The effect of daily protein supplementation, with or without resistance training for 1 year, on muscle size, strength, and function in healthy older adults: A randomized controlled trial

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ABSTRACT

Background: Protein supplementation alone or combined with resistance training has been proposed to be effective in counteracting age-related losses of muscle mass and strength.

Objectives: To investigate the effect of protein supplementation alone or combined with light-intensity or heavy-load resistance exercise on muscle size, strength, and function in older adults.

Methods: In a 1-y randomized controlled trial, 208 healthy older adults (>65 y) were randomly assigned to 1 of 5 interventions: *1*) carbohydrate supplementation (CARB); *2*) collagen protein supplementation (COLL); *3*) whey protein supplementation (WHEY); *4*) light-intensity resistance training 3–5 times/wk with whey protein supplementation (LITW); and *5*) heavy resistance training 3 times weekly with whey protein supplementation (HRTW). Protein supplements contained 20 g protein + 10 g carbohydrate, whereas CARB contained 30 g of carbohydrates. All intervention groups received the supplement twice daily. The primary outcome was change in the quadriceps cross-sectional area (qCSA). Secondary outcomes included measures of lower extremity strength and power, functional capabilities, and body composition.

Results: There were 184 participants who completed the study. COLL and WHEY did not affect any measured parameter compared to CARB. Compared to WHEY, HRTW improved the qCSA size (between-group difference, +1.68 cm^2; 95% CI, +0.41 to +2.95 cm^2; $P = 0.03$), as well as dynamic (+18.4 Nm; 95% CI, +10.1 to +26.6 Nm; $P < 10^{-4}$) and isometric knee extensor strength (+23.9 Nm; 95% CI, +14.2 to +33.6 Nm; $P < 10^{-5}$). LITW did not improve the qCSA size, but increased dynamic knee extensor strength compared to WHEY (+13.7 Nm; 95% CI, +5.3 and +22.1 Nm; $P = 0.01$).

Conclusions: Recommending protein supplementation as a standalone intervention for healthy older individuals seems ineffective in improving muscle mass and strength. Only HRTW was effective in both preserving muscle mass and increasing strength. Thus, we recommend that future studies investigate strategies to increase long-term compliance to heavy resistance exercise in healthy older adults. This trial was registered at clinicaltrials.gov as NCT02034760. *Am J Clin Nutr* 2021;113:790–800.

Keywords: protein supplementation, ageing, skeletal muscle, resistance training, randomized controlled trials, exercise

Introduction

It has often been suggested that the progressive age-related declines in muscle mass and function (1–3) can be counteracted by a higher protein intake and usage of muscle through exercise (4, 5). Cross-sectional and prospective cohort studies have shown that protein intake above the current RDA of 0.83 $g \cdot kg^{-1} \cdot d^{-1}$ (6) is associated with higher muscle mass (7–13), as well as better preservation of muscle mass, in older adults (>65 y) (14–16). The latter has led to increased recommendations of 1.1–1.3 g $protein \cdot kg^{-1} \cdot d^{-1}$ for older adults in the recent edition of the Nordic Nutrition Recommendations (17). However, intervention studies investigating the effects of increasing protein intake on muscle mass show mixed results (18–26). The duration of intervention studies is generally short (≤6 mo), and the discrepant findings might therefore be related to inadequate intervention lengths (27). Furthermore, the importance of protein quality [evaluated by the digestible indispensable amino acid score (28, 29)], when supplied as part of a mixed diet, is not known. Oikawa and colleagues (30) recently found that supplementation with a high-quality protein supplement (whey) induced greater increases in both acute and 6-d integrated muscle protein synthesis compared to a lower-quality protein supplement (collagen). However, to the present authors' knowledge, whether whey protein supplementation results in better preservation of muscle

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mass compared to collagen during long-term supplementation has not been investigated. Thus, the impact of increasing dietary protein intake on muscle mass and strength in older adults remains a debated topic, with an urgent need for long-term, well-conducted, human intervention studies (27, 31–34).

While heavy resistance training is the most potent exercise modality used to increase muscle mass and strength (35–38), some older adults prefer exercise interventions of lower intensity that are less expensive and situated in more convenient locations, like a home-based setting (39, 40). Lower-intensity training modalities can be effective in enhancing muscle mass (41–43) and, when accounting for adherence, a home-based low-intensity exercise program might therefore be an equally (or more) effective long-term exercise intervention compared to heavy-resistance exercise for older adults.

The aim of the present study was to investigate the effects of protein supplementation alone or combined with resistance training on muscle size and strength by conducting a 1-year randomized controlled trial. The hypotheses were:

1) Supplementation with higher-quality whey protein will benefit muscle size and strength more than supplementation with lower-quality collagen protein in healthy older adults.
2) Adherence to home-based, light-intensity resistance exercise is higher than adherence to center-based, heavy-resistance training, and thus exerts an equally beneficial long-term training strategy for gaining/preserving muscle mass and strength when combined with whey protein supplementation.

Methods

The Counteracting Age-Related Loss of Muscle Mass (CALM) trial was conducted at Bispebjerg Hospital, Copenhagen, Denmark, between 2014 and 2018. The design of the trial and detailed descriptions of methods and exclusion criteria have been published previously (44). The regional ethics committee approved the trial protocol (H-4-2013-070), and the subjects gave written informed consent to participate. The trial was registered at clinicaltrials.gov (NCT02034760).

Study participants

We recruited 208 community-dwelling adults aged 65 y and older. To be included, the participants were not allowed to partake in >1 of heavy resistance training per week at the time of inclusion. Participants were not included if they had any medical condition potentially preventing them from safely completing the 1-y intervention (e.g., diabetes mellitus, unstable cardiac arrhythmia, arthritis, etc.) (44).

Participant recruitment

Recruitment was done through advertisements in newspapers, magazines, and social media, as well as presentations at senior centers and public events. After a brief telephone screening for exclusion criteria, the participants underwent a physical examination, including blood samples, to determine whether they could perform the interventions safely. As part of the physical examination, measurements of blood pressure and a 30-s chair-stand test were also performed, with the latter used for stratifying randomization.

Randomization

Following screening and the health examination, participants were enrolled in the study and randomized into 1 of the following 5 groups using minimization software (MinimPy 0.3; http://minimpy.sourceforge.net/) (45): *1*) carbohydrate supplementation (CARB; 2 × 20 g maltodextrin + 10 g sucrose); *2*) whey protein supplementation (WHEY; 2 × 20 g whey protein hydrolysate + 10 g sucrose); *3*) collagen protein supplementation (COLL; 2 × 20 g bovine collagen protein hydrolysate + 10 g sucrose); *4*) light-intensity training with whey protein supplementation (LITW; 2 × 20 g whey protein hydrolysate + 10 g sucrose); or *5*) heavy resistance training with whey protein supplementation (HRTW; 2 × 20 g whey protein hydrolysate + 10 g sucrose). Randomization was done by an investigator not involved in the interventions and not sensitive to blinding. To account for the differences in group size (see sample size), we employed a stratified, biased coin minimization with 0.95 base probability, and used allocation ratios corresponding to the group sizes (46). In order to minimize between-group differences in muscle size at baseline, randomization was stratified by sex and number of completed repetitions on the 30-s chair stand test (<16 or ≥16) (47). The cut-off value of 16 repetitions on the 30-s chair stand test was chosen because this is the expected average performance in this test, based on previous findings in age-matched Danes (48).

Interventions

In all 5 intervention groups (CARB, COLL, WHEY, LITW, HRTW), participants were instructed to ingest the supplements

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Supplemental Tables 1–14 are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: CARB, carbohydrate supplementation; COLL, collagen protein supplementation; DPT, dynamic peak torque; EAA, essential amino acid; HRTW, heavy resistance training with whey protein supplementation; LITW, light-intensity resistance training with whey protein supplementation; LTM, lean tissue mass; MCS, mental component score; MVIC, maximal voluntary isometric contraction; PCS, physical component score; qCSA, quadriceps cross-sectional area; RFD, rate of force development; RM, repetition maximum; WHEY, Whey protein supplementation.

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twice daily, in the morning and at midday, preferably just before or during meals to increase satiety, thereby limiting potential excessive caloric intake. Participants randomized to HRTW or LITW were encouraged to ingest 1 of their daily supplements immediately after each training session. Supplements were provided to the participants in portion-sized packages of powder, developed and individually packaged by Arla Foods Ingredients Group P/S. Participants were instructed to dissolve the supplements in the fluid they preferred. Adherence to the supplements was continuously recorded by the participants in hard-copy diaries throughout the intervention.

Those assigned to HRTW performed heavy resistance training 3 times weekly (Monday, Wednesday, and Friday between 09:00 and 11:30) under the supervision of trained personnel. The training program consisted of 3 exercises for the lower extremities (leg extensions, leg press, and leg curls) and 2 upper-body exercises (pull-down, chest press), with each training session lasting ~1 h. Training was periodized into 3-mo cycles, increasing the load progressively from 3 sets of 12 repetitions at a 12 repetition maximum (RM) to 5 sets of 6 repetitions at a 6 RM in each cycle. Those assigned to LITW performed light-load home-based resistance 3–5 times weekly, using rubber bands (TheraBand, Hygenic Corp.) and their body weight for exercises chosen to mimic the muscle groups and movements used in training those assigned to HRTW [full details can be found elsewhere (44)]. Participants were allowed to perform the home-based training sessions whenever it fit their daily schedule best. To ensure proper execution, study personnel supervised LITW sessions once per week during the first month and once per month during the remainder of the intervention. Adherence to the training for HRTW was recorded by staff, whereas adherence to training for LITW was recorded by the participants in hard-copy diaries. All participants enrolled in the study were carefully instructed to not take up any new exercise regimens over the course of the intervention period, besides what was performed as part of the study for LITW and HRTW.

Primary outcome

The primary outcome was the change in the midthigh quadriceps cross-sectional area (qCSA) of the dominant leg, measured by MRI scans. MRI is considered the gold standard for measuring muscle size and detecting age-related atrophy (49, 50). MRI scans were performed in a Siemens Verio 3 Tesla scanner by blinded radiographers. Participants were scanned in a supine position using a dedicated 32-channel body coil, and a phantom was placed parallel to the femur during the scans. The following protocol was used: 3 plane gradient echo scout [matrix resolution, 1.2.0 × 1.6 × 6.0 mm; field of view (FOV), 330 mm; time to echo (TE), 3.69 ms; repetition time (TR), 7.8 ms; scan time, 27 s]; and axial T1 turbo spin echo (tse) from the medial tibia plateau to the pubic symphysis (matrix resolution, 0.8 × 0.8 × 8.0 mm; FOV, 400 mm; TE, 8.4 ms; TR, 500 ms; scan time, 3 min 26 s). Subjects were instructed to avoid vigorous physical activity for 48 h prior to the scans. Each scan consisted of 6 axial slices, with the first slice being placed in the medial tibia plateau. Each slice was 8 mm thick, separated by a 60-mm gap. Slice 4 on the dominant leg was used for assessing the qCSA. Using OsiriX v. 5.5.2 (OsiriX medical imaging software), each scan was analyzed twice by the same blinded investigator, showing a mean coefficient of variation between measurements of 0.7%. The mean of the 2 measurements was used for further analyses.

Secondary outcomes

To assess lower extremity strength, we measured dynamic peak torque (DPT) during concentric contractions of the knee extensors at movement speeds of 60°/s in a knee joint range of motion from 90° to 10° knee flexion (0° = full extension), as well as maximal voluntary isometric contractions (MVIC) of 70° knee flexion in an isokinetic dynamometer (Kinetic Communicator, model 500–11). From the isometric contractions, we also assessed rate of force development (RFD). RFD was measured as the average force development from 0–200 ms after the onset of a contraction in the MVIC measurements. Furthermore, leg extensor power was measured in the Nottingham Power Rig (Queens Medical Center, Nottingham University, UK) (51). The functional capabilities of the participants were assessed using the 400-m walk test (52). Assessments of gait speed, as well as measures of lower extremity strength and power, were all performed on the same day, typically the day after the MRI scan, and have been described in detail elsewhere (53). Self-perceived quality of life was measured using the Danish version of the 36-item Short-Form Health Survey (54). We report changes in the physical component score (PCS) and mental component score (MCS).

Body composition was assessed by dual-energy X-ray absorptiometry (Lunar iDXA, GE Medical Systems) using enCORE software (version 16). Study participants arrived fasting from 21:00 the night before and had refrained from strenuous activities for 48 h prior to the test. All scans were performed between 08:00 and 10:00, a week prior to the tests of strength and function. From these scans, we obtained measurements of lean tissue mass (LTM) and body fat percentage. Regions of interest for the extremities and visceral body parts were set based on the default definitions provided by the scanner software. The same examiner controlled the default positioning of all regions, with positions adjusted slightly when appropriate to take into account inter-individual differences in body placement and body size.

Daily activity levels were measured by attaching an accelerometer-based activity monitor (activPal 3, activPal 3c, or activPal micro; PAL technologies) on the anterior surface of the thigh (55). The monitor was worn for 96 continuous h, covering a full weekend. Data are represented as the average number of steps per day.

A detailed description of the dietary assessment can be found elsewhere (56). Briefly, participants weighed their dietary intake for 3 consecutive days (Wednesday to Friday), and wrote down the information in food logs. Trained staff then quantified nutrient intake using a dietary assessment tool (VITAKOST, MADLOG ApS). Dietary assessments were performed prior to the intervention and after 11 mo of the intervention. Nutrient intake was assessed for foods only. Total protein and energy intakes from the supplement were manually estimated by multiplying the compliance to the supplement with the dietary content of the supplement. However, if the participants used other fluids than water for dissolving the supplement, these fluids were

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registered in the food logs. For the participants who failed to report their compliance to the supplement, but who were still receiving the supplement, we used the median compliance rate from the respective groups.

Lastly, glycated hemoglobin, blood cholesterol, and triglycerides, as well as creatinine concentrations, were monitored to ensure that the supplementation did not cause the participants to develop insulin resistance, impaired kidney function, or hyperlipidemia. Results from these measurements can be found in **Supplemental Table 1.**

Blinding

Participants in the supplement-only groups (WHEY, COLL, CARB) were blinded to which supplement they received. Training interventions were not blinded to the participants. Staff performing and analyzing the MRI images, as well as the strength and functional tests, were blinded towards the interventions. Unblinded personnel performed DXA scans and blood sampling, but analyses and interpretation of the data output from these were done by blinded researchers.

Sample sizes

We aimed to be able to detect differences between any 2 groups in qCSA changes of 2% over the intervention period, corresponding to approximately 0.8 cm^2. Based on previous data from our lab (57), an SD of ~1.4 cm^2 for qCSA was expected. Thus, applying a level of significance of 0.05 and a power of 0.80, a group size of 30 participants was required. Anticipating a dropout rate of 15%, we assigned 36 participants each to HRTW, LITW, and CARB. Due to taste issues with the protein supplement, we expected a higher dropout rate in those assigned to WHEY and COLL, and therefore included 50 participants each in these groups (44).

Statistical analyses

Baseline data are summarized by group means ± SDs unless otherwise stated. Effects of the interventions were investigated within each study arm, separately. The individual treatment effects are reported as the mean changes and associated 95% CIs during the intervention. Between-treatment effects are reported as mean differences in treatment effects and associated 95% CIs. The level of significance was set to <0.05. The effects of the interventions were analyzed using a modified intention-to-treat analytic strategy, including all participants that completed at least 1 test at the 12-mo time point, irrespective of adherence to the interventions. As participants were not blinded to training interventions, we cannot exclude that participants dropped out of the study due to their allocated study arm; hence, missing data from participants who dropped out of the study were not imputed in the analysis. Effects of the interventions were also analyzed using a per-protocol analytic strategy, including participants with >75% adherence to the supplements, as well as >65% and >75% adherence to training for those assigned to HRTW and LITW, respectively. Results from the per-protocol analysis did not differ markedly from the results of the modified intention-to-treat analysis and can be found in Supplemental Table 1.

Changes from baseline to 12 mo were investigated in 2 separate analyses; an analysis of the effects of protein supplementation alone (CARB vs. COLL vs. WHEY), and an analysis of the effects of training combined with whey protein supplementation (WHEY vs. LITW vs. HRTW). These analyses were performed using a longitudinal mixed model with time (baseline and 12 mo) and intervention group (3 levels) as fixed predictors, including their interaction, and person as a random term. Treatment inferences were based on significance tests of the interaction terms, and further investigated by contrasts of intervention group changes from baseline to 12 mo between all pairs (CARB vs. COLL vs. WHEY; WHEY vs. LITW vs. HRTW) of group combinations. Analyses were not adjusted for covariates or multiple comparisons.

R (version 3.5.1; R Core Team) was used for data analysis, with the function lm() from the stats package (version 3.5.1), lmer() from the lme4 package (version 1.1-20), and glth() from the multcomp package (version 1.4-8).

Results

In total, we had 1285 contacts from potential participants, of which 1148 were screened via telephone. We scheduled 280 potential participants for an on-site screening visit, 39 of whom declined to participate. Another 33 potential participants were excluded prior to enrollment in the study (30 due to medications or diseases not discovered in the phone screening; 2 due to performing >1 h of heavy resistance training weekly; and 1 due to excessive alcohol intake). A Consolidated Standards of Reporting Trials (CONSORT) diagram is shown in **Figure 1**. In total, 208 participants were randomized and 184 completed the 12-mo tests. Characteristics of the included subjects are presented in **Table 1**. There were 24 participants who dropped out during the study: 11 due to illness or injury unrelated to the intervention, 5 due to disliking the supplement, 3 due to the testing being too extensive, and 5 due to personal reasons.

Adherence

Self-reported adherence to training was significantly higher for those assigned to LITW compared to the staff-registered adherence to training for those assigned to HRTW [median, LITW: 89% (IQR, 77%–96%); HRTW: 72% (IQR, 62%–78%); $P < 0.01$; see Supplemental Table 1]. Supplement adherence did not differ significantly between groups [median, CARB: 95% (IQR, 77%–97%); COLL: 96% (IQR, 86%–99%); WHEY: 88% (IQR, 82%–93%); $P = 0.11$]; however, a total of 34 participants failed to report their intake of the supplements throughout the intervention (**Supplemental Table 2**). These participants all came to the research facilities to receive additional supplements as planned, but they are not included in the adherence values due to their insufficient reporting of supplement intake.

In the supplementation-only analysis, changes in protein intake differed between groups (time∗group interaction, $P < 10^{-5}$; **Table 2**). Compared to those assigned to CARB, protein intake increased for those assigned to COLL (mean between-group difference, +32.3 g/d; 95% CI, +19.7 to +45.0 g/d; $P < 10^{-5}$) and WHEY (mean between-group difference, +27.2 g/d; 95% CI +14.4 to +39.9 g/d; $P < 10^{-4}$). In

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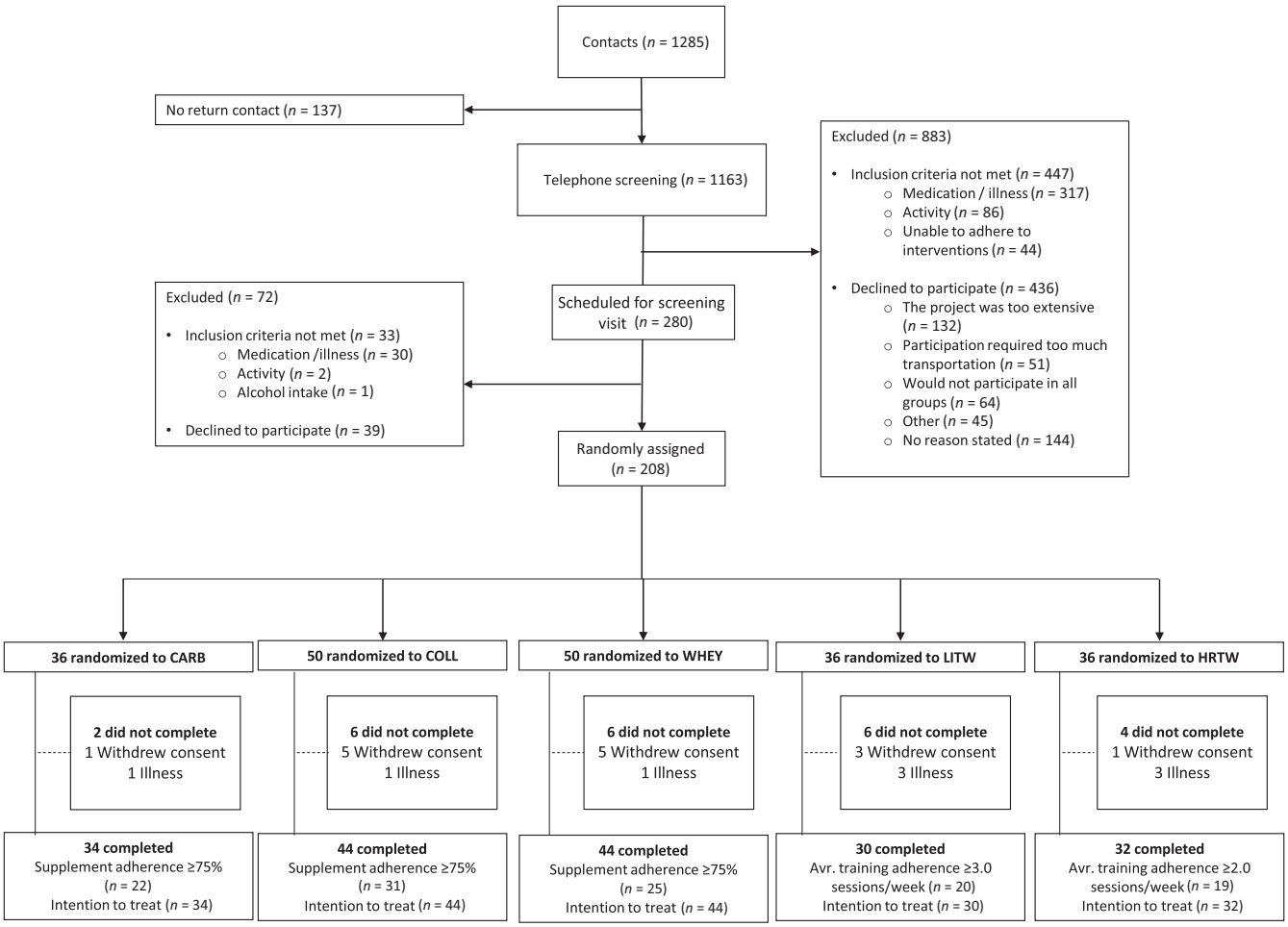

FIGURE 1 CONSORT diagram showing the flow of participants in the CALM trial. Abbreviations: CALM, Counteracting Age-Related Loss of Muscle Mass; CARB, carbohydrate supplementation; COLL, collagen protein supplementation; CONSORT: Consolidated Standards of Reporting Trials; HRTW, heavy resistance training with whey protein supplementation; LITW, light-intensity training with whey protein supplementation; WHEY, whey protein supplementation.

the combined training and supplementation analysis, changes in protein intake did not differ between groups (time∗group interaction, $P = 0.95$). Changes in energy intake did not differ significantly between groups in the supplementation-only analysis (time∗group interaction, $P = 0.49$) or in the combined training and supplementation analysis (time∗group interaction, $P = 0.69$).

Quadriceps size

In the supplementation-only analysis, we observed no between-group differences in changes in qCSA (time∗group interaction, $P = 0.17$; **Figure 2**A). In the combined training and supplementation analysis, the time∗group interaction term was significant ($P = 0.04$). HRTW was associated with a more positive change in qCSA compared to WHEY (mean between-group difference, +1.68 cm^2; 95% CI, +0.41 to +2.95 cm^2; $P = 0.03$), but not compared to LITW (mean between-group difference, +1.29 cm^2; 95% CI, -0.08 to +2.67 cm^2; $P = 0.16$). Changes in qCSA were not significantly different for those assigned to LITW compared to WHEY (mean between-group difference, +0.39 cm^2; 95% CI, -0.88 to +1.66 cm^2; $P = 0.82$). Investigating within-group changes in qCSA, neither those assigned to HRTW (0- to 12-mo change, +0.73 cm^2; 95% CI, -0.32 to +1.77 cm^2) nor those assigned to LITW (0- to 12-mo change, -0.54 cm^2; 95% CI, -1.70 to +0.62 cm^2) exhibited marked changes, whereas a decrease in qCSA was observed for those assigned to WHEY (0- to 12-mo change, -0.93 cm^2; 95% CI, -1.65 to -0.21 cm^2).

Lower body strength and power

No between-group differences were observed in the supplementation-only analysis for either MVIC (time∗group interaction, $P = 0.13$; Figure 2B), DPT ($P = 0.24$; Figure 2C), RFD ($P = 0.86$; Figure 2D), or leg extensor power ($P = 0.94$; Figure 2E). In the combined training and supplementations groups, changes in MVIC differed between groups (time∗group interaction, $P < 10^{-4}$), with HRTW inducing greater gains in MVIC compared to LITW (between-group difference, +16.8 Nm; 95% CI, +6.1 to +27.4 Nm; $P = 0.01$) and WHEY (between-group difference, +23.9 Nm; 95% CI, +14.2 to +33.6 Nm; $P < 10^{-5}$). However, changes in MVIC induced by LITW were not significantly different from those from WHEY

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TABLE 1 Baseline characteristics of the included participants by group

Variable	CARB $n = 36$	COLL $n = 50$	WHEY $n = 50$	LITW $n = 36$	HRTW $n = 36$
Demographics, mean ± SD					
Age, y	69.6 ± 3.9	70.4 ± 4.1	70.3 ± 4.3	70.4 ± 4.0	70.3 ± 3.1
Men/women, n	18/18	27/23	28/22	18/18	18/18
Body weight, kg	75.6 ± 12.3	75.1 ± 12.7	75.0 ± 13.6	75.4 ± 11.9	77.2 ± 13.8
BMI, kg/m^2	26.0 ± 3.9	25.4 ± 6.0	25.2 ± 3.6	25.7 ± 3.1	25.9 ± 3.5
Daily activity, steps/d	10894 ± 5165	10590 ± 3996	10118 ± 3590	10119 ± 3450	9777 ± 3574
Protein intake, g/kg/d	1.2 ± 0.3	1.2 ± 0.4	1.1 ± 0.3	1.0 ± 0.3	1.1 ± 0.4
Energy intake, kJ/d	8442 ± 1804	8150 ± 1952	8529 ± 2092	7445 ± 2220	8268 ± 2146
Body composition					
Fat free mass, kg	48.5 ± 7.8	49.2 ± 8.6	50.0 ± 8.5	48.1 ± 9.3	48.8 ± 9.9
Fat percentage, %	33.2 ± 9.3	32.0 ± 9.1	32.7 ± 7.5	34.3 ± 7.5	34.7 ± 7.1
Quadriceps size, cm^2	56.6 ± 11.3	56.0 ± 13.9	54.5 ± 11.0	56.7 ± 11.4	55.4 ± 13.1
Strength and function					
400 m gait time, s	248 ± 42	243 ± 38	242 ± 30	242 ± 30	251 ± 27
30 s chair stand, reps	19.9 ± 5.7	20.1 ± 5.3	19.4 ± 4.6	20.1 ± 4.6	18.9 ± 4.9
Leg extensor power, W	183.1 ± 56.2	191.2 ± 67.2	189.6 ± 59.6	190.8 ± 61.4	194.2 ± 65.8
MVIC, Nm	158.9 ± 41.1	169.0 ± 53.4	177.6 ± 47.0	171.5 ± 44.4	165.0 ± 50.8
DTP, Nm	145.2 ± 35.6	151.6 ± 45.3	156.4 ± 41.3	150.5 ± 37.1	149.9 ± 46.0
RFD, Nm/s	600.3 ± 225.2	636.4 ± 228.3	662.1 ± 238.0	615.7 ± 211.0	604.2 ± 208.1
SF-36					
MCS	59.3 ± 3.2	57.3 ± 4.3	57.6 ± 3.6	57.1 ± 4.7	57.5 ± 4.4
PCS	55.3 ± 4.7	56.0 ± 4.7	56.8 ± 3.1	56.4 ± 4.0	56.5 ± 4.2
Laboratory data					
HbA1c, mmol/mol	36.0 ± 2.2	35.8 ± 3.4	36.2 ± 3.5	35.8 ± 2.9	35.8 ± 2.7
Total cholesterol, mmol/l	5.6 ± 0.9	5.7 ± 1.0	6.0 ± 1.2	5.5 ± 1.0	5.8 ± 0.9
HDL cholesterol, mmol/l	1.9 ± 0.5	2.0 ± 0.6	1.8 ± 0.5	1.8 ± 0.5	1.8 ± 0.5
LDL cholesterol, mmol/l	3.1 ± 0.8	3.2 ± 1.0	3.4 ± 0.9	3.0 ± 1.0	3.4 ± 1.0
Triglycerides, mmol/l	1.3 ± 0.6	1.4 ± 0.8	1.7 ± 0.8	1.4 ± 0.6	1.4 ± 0.6
Creatinine, μmol/l	76.8 ± 14.7	81.4 ± 15.9	80.5 ± 11.6	78.8 ± 14.7	77.0 ± 12.7

Values are presented as means ± SDs. Abbreviations: CARB, carbohydrate supplementation; COLL, collagen protein supplementation; DTP, dynamic peak torque; HbA1c, glycated hemoglobin; HRTW, heavy resistance training with whey protein supplementation; LITW, light-intensity resistance training with whey protein supplementation; MCS, mental component score; MVIC, maximal voluntary isometric contraction; PCS, physical component score; RFD, rate of force development; SF-36, 36-item Short-Form Health Survey; WHEY, whey protein supplementation.

(between-group difference, +7.1 Nm; 95% CI, -2.8 to 17.1 Nm; $P = 0.34$). Changes in DPT differed between groups in the combined training and supplementation analysis (time∗group interaction: $P < 10^{-4}$). DPT increased in both those assigned to HRTW (between-group difference, +18.4 Nm; 95% CI, +10.1 to +26.6 Nm; $P < 10^{-4}$) and those assigned to LITW (between-group difference, +13.7 Nm; 95% CI, +5.3 to +22.1; $P = 0.01$) compared to results in those assigned to WHEY, but with no significant difference between those assigned to HRTW and LITW (between-group difference, +4.7 Nm; 95% CI, -4.4 to +13.7 Nm; $P = 0.57$). No between-group differences were observed in changes in RFD (time∗group interaction, $P = 0.12$) or leg extensor power ($P = 0.73$) in the combined training and supplementation analysis. However, when investigating within-group changes, HRTW increased RFD (0- to 12-mo change, +73.5 Nm/s; 95% CI, +24.6 to +122.4 Nm/s), with nominal increases from LITW (0- to 12-mo change, +52.1 Nm/s; 95% CI, -3.8 to +108.0 Nm/s) and no apparent change from WHEY (0- to 12-mo change, +12.2 Nm/s; 95% CI, -22.1 to +46.5 Nm/s).

Functional capabilities

In the supplementation-only analysis, no between-group differences were observed in changes in 400-m gait time (time∗group interaction, $P = 0.99$; Figure 2F), MCS ($P = 0.36$), or PCS ($P = 0.38$; Table 2). In the combined training and supplementation analysis, changes in 400-m gait times were not significantly different between groups (time∗group interaction, $P = 0.14$). However, when investigating within-group changes, gait times decreased for those assigned to HRTW (0- to 12-mo change, -7.8 s; 95% CI, -15.1 to -0.45 s) and decreased nominally for those assigned to LITW (0- to 12-mo change, -4.7 s; 95% CI, -9.9 to +0.6 s), with no apparent change in those assigned to WHEY (0- to 12-mo change, +0.1 s; 95% CI, -5.0 to +5.2 s). No between-group differences were observed in changes in MCS (time∗group interaction, $P = 0.83$) or PCS ($P = 0.49$; Table 2) in the combined training and supplementation analysis.

Body composition

In the supplementation-only analysis, changes in body weight (time∗group interaction, $P = 0.46$), fat percentage ($P = 0.95$), and LTM ($P = 0.29$) did not differ between groups. However, when investigating within-group changes, increases in fat percentages were observed in all supplementation-only groups, with no marked changes in LTM or body weight (Table 2). In the combined training and supplementation analysis, changes in LTM were not significantly different between groups (time∗group interaction, $P = 0.09$). Investigating within-group

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TABLE 2 Changes in dietary intake, activity level, self-perceived health, and body composition

Changes from 0–12 m, mean (95% CI)	CARB $n = 34$	COLL $n = 44$	WHEY $n = 44$	LITW $n = 30$	HRTW $n = 32$
Diet					
Estimated total protein intake, g/d	-4.9 (-15.8 to 6.1)	+29.0 (+21.1 to +36.8)*	+25.7 (+15.6 to +35.8)*	+23.9 (+15.2 to +32.5)	+26.7 (+18.9 to +34.5)
Protein intake excluding supplement, g/d	-4.9 (-15.8 to +6.1)	-8.3 (-15.5 to -1.1)	-6.4 (-15.1 to +2.3)	-9.6 (-17.7 to -1.5)	-5.8 (-12.3 to +0.7)
Estimated total energy intake, kJ/d	+949 (+62 to +1835)	+408 (-130 to +947)	+518 (-322 to +1358)	+474 (-427 to +1375)	-41 (-707 to +625)
Energy intake excluding supplement, g/d	-81 (-960 to +798)	-649 (-1176 to -122)	-389 (-1197 to +419)	-472 (-1351 to +406)	-961 (-1609 to -314)
Activity level					
Daily activity, steps/d	-1662 (-3505 to +180)	+330 (-869 to +1529)	-91 (-1217 to +1035)	-322 (-1521 to +878)	-368 (-1210 to +475)
SF-36					
MCS	-1.1 (-2.9 to +0.8)	+0.5 (-1.0 to +2.0)	-0.2 (-2.5 to +2.1)	+0.4 (-1.1 to +1.9)	-1.0 (-3.9 to +2.0)
PCS	-2.6 (-5.0 to -0.2)	-0.6 (-2.4 to + 1.1)	-0.4 (-1.6 to +0.8)	-0.4 (-1.7 to +0.8)	0.0 (-1.6 to +1.6)
Body composition					
Body weight, kg	+1.2 (0.0 to +2.3)	+0.7 (-0.1 to +1.5)	+0.4 (-0.3 to +1.1)	+0.7 (-0.5 to +1.8)	-0.2 (-1.3 to +1.0)
Lean tissue mass, kg	+0.2 (-0.2 to +0.5)	+0.0 (-0.3 to +0.3)	-0.2 (-0.5 to +0.1)	+0.1 (-0.3 to +0.5)	+0.4 (0 to +0.8)
Fat percentage, pp	+0.7 (0.0 to +1.5)	+0.6 (0.0 to +1.2)	+0.7 (+0.1 to + 1.2)	+0.5 (-0.4 to +1.3)	-0.4 (-1.3 to +0.5)

Values are presented as mean changes from 0–12 mo with 95% CIs. Data were analyzed using a mixed-model analysis with time (baseline and 12 mo) and intervention group (3 levels) as fixed predictors. If the time∗group interaction term was significant ($P < 0.05$), between-group differences were further investigated using pairwise contrast analysis. *Significantly different compared to CARB in a pairwise analysis. Abbreviations: CARB, carbohydrate supplementation; COLL, collagen protein supplementation; LITW, light-intensity resistance training with whey protein supplementation; HRTW, heavy resistance training with whey protein supplementation; MCS, mental component score; PCS, physical component score; pp, percentage points; SF-36, 36-item Short-Form Health Survey; WHEY, whey protein supplementation.

changes in LTM, nominal increases in LTM were observed for those assigned to HRTW (0- to 12-mo change, +0.39 kg; 95% CI, -0.01 to +0.79 kg), whereas no apparent change was observed for those assigned to LITW (0- to 12-mo change, +0.10 kg; 95% CI, -0.33 to +0.54 kg). Changes in fat percentages (time∗group interaction: $P = 0.10$) and body weight ($P = 0.57$) did not differ between groups in the combined training and supplementation analysis.

Discussion

This study investigated the effects of 2 modifiable strategies to counteract age-related loss of muscle mass in older adults: protein supplementation alone and/or combined with resistance exercise. Increasing daily protein intake from ∼1.1 $g \cdot kg^{-1}$ to ∼1.5 $g \cdot kg^{-1}$ by providing daily protein supplements to healthy, home-dwelling, older individuals had no beneficial effects in any of the performed measures. These results provide strong evidence that an increase in protein intake alone does not add a benefit in preserving muscle mass or strength in healthy older adults living independently and eating in accordance with current guidelines. Increasing protein content in an iso-caloric diet has been shown to result in the loss of fat mass (18), but in the present study supplementation of any kind was associated with an increase in the fat percentage, with no marked change in LTM or body weight. Although this finding was not controlled against normal eating behavior, the increasing fat percentage could indicate that the older adults in the present study did not adjust energy intake and/or expenditure sufficiently when supplemented with extra calories, irrespective of the source of supplemented calories (protein/carbohydrate). As muscle protein synthesis mainly seems to be regulated by the essential amino acid (EAA) content of the ingested protein (58), future studies could consider supplementing EAAs alone to avoid the additional calories.

Contrary to our hypothesis, WHEY was not associated with more positive changes in qCSA compared to COLL or CARB. This finding is surprising and contradicts our hypothesis that supplements with high-quality protein should be superior to lower-quality protein supplements in maintaining muscle mass. In a recent study from Oikawa and colleagues (30), it was found that whey protein supplementation induced greater acute and 6-d integrated muscle protein synthesis compared to collagen supplementation in healthy older women. While these findings are contradictory, it should be noted that acute changes in muscle protein synthesis are not well correlated with long-term changes in muscle mass (59). Thus, while whey protein supplementation might increase muscle protein turnover to a greater extent than collagen protein supplementation, the present results indicate that this has no functional long-term effect in healthy older adults.

The impact of resistance exercise on top of whey supplementation was also investigated. The effects of LITW were sparse and inferior to those of HRTW, despite the higher adherence to LITW. While HRTW was effective in increasing muscle strength and the increments in MVIC and DTP were comparable to what has been previously observed (37, 60–62), the lack of change in muscle mass was unexpected. Surprisingly, 1 y of supervised resistance training did not elicit significant increases in qCSA, although several studies have reported 5–10% increases in qCSA after 3–4 mo of training (63–65). However, a number of other studies have

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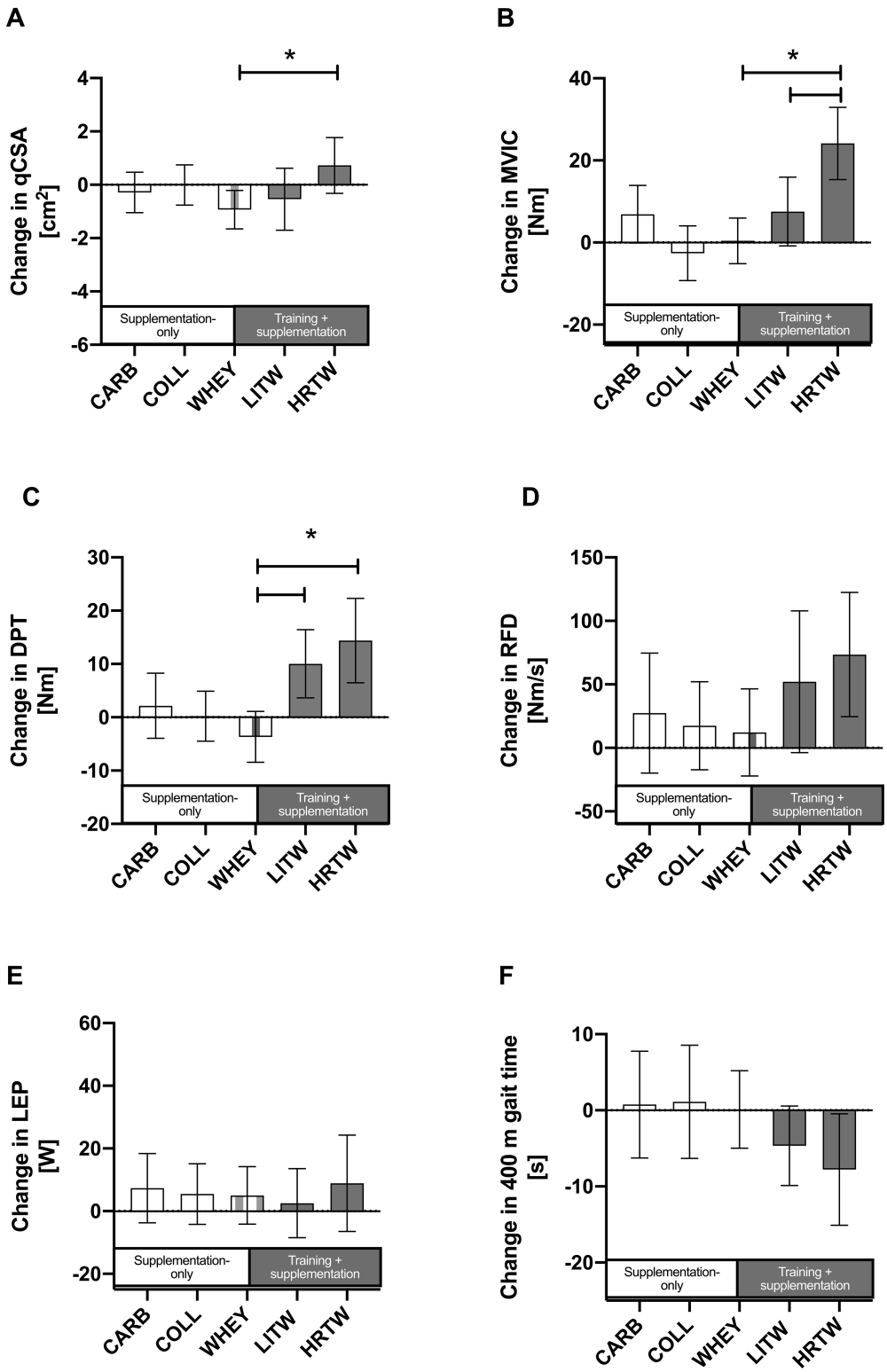

FIGURE 2 Changes in muscle size, strength, and function over the intervention period. Changes are shown from baseline to 12 mo in (A) qCSA; (B) knee extensor MVIC; (C) DTP of the knee extensors; (D) RFD of the knee extensors; (E) LEP; and (F) 400 m gait time. Results are shown as mean changes from baseline to 12 mo of intervention. Error bars indicate 95% CIs. Data were analyzed using a mixed-model analysis with time (baseline and 12 mo) and intervention group (3 levels) as fixed predictors. If the time∗group interaction term was significant ($P < 0.05$), between-group differences were further investigated using a pairwise contrast analysis. *Significant between-group difference in changes over the intervention period. There were 34 participants in the CARB group, 44 in the COLL group, 32 in the HRTW group, 30 in the LITW group, and 44 in the WHEY group. Abbreviations: CARB, carbohydrate supplementation; COLL, collagen protein supplementation; DTP, dynamic peak torque; HRTW, heavy resistance training with whey protein supplementation; LEP, leg extensor power; LITW, light-intensity training with whey protein supplementation; MVIC, maximal voluntary isometric contraction; qCSA, quadriceps cross-sectional area; RFD, rate of force development; WHEY, whey protein supplementation.

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also struggled to induce muscle hypertrophy in older adults (66–70). In the present study, median training adherence corresponded to an average of ∼2 training sessions per week in HRTW, which has been shown previously to induce hypertrophy in older adults (71). However, during the present study, most participants went on vacation for 3–4 weeks during the intervention, causing prolonged breaks from the heavy resistance training. These breaks from training are likely to attenuate the increases in muscle size, and thus could potentially explain the insignificant hypertrophy observed in the present results. Compared to the very intense 3–4-mo training studies previously reported (63–65), we suggest that the present results are more realistic estimates of the effects when recommending that older adults complete resistance training for prolonged periods of time.

While our statistical analysis revealed no between-group differences in changes in functional capabilities, it should be noted that we observed that HRTW improved 400-m gait times. The 400-m gait test has previously been shown to be a strong predictor of both functional capabilities and the risk of future mobility limitations in healthy older adults (52). Furthermore, we have previously shown that strength is a good predictor of functional capabilities in our cohort of older adults (53). Albeit speculative in relation to the present results, our findings suggest that heavy resistance exercise combined with protein supplementation is capable of improving functional capacity even in active older adults.

Limitations

We recruited well-functioning, home-dwelling, healthy older adults with a rather active lifestyle. As a group, they were well nourished and ingested protein, on average, above the current RDA in their habitual diet (56). Hence, the present data cannot be extrapolated to other, more frail elderly people and/or those eating less energy/protein in their normal diet.

Unfortunately, a relatively high number of participants in the present study did not report their adherence to the dietary supplement. The estimated total energy and protein intakes including the supplements in the modified intention-to-treat analysis should therefore be interpreted with caution. Future studies should consider continuous monitoring of adherence in order to minimize the number of missing cases.

Our study did not include training groups not receiving protein supplementation. Therefore, the obtained results in the training groups may not be solely attributed to the training per se, and any interaction between protein supplementation and resistance training cannot be derived from the present study. However, while protein supplementation has been shown to be effective in improving adaptations to resistance training in young individuals (38), the additive effects seem to be minor in older adults (38, 72).

Conclusion

This 1-y intervention study does not support the hypothesis that protein supplementation alone benefits preservation of muscle mass and strength in healthy older adults. Despite seemingly higher compliance, light-resistance, home-based training is not as effective as heavy-load resistance training in increasing muscle size and strength, when combined with whey protein supplementation. Future research and innovation efforts should focus on improving long-term compliance to heavy-resistance exercise in healthy older adults to obtain greater muscular benefits.

The authors' responsibilities were as follows – KHM, MAR: had full access to all the data in the present study, and take responsibility for the integrity of the data analysis; SR, RB, GH, DSN, SBE, APJ, MVL, URM, LH: designed the study; KHM, SR, RB, JB, GH, MJ: acquired the data; KHM, SRS, IT, MAR, LH: analyzed and interpreted the data; KHM, LH: drafted the manuscript; MAR: conducted the statistical analysis; RB, SR: conducted the randomization procedure; LH, APJ, DSN, SBE: obtained funding; and all authors: critically revised the manuscript, had the opportunity to comment on the interpretation of results, and read and approved the final manuscript.

Data Availability

Data described in the article will be made available upon request pending application to the Counteracting Age-Related Loss of Muscle Mass (CALM) trial.

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