Clinical Nutrition 41 (2022) 1034–1045

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Randomized Control Trials

Effects of an increased habitual dietary protein intake followed by resistance training on fitness, muscle quality and body composition of seniors: A randomised controlled trial

Sandra Unterberger [a, b], Rudolf Aschauer [a, b], Patrick A. Zöhrer [a, c], Agnes Draxler [c], Bernhard Franzke [a, c], Eva-Maria Strasser [d], Karl-Heinz Wagner [a, c], Barbara Wessner [a, b, *]

[a] Research Platform Active Ageing, University of Vienna, Althanstraße 14, 1090 Vienna, Austria
[b] Centre for Sport Science and University Sports, University of Vienna, Auf der Schmelz 6, 1150 Vienna, Austria
[c] Faculty of Life Sciences, Department of Nutritional Sciences, University of Vienna, Althanstraße 14, 1090 Vienna, Austria
[d] Karl Landsteiner Institute for Remobilization and Functional Health/Institute for Physical Medicine and Rehabilitation, Kaiser Franz Joseph Hospital, Social Medical Center South, Vienna, Austria

ARTICLE INFO

Article history:
Received 26 July 2021
Accepted 18 February 2022

Keywords:
Protein supplementation
High protein diet
Resistance training
Muscle quality
Physical function
Older adults

SUMMARY

Background & aims: Resistance training and a sufficient amount of dietary protein have been suggested to build up and maintain muscle mass, strength and function into old age. As there is still no consensus on the optimum amount of protein intake in older people, this study aims to evaluate first whether it is achievable to double the recommended amount, which is 1 g/kg BW/d in German speaking countries, via food administration and secondly whether this would lead to stronger improvements when subsequently combined with resistance training.

Methods: In total, 136 community-dwelling older adults (54% females, 72.9 ± 4.8 yrs) were randomly assigned to one of the three study groups: observational control (CON), recommended protein (RP + T) and high protein (HP + T) intake groups. After six weeks of observation or nutritional counselling to achieve the respective protein target levels, eight weeks of resistance training (2x/week) were applied in RP + T and HP + T groups. Parameters indicative for muscle mass, strength and function were measured at baseline (t1), before (t2) and after the training period (t3).

Results: Baseline protein intake for the different groups were 0.83 (CON), 0.97 (RP + T) and 0.78 (HP + T) g/kg BW/d and increased by 0.18 ± 0.31 (RP + T, $p = 0.003$) and 0.83 ± 0.33 (HP + T, $p > 0.001$) g/kg BW/d between t1 and t3 while CON remained unchanged. Most of the physical performance parameters improved over time, but no interaction effects between group and time could be observed. While body fat mass initially increased from t1 to t2 (0.8 ± 2.3 kg, $p = 0.001$), skeletal muscle mass decreased (−0.5 ± 1.9 kg, $p = 0.025$), a trend which was reversed from t2 to t3 only in HP + T group (body fat mass: −0.47 ± 2.12 kg, $p = 0.041$; muscle mass: 0.51 ± 1.57 kg, $p = 0.021$).

Conclusion: The findings suggest that a substantial increase of habitual protein intake above the currently recommended levels is achievable within 17 weeks in community-dwelling older adults, whereby the extra amount of protein led to minor changes in body composition but not physical performance or muscle quality (NCT04023513).

1. Introduction

The United Nations predict the proportion of people over 60 years of age to be doubled by 2050 [1] and both society and individuals are interested in spending the concomitant general increase in life expectancy in the best physical condition [2]. Ageing is related to a decrease in fat-free mass, which results in reduced power, strength, skeletal muscle mass (SM) and accordingly decreased muscle quality

* Corresponding author. Centre for Sport Science and University Sports, University of Vienna, Auf der Schmelz 6, A-1150 Vienna, Austria.
E-mail addresses: sandra.unterberger@univie.ac.at (S. Unterberger), rudolf.aschauer@univie.ac.at (R. Aschauer), patrick.zoehrer@univie.ac.at (P.A. Zöhrer), agnes.draxler@univie.ac.at (A. Draxler), bernhard.franzke@univie.ac.at (B. Franzke), eva-maria.strasser@wienkav.at (E.-M. Strasser), karl-heinz.wagner@univie.ac.at (K.-H. Wagner), barbara.wessner@univie.ac.at (B. Wessner).

https://doi.org/10.1016/j.clnu.2022.02.017

(MQ, muscle strength or power relative to muscle mass) [3–5]. This is of particular importance as these factors are key components for mobility and daily living activities (ADLs) [6]. Resistance training and food protein intake are non-pharmacological strategies that can potentially reverse or mitigate especially musculoskeletal declines [7,8], yet, older individuals show a reduced muscle protein synthesis response not only to resistance training but also to administered amino acids, a situation known as anabolic resistance [9,10]. Hence, there is growing interest in developing effective strategies to counteract these age-associated changes and to overcome the accompanied gradual decline in physical capabilities [11]. Besides total protein intake supplied per day [12,13], also protein quality [14,15], protein dose per meal [16], and its timing [17] are important factors to be considered.

The PROT-AGE study group and the ESPEN Expert group recommend an average daily intake in the range of 1.0 to 1.2 g/kg body weight (BW)/d for healthy adults, whereas most older adults who have acute or chronic diseases would need even more dietary protein (1.2 to 1.5 g/kg BW/d) [18,19]. Recommendations for protein intake in Austria and other German speaking countries amount to 1.0 g/kg BW/d for older adults [20]. However, these and even lower recommendations of 0.8 g/kg BW/d in U.S [21] are not met by many older adults through their regular diet due to reduced appetite coupled with a decline in biological and physiological functions such as changes in fluid electrolyte regulation, delayed gastric emptying and diminished senses of smell and taste [22–27]. Diets enriched with common protein-rich foods may be an effective strategy to increase total protein intake and hence to maintain SM, function and MQ [28]. Thereby, long-term consumption of $\leq$2 g/kg BW/d has been shown to be safe for healthy adults with little evidence of intestinal, hepatic, renal or cardiovascular dysfunction in healthy people [29,30].

Protein supplementation alone or in combination with resistance training has been shown to improve SM, MQ, physical function, and strength in some studies [15,31,32], but numerous well-designed trials reported opposing results [33–35]. Thus, it is still uncertain whether increasing protein intake, especially when achieved mainly via habitual food supply and combined with resistance training, can be seen as an effective strategy to maintain or increase physical performance of older individuals.

The aim of the present study was to investigate whether adjusting the habitual protein intake to either the Austrian recommended dose of 1.0 g/kg BW/day for this age group or to a double dose of about 2.0 g/kg BW/day, either alone (6 weeks) or in combination with 8 weeks of resistance training would affect body composition, functional performance or MQ of people aged 65 to 85 years.

2. Material & methods

2.1. Study design

The study was designed as a randomized, controlled, observer-blind trial with three groups: control group (CON, observation only), recommended or high protein intake plus resistance training groups (RP + T, HP + T). Inclusion and exclusion criteria were screened at the medical visit before the beginning of the study (t0). The study included two phases, a six-week period focusing on the nutritional observation/counselling followed by an eight-week lasting period where nutritional counselling was accompanied by a progressive resistance exercise training programme in RP + T and HP + T. Data were collected at baseline (t1), interim in week 8 (t2) and at the end of the intervention in week 17 (t3). All assessments were conducted at the Centre for Sport Science and University Sports, University of Vienna, Austria between July and December 2018.

2.2. Participants

Participants were recruited via local media advertisements as well as during information events at local senior citizens meeting centres. Inclusion was possible for community-dwelling males and females aged between 65 and 85 years who did not perform any regular resistance training during the last six months. Cognitive impairment (Mini Mental State Examination Score <23), acute and chronic diseases that would contraindicate resistance training, serious cardiovascular diseases, diabetic retinopathy, manifest osteoporosis, anticoagulant or cortisone medication, a frailty index $\geq$3 or the need for walking aids comprised the exclusion criteria. Pre-existing diseases such as hyperlipidaemia, diabetes mellitus type 2, osteoporosis, heart disease and history of cancer were evaluated during the medical examination at t0. These diseases did not represent a cause for exclusion as long as they did not directly interact with the physical performance tests. Obesity (BMI $\geq$30 kg/m^2) and hypertension (blood pressure above 140/90 mm Hg) were defined according to World Health Organization criteria [36].

A written informed consent form was signed by all participants before participation in the study. The study was performed in accordance with the Declaration of Helsinki, approved by the Ethics Committee of the University of Vienna (Reference Number: 00322) and registered at https://clinicaltrials.gov (NCT04023513).

2.3. Interventions

2.3.1. Nutritional intervention

While participants of CON did not receive any nutritional intervention, the food-based interventions of RP + T and HP + T were personalised based on regular individual dietary assessments (see 2.4.1 for details). For RP + T a target protein intake of 1.0 g/kg BW/d should be achieved based on the recommendation of the D-A-CH reference values [20]. For HP + T the target protein intake was set to 2.0 g/kg BW/d. The additional protein intake in the HP + T group should not be reached by supplements but via nutritional counselling and commercially available protein-rich foods (e.g. protein-rich milk products, bars, puddings, protein-rich bread, bacon crisps, protein rich soups, pea protein sticks as well as recipes for self-prepared foods). To support the participants of the HP + T group in increasing their habitual protein intake, a selection of different protein-rich food products was provided by the study team, but the participants were free to use them. The RP + T group received food alternatives with moderate protein content (e.g., milk products, bars, bread, puddings, self-made vegetable muffins). The protein intake was personalised and calculated on the respective BW and monitored over the whole study period. The subjects received every fortnight the food for the calculated additional protein intake for 2 weeks via special delivery service. From week 9 to week 17 (training phase) HP + T and RP + T continued their dietary regimen and in addition HP + T received 40 g of veganeo hazelnut-chocolate drink (AnovonA®, Laufen, Germany: 146 kcal, 1.5 g fat, 1.4 g carbohydrates, 32 g protein (leucine 3.1 g, isoleucine 1.6 g, valine 2.0 g) or 40 g veganeo vanilla (AnovonA®, Laufen, Germany: 146 kcal, 1.1 g fat, 1.2 g carbohydrates, 32 g protein (leucine 3.2 g, isoleucine 1.7 g, valine 2.1 g)) dissolved in 300 ml water twice a week directly after each training session. To balance the additional caloric supply, RP + T received an equivalent isocaloric, carbohydrate-containing drink (40 g bulkpowders.com, pure series, (Colchester, UK): 152 kcal per serving; 0 g fat, 38 g carbohydrates (1:1, cyclical dextrin:dextrose & maltodextrin), 0 g protein with 300 ml water) after the training sessions. For CON, the habitual dietary intake was observed but not adapted during the whole study period. The sex-specific target range for total energy intake for HP + T and RP + T was based on the D-A-CH reference

values for this age group for an estimated physical activity level (PAL) between 1.4 and 1.6 (females: 1.700–1.900 kcal/d; males: 2.100–2.500 kcal/d).

2.3.2. Resistance training

Resistance training was performed between t2 and t3 for eight weeks, twice a week on non-consecutive days following the guidelines of the American College of Sport Medicine (ACSM) [37]. Both groups, RP + T and HP + T, completed the same training sessions, which included 5–10 min of warm-up followed by 45–60 minutes of resistance exercise training for the major muscle groups. The sessions ended with 5–10 min of cool-down. The training was carried out in selected gyms, all equipped with the same training devices (TECHNOGYM Selection 700 & 900, Italy). The resistance training consisted of five machine-guided exercises (leg press/leg curl/latissimus pulldown/rowing/chest press), two free weight exercises (goblet box squat/dumbbell shoulder press) and one body weight exercise (front plank with alternating single leg raise). In the training sessions, participants of RP + T and HP + T were mixed. Subjects were instructed to spend 1–2 seconds on the concentric and 3–4 seconds on the eccentric phase. Intensity was controlled subjectively by using the OMNI Rate of Perceived Exertion scale (RPE 0–10) [37,38]. During the first two weeks, the participants performed one to two sets of 15–20 repetitions at submaximal load (RPE 3–4) for familiarisation. From week three, the subjects performed two sets, increased the weight and decreased the number of repetitions to 10–15 trials with an RPE of 4–6. From week 6, the intensity was further increased to an RPE of 6–7, whereby the number of repetitions was decreased to 8–12 repetitions and the number of sets was increased to three. The weight was individually adjusted based on the self-reported RPE and load was increased if RPE fell below 4 or if more than 12 repetitions could be completed. The sessions were supervised by sport scientists who monitored the correct and safe execution of the exercise tasks and adapted the intensity if necessary.

2.4. Outcomes

Assessment of outcomes followed a standardized procedure and was carried out during weeks 1, 8 and 17 by trained research staff blinded to the group allocation. Protein intake levels and 30-second chair stand test were considered as primary outcomes. Secondary outcomes were total energy, carbohydrates and fat intakes as well as body composition, handgrip strength, 30-second arm curl test, 6-min walk test, gait speed, timed up and go, chair sit-and-reach, back scratch and MQ.

2.4.1. Dietary intake assessment

To estimate the habitual as well as the additional protein-rich food intake, the participants completed 24-h dietary recalls every 7–10 days during the whole study period. Finally, 9 ± 1 interviews per participant were conducted and evaluated within 2 days on average. Baseline food intake data comprise two 24-h dietary recalls with 10 days in between them. Seven out of nine interviews referred to weekdays while two were based on weekend days. At least four interviews were conducted in a face-to-face setting before participants could choose to switch to a telephone interview, since the effort to meet for a face-to-face interview was very high for this age group and the subjects were already trained in the first four interviews. Dietary intake was conducted by Globodiet®, formerly EPICSoft. GloboDiet was developed by the International Agency for Research on Cancer and further adapted for Austria at the Department of Nutritional Sciences in Vienna [39]. Every participant received a photo book to support the estimation of the portion size. The reported foods collected during the interviews were linked to the German food composition database (Bundeslebensmittelschlüssel) version 3.02 [40]. Total energy intake (kcal), carbohydrates (g/kg BW/d), fat (g/kg BW/d) and protein (g/kg BW/d) were estimated. The consumption of provided foods was reported in a food diary. These logs were collected and reviewed during every 24-h recall. Furthermore, participants were trained and supported with an info booklet to estimate protein content of various products. Two days after the respective interview potentially necessary adjustments were communicated to the participants.

2.4.2. Anthropometry and body composition

Anthropometric and body composition measurements were carried out in the morning after an overnight fast. A stadiometer attached to a digital scale (seca 217 + 877, Seca GmbH & Co KG, Hamburg, Germany) was used to measure body height (to 0.01 m) and mass (to 0.1 kg). Participants were lightly clothed and barefoot. Body mass index (BMI) was calculated from the ratio of body weight (in kg) and height (in m) squared.

Body composition was determined by body impedance analysis (BIA, Nutriguard-MS + NutriPlus-Software, Version 5.1, Data-Input, GmbH, Germany). Data output included resistance (R, Ω), reactance (Xc, Ω), fat mass (kg and percentage) and phase angle (°). Skeletal muscle mass (SM) was calculated by: $SM\ (kg) = [(Ht^2/R \times 0.401) + (sex \times 3.825) + (age \times -0.071)] + 5.102$ where Ht is height in cm, R is resistance in Ω; sex = 1 for men and sex = 0 for women, and age is indicated in years [41]. Appendicular skeletal muscle mass (ASMM) was calculated by: $ASMM\ (kg) = -3.964 + (0.227 \times RI) + (0.095 \times weight) + (1.384 \times sex) + (0.064 \times Xc)$ where RI is resistance index (resistance/height^2), sex = 1 for men and sex = 0 for women, and Xc is reactance in Ω [42].

2.4.3. Physical function

Physical function was assessed with the Senior Fitness Test Battery [43], the timed up and go test [44] and gait speed [1]. The Senior Fitness Test Battery consists of the following subtests:

2.4.3.1. 30-Second chair stand test. Lower body strength (endurance) was assessed with the 30-s chair stand test. The reliability of this test has been shown to be excellent (ICC = 0.89) [43]. Subjects were instructed to sit on a chair with both legs at 90°, feet flat on the floor and arms crossed in front of the chest. Participants were asked to stand up and sit down as many times as possible within 30 s. A repetition was completed when the person fulfilled a cycle of standing up (knees and hips fully extended) and sitting down again. In the last second, an effort was considered valid if more than 50% of the range of motion had been achieved. To perform the technique correctly, one to two repetitions were executed before the test [43]. From the number of repetitions after 20 s, power of the lower extremities was estimated by using the following formula: [−715.218 + 13.915 × body weight (kg) + 33.425 × repetitions from 20-s chair stand test] [45].

2.4.3.2. 30-Second arm curl test. Strength (endurance) of the upper extremities were measured with the 30-s arm curl test which has been shown to have a good reliability (ICC = 0.81) [43]. This test was carried out in a sitting position, holding a 2.3 and 3.6 kg dumbbell in the dominant hand, respectively for women and men. Participants started with a full elbow extension and consequently bent the elbow to full flexion. After a brief demonstration and practice, the participant was asked to perform as many arm curls as fast as possible. The total number of curls within 30 s were counted [43].

2.4.3.3. Six-Minute walk test. Aerobic endurance was measured with the 6-min walk test. The reliability of this test was reported as excellent (ICC = 0.94) [43]. The participants walked up and

down for six minutes as fast as possible on a 30-meter corridor. The covered distance was measured to the nearest cm and expressed in m [46].

2.4.3.4. Chair sit-and-reach. The chair sit-and-reach test was used to measure lower limb flexibility. The test has an excellent reliability (ICC = 0.95) [43]. With one leg extended and the other bent at an angle of 90°, participants sat on the edge of a chair. They were asked to reach the toes of the extended leg with their fingers, keeping their back and leg straight. The final point had to be held for two seconds and the distance between fingertips and toes was measured in cm. Negative values denote trials where participants did not reach the toes, whereas positive values mark trials where fingers extend the toes. The test was performed twice, and the best result was noted [43].

2.4.3.5. Back scratch. Shoulder flexibility was measured by back scratch test. Reliability for this test is considered as high (ICC = 0.96) [43]. In a standing position, participants placed the preferred hand behind over the same shoulder, palms turned inwards, fingers pointing downwards and reach down the middle of the back as far as possible. Then the other arm was placed behind the back, palms facing outwards and fingers pointing upwards. Participants were asked to reach as far as possible, attempting to overlap the middle fingers of both hands. The distance between the fingers was measured as positive when the fingers overlapped and as negative when the fingers did not overlap. Two trials were performed, the better result was recorded [43].

2.4.3.6. Timed up and go test. To assess mobility, the timed up and go test was used which has an excellent reliability (ICC = 0.98) [43]. Participants were required to sit on a chair, then stand up as quickly as possible, walk three metres, turn around a cone, return to the chair and sit down again. Before the test was conducted, the subjects were allowed to take one practice run to familiarise themselves with the procedure. The required time was measured in seconds and the faster one of two runs was reported [44].

2.4.3.7. Gait speed. Participants were instructed to walk ten metres, first at a self-selected speed and then at their maximum speed. Two rounds were carried out at each speed, with a break of one minute in between. Light barriers (Brower Timing Systems, USA) were attached to measure the time in seconds needed to cover the distance between two and eight metres. The faster time of the two trials was used for calculating gait speed expressed in m/s. The reliability for gait speed for self-selected and maximal was reported as being high (ICC = 0.90–0.96) (26).

2.4.4. Handgrip strength

Handgrip strength represents the maximal isometric force achieved on a handgrip dynamometer (SAEHAN Corporation, Korea). This test was administered in a sitting position by holding the dynamometer at a 90° flexion of the elbows in the dominant hand. The higher result of two trails was used as a result [47].

2.4.5. Muscle quality

Muscle Quality (MQ) is defined as muscle strength or power per unit of SM [48]. MQ of the upper body (MQ_{UBS}) was calculated from the ratio between handgrip strength (kg) and SM (kg). MQ of the lower body (MQ_{LBP}) was revealed by dividing the estimated lower body power (W) by SM (kg).

2.5. Statistics

2.5.1. Sample size

Sample size calculation was performed by using the statistical power analyses tool G*Power [49] and was based on 30-s chair stand test results from a previous study [50]. An a priori power analysis showed that with an assumed α of 0.05, a power (1-β) of 0.85 and a moderate effect size (partial η^2) of 0.13, a total sample size of 93 subjects would be necessary to be included in the study. Drop-out rate was estimated to be 40%. Therefore, a total of about 130 subjects were aimed to be included in the study.

2.5.2. Randomisation and stratification

Participants who met the inclusion criteria were randomly assigned to one of the three intervention groups (1:1:1) using an academic randomisation tool and random permuted blocks (6, 3; random number seed: 126277972, https://randomizer.at/, Institute of Medical Informatics, Statistics and Documentation, Medical University of Graz, Austria). Rejections were compensated. As physical performance might be influenced by age and sex, subjects were stratified by age groups (65–69.9; 70–74.9; 75–79.9; 80 to <85 yrs) and sex (female; male) to achieve similar baseline conditions. This resulted in eight different strata. Block randomisation was performed for each stratum. The random allocation sequence and assignments to groups was done by an independent researcher. The assessors of physical performance and anthropometric tests were blinded to group allocations.

2.5.3. Statistical analyses

Data acquisition and data analyses were performed using commercial software with data files coded and anonymized. All statistical analyses were carried out using SPSS (IBM Corp, New York, NY, USA, version 26). One-way ANOVA (continuous variables) and Chi-square (categorical variables) tests were used to compare differences between groups at baseline. Main time and group effects as well as time*group interactions were determined using a two-way-mixed ANOVA with Bonferroni-corrected post-hoc tests. If sphericity (Mauchly's W) was violated, the Greenhouse Geisser corrected values were used. All data are expressed as mean ± standard deviation. Significance was set to $\alpha = 0.05$. Effect sizes were calculated using partial η^2 and were defined as small, moderate or large based upon 0.02, 0.13, and 0.26, respectively [51].

2.5.4. Missing data

At baseline some data are missing for dietary intake assessment as nine participants left the study already before their first 24-hour recall. Additionally, one person could not be interviewed within the first two weeks. Therefore, dietary intake data are available from 124 subjects at baseline. Furthermore, baseline body composition data are missing from five participants due to technical issues. At baseline, none of the physical performance data are missing.

Finally, 116 subjects, who participated in at least one of the physical performance tests at all three time points, were included in the per-protocol analyses. From these, nine data sets are missing for body composition (again due to technical issues) and two and three persons respectively refused some particular tests due to a certain discomfort in either the upper or the lower extremities. Actual numbers are provided with the respective tables.

3. Results

3.1. Participants' flow

A total of 632 people were interested in the study. After pre-screening of general inclusion and exclusion criteria 183 persons underwent the medical pre-examination. Finally, 136 persons met the eligibility criteria and were included in the study. These 136 participants were randomly allocated to CON (n = 47), RP + T (n = 48) and HP + T (n = 41). Two participants withdrew due to medical reasons after randomization and before t1. Therefore, 134 individuals were included in the baseline assessments and represented the final study population. In the nutritional intervention phase, from t1 to t2, eight people dropped out of the study due to medical reasons and seven due to loss of interest (CON: n = 41, 87.2%; RP + T: n = 37, 92.5%; HP + T: n = 41, 87.2%). Finally, a total of 116 (86.5%) participants (CON: n = 41, 87.2%; RP + T: n = 36, 90.0%; HP + T: n = 39, 83.0%) completed the study after 17 weeks. None of the dropouts left the study due to injury or adverse reactions to the interventions and there was no difference between the groups (X^2 (4) = 2.740, p = 0.602). Details of the participant flow are shown in (Fig. 1).

3.2. Baseline characteristics

Groups were comparable regarding age, anthropometric data, body composition, and comorbidities. At least one comorbidity was reported by 65.7% of the subjects. The main reported illnesses were adiposity (14.7%), arterial hypertension (47.0%), hyperlipidemia (8.2%), diabetes mellitus type 2 (6.0%), history of cardiac diseases (3.0%), osteoporosis (6.7%) and history of cancer (11.2%).

Total energy, protein, carbohydrate and fat intake were comparable between groups at baseline (Table 1). Protein intake was 0.85 g/kg BW/d (14 ± 4 E%) for the whole study population, 0.83 g/kg BW/d (14 ± 4 E%), for CON, 0.97 g/kg BW/d (14 ± 4 E%) for RP + T and 0.78 g/kg BW/d (13 ± 3 E%) for the HP + T group.

3.3. Macronutrient and energy intake

For protein intake, a time*group interaction was found (p < 0.001), therefore simple main effects for time and group have been calculated. Protein intake was increased by 0.18 ± 0.31 and 0.83 ± 0.33 g/kg BW/d (p = 0.003; p < 0.001) between t1 and t3 in RP + T (t1: 14 ± 4 E%, t3: 15 ± 3 E%) and HP + T (t1: 13 ± 3 E%, t3: 24 ± 4 E%), respectively, while CON remained unchanged (0.02 ± 0.38, p > 0.050; t1: 14 ± 4 E%, t3: 13 ± 3 E%). At t2 and t3 HP + T had higher protein intake levels in comparison to both, RP + T and CON (p < 0.001). At t3, also RP + T was significantly higher than CON at t3 (p = 0.004).

No time*group interaction was found for the secondary outcomes energy, carbohydrate and fat intake (p > 0.050). Energy intake increased significantly within the study period (p = 0.002) by 175 ± 551 kcal/d from t1 to t2 (p = 0.003) and by 142 ± 619 kcal/d from t1 to t3 (p = 0.046) but remained unchanged between t2 and t3. There were no differences in energy intake between groups (p = 0.273). No differences between groups or changes over time were observed for carbohydrate or fat intake (p > 0.050), (Table 3).

3.4. Physical function

Changes in physical function outcomes are reported in Table 4. Muscle strength for the lower and upper extremities as assessed by 30-s chair stand and 30-s arm curl tests did not reveal any significant group*time interactions (p > 0.050), whereas general changes over time were found for both tests (p < 0.001). Post hoc analyses

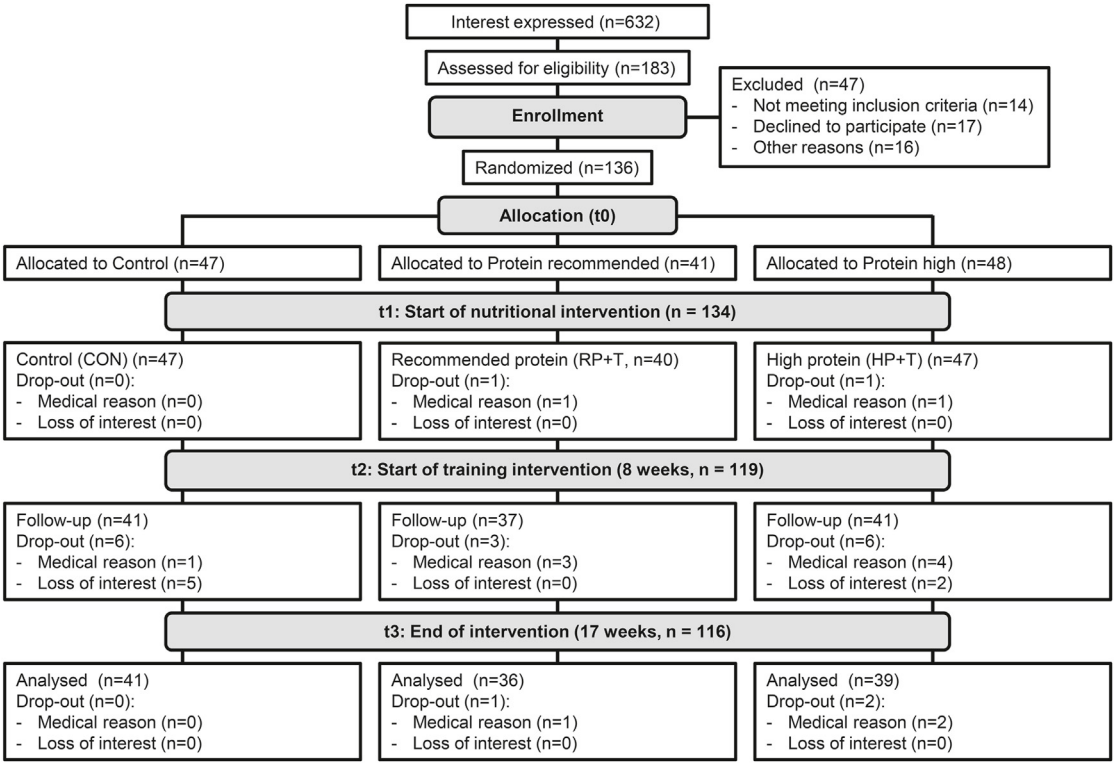

Fig. 1. Flow diagram.

Table 1
Baseline characteristics of the participants.

	Total	CON	RP + T	HP + T
Sex [f/m], (% females)]	73/63 (54.4%)	24/23 (53.2%)	21/20 (51.2%)	28/20 (58.3%)
Age [years], n = 134	72.9 ± 4.8	73.0 ± 4.9	72.4 ± 4.3	73.2 ± 5.2
Body weight [kg], n = 134	74.3 ± 13.6	74.3 ± 13.0	75.3 ± 15.5	73.4 ± 12.7
Height [m], n = 134	1.68 ± 0.10	1.69 ± 0.10	1.69 ± 0.09	1.67 ± 0.09
BMI [kg/m^2], n = 134	26.2 ± 3.9	26.0 ± 4.0	26.3 ± 4.3	26.2 ± 3.6
Body fat [kg], n = 129	18.0 ± 7.1	17.8 ± 6.8	18.1 ± 7.6	18.0 ± 7.0
Body fat [%], n = 129	24.1 ± 7.6	24.0 ± 7.9	24.1 ± 7.3	24.3 ± 7.5
Skeletal muscle mass [kg], n = 129	25.5 ± 7.1	25.8 ± 7.3	25.6 ± 7.6	25.1 ± 6.8
ASMM [kg], n = 129	20.0 ± 4.5	20.2 ± 4.5	20.2 ± 4.9	19.7 ± 4.2
Phase angle [°], n = 129	5.1 ± 0.7	5.2 ± 0.6	5.2 ± 0.8	5.1 ± 0.6
Protein intake [g/kg BW/d], n = 124	0.85 ± 0.43	0.83 ± 0.40	0.97 ± 0.55	0.78 ± 0.33
Fat intake [g/kg BW/d], n = 124	1.07 ± 0.51	1.08 ± 0.56	1.12 ± 0.45	1.02 ± 0.53
Carbohydrate intake [g/kg BW/d], n = 124	2.61 ± 1.24	2.68 ± 1.38	2.76 ± 1.09	2.43 ± 1.22
Energy intake [kcal/d], n = 124	1845 ± 714	1838 ± 726	2000 ± 693	1719 ± 710
Comorbidities, n = 134				
Adiposity (≥30 kg/m^2) [number (% of total)]	20 (14.7%)	8 (17.0%)	5 (12.5%)	7 (12.5%)
Hypertension [number (% of total)]	63 (47.0%)	23 (48.9%)	21 (52.5)	19 (40.4%)
Hyperlipidemia [number (% of total)]	11 (8.2%)	4 (8.5%)	2 (5.0%)	5 (10.6%)
Diabetes mellitus type 2 [number (% of total)]	8 (6.0%)	3 (6.4%)	2 (5.0%)	3 (6.4%)
History of cardiac diseases [number (% of total)]	4 (3.0%)	3 (6.4%)	0 (0.0%)	1 (2.1%)
Osteoporosis [number (% of total)]	9 (6.7%)	5 (10.6%)	2 (5.0%)	2 (4.3%)
History of cancer [number (% of total)]	15 (11.2%)	7 (14.9%)	3 (7.5%)	5 (10.6%)

Values are shown as mean ± standard deviation. CON (control group = observation only); RP + T (recommended protein group + resistance training); HP + T (high protein group + resistance training); BMI (body mass index); ASMM (appendicular skeletal muscle mass); BW (body weight).
In addition, no meaningful differences between groups were detected for physical performance and MQ parameters (Table 2).

Table 2
Physical function, handgrip strength and muscle quality at baseline.

	Total	CON	RP + T	HP + T
30-s chair stand [reps], n = 134	13.2 ± 3.4	13.0 ± 3.6	13.7 ± 3.0	13.1 ± 3.6
30-s arm curl [reps], n = 134	15.9 ± 3.6	15.4 ± 3.5	16.6 ± 3.9	15.9 ± 3.5
6-min walk test [m], n = 134	581 ± 85	581 ± 74	599 ± 91	567 ± 90
Self-selected gait speed [m/s], n = 134	1.5 ± 0.3	1.5 ± 0.3	1.5 ± 0.3	1.5 ± 0.2
Maximal gait speed [m/s], n = 134	2.1 ± 0.4	2.1 ± 0.3	2.3 ± 0.4	2.0 ± 0.3
Timed up and go [s], n = 134	5.9 ± 1.1	5.8 ± 1.0	5.8 ± 1.1	6.0 ± 1.2
Chair sit-and-reach [cm], n = 134	−2.1 ± 11.1	−2.8 ± 10.6	−1.2 ± 13.1	−2.2 ± 10.0
Back scratch [cm], N = 134	−8.1 ± 11.0	−7.2 ± 10.9	−8.3 ± 11.4	−8.7 ± 11.0
Handgrip strength dom [kg], n = 134	32.3 ± 9.3	32.6 ± 10.0	33.8 ± 9.6	30.8 ± 8.4
MQ_{UBS} [kg/kg], n = 129	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.2	1.2 ± 0.3
MQ_{LBP} [W/kg], n = 129	24.2 ± 5.2	23.9 ± 5.2	24.6 ± 5.0	24.1 ± 5.4

Values are shown as mean ± standard deviation. CON (control group = observation only); RP + T (recommended protein group + resistance training); HP + T (high protein group + resistance training); dom (dominant hand); MQ_{UBS} (muscle quality upper body strength); MQ_{LBP} (muscle quality lower body power).

revealed an improvement in 30-s chair stand test from t1 to t2 (0.8 ± 1.9 reps, $p < 0.001$) and from t2 to t3 (1.0 ± 2.0 reps, $p < 0.001$) and hence from t1 to t3 (1.9 ± 2.0 reps, $p < 0.001$). An increased performance was also detected for 30-s arm curl test which improved from t1 to t2 (1.4 ± 2.7 reps, $p < 0.001$) and within the overall period from t1 to t3 (1.5 ± 3.1 reps, $p < 0.001$), but not from t2 to t3 ($p > 0.050$). A main effect for groups was not found in 30-s chair stand ($p = 0.297$) but for 30-s arm curl ($p = 0.037$), however, Bonferroni-corrected post hoc analyses showed no differences between groups ($p > 0.050$).

Aerobic endurance was assessed by 6-min walk test, where neither time*group interaction nor group effects have been observed ($p > 0.050$). Again, a main effect of time was detected irrespective of group allocation ($p = 0.002$). Bonferroni-corrected post hoc analyses revealed similar walking distance from t1 to t2 ($p = 0.622$), but an improvement in phase 2 of the study from t2 to t3 (8.8 ± 34.3 m, $p = 0.023$) leading to an overall improvement from t1 to t3 (14.1 ± 43.1 m, $p = 0.002$).

No time*group interaction was detected for self-selected and maximal gait speed ($p > 0.050$). However, gait speed changed over time regardless of group assignment (self-selected: $p < 0.001$; maximal: $p = 0.016$). Bonferroni-corrected post-hoc analyses for self-selected gait speed showed no difference from t1 to t2 ($p < 0.050$), while similarly to aerobic performance an increase was found from t2 to t3 (0.1 ± 0.3 m/s, $p < 0.001$) and hence also from t1 to t3 (0.1 ± 0.4 m/s, $p = 0.007$). Also, maximal gait speed did not differ between t1 and t2 ($p > 0.050$) but increased in the training phase from t2 to t3 (0.1 ± 0.2 m/s, $p = 0.026$). However, no difference was detected from t1 to t3 ($p > 0.050$).

No interaction, time or group effect was found for handgrip strength, flexibility or mobility measured by sit-and-reach, back scratch and timed up and go tests ($p > 0.050$).

3.5. Muscle quality

Neither in the upper nor in the lower extremity a time*group interaction or group effect was detected for MQ as assessed by dividing strength or power by SM ($p > 0.050$). However, MQ_{LBP} changed over time, irrespective of group allocation ($p < 0.001$), while MQ_{UBS} remained unchanged ($p = 0.500$). Bonferroni-corrected post hoc analyses for MQ_{LBP} revealed that there was a significant increase for all groups from t1 to t2 (1.5 ± 2.7 W/kg, $p < 0.001$) and from t2 to t3 (1.2 ± 2.6 W/kg, $p < 0.001$), as well as from t1 to t3 (2.6 ± 3.2 W/kg, $p < 0.001$) (Table 4).

3.6. Anthropometry and body composition

Changes in anthropometry and body composition parameters are shown in Table 5. For body weight and BMI a time*group interaction was detected (body weight: $p = 0.001$; BMI: $p = 0.002$). While body weight did not change over time in CON and HP + T

Table 3
Intervention effects on macronutrient and energy intake.

Parameter	Group	Mean (95% confidence interval)			Time p-value (partial η^2)	Group p-value (partial η^2)	Time x group p-value (partial η^2)	Phase differences	
		Baseline (t1)	8 weeks (t2)	17 weeks (t3)				Δ (t2 − t1)	Δ (t3 − t2)
Protein [g/kg BW/d], n = 115	CON	0.83 ± 0.40	0.91 ± 0.40	0.85 ± 0.26	< **0.001** (0.426)	< **0.001** (0.347)	< **0.001** (0.409)	0.08 ± 0.32	−0.05 ± 0.30
	RP + T	0.89 ± 0.28	1.09 ± 0.33**	1.06 ± 0.26**				0.20 ± 0.35	−0.02 ± 0.27$^{\#}$
	HP + T	0.80 ± 0.32	1.54 ± 0.36***	1.63 ± 0.37***				0.74 ± 0.36	0.09 ± 0.31$^{\#\#\#}$
Carbohydrates [g/kg BW/d], n = 115	CON	2.68 ± 1.38	2.70 ± 1.09	2.82 ± 1.06	0.740 (0.002)	0.338 (0.019)	0.664 (0.010)	0.02 ± 0.88	0.11 ± 0.75
	RP + T	2.71 ± 1.12	2.84 ± 0.96	2.74 ± 0.76				0.14 ± 0.93	−0.10 ± 0.69
	HP + T	2.50 ± 1.21	2.52 ± 0.88	2.41 ± 0.89				0.02 ± 0.90	−0.11 ± 0.61
Fat [g/kg BW/d], n = 115	CON	1.08 ± 0.56	1.18 ± 0.44	1.06 ± 0.39	0.138 (0.018)	0.901 (0.002)	0.878 (0.004)	0.10 ± 0.47	−0.12 ± 0.38$^{\#}$
	RP + T	1.08 ± 0.40	1.13 ± 0.37	1.05 ± 0.34				0.05 ± 0.38	−0.07 ± 0.32
	HP + T	1.04 ± 0.53	1.11 ± 0.38	1.07 ± 0.35				0.07 ± 0.47	−0.03 ± 0.26
Energy intake [kcal/d], n = 115	CON	1838 ± 726	1961 ± 578	1898 ± 528	**0.002** (0.057)	0.334 (0.019)	0.693 (0.009)	123.3 ± 559.1	−63.2 ± 500.8
	RP + T	1962 ± 691	2115 ± 639	2107 ± 566				153.1 ± 582.2	−7.9 ± 440.4
	HP + T	1752 ± 704	2002 ± 510*	1975 ± 498*				249.4 ± 517.6	−26.8 ± 368.1$^{\#}$

Values are shown as mean ± standard deviation. p-values refer to main effects of time and group as well as time*group interactions (two-way mixed ANOVA). Significant effects ($p < 0.05$) are shown in bold. Effect size is given as partial η^2, whereby 0.02, 0.13, and 0.26 represent small, moderate, or large effects. CON (control group = observation only); RP + T (recommended protein group + resistance training); HP + T (high protein group + resistance training). In case of significant overall time effects, Bonferroni-corrected post hoc analyses were performed individually for groups, whereby asterisks indicate significant differences to t1. Additionally, significant differences between phase 1 and phase 2 as calculated by paired t-tests are marked by hashes. $^{***,\#\#\#}$($p < 0.001$); **($p < 0.01$); $^{*,\#}$($p < 0.05$).

($p > 0.050$), an increase of body weight was observed for RP + T during the training phase from t2 to t3 (+0.92 ± 1.35 kg, $p < 0.001$) resulting in an overall increase during the study period from t1 to t3 (+1.29 ± 1.31 kg, $p < 0.001$). For RP + T this gain in body weight was paralleled by an increase in BMI from t2 to t3 (+0.26 ± 0.43 kg/m^2, $p < 0.001$) and hence from t1 to t3 (+0.38 ± 0.46 kg/m^2, $p = 0.002$). BMI slightly increased also in CON from t1 to t2 (+0.2 ± 0.51 kg/m^2, $p = 0.047$), but not in HP + T ($p > 0.050$). There was no overall difference in body weight and BMI between groups at any of the time points ($p > 0.050$) (Table 4).

Table 4
Intervention effects on physical function, handgrip strength and muscle quality parameters.

Parameter	Group	Mean (95% confidence interval)			Time p-value (partial η^2)	group p-value (partial η^2)	time x group p-value (partial η^2)	Phase differences	
		Baseline (t1)	8 weeks (t2)	17 weeks (t3)				Δ (t2 − t1)	Δ (t3 − t2)
30-s chair stand [reps], n = 113	CON	12.6 ± 3.6	13.7 ± 3.8***	14.2 ± 3.6$^{***,\circ}$	< **0.001** (0.295)	0.489 (0.013)	0.410 (0.018)	1.05 ± 1.63	0.55 ± 1.32
	RP + T	13.6 ± 3.2	14.2 ± 3.7	15.6 ± 4.4$^{***,\circ\circ}$				0.53 ± 1.94	1.44 ± 1.65$^{\#}$
	HP + T	13.2 ± 3.8	14.2 ± 4.3*	15.3 ± 4.1$^{***,\circ}$				0.95 2.13	1.13 ± 2.60
30-s arm curl [reps], n = 113	CON	14.9 ± 3.5	16.3 ± 3.3*	16.5 ± 3.6**	< **0.001** (0.151)	**0.037** (0.058)	0.840 (0.006)	1.35 ± 2.80	0.23 ± 2.39
	RP + T	16.6 ± 4.0	18.1 ± 4.5*	17.7 ± 3.7				1.41 ± 2.87	−0.32 ± 3.14
	HP + T	16.5 ± 3.2	18.0 ± 3.2**	18.3 ± 3.5**				1.49 ± 2.61	0.28 ± 2.44
6-min walk test [m], n = 113	CON	580 ± 76	570 ± 80	583 ± 77°	**0.002** (0.059)	0.157 (0.033)	0.063 (0.041)	−10.1 ± 44.6	12.9 ± 31.4$^{\#}$
	RP + T	600 ± 97	617 ± 90	622 ± 96**				16.2 ± 50.2	5.0 ± 38.4
	HP + T	582 ± 81	592 ± 66	601 ± 65*				9.5 ± 35.8	8.6 ± 33.8
Self-selected gait speed [m/s], n = 113	CON	1.5 ± 0.3	1.5 ± 0.3	1.6 ± 0.3°	< **0.001** (0.087)	0.843 (0.003)	0.633 (0.011)	−0.02 ± 0.30	0.09 ± 0.24
	RP + T	1.5 ± 0.3	1.5 ± 0.2	1.6 ± 0.4				0.02 ± 0.35	0.12 ± 0.34
	HP + T	1.5 ± 0.2	1.4 ± 0.2	1.6 ± 0.3$^{\circ\circ}$				−0.07 ± 0.26	0.17 ± 0.25$^{\#\#}$
Maximal gait speed [m/s], n = 113	CON	2.1 ± 0.3	2.1 ± 0.3	2.1 ± 0.4	**0.016** (0.037)	0.070 (0.047)	0.487 (0.015)	−0.02 ± 0.27	0.07 ± 0.22
	RP + T	2.3 ± 0.5	2.2 ± 0.4	2.3 ± 0.4				−0.09 ± 0.28	0.05 ± 0.23
	HP + T	2.1 ± 0.3	2.1 ± 0.3	2.1 ± 0.4				−0.02 ± 0.16	0.08 ± 0.21$^{\#}$
Timed up and go [s], n = 112	CON	5.8 ± 1.0	5.7 ± 1.0	5.7 ± 1.0	0.061 (0.025)	0.996 (0.000)	0.927 (0.004)	−0.10 ± 0.66	−0.01 ± 0.59
	RP + T	5.8 ± 1.1	5.6 ± 1.2	5.7 ± 1.3				−0.17 ± 0.59	0.11 ± 0.52
	HP + T	5.8 ± 0.8	5.6 ± 0.8	5.6 ± 0.8				−0.14 ± 0.73	0.01 ± 0.69
Chair sit-and-reach [cm], n = 114	CON	−2.3 ± 9.6	−2.0 ± 10.2	−2.2 ± 10.6	0.118 (0.019)	0.887 (0.002)	0.635 (0.011)	0.29 ± 3.66	−0.24 ± 5.20
	RP + T	−1.7 ± 12.7	−1.4 ± 12.8	−0.3 ± 12.1				0.24 ± 5.34	1.11 ± 5.36
	HP + T	−1.8 ± 9.2	−1.2 ± 9.1	−0.5 ± 9.2				0.65 ± 4.12	0.67 ± 4.76
Back scratch [cm], n = 114	CON	−7.4 ± 10.5	−7.4 ± 11.1	−7.8 ± 11.2	0.073 (0.023)	0.806 (0.004)	0.266 (0.023)	−0.04 ± 3.21	−0.40 ± 3.21
	RP + T	−8.6 ± 11.9	−8.1 ± 12.4	−9.5 ± 12.7				0.53 ± 5.00	−1.37 ± 4.31
	HP + T	−10.0 ± 11.0	−8.4 ± 10.8*	−9.1 ± 10.5				0.92 ± 6.38	−0.71 ± 3.53
Dom handgrip strength [kg], n = 114	CON	31.9 ± 9.6	31.8 ± 10.3	31.9 ± 10.4	0.675 (0.004)	0.329 (0.020)	0.704 (0.010)	−0.10 ± 3.37	0.13 ± 3.29
	RP + T	34.4 ± 9.2	34.5 ± 8.5	33.8 ± 8.5				0.09 ± 2.93	−0.66 ± 2.87
	HP + T	31.4 ± 8.9	30.9 ± 9.4	31.2 ± 9.6				−0.46 ± 2.49	0.26 ± 3.23
MQ_{UBS} [kg/kg], n = 107	CON	1.3 ± 0.3	1.3 ± 0.3	1.3 ± 0.3	0.500 (0.007)	0.350 (0.020)	0.637 (0.012)	0.01 ± 0.16	0.02 ± 0.17
	RP + T	1.3 ± 0.2	1.3 ± 0.2	1.3 ± 0.2				0.03 ± 0.15	−0.04 ± 0.15
	HP + T	1.2 ± 0.3	1.2 ± 0.3	1.2 ± 0.2				0.01 ± 0.14	−0.02 ± 0.16
MQ_{LBP} [W/kg], n = 106	CON	23.7 ± 5.4	25.6 ± 5.8***	26.5 ± 5.6$^{***,\circ}$	< **0.001** (0.303)	0.517 (0.013)	0.268 (0.025)	1.92 ± 2.51	0.85 ± 2.04
	RP + T	24.8 ± 4.7	25.7 ± 4.6	27.7 ± 5.3$^{***,\circ\circ}$				0.83 ± 2.12	2.03 ± 2.10$^{\#}$
	HP + T	23.4 ± 5.1	25.0 ± 5.0*	25.7 ± 4.8**				1.62 ± 3.24	0.65 ± 3.28

Values are shown as mean ± standard deviation. p-values refer to main effects of time and group as well as time*group interactions (two-way mixed ANOVA). Significant effects ($p < 0.05$) are shown in bold. Effect size is given as partial η^2, whereby 0.02, 0.13, and 0.26 represent small, moderate, or large effects. CON (control group = observation only); RP + T (recommended protein group + resistance training); HP + T (high protein group + resistance training). MQ_{UBS} (muscle quality upper body strength); MQ_{LBP} (muscle quality lower body power) In case of significant overall time effects, Bonferroni-corrected post hoc analyses were performed individually for groups, whereby asterisks indicate significant differences to t1 and circles to t2. Additionally, significant differences between phase 1 and phase 2 as calculated by paired t-tests are marked by hashes. $^{***,\circ\circ}$ ($p < 0.001$); $^{**,\circ,\#\#}$ ($p < 0.01$); $^{*,\circ,\#}$ ($p < 0.05$).

Table 5
Intervention effects on anthropometry and body composition parameters.

Parameter	Group	Mean (95% confidence interval)			time p-value (partial η^2)	group p-value (partial η^2)	time x group p-value (partial η^2)	Phase differences	
		Baseline (t1)	8 weeks (t2)	17 weeks (t3)				Δ (t2 − t1)	Δ (t3 − t2)
Body weight [kg], n = 116	CON	73.7 ± 12.4	74.2 ± 12.4	74.2 ± 12.2	< **0.001** (0.110)	0.598 (0.009)	**0.001** (0.078)	0.47 ± 1.33	0.03 ± 1.07
	RP + T	75.9 ± 15.6	76.2 ± 15.3	77.2 ± 15.9$^{***,\circ}$				0.37 ± 1.24	0.92 ± 1.35
	HP + T	73.3 ± 13.4	73.2 ± 13.1	73.5 ± 13.1				0.15 ± 1.82	0.24 ± 1.29
BMI [kg/m^2], n = 116	CON	26.0 ± 3.9	26.2 ± 3.9*	26.2 ± 3.8	**0.001** (0.060)	0.764 (0.005)	**0.002** (0.077)	0.20 ± 0.51	−0.02 ± 0.50
	RP + T	26.4 ± 4.3	26.5 ± 4.2	26.7 ± 4.4$^{***,\circ}$				0.12 ± 0.41	0.26 ± 0.43
	HP + T	25.9 ± 3.6	25.8 ± 3.6	25.9 ± 3.6				0.03 ± 0.73	0.03 ± 0.49
Body fat [%], n = 109	CON	24.8 ± 7.7	25.7 ± 7.5*	26.1 ± 7.5**	< **0.001** (0.057)	0.476 (0.014)	0.592 (0.013)	1.00 ± 2.47	0.37 ± 2.25
	RP + T	24.7 ± 7.2	25.4 ± 6.4	25.8 ± 7.5				0.72 ± 2.51	0.35 ± 2.73
	HP + T	22.9 ± 7.2	24.3 ± 7.9	23.7 ± 7.9				1.37 ± 4.29	−0.61 ± 2.79
Body fat [kg], n = 109	CON	18.3 ± 6.8	19.2 ± 6.8*	19.4 ± 6.7*	< **0.001** (0.094)	0.495 (0.013)	0.379 (0.020)	0.86 ± 2.06	0.24 ± 1.74
	RP + T	18.7 ± 7.7	19.3 ± 7.1	19.9 ± 8.1*				0.58 ± 1.83	0.59 ± 2.21
	HP + T	17.0 ± 6.7	18.0 ± 7.1	17.5 ± 6.9				1.02 ± 2.85	−0.47 ± 2.12$^{\#}$
SM [kg], n = 109	CON	25.0 ± 6.8	24.6 ± 6.6	24.4 ± 6.7*	0.018 (0.039)	0.829 (0.004)	0.333 (0.021)	−0.45 ± 1.60	−0.13 ± 1.22
	RP + T	25.6 ± 7.7	25.4 ± 7.5	25.6 ± 7.8				−0.20 ± 1.65	0.22 ± 1.59
	HP + T	25.9 ± 7.1	25.1 ± 7.0	25.6 ± 7.4				−0.80 ± 2.27	0.51 ± 1.57$^{\#}$
ASMM [kg], n = 109	CON	19.8 ± 4.2	19.6 ± 4.1	19.5 ± 4.1*	0.124 (0.020)	0.826 (0.004)	0.217 (0.027)	−0.20 ± 0.87	−0.08 ± 0.58
	RP + T	20.2 ± 5.0	20.2 ± 5.0	20.5 ± 5.1				−0.06 ± 1.03	0.27 ± 0.84
	HP + T	20.1 ± 4.5	19.8 ± 4.4	20.0 ± 4.6				−0.28 ± 1.16	0.23 ± 0.78
Phase angle [°], n = 109	CON	5.2 ± 0.6	5.1 ± 0.6	5.1 ± 0.6	0.716 (0.003)	0.730 (0.006)	0.311 (0.022)	−0.03 ± 0.39	−0.06 ± 0.34
	RP + T	5.2 ± 0.8	5.2 ± 0.8	5.3 ± 0.9				0.01 ± 0.67	0.11 ± 0.41
	HP + T	5.1 ± 0.6	5.2 ± 0.7	5.1 ± 0.7				0.12 ± 0.62	−0.06 ± 0.34

Values are shown as mean ± standard deviation. p-values refer to main effects of time and group as well as time*group interactions (two-way mixed ANOVA). Significant effects ($p < 0.05$) are shown in bold. Effect size is given as partial η2, whereby 0.02, 0.13, and 0.26 represent small, moderate, or large effects. CON (control group = observation only); RP + T (recommended protein group + resistance training); HP + T (high protein group + resistance training). SM (skeletal muscle mass); ASMM (appendicular skeletal muscle mass). In case of significant overall time effects, Bonferroni-corrected post hoc analyses were performed individually for groups, whereby asterisks indicate significant differences to t1 and circles to t2. Additionally, significant differences between phase 1 and phase 2 as calculated by paired t-tests are marked by hashes. *** ($p < 0.001$); $^{**,\circ}$ ($p < 0.01$); $^{*,\#}$ ($p < 0.05$).

Body fat and SM revealed no time*group interaction or group effect ($p > 0.050$). However, body fat and SM changed over time, irrespective of group allocation ($p < 0.050$). Bonferroni-corrected post hoc analyses for body fat percentage and mass revealed that there was a significant increase from t1 to t2 (1.0 ± 3.2%, $p = 0.004$; 0.8 ± 2.3 kg, $p = 0.001$), but not from t2 to t3 ($p > 0.050$). Over the duration of the study (t1 to t3), body fat increased (1.1 ± 3.2%, $p = 0.003$; 0.9 ± 2.5 kg, $p < 0.001$). Post hoc analyses for SM showed a decrease from t1 to t2 (−0.5 ± 1.9 kg, $p = 0.025$) but not for the other time points. Phase angle and appendicular muscle mass did not reveal any group*time interactions, main effects of group, nor was there a main effect of time ($p > 0.050$).

Interestingly, when comparing changes during the nutritional phase (Δ (t2 − t1)) to those elicited during the training phase (Δ (t3 − t2)), it seems to be noteworthy that the initial increases in body fat and decreases in SM could be reversed only in the HP + T which showed a significant difference to phase 1 with body fat mass being decreased (−0.47 ± 2.12 kg, $p = 0.041$) while SM being increased (+0.51 ± 1.57 kg, $p = 0.021$).

3.7. Linear regression

In order to assess whether a higher protein intake in the training phase would be associated with changes in strength, muscle mass or MQ, differences between t2 and t3 in these parameters were correlated to protein intake in phase 2 (only for participants allocated to RP + T or HP + T). Protein intake amounts were not associated with changes in 30-s chair stand test ($r = 0.036$, $p = 0.760$), 30-s arm curl test ($r = 0.182$, $p = 0.123$), muscle mass ($r = -0.002$, $p = 0.986$), MQ_{UBS} ($r = 0.165$, $p = 0.166$), MQ_{LBP} ($r = -0.036$, $p = 0.769$). However, a weak but positive association between protein intake and changes in handgrip strength from t2 to t3 was observed ($r = 0.245$, $p = 0.035$). Irrespective of group allocation, a linear regression established that daily protein intake significantly predicts an increase in relative handgrip strength: y (change in handgrip strength) = 1.74 * x (protein intake) - 2.54. Thereby, the daily amount of protein intake accounted for 6.8% of the declared variability in handgrip strength changes (Fig. 2).

4. Discussion

The main aim of our study was to investigate whether increasing the protein intake to or above the recommended level would ameliorate the muscular response to resistance training in older adults. We were able to reach the recommended daily level of 1.0 g protein per kg BW in RP + T, whereas the high protein group doubled their baseline intake levels to 1.6 g protein per kg BW. However, protein intake did not modulate the response to resistance training with respect to physical performance parameters, but showed a potential impact on body composition.

In many of the previously conducted randomized controlled studies, an increase in protein intake was achieved by administering protein supplements [52–55]. This is in contrast to our study, as we decided to provide the extra protein mainly through regular food, which turned out to provide a big challenge compared to supplementation with respect to protein intake calculation, macronutrient distribution and energy intake calculation, particularly for subjects with a higher body weight [56]. Nevertheless, all subjects from HP + T without exception increased their individual protein intakes, whereby the study target level of 2 g/kg BW/d was reached by eight individuals (20.5%). Within 17 weeks, the average protein intake in the HP + T doubled and energy provided via protein accounted for 24 E% being well above the general recommendations up to 15 E% [20]. The difficulty to reach a certain target amount by regular food has also been shown in another study with healthy older men and women (61 ± 1 yrs), where a protein intake of 1.6 g/kg BW/d in the high protein group was intended, but after 10 weeks, protein intake could only be increased from initially 1.1 to 1.2 g/kg BW/d [34]. A meta-analysis revealed that the amount of 1.6 g/kg BW/d, which was achieved in the current study might be

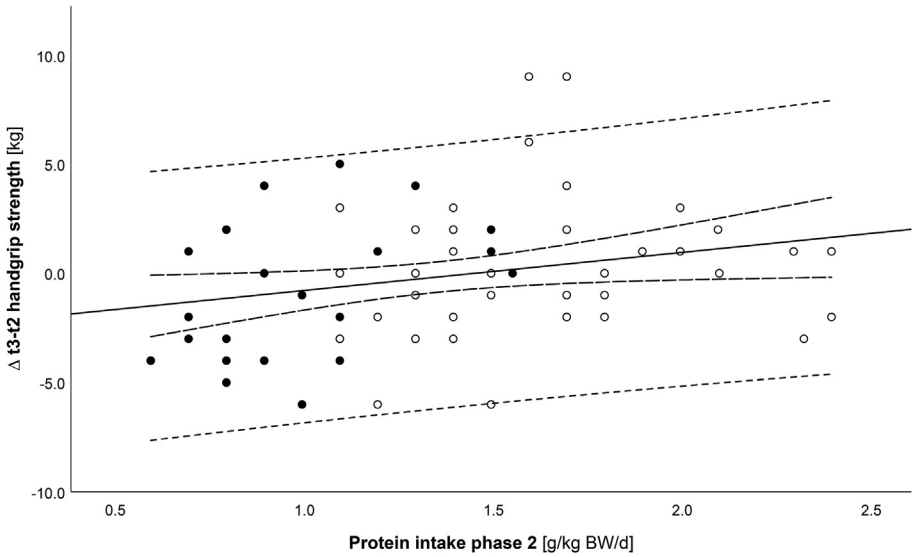

Fig. 2. Association between protein intake and change in handgrip strength.
Different symbols represent the assignment to the intervention groups (● black dot, recommended protein group + resistance training (RP + T); ○ white dot, high protein group + resistance training (HP + T)). Solid line, linear regression line; bold dashed line, prediction intervals; dashed line, lower and upper limits of the 95% confidence interval.

optimal to increase strength and lean mass, whereas higher doses would not exert additional effects [12]. However, a regression analyses performed independently of group allocation in our study revealed a weak but positive association between protein intake and changes in handgrip strength during the training phase, whereby no levelling off at 1.6 g/kg BW/d was observed. Therefore, future studies might be needed to confirm whether an increase above this level would be associated with further benefits on physical performance.

It is noteworthy that the additional offer of high-protein (HP + T) or isocaloric, high-carbohydrate (RP + T) foods initially led to an increase in energy intake especially in phase 1, which was most pronounced in the HP + T group. This observation is paralleled by changes in body composition in all groups. However, only the high-protein group was able to reverse the trend by decreasing body fat and increasing muscle mass in phase 2 of the study. As training load was equal in RP + T and HP + T, the additional amount of protein could be the reason for this observation which might be used more efficiently for muscle protein synthesis. An adequate intake of protein is important to maintain skeletal muscle mass and quality with aging and compensate for reduced muscle protein synthesis [13,57,58]. For optimising muscle protein synthesis, it is recommended to consume 20–40 g protein per meal [16], which is however not easy to achieve in an old population due to age-associated changes in appetite, difficulties in chewing fibrous foods, use of medication and polypharmacy, noticed food intolerances, the elevated cost of more nutrient-dense foods, and fear of eating too much fat and cholesterol [59]. In order to facilitate protein intake in close proximity to strength training, additional protein drinks containing 32 g protein were provided twice a week directly after the training session. This amount accounts only for about 8% of the total protein intake per week in the HP + T group, we only can hypothesize whether this has exerted a special stimulus on muscle protein synthesis and hence body composition. Although the ingestion of whole foods, which contain a food matrix rich in dietary protein, vitamins, minerals, and other macronutrients may provide improvements in overall diet quality and have a direct influence on changes of post-exercise muscle protein synthesis rates, obtaining dietary protein exclusively from whole foods may not always be convenient with respect to availability and food volume directly after the exercise session [60]. Furthermore, a comparison of different dietary protein sources such as skim milk, soy milk, beefsteak, boiled egg, and a liquid meal supplement revealed that area-under-the-curve values for total plasma amino acids were similar between the different protein sources, but liquid forms of protein achieved peak concentrations twice as quickly after ingestion as solid protein-rich foods [61].

Strength training was performed only in phase 2 and only for RP + T and HP + T. Nevertheless, many of the physical performance parameters such as 30-s chair stand, 30-s arm curl, 6-min walk test, self-selected and maximal gait speed, as well as in MQ_{LBP} increased over time. While a high test-retest intraclass correlation was confirmed for all the applied tests for physical function in older people [1,43,44], a learning effect was reported for repeated measurements [62]. To counteract the learning effect, we asked the participants to perform all tests with the exception of the 6-min walk test before the start of the measurement to familiarize themselves with the correct exercise sequence in order to make a possible familiarisation effect as small as possible. Despite the general amelioration of functional performance, a steeper increase in the second study phase was observed from t2 to t3 for 30-s chair stand test, gait speed, and especially for muscle quality of the lower extremities hinting to the fact that the applied resistance training was able to overcome a potential learning effect in these parameters. Special care was taken to match the physical performance tests and the movement patterns of the resistance training exercises. In this respect it has been shown that chair stand performance correlates well with leg-press performance for both men and women [43]. Although we used a very comprehensive test battery for the functional tests, we cannot exclude the possibility that functional changes may have occurred in other areas that we were unable to detect.

With respect to efficacy of the training programme, it seems that the applied strength training was more effective to target lower body performance than upper body performance. It is already known that ageing affects power and strength of the lower body to a higher extent than those of the upper body [63], but it is unclear whether this could account for differences in trainability between upper and lower body [64]. It has been suggested that muscles being elicited more frequently would have

a smaller potential to gain strength at older age [65]. With respect to training progression, we have used the RPE method which appear equally effective to other methods for increasing training loads, such as using percentages of the one repetition maximum, a target number of repetitions, or the repetitions in reserve. Furthermore, the RPE method is considered optimal for older adults as it is likely to be perceived as the most tolerable one [66]. In the current study progression of volume load was achieved by 143.9 ± 148.3% for leg press and 82.7 ± 73.7% for chest press which could explain both the potential differences between upper and lower body as well as the somewhat lower improvements in physical performance in comparison to other studies [67]. However, compliance levels of the participants was very high as estimated from training session attendance (RP + T = 89.6 ± 8.6%; HP + T = 90.7 ± 12.7%). Although this might have led to a somewhat lower absolute load it has been shown that hypertrophic responses can be elicited with both low-loads and high-loads, yet only when maximal effort is achieved [68–70]. Furthermore, intensity plays an important role in the effectiveness and safety of resistance training programmes, especially in older adults. A previous report suggested that moderate resistance training may be related to not only to higher acceptance and compliance, but also to lower injury rates in older adults [71]. As no injury in response to the training programme was reported in our study, we consider this programme to be safe which could be continued for even longer periods similar to other studies applying resistance training for 11 weeks or more [15,31,52,72].

The randomized controlled study design, the close nutritional support for each participant which allowed the modulation of overall protein intake in a field setting, together with a supervised strength training programme based on ACSM guidelines represent the main strength of our study. Nevertheless, some limitations such as the use of BIA for the assessment of body composition need to be addressed as well. We believe that in the context of our study BIA is a valid tool to assess changes in muscle mass and body fat as we and others have observed strong correlations to DEXA with respect to fat and lean mass [73,74]. However, we need to keep in mind that different methods differ not only in accuracy or precision but also in measuring different compartments making comparisons difficult [75]. Although the study was conducted in a very controlled environment, we were not able to completely assess every lifestyle aspect of the participants leaving the possibility of residual confounding.

5. Conclusion

In conclusion the current study clearly indicates that a substantial increase of habitual protein intake above the currently recommended levels is achievable within 17 weeks in community-dwelling older adults, at least when nutritional guidance of the participants is provided. Although the extra amount of protein did not modulate the response to resistance training, minor changes in body composition towards a higher skeletal muscle mass and lower body fat mass have been observed especially during the training phase which might depend upon higher protein availability. However, there is still a need to find the optimal combination of protein intake and training variables to efficiently support muscle function of older adults.

Funding

This work was supported by the University of Vienna, by funding the Research Platform Active Ageing, and the EU-program Interreg SK-AT (NutriAging). This article was supported by the Open Access Publishing Fund of the University of Vienna.

Author contributions

Sandra Unterberger: Data curation, Formal analysis, Investigation, Software, Visualization, Roles/Writing - original draft **Rudolf Aschauer:** Data curation, Formal analysis, Investigation, Visualization **Patrick A. Zöhrer:** Data curation, Investigation **Agnes Draxler:** Investigation **Bernhard Franzke:** Conceptualization, Investigation, Methodology, Writing - review & editing **Eva-Maria Strasser:** Conceptualization, Methodology, Writing - review & editing **Karl-Heinz Wagner:** Conceptualization, Funding acquisition, Methodology, Project administration, Resources, Supervision, Writing - review & editing **Barbara Wessner:** Conceptualization, Formal analysis, Funding acquisition, Resources, Supervision, Validation, Visualization, Writing - review & editing.

Conflict of interest

The authors declare no conflict of interest. The acknowledged providers of nutritional products as well as the funding bodies had no role in the design of the study; in the collection, analyses, or interpretation of data; in the writing of the manuscript, or in the decision to publish the results.

Acknowledgements

The authors want to thank all participants and students who supported this project as well as Holmes Place Wien GmbH and Stars Fitness GmbH for providing their training locations. We further thank the companies Chiefs AG, Rudolf Ölz Meisterbäcker GmbH & Co KG, Neoh (Alpha Republic GmbH), AnovonA Medsupps GmbH, NÖM AG, Gittis Naturprodukte GmbH, GoVital (Findus Sverige AB), Nomad Foods Europe, Fleischwaren Berger GmbH & Co KG and Handl Tyrol GmbH for providing us with the protein-rich and standard food products.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.clnu.2022.02.017.

References

[1] Steffen TM, Hacker TA, Mollinger L. Age- and gender-related test performance in community-dwelling elderly people: six-minute walk test, berg balance scale, timed up & go test, and gait speeds. Phys Ther 2002;82:128–37. https://doi.org/10.1093/ptj/82.2.128.
[2] Beard JR, Officer A, de Carvalho IA, Sadana R, Pot AM, Michel JP, et al. The World report on ageing and health: a policy framework for healthy ageing. Lancet 2016;387:2145–54. https://doi.org/10.1016/S0140-6736(15)00516-4.
[3] Goodpaster BH, Park SW, Harris TB, Kritchevsky SB, Nevitt M, Schwartz AV, et al. The loss of skeletal muscle strength, mass, and quality in older adults: the health, aging and body composition study. J Gerontol A Biol Sci Med Sci 2006;61:1059–64. https://doi.org/10.1093/gerona/61.10.1059.
[4] Koster A, Ding J, Stenholm S, Caserotti P, Houston DK, Nicklas BJ, et al. Does the amount of fat mass predict age-related loss of lean mass, muscle strength, and muscle quality in older adults? J Gerontol A Biol Sci Med Sci 2011;66:888–95. https://doi.org/10.1093/gerona/glr070.
[5] Suetta C, Haddock B, Alcazar J, Noerst T, Hansen OM, Ludvig H, et al. The Copenhagen Sarcopenia Study: lean mass, strength, power, and physical function in a Danish cohort aged 20-93 years. J Cachexia Sarcopenia Muscle 2019;10:1316–29. https://doi.org/10.1002/jcsm.12477.
[6] McGregor RA, Cameron-Smith D, Poppitt SD. It is not just muscle mass: a review of muscle quality, composition and metabolism during ageing as determinants of muscle function and mobility in later life. Longev Heal 2014;3:9. https://doi.org/10.1186/2046-2395-3-9.
[7] Romero-Arenas S, Blazevich AJ, Martinez-Pascual M, Perez-Gomez J, Luque AJ, Lopez-Roman FJ, et al. Effects of high-resistance circuit training in an elderly population. Exp Gerontol 2013;48:334–40. https://doi.org/10.1016/j.exger.2013.01.007.
[8] Van Roie E, Delecluse C, Coudyzer W, Boonen S, Bautmans I. Strength training at high versus low external resistance in older adults: effects on muscle

volume, muscle strength, and force-velocity characteristics. Exp Gerontol 2013;48:1351–61. https://doi.org/10.1016/j.exger.2013.08.010.
[9] Breen L, Phillips SM. Interactions between exercise and nutrition to prevent muscle waste during ageing. Br J Clin Pharmacol 2013;75:708–15. https://doi.org/10.1111/j.1365-2125.2012.04456.x.
[10] Franzke B, Neubauer O, Cameron-Smith D, Wagner KH. Dietary protein, muscle and physical function in the very old. Nutrients 2018;10. https://doi.org/10.3390/nu10070935.
[11] Shin H, Panton LB, Dutton GR, Ilich JZ. Relationship of physical performance with body composition and bone mineral density in individuals over 60 Years of age: a systematic review. J Aging Res 2011;2011:191896. https://doi.org/10.4061/2011/191896.
[12] Morton RW, Murphy KT, McKellar SR, Schoenfeld BJ, Henselmans M, Helms E, et al. A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults. Br J Sports Med 2018;52:376–84. https://doi.org/10.1136/bjsports-2017-097608.
[13] Moore DR, Churchward-Venne TA, Witard O, Breen L, Burd NA, Tipton KD, et al. Protein ingestion to stimulate myofibrillar protein synthesis requires greater relative protein intakes in healthy older versus younger men. J Gerontol A Biol Sci Med Sci 2015;70:57–62. https://doi.org/10.1093/gerona/glu103.
[14] Pennings B, Boirie Y, Senden JM, Gijsen AP, Kuipers H, van Loon LJ. Whey protein stimulates postprandial muscle protein accretion more effectively than do casein and casein hydrolysate in older men. Am J Clin Nutr 2011;93:997–1005. https://doi.org/10.3945/ajcn.110.008102.
[15] Hamarsland H, Handegard V, Kashagen M, Benestad HB, Raastad T. No difference between spray dried milk and native whey supplementation with strength training. Med Sci Sports Exerc 2019;51:75–83. https://doi.org/10.1249/MSS.0000000000001758.
[16] Jager R, Kerksick CM, Campbell BI, Cribb PJ, Wells SD, Skwiat TM, et al. International society of sports nutrition position stand: protein and exercise. J Int Soc Sports Nutr 2017;14:20. https://doi.org/10.1186/s12970-017-0177-8.
[17] Andersen LL, Tufekovic G, Zebis MK, Crameri RM, Verlaan G, Kjaer M, et al. The effect of resistance training combined with timed ingestion of protein on muscle fiber size and muscle strength. Metabolism 2005;54:151–6. https://doi.org/10.1016/j.metabol.2004.07.012.
[18] Bauer J, Biolo G, Cederholm T, Cesari M, Cruz-Jentoft AJ, Morley JE, et al. Evidence-based recommendations for optimal dietary protein intake in older people: a position paper from the PROT-AGE Study Group. J Am Med Dir Assoc 2013;14:542–59. https://doi.org/10.1016/j.jamda.2013.05.021.
[19] Deutz NE, Bauer JM, Barazzoni R, Biolo G, Boirie Y, Bosy-Westphal A, et al. Protein intake and exercise for optimal muscle function with aging: recommendations from the ESPEN Expert Group. Clin Nutr 2014;33:929–36. https://doi.org/10.1016/j.clnu.2014.04.007.
[20] DGE, ÖGE, SGE. *D-A-C-H-Referenzwerte für die Nährstoffzufuhr* 2. 2019. Bonn.
[21] Trumbo P, Schlicker S, Yates AA, Poos M, Food, Nutrition Board of the Institute of Medicine, T.N.A. Dietary reference intakes for energy, carbohydrate, fiber, fat, fatty acids, cholesterol, protein and amino acids. J Am Diet Assoc 2002;102:1621–30. https://doi.org/10.1016/s0002-8223(02)90346-9.
[22] Gaytan-Gonzalez A, Ocampo-Alfaro MJ, Torres-Naranjo F, Gonzalez-Mendoza RG, Gil-Barreiro M, Arroniz-Rivera M, et al. Dietary protein intake patterns and inadequate protein intake in older adults from four countries. Nutrients 2020;12. https://doi.org/10.3390/nu12103156.
[23] Pasiakos SM, Agarwal S, Lieberman HR, Fulgoni 3rd VL. Sources and amounts of animal, dairy, and plant protein intake of US adults in 2007-2010. Nutrients 2015;7:7058–69. https://doi.org/10.3390/nu7085322.
[24] Seemer J, Volkert D, Fleckenstein-Sussmann D, Bader-Mittermaier S, Sieber CC, Kiesswetter E. Usual protein intake amount and sources of nursing home residents with (risk of) malnutrition and effects of an individualized nutritional intervention: an enable study. Nutrients 2021;13. https://doi.org/10.3390/nu13072168.
[25] Wolfe RR, Miller SL, Miller KB. Optimal protein intake in the elderly. Clin Nutr 2008;27:675–84. https://doi.org/10.1016/j.clnu.2008.06.008.
[26] Ahmed T, Haboubi N. Assessment and management of nutrition in older people and its importance to health. Clin Interv Aging 2010;5:207–16. https://doi.org/10.2147/cia.s9664.
[27] Fulgoni 3rd VL. Current protein intake in America: analysis of the national health and nutrition examination survey, 2003-2004. Am J Clin Nutr 2008;87:1554S–7S. https://doi.org/10.1093/ajcn/87.5.1554S.
[28] Mitchell CJ, Milan AM, Mitchell SM, Zeng N, Ramzan F, Sharma P, et al. The effects of dietary protein intake on appendicular lean mass and muscle function in elderly men: a 10-wk randomized controlled trial. Am J Clin Nutr 2017;106:1375–83. https://doi.org/10.3945/ajcn.117.160325.
[29] Pedersen AN, Kondrup J, Borsheim E. Health effects of protein intake in healthy adults: a systematic literature review. Food Nutr Res 2013;57. https://doi.org/10.3402/fnr.v57i0.21245.
[30] Wu G. Dietary protein intake and human health. Food Funct 2016;7:1251–65. https://doi.org/10.1039/c5fo01530h.
[31] Gryson C, Ratel S, Rance M, Penando S, Bonhomme C, Le Ruyet P, et al. Four-month course of soluble milk proteins interacts with exercise to improve muscle strength and delay fatigue in elderly participants. J Am Med Dir Assoc 2014;15:958 e951–959. https://doi.org/10.1016/j.jamda.2014.09.011.
[32] Mori H, Tokuda Y. Effect of whey protein supplementation after resistance exercise on the muscle mass and physical function of healthy older women: a randomized controlled trial. Geriatr Gerontol Int 2018;18:1398–404. https://doi.org/10.1111/ggi.13499.
[33] Arnarson A, Gudny Geirsdottir O, Ramel A, Briem K, Jonsson PV, Thorsdottir I. Effects of whey proteins and carbohydrates on the efficacy of resistance training in elderly people: double blind, randomised controlled trial. Eur J Clin Nutr 2013;67:821–6. https://doi.org/10.1038/ejcn.2013.40.
[34] Iglay HB, Apolzan JW, Gerrard DE, Eash JK, Anderson JC, Campbell WW. Moderately increased protein intake predominately from egg sources does not influence whole body, regional, or muscle composition responses to resistance training in older people. J Nutr Health Aging 2009;13:108–14. https://doi.org/10.1007/s12603-009-0016-y.
[35] Lichtenberg T, von Stengel S, Sieber C, Kemmler W. The favorable effects of a high-intensity resistance training on sarcopenia in older community-dwelling men with osteosarcopenia: the randomized controlled FrOST study. Clin Interv Aging 2019;14:2173–86. https://doi.org/10.2147/CIA.S225618.
[36] World Health Organisation. Obesity: preventing and managing the global epidemic. World Health Organization; 2000. 9789241208949.
[37] Riebe D, Ehrman JK, Liguori G, Magal M. ACSM's guidelines for exercise testing and prescription. Lippincott Williams & Wilkins; 2018.
[38] Morishita S, Tsubaki A, Nakamura M, Nashimoto S, Fu JB, Onishi H. Rating of perceived exertion on resistance training in elderly subjects. Expert Rev Cardiovasc Ther 2019;17:135–42. https://doi.org/10.1080/14779072.2019.1561278.
[39] Slimani N, Casagrande C, Nicolas G, Freisling H, Huybrechts I, Ocke MC, et al. The standardized computerized 24-h dietary recall method EPIC-Soft adapted for pan-European dietary monitoring. Eur J Clin Nutr 2011;65(Suppl 1):S5–15. https://doi.org/10.1038/ejcn.2011.83.
[40] Powe CE, Evans MK, Wenger J, Zonderman AB, Berg AH, Nalls M, et al. Vitamin D-binding protein and vitamin D status of black Americans and white Americans. N Engl J Med 2013;369:1991–2000. https://doi.org/10.1056/NEJMoa1306357.
[41] Janssen I, Heymsfield SB, Baumgartner RN, Ross R. Estimation of skeletal muscle mass by bioelectrical impedance analysis. J Appl Physiol 2000;89:465–71. https://doi.org/10.1152/jappl.2000.89.2.465. 1985.
[42] Sergi G, De Rui M, Veronese N, Bolzetta F, Berton L, Carraro S, et al. Assessing appendicular skeletal muscle mass with bioelectrical impedance analysis in free-living Caucasian older adults. Clin Nutr 2015;34:667–73. https://doi.org/10.1016/j.clnu.2014.07.010.
[43] Rikli RE, Jones CJ. Development and validation of a functional fitness test for community-residing older adults. J Aging Phys Activ 1999;7:129–61.
[44] Podsiadlo D, Richardson S. The timed "Up & Go": a test of basic functional mobility for frail elderly persons. J Am Geriatr Soc 1991;39:142–8. https://doi.org/10.1111/j.1532-5415.1991.tb01616.x.
[45] Smith WN, Del Rossi G, Adams JB, Abderlarahman KZ, Asfour SA, Roos BA, et al. Simple equations to predict concentric lower-body muscle power in older adults using the 30-second chair-rise test: a pilot study. Clin Interv Aging 2010;5:173–80. https://doi.org/10.2147/cia.s7978.
[46] Laboratories, A.T.S.C.o.P.S.f.C.P.F. ATS statement: guidelines for the six-minute walk test. Am J Respir Crit Care Med 2002;166:111–7. https://doi.org/10.1164/ajrccm.166.1.at1102.
[47] Mijnarends DM, Meijers JM, Halfens RJ, ter Borg S, Luiking YC, Verlaan S, et al. Validity and reliability of tools to measure muscle mass, strength, and physical performance in community-dwelling older people: a systematic review. J Am Med Dir Assoc 2013;14:170–8. https://doi.org/10.1016/j.jamda.2012.10.009.
[48] Barbat-Artigas S, Filion ME, Plouffe S, Aubertin-Leheudre M. Muscle quality as a potential explanation of the metabolically healthy but obese and sarcopenic obese paradoxes. Metab Syndr Relat Disord 2012;10:117–22. https://doi.org/10.1089/met.2011.0092.
[49] Faul F, Erdfelder E, Lang AG, Buchner AG. *Power 3: a flexible statistical power analysis program for the social, behavioral, and biomedical sciences. Behav Res Methods 2007;39:175–91. https://doi.org/10.3758/bf03193146.
[50] Oesen S, Halper B, Hofmann M, Jandrasits W, Franzke B, Strasser EM, et al. Effects of elastic band resistance training and nutritional supplementation on physical performance of institutionalised elderly–A randomized controlled trial. Exp Gerontol 2015;72:99–108. https://doi.org/10.1016/j.exger.2015.08.013.
[51] Cohen J. Statistical power analysis for the behavioral sciences,. 2nd ed. New Jersey: Lawrence Erlbaum Associates; 1998. vol. 1998.
[52] Holwerda AM, Overkamp M, Paulussen KJM, Smeets JSJ, van Kranenburg J, Backx EMP, et al. Protein supplementation after exercise and before sleep does not further augment muscle mass and strength gains during resistance exercise training in active older men. J Nutr 2018;148:1723–32. https://doi.org/10.1093/jn/nxy169.
[53] Bo Y, Liu C, Ji Z, Yang R, An Q, Zhang X, et al. A high whey protein, vitamin D and E supplement preserves muscle mass, strength, and quality of life in sarcopenic older adults: a double-blind randomized controlled trial. Clin Nutr 2019;38:159–64. https://doi.org/10.1016/j.clnu.2017.12.020.
[54] Gade J, Beck AM, Andersen HE, Christensen B, Ronholt F, Klausen TW, et al. Protein supplementation combined with low-intensity resistance training in geriatric medical patients during and after hospitalisation: a randomised, double-blind, multicentre trial. Br J Nutr 2019;122:1006–20. https://doi.org/10.1017/S0007114519001831.
[55] Amasene M, Besga A, Echeverria I, Urquiza M, Ruiz JR, Rodriguez-Larrad A, et al. Effects of leucine-enriched whey protein supplementation on physical function in post-hospitalized older adults participating in 12-weeks of

resistance training program: a randomized controlled trial. Nutrients 2019;11. https://doi.org/10.3390/nu11102337.
[56] Beasley JM, Deierlein AL, Morland KB, Granieri EC, Spark A. Is meeting the recommended dietary allowance (RDA) for protein related to body composition among older adults?: results from the cardiovascular health of seniors and built environment study. J Nutr Health Aging 2016;20:790–6. https://doi.org/10.1007/s12603-015-0707-5.
[57] Houston DK, Nicklas BJ, Ding J, Harris TB, Tylavsky FA, Newman AB, et al. Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. Am J Clin Nutr 2008;87:150–5. https://doi.org/10.1093/ajcn/87.1.150.
[58] Wall BT, Gorissen SH, Pennings B, Koopman R, Groen BB, Verdijk LB, et al. Aging is accompanied by a blunted muscle protein synthetic response to protein ingestion. PLoS One 2015;10:e0140903. https://doi.org/10.1371/journal.pone.0140903.
[59] Clegg ME, Williams EA. Optimizing nutrition in older people. Maturitas 2018;112:34–8. https://doi.org/10.1016/j.maturitas.2018.04.001.
[60] Vliet SV, Beals JW, Martinez IG, Skinner SK, Burd NA. Achieving optimal post-exercise muscle protein remodeling in physically active adults through whole food consumption. Nutrients 2018;10. https://doi.org/10.3390/nu10020224.
[61] Burke LM, Winter JA, Cameron-Smith D, Enslen M, Farnfield M, Decombaz J. Effect of intake of different dietary protein sources on plasma amino acid profiles at rest and after exercise. Int J Sport Nutr Exerc Metabol 2012;22:452–62. https://doi.org/10.1123/ijsnem.22.6.452.
[62] Tager IB, Swanson A, Satariano WA. Reliability of physical performance and self-reported functional measures in an older population. J Gerontol A Biol Sci Med Sci 1998;53:M295–300. https://doi.org/10.1093/gerona/53a.4.m295.
[63] Candow DG, Chilibeck PD. Differences in size, strength, and power of upper and lower body muscle groups in young and older men. J Gerontol A Biol Sci Med Sci 2005;60:148–56. https://doi.org/10.1093/gerona/60.2.148.
[64] Gentil P, Ferreira-Junior JB, Bemben MG, Ferreira DV, Bottaro M. The effects of resistance training on lower and upper body strength gains in young women. Int J Kinesiol Sports Sci 2015;3:18–23.
[65] Sousa N, Mendes R, Abrantes C, Sampaio J. Differences in maximum upper and lower limb strength in older adults after a 12 week intense resistance training program. J Hum Kinet 2011;30:183–8. https://doi.org/10.2478/v10078-011-0086-x.
[66] Buskard ANL, Jacobs KA, Eltoukhy MM, Strand KL, Villanueva L, Desai PP, et al. Optimal approach to load progressions during strength training in older adults. Med Sci Sports Exerc 2019;51:2224–33. https://doi.org/10.1249/MSS.0000000000002038.
[67] Raymond MJ, Bramley-Tzerefos RE, Jeffs KJ, Winter A, Holland AE. Systematic review of high-intensity progressive resistance strength training of the lower limb compared with other intensities of strength training in older adults. Arch Phys Med Rehabil 2013;94:1458–72. https://doi.org/10.1016/j.apmr.2013.02.022.
[68] Mitchell CJ, Churchward-Venne TA, West DW, Burd NA, Breen L, Baker SK, et al. Resistance exercise load does not determine training-mediated hypertrophic gains in young men. J Appl Physiol 2012;113:71–7. https://doi.org/10.1152/japplphysiol.00307.2012. 1985.
[69] Takarada Y, Takazawa H, Sato Y, Takebayashi S, Tanaka Y, Ishii N. Effects of resistance exercise combined with moderate vascular occlusion on muscular function in humans. J Appl Physiol 2000;88:2097–106. https://doi.org/10.1152/jappl.2000.88.6.2097. 1985.
[70] Kalapotharakos VI, Michalopoulos M, Tokmakidis SP, Godolias G, Gourgoulis V. Effects of a heavy and a moderate resistance training on functional performance in older adults. J Strength Condit Res 2005;19:652–7. https://doi.org/10.1519/15284.1.
[71] Vincent KR, Braith RW, Feldman RA, Magyari PM, Cutler RB, Persin SA, et al. Resistance exercise and physical performance in adults aged 60 to 83. J Am Geriatr Soc 2002;50:1100–7. https://doi.org/10.1046/j.1532-5415.2002.50267.x.
[72] Hofmann M, Schober-Halper B, Oesen S, Franzke B, Tschan H, Bachl N, et al. 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 2016;116:885–97. https://doi.org/10.1007/s00421-016-3344-8.
[73] Shah P, Abel AAI, Boyalla V, Pellicori P, Kallvikbacka-Bennett A, Sze S, et al. A comparison of non-invasive methods of measuring body composition in patients with heart failure: a report from SICA-HF. ESC Heart Fail 2021;8:3929–34. https://doi.org/10.1002/ehf2.13402.
[74] Strasser EM, Hofmann M, Franzke B, Schober-Halper B, Oesen S, Jandrasits W, et al. Strength training increases skeletal muscle quality but not muscle mass in old institutionalized adults: a randomized, multi-arm parallel and controlled intervention study. Eur J Phys Rehabil Med 2018;54:921–33. https://doi.org/10.23736/S1973-9087.18.04930-4.
[75] Borga M, West J, Bell JD, Harvey NC, Romu T, Heymsfield SB, et al. Advanced body composition assessment: from body mass index to body composition profiling. J Invest Med 2018;66:1–9. https://doi.org/10.1136/jim-2018-000722.