A Systematic Review. *J Am Med Dir Assoc* **17,** 10, 959.e1-9.

12. Santesso N, Akl EA, Bianchi M *et al.* (2012) Effects of higher- versus lower-protein diets on health outcomes: A systematic review and meta-analysis. *Eur J Clin Nutr* **66,** 7, 780–788.
13. Laeger T, Henagan TM, Albarado DC *et al.* (2014) FGF21 is an endocrine signal of protein restriction. *J Clin Invest* **124**, 3913–3922.
14. Levine ME, Suarez JA, Brandhorst S *et al.* (2014) Low protein intake is associated with a major reduction in IGF-1, cancer, and overall mortality in the 65 and younger but not older population. *Cell Metab* **19**, 407–417.
15. Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group (2009). Preferred Reporting Items for Systematic Reviews and Meta-Analyses: The PRISMA Statement. *J Clin Epidemiol*. Published online: 27 July 2009. doi: 10.1016/j.jclinepi.2009.06.005.
16. Higgins JP, Altman DG, Gotzsche PC *et al.* (2011) The Cochrane Collaboration's tool for assessing risk of bias in randomised trials. *BMJ* **343**, d5928.
17. Hofmann M, Schober-Halper B, Oesen S *et al.* (2016) Effects of elastic band resistance training and nutritional supplementation on muscle quality and circulating muscle growth and degradation factors of institutionalized elderly women: The Vienna Active Ageing Study (VAAS). *Eur J Appl Physiol* **116,** 5, 885–897.
18. Hozo SP, Djulbegovic B, Hozo I (2005) Estimating the mean and variance from the median, range, and the size of a sample. *BMC Med Res Methodol* **5**, 13.
19. Kirn DR, Koochek A, Reid KF *et al.* (2015) The Vitality, Independence, and Vigor in the Elderly 2 Study (VIVE2): Design and methods. *Contemp Clin Trials* **43**, 164–171.
20. Bechshoft RL, Reitelseder S, Hojfeldt G *et al.* (2016) Counteracting Age-related Loss of Skeletal Muscle Mass: A clinical and ethnological trial on the role of protein supplementation and training load (CALM Intervention Study): study protocol for a randomized controlled trial. *Trials* **17,** 1, 397.
21. Kraemer WJ, Hatfield DL, Volek JS *et al.* (2009) Effects of amino acids supplement on physiological adaptations to resistance training. *Med Sci Sports Exerc* **41,** 5, 1111–1121.

22. Ikeda T, Aizawa J, Nagasawa H *et al.* (2016) Effects and feasibility of exercise therapy combined with branched-chain amino acid supplementation on muscle strengthening in frail and pre-frail elderly people requiring long-term care: A crossover trial. *Appl Physiol Nutr Metab* **41,** 4, 438–445.
23. Candow DG, Burke NC, Smith-Palmer T *et al.* (2006) Effect of Whey and Soy Protein Supplementation Combined with Resistance Training in Young Adults. *Int J Sport Nutr Exerc Metab* **16,** 3, 233–244.
24. Andersen LL, Tufekovic G, Zebis MK *et al.* (2005) The effect of resistance training combined with timed ingestion of protein on muscle fiber size and muscle strength. *Metabolism* **54,** 2, 151–156.
25. Trabal J, Forga M, Leyes P *et al.* (2015) Effects of free leucine supplementation and resistance training on muscle strength and functional status in older adults: A randomized controlled trial. *Clin Interv Aging* **10**, 713–723.
26. Arnarson A, Gudny Geirsdottir O, Ramel A *et al.* (2013) Effects of whey proteins and carbohydrates on the efficacy of resistance training in elderly people: Double blind, randomised controlled trial. *Eur J Clin Nutr* **67,** 8, 821–826.
27. Chale A, Cloutier GJ, Hau C *et al.* (2013) Efficacy of Whey Protein Supplementation on Resistance ExerciseInduced Changes in Lean Mass, Muscle Strength, and Physical Function in Mobility-Limited Older Adults. *J Gerontol A Biol Sci Med Sci* **68,** 6, 682–690.
28. Godard MP, Williamson DL, Trappe SW (2002) Oral amino-acid provision does not affect muscle strength or size gains in older men. *Med Sci Sports Exerc* **34,** 7, 1126–1131.
29. Karelis AD, Messier V, Suppere C *et al.* (2015) Effect of cysteine-rich whey protein (Immunocal®) supplementation in combination with resistance training on muscle strength and lean body mass in non-frail elderly subjects: A randomized, double-blind controlled study. *J Nutr Health Aging* **19,** 5, 531–536.
30. Kim HK, Suzuki T, Saito K *et al.* (2012) Effects of exercise and amino acid supplementation on body composition and physical function in community-

dwelling elderly Japanese sarcopenic women: A randomized controlled trial. *J Am Geriatr Soc* **60,** 1, 16–23.

31. Antonio J, Sanders MS, Ehler LA *et al.* (2000) Effects of exercise training and amino-acid supplementation on body composition and physical performance in untrained women. *Nutrition* **16,** 11-12, 1043–1046.
32. Coburn JW, Housh DJ, Housh TJ *et al.* (2006) Effects of leucine and whey protein supplementation during eight weeks of unilateral resistance training. *J Strength Cond Res* **20,** 2, 284–291.
33. Farup J, Rahbek SK, Vendelbo MH *et al.* (2014) Whey protein hydrolysate augments tendon and muscle hypertrophy independent of resistance exercise contraction mode. *Scand J Med Sci Sports* **24,** 5, 788–798.
34. Herda AA, Herda TJ, Costa PB *et al.* (2013) Muscle performance, size, and safety responses after eight weeks of resistance training and protein supplementation: A randomized, double-blinded, placebo-controlled clinical trial. *J Strength Cond Res* **27,** 11, 3091–3100.
35. Hoffman JR, Ratamess NA, Tranchina CP *et al.* (2009) Effect of Protein-Supplement Timing on Strength, Power, and Body-Composition Changes in Resistance-Trained Men. *Int J Sport Nutr Exerc Metab* **19,** 2, 172–185.
36. Hulmi JJ, Kovanen V, Selänne H *et al.* (2009) Acute and long-term effects of resistance exercise with or without protein ingestion on muscle hypertrophy and gene expression. *Amino acids* **37,** 2, 297–308.
37. Ispoglou T, King RFGJ, Polman RCJ *et al.* (2011) Daily L-Leucine Supplementation in Novice Trainees during a 12-Week Weight Training Program. *Int J Sport Physiol* **6,** 1, 38–50.
38. Joy JM, Lowery RP, Wilson JM *et al.* (2013) The effects of 8 weeks of whey or rice protein supplementation on body composition and exercise performance. *Nutr J* **12**, 86.
39. Kerksick CM, Rasmussen CJ, Lancaster SL *et al.* (2006) The effects of protein and amino acid supplementation on performance and training adaptations during ten weeks of resistance training. *J Strength Cond Res* **20,** 3, 643–653.

40. Slater G, Jenkins D, Logan P *et al.* (2001) Beta-hydroxy-beta-methylbutyrate (HMB) supplementation does not affect changes in strength or body composition during resistance training in trained men. *Int J Sport Nutr Exerc Metab* **11,** 3, 384–396.
41. Volek JS, Volk BM, Gómez AL *et al.* (2013) Whey protein supplementation during resistance training augments lean body mass. *J Am Coll Nutr* **32,** 2, 122–135.
42. Willoughby DS, Stout JR, Wilborn CD (2007) Effects of resistance training and protein plus amino acid supplementation on muscle anabolism, mass, and strength. *Amino acids* **32,** 4, 467–477.
43. Higgins JP, Thompson SG, Deeks JJ *et al.* (2003) Measuring inconsistency in meta-analyses. *BMJ* **327,** 7414, 557–560.
44. Reidy PT & Rasmussen BB (2016) Role of Ingested Amino Acids and Protein in the Promotion of Resistance Exercise–Induced Muscle Protein Anabolism123. *J Nutr* **146,** 2, 155–183.
45. Hamarsland H, Nordengen AL, Aas SN *et al.* (2017) Native whey protein with high levels of leucine results in similar post-exercise muscular anabolic responses as regular whey protein: a randomized controlled trial. *J Int Soc Sports Nutr* **14,** 43.

9. Appendix

The following figures represent the forest plots for the anthropometric outcome parameters (body weight, lean body mass, fat mass and % fat mass) without classification into subgroups.

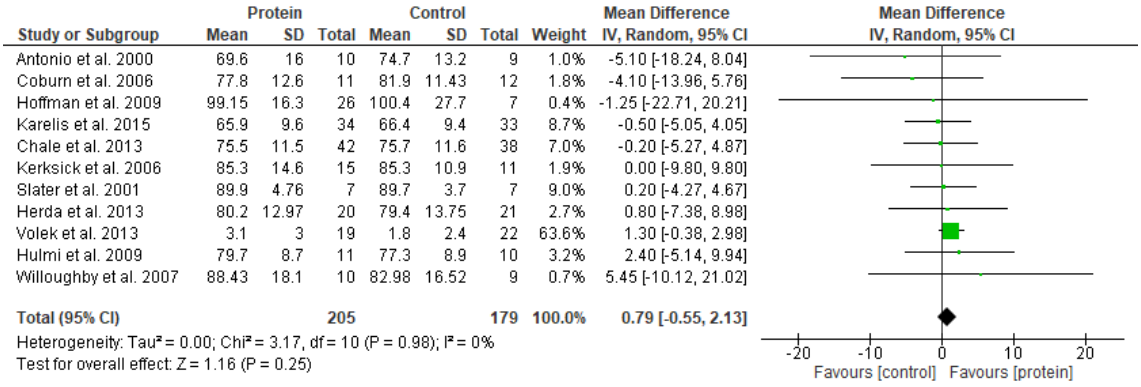

Figure 34: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for body weight in kilogram (kg) of 11 randomized controlled trials comparing the effect of protein supplementation versus control treatments. The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond at the bottom of the graph represents the pooled weighted mean differences including 95% CI.

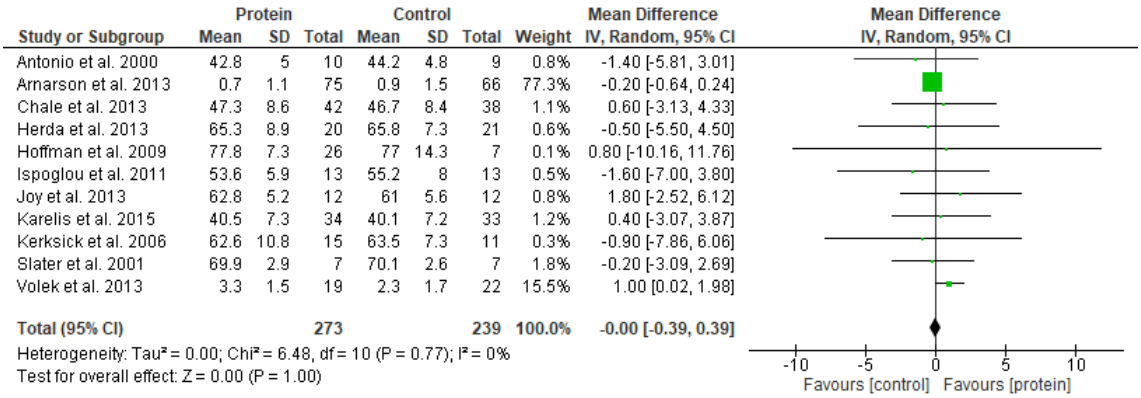

Figure 35: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for lean body mass in kilogram (kg) of 11 randomized controlled trials comparing the effect of protein supplementation versus control treatments. The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond at the bottom of the graph represents the pooled weighted mean differences including 95% CI.

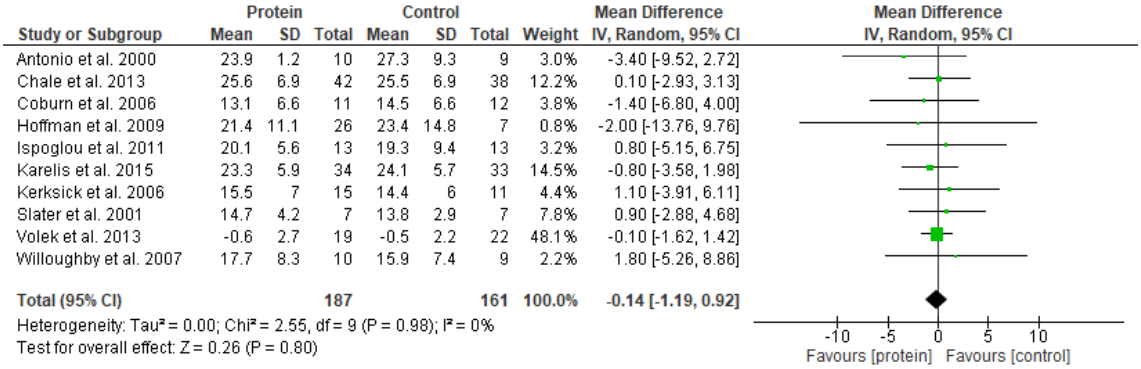

Figure 36: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for fat mass in kilogram (kg) of 10 randomized controlled trials comparing the effect of protein supplementation versus control treatments. The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond at the bottom of the graph represents the pooled weighted mean differences including 95% CI.

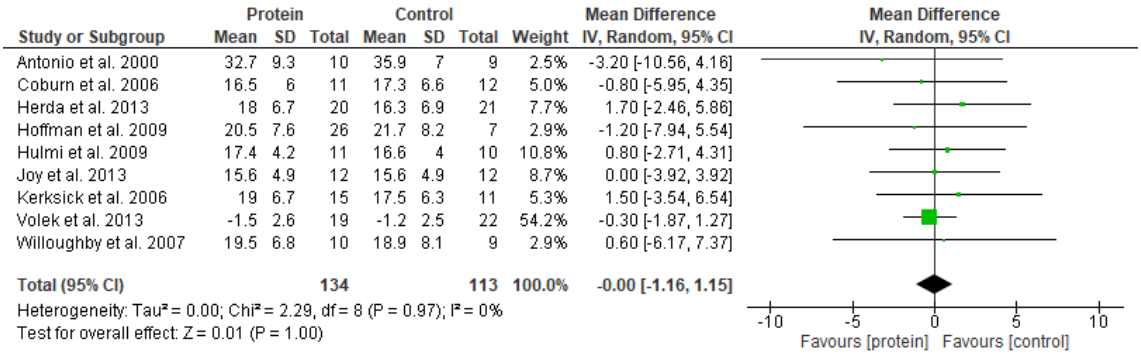

Figure 37: Forest plot showing pooled weighted mean differences with associated 95% confidence interval (CI) for fat mass in percentage (%) of 9 randomized controlled trials comparing the effect of protein supplementation versus control treatments. The horizontal line shows the 95% CI of these effects. The area of the square reflects the (relative) weight of each study within the meta-analysis. The diamond at the bottom of the graph represents the pooled weighted mean differences including 95% CI.

The tables below deal with the risk of bias assessment, structured by bias domain for each included study. The seven bias domains were justified by quotations or comments, and classified as low, unclear or high risk [16].

Table 21: Risk of bias table for Andersen et al. 2005 [24]

Bias Domain	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Quote: "The subjects were ranked according to the maximal isometric torque of the knee extensor muscles, which was determined on a screening visit to the laboratory, matched accordingly in pairs, and randomly assigned to either the protein group or the carbohydrate group."
Allocation concealment (selection bias)	Low risk	Quote: "The protein and carbohydrate supplements were stored in identiacl opaque sachets and heavily flavored with vanilla to render identification of the respective supplements difficult."
Blinding of participants and personnel (performance bias)	Low risk	Quote: "To keep the study double blinded, neither the subjects nor any of the involved researchers knew shich group the subjects belonged to."
Blinding of outcome assessment (detection bias)	Low risk	Quote: "the biopsy specimes were blinded" Comment: Randomized and double-blind approach.
Incomplete outcome data (attrition bias)	Unclear risk	Comment: Possible dropouts and their reasons are not mentioned.
Selective reporting (reporting bias)	Unclear risk	Quote: "only the protein group showed muscle fiber hypertrophy of the trained leg muscles. Type I and type II muscle fCSA of the vastus lateralis increased by 18% ± 5% (P< .01) and 26% ± 5% (P< .01), respectively, in the protein group, whereas no significant change occured in the carbohydrate group." Comment: Data from the control group not mentioned.
Other bias	Unclear risk	Quote: "This study was supported by Numico Research BV (Wageningen, the Netherlands), Danish Research Council (Copenhagen, Denmark) grant 22010254." Comment: Whether there is a potential conflict of interest is not apparent.

Table 22: Risk of bias table for Antonio et al. 2000 [31]

Bias Domain	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "subjects were randomly assigned to an EAA or a placebo group."
Allocation concealment (selection bias)	Low risk	Comment: Type of allocation not described.
Blinding of participants and personnel (performance bias)	Low risk	Quote: "Subjects in the placebo group ingested an equal number of identical-looking pills.", "double-blind"
Blinding of outcome assessment (detection bias)	Low risk	Quote: "double-blind, placebo-controlled study."
Incomplete outcome data (attrition bias)	Low risk	Quote: "Two women dropped out from the study due to personal reasons."
Selective reporting (reporting bias)	Unclear risk	Comment: Insufficient information to permit judgement.
Other bias	High risk	Quote: "This study was funded by Chem International, Inc., Hillsdale, New Jersey, USA.", "Subjects were compensated financially for completing the study."

Table 23: Risk of bias table for Arnarson et al. 2013 [26]

Bias Domain	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "Treatment assignment was randomized and double-blinded."
Allocation concealment (selection bias)	Low risk	Quote: "Participants were randomly allocated to treatment groups following a stratified randomization procedure based on a computer-generated list of random numbers."
Blinding of participants and personnel (performance bias)	Low risk	Quote: "The supplement drinks were provided in identical brick-style cartons and each of the two supplements had a specific three-digit-labelling. Investigators and other staff were kept blind to supplement assignment by the producer of the supplement until the intervention was completed."
Blinding of outcome assessment (detection bias)	Low risk	Quote: "double blind, randomised controlled trial"
Incomplete outcome data (attrition bias)	Low risk	Quote: "The flow diagram shows the number of participants who quit participation due to health complications or lack of motivation during the course of the study." Comment: 20 out of 161 withdrew from the study.
Selective reporting (reporting bias)	Unclear risk	Comment: Biochemical analyzes are collected at baseline and end point, but only the baseline data are reported.
Other bias	Low risk	Quote: "The authors declare no conflict of interest."

Table 24: Risk of bias table for Candow et al. 2006 [23]

Bias Domain	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "Twenty-seven untrained healthy subjects (18 female, 9 male) age 18 to 35 y were randomly assigned."
Allocation concealment (selection bias)	Low risk	Quote: "Entry and analysis of data was performed by analyzing coded groups."
Blinding of participants and personnel (performance bias)	Low risk	Quote: "An individual, who was not involved in the study, was responsible for randomizing the subjects and coding the supplements to ensure all subjects and investigators remained blinded throughout the study."
Blinding of outcome assessment (detection bias)	Low risk	Quote: "The study used a double-blind repeated measures design."
Incomplete outcome data (attrition bias)	Low risk	Quote: "Of the original 31 subjects who volunteered, 27 completed the study. One subject from the W group withdrew because of shoulder and back pain and one subject in each group withdrew because of time constraints."
Selective reporting (reporting bias)	Unclear risk	Comment: Baseline data for LBM and strength are not specified.
Other bias	Unclear risk	Comment: Whether there is a potential conflict of interest is not apparent.

Table 25: Risk of bias table for Chale et al. 2013 [27]

Bias Domain	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "a research assistant unaffiliated with this study who maintained the randomization schedule and communicated the randomization to the research dietitian."
Allocation concealment (selection bias)	Unclear risk	Quote: "The randomization schedule was developed by the study statistician." Comment: Type of allocation not described.
Blinding of participants and personnel (performance bias)	Low risk	Quote: "Participants were randomized to receive either 40 g/day of WPC...", "a research dietitian, blinded to the randomization schedule provided by the study statistician, distributed the supplements..."
Blinding of outcome assessment (detection bias)	Low risk	Quote: "randomized, double-blind, controlled study." Comment: Probably done.
Incomplete outcome data (attrition bias)	Low risk	6 months: 3/42 withdrew from intervention group; 2/38 withdrew from control group, "all data presented are based on th "Intent-to-Treat" analyses." Comment: Unequal distribution of dropouts but the missing data were imputed using an appropriate statistical method.
Selective reporting (reporting bias)	Unclear risk	Quote: "Further examination of data from the “completers” analysis resulted in improvements in all measures of physical functioning in both groups over time but including 400-m walk time ($p = .01$; data not shown)."
Other bias	Low risk	Quote: "Any opinions, findings, conclusion, or recommendations expressed in this publication are those of the authors and do not necessarily reflect the view of the U.S. Department of Agriculture."

Table 26: Risk of bias table for Coburn et al. 2006 [32]

Bias Domain	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "Using a double-blind design for the supplement (SUPP) and placebo groups (PL), the subjects were randomly assigned into 1 of 3 groups."
Allocation concealment (selection bias)	Unclear risk	Comment: Type of allocation not described.
Blinding of participants and personnel (performance bias)	Low risk	Quote: "Two of the investigators, who were unaware of group membership or time of testing performed all CSA measurements.", "double-blind design"
Blinding of outcome assessment (detection bias)	Low risk	Quote: "A randomized, double-blind design was used."
Incomplete outcome data (attrition bias)	Unclear risk	Comment: No information about the dropouts and their reasons.
Selective reporting (reporting bias)	Unclear risk	Quote: "Intratester reliability and intertester objectivity of the CSA measurements were determined by measuring images from 10 randomly selected subjects." Comment: Out of 33 participants, only 10 were selected.
Other bias	Low risk	Quote: "The results of this study do not constitute endorsement of the product by the authors or the National Strength and Conditioning Association."

Table 27: Risk of bias table to Farup et al. 2014 [33]

Bias Domain	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Unclear risk	Quote: "subjects were equally allocated into either a high-leucine whey protein hydrolysate + carbohydrate group (WHD, n=11) or an isoenergetic placebo group (PLA, n=11)." Comment: Whether the sequence generation was randomized can not be clearly proven.
Allocation concealment (selection bias)	Unclear risk	Comment: Type of allocation not described.
Blinding of participants and personnel (performance bias)	Low risk	Quote: "This long-term training study design was conducted in a double-blinded fashion."
Blinding of outcome assessment (detection bias)	Low risk	Quote: "double-blinded" Comment: Probably done.
Incomplete outcome data (attrition bias)	Unclear risk	Comment: No information for missing data or dropouts provided.
Selective reporting (reporting bias)	Unclear risk	Comment: Insufficient information to permit judgement.
Other bias	Low risk	Quote: "There is no conflict of interest declared by the authors."

Table 28: Risk of bias table for Godard et al. 2002 [28]

Bias Domain	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "older men were randomly assigned to either the experimental (EX) or control (CN) groups."
Allocation concealment (selection bias)	Unclear risk	Comment: Type of allocation not described.
Blinding of participants and personnel (performance bias)	Unclear risk	Quote: "The control group received no provision during the study, but completed all testing and training in the same order and fashion as the experimental group."
Blinding of outcome assessment (detection bias)	Unclear risk	Comment: No information on blinding.
Incomplete outcome data (attrition bias)	Unclear risk	Comment: No information about the dropouts and their reasons.
Selective reporting (reporting bias)	Unclear risk	Comment: Insufficient information to permit judgement.
Other bias	Unclear risk	Quote: "This investigation was supported by National Institutes of Health/National Istitutes on Aging grant AG154876." Comment: Whether there is a potential conflict of interest is not apparent.

Table 29: Risk of bias table for Herda et al. 2013 [34]

Bias Domain	**Authors' judgement**	**Support for judgement**
Random sequence generation (selection bias)	Low risk	Quote: "After the pretesting assessments, the subjects were randomly assigned to 1 of 5 treatment groups."
Allocation concealment (selection bias)	Unclear risk	Comment: Type of allocation not described.
Blinding of participants and personnel (performance bias)	Low risk	Quote: "Each serving of all supplements were chocolate flavored power packaged individually in opaque white disposable packets with no writing other than lables with removable stickers showing the subject number, clinical trial number, and mixing instructions. The stickers were removed and placed in each subject's case report form to document that the supplement had been consumed."
Blinding of outcome assessment (detection bias)	Low risk	Quote: "A randomized, double-blinded, placebo-controlled clinical trial."
Incomplete outcome data (attrition bias)	Unclear risk	Comment: No information for missing data or dropouts provided.
Selective reporting (reporting bias)	Unclear risk	Comment: Insufficient information to permit judgement.
Other bias	Low risk	Quote: "The results of this study do not constitute endorsement of the product by the authors or the National Strength and Conditioning Association."

Table 30: Risk of bias table for Hoffman et al. 2009 [35]

Bias Domain	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	High risk	Quote: "Participants were randomly assigned.", "7 participants agreed to serve as a control group." Comment: Randomization probably not met.
Allocation concealment (selection bias)	Unclear risk	Quote: "Each supplement was prepackaged in a spherical tube." Comment: Type of packaging is unclear.
Blinding of participants and personnel (performance bias)	Unclear risk	Quote: "7 participants agreed to serve as a control group (n = 7; 20.7 ± 1.1 years, 179.4 ± 9.4 cm, 100.1 ± 27.2 kg) and did not use any protein or other nutritional supplement.", "Each supplement was prepackaged in a spherical tube." Comment: Type of blinding not specified.
Blinding of outcome assessment (detection bias)	Unclear risk	Comment: No information on blinding.
Incomplete outcome data (attrition bias)	Unclear risk	Comment: No information on possible dropouts.
Selective reporting (reporting bias)	Unclear risk	Comment: No data shown from the urine measurements.
Other bias	Unclear risk	Quote: "This study was supported by a grant from IDS Sports." Comment: Whether there is a potential conflict of interest is not apparent.

Table 31: Risk of bias table for Hofmann et al. 2016 [17]

Bias Domain	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "Eligible participants were randomly, but stratified by gender, assigned to one of the three intervention groups."
Allocation concealment (selection bias)	Unclear risk	Quote: "Eligible participants were randomly assigned to one of the three intervention groups." Comment: Type of allocation not described.
Blinding of participants and personnel (performance bias)	Unclear risk	Comment: Only one intervention group receives a supplement.
Blinding of outcome assessment (detection bias)	Low risk	Quote: "a randomized, controlled intervention study." Comment: Probably done.
Incomplete outcome data (attrition bias)	Unclear risk	Quote: " Blood samples were available from 70/91 women and MQ could be assessed from 59/91 after 6 months." Comment: No information regarding the dropouts and their reasons.
Selective reporting (reporting bias)	Unclear risk	Quote: "Sekeletal muscle mass and handgrip strength did not change over 6 months of intervention in any of the intervention groups. (data not shown)."
Other bias	Low risk	Quote: "The authors have no confict of interest."

Table 32: Risk of bias table for Hulmi et al. 2009 [36]

Bias Domain	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "The subjects were randomly assigned after control testing sessions to either the whey protein group (n=13), placebo group (n=14) or control group (n=11).
Allocation concealment (selection bias)	Unclear risk	Comment: Type of allocation not described.
Blinding of participants and personnel (performance bias)	Low risk	Quote: "The drinks were provided for the subjects in a double-blind fashion. The drinks were made in our own laboratory by the personnel who coded the drinks.", "supplements looked and tasted as identical as possible."
Blinding of outcome assessment (detection bias)	Low risk	Quote: "double-blind" Comment: Probably done.
Incomplete outcome data (attrition bias)	Unclear risk	Quote: "The number of subjects who completes the study was 31." Comment: Type of dropouts not described.
Selective reporting (reporting bias)	Unclear risk	Quote: "The cross-sectional area (CSA) of the quadriceps femoris (QF) increased significantly after 21 weeks of RT in both protein and placebo groups but not in the control group." Comment: The exact data are not given, it is referred to a figure.
Other bias	Unclear risk	Comment: Whether there is a potential conflict of interest is not apparent.

Table 33: Risk of bias table for Ikeda et al. 2016 [22]

Bias Domain	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "The subjects were randomly divided into two groups."
Allocation concealment (selection bias)	Low risk	Quote: "the assignment list using computer-generated random numbers in advance."
Blinding of participants and personnel (performance bias)	High risk	Quote: "Investigators were not blind to group allocation", "there was a possibility that the participants guessed the condition of supplementation."
Blinding of outcome assessment (detection bias)	Low risk	Quote: "single-blinded, randomized, crossover experimental design"
Incomplete outcome data (attrition bias)	Low risk	Quote: "An intention-to-treat analysis was conducted in both groups by order of dosage and supplementation." Comment: Likewise, the dropouts and their reasons are described.
Selective reporting (reporting bias)	Unclear risk	Comment: Insufficient information to permit judgement.
Other bias	High risk	Quote: " a crossover trial." Comment: potential source of bias related to the specific study design used (carry over effect).

Table 34: Risk of bias table for Ispoglou et al. 2011 [37]

Bias Domain	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "Thus, the required number of 13 participants per group (determined by power calculation using David Machin's software version 2) was met.", "The design of the study was placebo-controlled and double blind."
Allocation concealment (selection bias)	Low risk	Quote: "Powders were dispensed in plastic food bags that were sealed, placed in opaque envelopes, which were labeled as A, or B."
Blinding of participants and personnel (performance bias)	Low risk	Quote: "Furthermore, as the experimental groups were discreet groups, neither the participants in the leucine group nor the participants in the placebo group knew what the drink tasted like in the opposite group."
Blinding of outcome assessment (detection bias)	Low risk	Quote: "The design of the study was placebo-controlled and double blind." Comment: Probably done.
Incomplete outcome data (attrition bias)	Unclear risk	Quote: "only 26 out of 40 participants completed the required number of training sessions. This resulted in a dropout of 35%." Comment: Missing data evenly distributed, reasons of dropouts not specified.
Selective reporting (reporting bias)	Unclear risk	Comment: No data shown for ratings of perceived exertion during each hypertrophy workout, blood analyses or the physical activity recall.
Other bias	Unclear risk	Quote: "The research was funded by the Greek National Foundation Scholarships (IKY)." Comment: Whether there is a potential conflict of interest is not apparent.

Table 35: Risk of bias table for Joy et al. 2013 [38]

Bias Domain	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "Subjects were randomly and equally divided into two groups."
Allocation concealment (selection bias)	Unclear risk	Comment: Type of allocation not described.
Blinding of participants and personnel (performance bias)	Low risk	Quote: "The protein supplement was administered under supervision of a laboratory assistant following resistance training, and it consisted of either 48 g of whey protein isolate (Nutra Bio Whey Protein Isolate (Dutch Chocolate), Middlesex, NJ) or 48 g of rice protein isolate.", "The amino acid profile of the study material was analyzed by an independent analytical laboratory."
Blinding of outcome assessment (detection bias)	Low risk	Quote: "Our study consisted of a randomized, double blind protocol."
Incomplete outcome data (attrition bias)	Unclear risk	Comment: No information for missing data or dropouts provided.
Selective reporting (reporting bias)	Unclear risk	Comment: Insufficient information to permit judgement.
Other bias	Low risk	Quote: "The authors declare that they have no competing interests."

Table 36: Risk of bias table for Karelis et al. 2015 [29]

Bias Domain	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "Participants were randomly assigned into two groups."
Allocation concealment (selection bias)	Unclear risk	Comment: No statement regarding the nature of the allocation.
Blinding of participants and personnel (performance bias)	Low risk	Quote: "subjects and the research team at IRCM (principal investigators, study coordinators and trainers) were blinded as to which supplement was given until the end of the trial."
Blinding of outcome assessment (detection bias)	Low risk	Quote: "Randomized double-blind controlled intervention study."
Incomplete outcome data (attrition bias)	Low risk	Quote: "Subjects dropped out for the following reasons: health problems not related to training (n = 2), minor injury related to training (n= 3), did not like the training program (n = 2), did not tolerate the study product (n = 2), conflicting time schedules (n = 1), travel distance to the research unit (n = 1) and unspecified reasons (n = 4)
Selective reporting (reporting bias)	Unclear risk	Comment: Insufficient information to permit judgement.
Other bias	High risk	Quote: "Conflict of interest: Immunotec Inc. provided funds for this study."

Table 37: Risk of bias table for Kerksick et al. 2006 [39]

Bias Domain	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "In a double-blind and randomized manner, subjects were assigned to."
Allocation concealment (selection bias)	Unclear risk	Comment: No statement regarding the nature of the allocation.
Blinding of participants and personnel (performance bias)	Low risk	Quote: "Supplements were prepared in powder form with identical texture, taste, and appearance and were independently packaged and labeled in single-serving foil packets for double-blind administration."
Blinding of outcome assessment (detection bias)	Low risk	Quote: "This study was conducted as a double-blind, placebo-controlled, randomized trial." Comment: Probably done.
Incomplete outcome data (attrition bias)	Unclear risk	Comment: No information regarding the dropouts and their reasons.
Selective reporting (reporting bias)	Unclear risk	Quote: "As a result of the insufficient number of female participants (n=8) who volunteered and completed the protocol, statistical analysis was completed only on the 36 remaining male participants."
Other bias	Low risk	Quote: "This study was funded by a research grant from Royal Numico. Investigators in the Exercise and Sport Nutrition Laboratory independently collected, analyzed, and interpreted the results from this study and have no financial interests in the results of this study."

Table 38: Risk of bias table for Kim et al. 2012 [30]

Bias Domain	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "randomly assigned to one of four groups"
Allocation concealment (selection bias)	Low risk	Quote: "computer-generated random numbers."
Blinding of participants and personnel (performance bias)	Unclear risk	Quote: "co-investigators were blind to the randomization procedure and group allocations", "placebo treatments were not provided in this study" Comment: Control group: only exercises.
Blinding of outcome assessment (detection bias)	Low risk	Quote: "A randomized controlled trial.", "co-investigators were blind to the randomization procedure and group allocations."
Incomplete outcome data (attrition bias)	Low risk	Quote: "Eleven participants (exercise + AAS = 4, exercise = 3, AAS = 2, HE = 2) were unable to complete the study after randomization because of spouse care (n = 3), admission to nursing home (n = 2), lack of motivation (n = 2), severe knee or back pain (n = 1), death (n = 1), falls and hip fracture (n = 1), and hospitalization (n = 1)."
Selective reporting (reporting bias)	Unclear risk	Comment: Insufficient information to permit judgement.
Other bias	Low risk	Quote: "The authors have no conflict of interest to disclose."

Table 39: Risk of bias table for Kraemer et al. 2009 [21]

Bias Domain	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "were matched and randomized into two groups."
Allocation concealment (selection bias)	Unclear risk	Comment: Type of allocation not described.
Blinding of participants and personnel (performance bias)	Low risk	Quote: "double-blind.", "Each volunteer received the supplements in plain, white blinded packets."
Blinding of outcome assessment (detection bias)	Low risk	Quote: "A randomized, double-blind, placebo-controlled design."
Incomplete outcome data (attrition bias)	Unclear risk	Comment: No information regarding the dropouts and their reasons.
Selective reporting (reporting bias)	Unclear risk	Comment: Insufficient information to permit judgement.
Other bias	High risk	Quote: "This work was supported by a grant from Abbott Laboratories (Abbott Park, IL), the makers of Muscle Armor™. The results of the present study do not constitute endorsement by the American College of Sports Medicine."

Table 40: Risk of bias table for Slater et al. 2001 [40]

Bias Domain	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "subjects were matched according to total strength combined across the three lifts and in a randomized, double-blind manner, allocated to one of three groups."
Allocation concealment (selection bias)	Unclear risk	Comment: No statement regarding the nature of the allocation.
Blinding of participants and personnel (performance bias)	Low risk	Quote: "The supplement was provided in labeled, sealed, plastic bottles.", "Treatment codes for the 6-week trial were maintained by an independent third party who did not reveal the code until final analysis had been completed."
Blinding of outcome assessment (detection bias)	Low risk	Quote: "double-blind, parallel-design supplementation trial."
Incomplete outcome data (attrition bias)	Unclear risk	Comment: No information about the dropouts and their reasons.
Selective reporting (reporting bias)	Unclear risk	Quote: "For subjects with missing data from a specific test at week 3, a mean substitution method was employed to account for the missing value. For all other missing data, subjects test results were removed from analysis for that specific test only.", "It was considered important to obtain information relating to effect size so that any medium to large effects were not missed due to the relatively small subject pool.", "Three of these subjects were removed from analysis of urinary parameters due to incomplete 24-hour urine collection during one of the testing periods."
Other bias	Unclear risk	Quote: "This investigation was supported by grants from Experimental and Applied Sciences, Inc., Golden, CO, and the Olympic Athlete Program, Australian Sports Commission." Comment: Whether there is a potential conflict of interest is not apparent.

Table 41: Risk of bias table for Trabal et al. 2015 [25]

Bias Domain	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "Thirty older adults were randomly assigned to."
Allocation concealment (selection bias)	Unclear risk	Comment: Type of allocation not described.
Blinding of participants and personnel (performance bias)	Low risk	Quote: "provided in a double-blinded manner", "Both supplement and placebo were accompanied with lemon and lime flavor (Flavor Sachets; Nutricia) to disguise the characterstic taste of leucine."
Blinding of outcome assessment (detection bias)	Low risk	Quote: "a randomized, double-blind, placebo-controlled, parallel study with two intervention groups."
Incomplete outcome data (attrition bias)	Low risk	Quote: "missing data at 12 weeks were assumed to be missing at random and were adequately extrapolated by the MMRM model." Comment: Dropouts and their reasons are described.
Selective reporting (reporting bias)	Unclear risk	Quote: "the analysis of the GDS-15 scores did not find any significant changes on this outcome (data not shown)."
Other bias	Low risk	Quote: "The authors report no conflicts of interest in this work."

Table 42: Risk of bias table for Volek et al. 2013 [41]

Bias Domain	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "Healthy men and women were randomly assigned in a double-blind manner to supplement daily with whey protein (whey), soy protein (soy), or carbohydrate (carb)."
Allocation concealment (selection bias)	Unclear risk	Comment: Type of allocation not described.
Blinding of participants and personnel (performance bias)	Low risk	Quote: "Using a double-blind protocol, the carbohydrate, whey, and soy powdered supplements were provided in identical individualized packets."
Blinding of outcome assessment (detection bias)	Low risk	Quote: "double-blind manner"
Incomplete outcome data (attrition bias)	Unclear risk	Quote: "only subjects who completed the required training sessions and were compliant with the supplement protocol (>90%) were analyzed." Comment: Type of dropouts not described.
Selective reporting (reporting bias)	Unclear risk	Comment: Insufficient information to permit judgement.
Other bias	Unclear risk	Comment: Whether there is a potential conflict of interest is not apparent.

Table 43: Risk of bias table for Willoughby et al. 2007 [42]

Bias Domain	Authors' judgement	Support for judgement
Random sequence generation (selection bias)	Low risk	Quote: "participants were matched by age, total body mass, and leg press strength, and then randomly assigned, in a double blind fashion."
Allocation concealment (selection bias)	Unclear risk	Comment: Type of allocation not described.
Blinding of participants and personnel (performance bias)	Low risk	Quote: "double blind", "independently prepared in individually blinded packages."
Blinding of outcome assessment (detection bias)	Low risk	Quote: "double blind" Comment: Probably done.
Incomplete outcome data (attrition bias)	Low risk	Quote: "However, one subject was forced to withdraw from the study due to illness unrelated to the study."
Selective reporting (reporting bias)	Unclear risk	Comment: Insufficient information to permit judgement.
Other bias	Unclear risk	Comment: Whether there is a potential conflict of interest is not apparent.

Figure 38 summarizes the evaluation for each individual domain and study. Classification of risk of bias was done according to the Cochrane Risk of Bias Tool for Randomized Controlled Trials using the colors of a traffic light (green = low risk, yellow = unclear risk and red = high risk) [16].

Figure 38: Cochrane risk of bias summary of included trials (green = low risk, yellow = unclear risk and red = high risk)