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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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Abbreviations:

CARB: Carbohydrate supplementation

CI: Confidence interval

COLL: Collagen protein supplementation

DPT: Dynamic peak torque

HbA1c: Haemoglobin A1c

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

mITT: modified intention-to-treat

MRI: Magnetic resonance imaging

MVIC: Maximal voluntary isometric contraction

PCS: Physical component score

pp: Percentage points

PP: Per protocol

qCSA: Quadriceps cross-sectional area

RDA: Recommended daily allowance

RFD: Rate of force development

RM: Repetition maximum

SD: Standard deviation

WHEY: Whey protein supplementation

ABSTRACT

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

Objective: 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-year randomized controlled trial, 208 healthy older adults (>65 years) were randomly assigned to one of five interventions: 1) Carbohydrate supplementation (CARB), 2) Collagen protein supplementation (COLL), 3) Whey protein supplementation (WHEY), 4) Light-intensity resistance training 3-5 times/week with whey protein supplementation (LITW), 5) Heavy resistance training 3 times weekly with whey protein supplementation (HRTW). Protein supplements contained 20 g protein + 10 g carbohydrate, whereas CARB received 30 g of carbohydrates. All intervention groups received the supplement twice daily. The primary outcome was change in quadriceps cross-sectional area (qCSA), assessed by magnetic resonance imaging. Secondary outcomes included measures of lower extremity strength and power, functional capabilities, and body composition.

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

Conclusions: Recommending protein supplementation as a stand-alone intervention for healthy older individuals appears to be 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

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

BACKGROUND

The progressive age-related decline in muscle mass and function (1–3) has extensively been suggested to 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 recommended daily allowance (RDA) of 0.83 $g \cdot kg^{-1} \cdot day^{-1}$ (6) is associated with higher muscle mass (7–13), as well as a better preservation of muscle mass in older adults (>65 years) (14–16). The latter leading to increased recommendations of 1.1-1.3 g $protein \cdot kg^{-1} \cdot day^{-1}$ for older adults in the recent edition of the Nordic Nutrition Recommendations (17). However, intervention studies investigating the effect of increasing protein intake on muscle mass show mixed results (18–26). The duration of intervention studies are generally short (≤6 months), 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-days integrated muscle protein synthesis compared to a lower-quality protein supplement (collagen). However, to the present authors´ knowledge, it has not been investigated whether whey protein supplementation results in better preservation of muscle mass compared to collagen during long-term supplementation. 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 to increase muscle mass and strength (35–38), some older adults prefer exercise interventions of lower intensity, expensiveness, 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 as heavy resistance exercise for older adults.

The aim of the present study was to investigate the effect 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 has been published previously (44). The regional ethics committee approved the trial protocol (H-4-2013-070), and the subjects gave their written informed consent to participate. The trial was registered at Clinicaltrials.gov (Identifier: NCT02034760).

Study participants:

208 community-dwelling adults aged 65 years and older were recruited. To be included the participants were not allowed to partake in >1 hour 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-year intervention (e.g. diabetes mellitus, unstable cardiac arrhytmia, arthritis, etc.) (44).

Participant recruitment:

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

Randomization:

Following screening and health examination, participants were enrolled in the study and randomized into one of the following five groups using minimization software (MinimPy 0.3; http://minimpy.sourceforge.net/) (45): 1) Carbohydrate supplementation (CARB; 2x20 g maltodextrin + 10 g sucrose), 2) Whey protein supplementation (WHEY; 2x20 g whey protein hydrolysate + 10 g sucrose), 3) Collagen protein supplementation (COLL; 2x20 g bovine collagen protein hydrolysate + 10 g sucrose), 4) Light-intensity training with whey protein supplementation (LITW; 2x20 g whey protein hydrolysate + 10 g sucrose), 5) Heavy resistance training with whey protein supplementation (HRTW; 2x20 g whey protein hydrolysate + 10 g sucrose). Randomization was

done by an investigator not involved in interventions or 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). 16 repetitions were chosen as the cut-off value on the 30-s chair stand test as this is the expected average performance in this test, based on previous findings in age-matched Danes (48).

Interventions:

In all five intervention groups (CARB, COLL, WHEY, LITW, HRTW), participants were instructed to ingest the supplements twice daily, at morning and 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 one 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, Viby J, Denmark. 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.

HRTW performed heavy resistance training 3 times weekly (Monday, Wednesday, and Friday between 9 AM and 11:30 AM under 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 have a duration of ~1 hour. Training was periodized into 3-month cycles, increasing the load progressively from 3 sets of

12 repetitions at 12 repetition maximum (RM) to 5 sets of 6 repetitions at 6 RM in each cycle. LITW performed light load home-based resistance 3-5 times weekly, using rubber bands (TheraBand®, Hygenic Corp., Akron, OH, USA) and bodyweight for exercise chosen to mimic the muscle groups and movements training by HRTW (full details can be found elsewhere (44)). Participants were allowed to perfom 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 change in midthigh quadriceps cross-sectional area (qCSA) of the dominant leg, measured by magnetic resonance imaging (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 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 GRE scout (matrix res. 1.2.0x1.6x6.0 mm, FOV 330mm, TE 3.69ms, TR 7.8ms, scan time 27s); Axial T1 tse from the medial tibia plateau to the pubic symphysis (matrix res. 0.8x0.8x8.0mm, FOV 400mm, TE 8.4ms, TR 500, scan time 3:26). Subjects were instructed to avoid vigorous physical activity for 48 hours prior to the scans. Each scan consisted of six 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 quadriceps cross-sectional area (qCSA). Using OsiriX v. 5.5.2 (OsiriX medical imaging software, Geneva, Switzerland) each scan was analysed twice by the same blinded investigator, showing a mean coefficient of variation between measurements of 0.7%. The mean of the two measurements were used for further analysis.

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 at 70° knee flexion in an isokinetic dynamometer (Kinetic Communicator, model 500-11, Chattanooga, TN, USA). 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 onset of 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 (PCS) and mental component scores (MCS).

Body composition was assessed by dual-energy X-ray absorptiometry (Lunar iDXA, GE Medical Systems, Pewaukee, WI, USA) using enCORE software (version 16). Study participants arrived fasting from 21:00 the night before and refrained from strenuous activities for 48 hours 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 lean tissue mass (LTM) as well as 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, which were 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^{TM}, activPal 3c^{TM}, or activPal micro; PAL technologies, Glasgow, UK) on the anterior surface of the thigh (55). The monitor was worn for 96 continuous hours 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 three 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^{TM}, MADLOG ApS, Kolding, Denmark). Dietary assessments were performed prior to the intervention, and after 11 months of the intervention. Nutrient intake was assessed for foods only. Total protein and energy intake from the supplement was 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 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, HbA1c, blood cholesterol and triglycerides, as well 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 **Supplementary Table 2.**

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 analysing 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 two 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 included 36 participants in HRTW, LITW and CARB groups. Due to taste issues with the protein supplement we expected a higher dropout rate in WHEY and COLL, and therefore included 50 participants in these groups (44).

Statistical analyses:

Baseline data are summarized by group means ± standard deviations (SD) unless otherwise stated. Effects of the interventions were investigated within each study arm, separately. The individual treatment effects are reported as the mean change and associated 95% confidence intervals (CI)

during the intervention. Between-treatment effects are reported as mean difference in treatment effect and associated 95% CI. The level of significance was set to <0.05. The effects of the interventions were analysed using a modified intention-to-treat analytic strategy, including all participants that completed at least one test at the 12-month timepoint, 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, and hence missing data from participants who dropped out of the study was not imputed in the analysis. Effects of the interventions were also analysed using per protocol analytic strategy, including participants with >75% adherence to the supplements, as well as >65% and >75% adherence to training for 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 **Supplementary Table 1 and Supplementary Table 2.**

Changes from baseline to 12 months were investigated in two 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 month) and intervention group (three levels) as fixed predictors, including their interaction, and person as random term. Treatment inferences were based on significance test of the interaction term, and further investigated by contrasts of intervention group changes from baseline to 12 months between all pairs (CARB vs COLL vs WHEY, and WHEY vs LITW vs HRTW) of group combinations. Analyses were not adjusted for covariates or multiple comparisons.

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

RESULTS

In total, we had 1285 contacts from potential participants of which 1148 were screened via telephone. 280 participants were scheduled for an on-site screening visit of which 39 participants declined to participate. 33 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 hour of heavy resistance training weekly, and 1 due to excessive alcohol intake). Consort diagram is shown in **Figure 1**. 208 participants were randomized and 184 completed the 12-month tests characteristics of the included subjects are presented in Table 1. 24 participants 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 LITW compared to the staff-registered adherence to training in HRTW ([Median [Interquartile range]], LITW: 89% [77%, 96%]; HRTW: 72% [62%, 78%]; $P < 0.01$) (see **Supplementary Table 1**). Supplement adherence did not differ significantly between groups (CARB: 95% [77%, 97%]; COLL: 96% [86%, 99%]; WHEY: 88% [82%, 93%], P=0.11), however, a total of 34 participants failed to report their intake of the supplements throughout the intervention (supplemental table 1). 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.

Protein intake increased for COLL ([mean (95% CI)] +29.0 g/day (+21.1, +36.8)), WHEY (+25.7 g/day (+15.6, +35.8)), LITW (+23.9 g/day (+15.2, +32.5)), and HRTW (+26.7 g/day (+18.9, +34.5)) over the intervention period, while energy intake did not change significantly (COLL: +408 kJ/day (-130, +947); WHEY: +518 kJ/day (-322, +1358); LITW: +474 kJ/day (-427, +1375); HRTW: -41 kJ/day (-707, +625)) (see **Table 2**). Energy intake increased for CARB, with no change in protein intake (Energy: +948 kJ/day (+62, +1835); Protein: -4.9 g/day (-15.8, +6.1)).

Quadriceps size

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

Lower body strength and power

No between-group differences were observed in the supplementation-only analysis for neither MVIC (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, with HRTW inducing greater gains in MVIC compared to LITW (Between-group difference: +16.8 Nm (+6.1, +27.4), P = 0.01) and WHEY (+23.9 Nm (+14.2, +33.6), $P< 10^{-5}$). However, changes in MVIC for LITW were not significantly different from WHEY (+7.1 Nm,

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

Functional capabilities

In the supplementation-only analysis, no between-group differences were observed in changes in 400 m gait time (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 (P = 0.14). However, when investigating within-group changes, gait times decreased for HRTW (0-12 months change: -7.8 s (-15.1, -0.45)) and decreased nominally for LITW (-4.7 s (-9.9, +0.6)), with no apparent change in WHEY (+0.1 (-5.0, +5.2)). No between-group differences were observed in changes in MCS (P = 0.83) or PCS (P = 0.49) in the combined training and supplementation analysis.

Body composition

In the supplementation-only analysis, changes in body weight (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 percentage 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 (P = 0.09). Investigating within-group changes in LTM, nominal increases in LTM were observed in HRTW (0-12 month change: +0.39 kg (-0.01, +0.79)), whereas no apparent change was observed for LITW (+0.10 kg (-0.33, +0.54)). Changes in fat percentage (P = 0.10) as well as body weight (P = 0.57) did not differ between groups in the combined training and supplementation analysis.

DISCUSSION

This study investigated the effect of two 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 loss of fat mass (18), but in the present study supplementation of any kind was associated with an increase in fat percentage, with no marked change in LTM or body weight. Although this finding was not controlled against normal eating behavior, 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 the regulation of muscle protein synthesis mainly seem 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 the 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-day 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 year of supervised resistance training did not elicit significant increases in qCSA, which have been shown in several studies reporting 5-10% increments in qCSA after 3-4 months of training (63–65). However, a number of other studies have 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 month training studies previously reported (63–65), we suggest that the present results are more realistic estimates of the effects when recommending older adults to 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 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 on average above current RDA of protein in their habitual diet (56). Hence, the present data cannot be extrapolated to other, more frail elderly people and/or some 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 mITT analysis should therefore be interpreted with caution. Future studies should consider continuous monitoring of adherence registrations 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 therefore 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-year 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.

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382 Mikkelsen is an employee at Arla Foods Ingredients P/S. All other authors report no conflict of
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FIGURE LEGENDS

Figure 1: **CONSORT diagram showing the flow of participants in the CALM trial.**

CARB: Carbohydrate supplementation; COLL: Collagen protein supplementation; HRTW: Heavy resistance training with whey protein supplementation. LITW: Light-intensity training with whey protein supplementation; WHEY: Whey protein supplementation.

Figure 2: **Changes in muscle size, strength and function over the intervention period.**

Changes from baseline to 12 months in A) Quadriceps cross-sectional area (qCSA). B) Knee extensor maximal voluntary isometric contraction (MVIC) C) Dynamic peak torque (DTP) of the knee extensors. D) Rate of force development (RFD) of the knee extensors. E) Leg extensor power. F) 400 m gait time. Results are shown as mean changes from baseline to 12 months of intervention. Error bars indicate 95% confidence intervals. Data were analyzed using a mixed model analysis with time (baseline and 12 months) and intervention group (three levels) as fixed predictors. If the time x group interaction term was significant ($P < 0.05$), between-group differences were further investigated using pairwise contrast analysis. *: Significant between-group difference in changes over the intervention period. CARB: Carbohydrate supplementation (n = 34); COLL: Collagen protein supplementation (n = 44); HRTW: Heavy resistance training with whey protein supplementation (n = 32). LITW: Light-intensity training with whey protein supplementation (n = 30); WHEY: Whey protein supplementation (n = 44).

Table 1. Baseline characteristics of the included participants by group.

	CARB	*COLL*	*WHEY*	*LITW*	*HRTW*
Variable	*(n = 36)*	*(n = 50)*	*(n = 50)*	*(n = 36)*	*(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)*
Sex (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/day	*10894 (5165)*	*10590 (3996)*	*10118 (3590)*	*10119 (3450)*	*9777 (3574)*
Protein intake, g/kg/day	*1.2 (0.3)*	*1.2 (0.4)*	*1.1 (0.3)*	*1.0 (0.3)*	*1.1 (0.4)*
Energy intake, kJ/day	*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 mean (SD). BMI: body mass index; CARB: carbohydrate supplementation; COLL: collagen protein supplementation; DTP: dynamic peak torque; HbA1c: haemoglobin A1c; HDL: high-density lipoprotein; HRTW: heavy resistance training with whey protein supplementation; LDL: low-density lipoprotein; 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: short form 36; WHEY: whey protein supplementation.

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

Changes from 0-12m [Mean (95% CI)]	CARB (n = 34)	COLL (n = 44)	WHEY (n = 44)	LITW (n = 30)	HRTW (n = 32)
Diet					
Estimated total protein intake, g/day	-4.9 (-15.8, 6.1)	+29.0 (21.1, 36.8)*	+25.7 (15.6, 35.8)*	+23.9 (15.2, 32.5)	+26.7 (18.9, 34.5)
Protein intake excluding supplement, g/day	-4.9 (-15.8, 6.1)	-8.3 (-15.5, -1.1)	-6.4 (-15.1, 2.3)	-9.6 (-17.7, -1.5)	-5.8 (-12.3, 0.7)
Estimated total energy intake, kJ/day	+949 (62, 1835)	+408 (-130, 947)	+518 (-322, 1358)	+474 (-427, 1375)	-41, (-707, 625)
Energy intake excluding supplement, g/day	-81 (-960, 798)	-649 (-1176, -122)	-389 (-1197, 419)	-472 (-1351, 406)	-961 (-1609, -314)
Activity level					
Daily activity, Steps/day	-1662 (896)	+330 (589)	-91 (-1217, 1035)	-322 (-1521, 878)	-368 (-1210, 475)
SF-36					
MCS	-1.1 (-2.9, 0.8)	+0.5 (-1.0, +2.0)	-0.2 (-2.5, +2.1)	+0.4 (-1.1, +1.9)	-1.0 (-3.9, +2.0)
PCS	-2.6 (-5.0, -0.2)	-0.6 (-2.4, + 1.1)	-0.4 (-1.6, +0.8)	-0.4 (-1.7, +0.8)	0.0 (-1.6, +1.6)
Body Composition					
Body weight, kg	+1.2 (0.0, +2.3)	+0.7 (-0.1, +1.5)	+0.4 (-0.3, +1.1)	+0.7 (-0.5, +1.8}	-0.2 (-1.3, +1.0)
Lean tissue mass, kg	+0.2 (-0.2, 0.5)	+0.0 (-0.3, 0.3)	-0.2 (-0.5, 0.1)	+0.1 (-0.3, 0.5)	+0.4 (0, 0.8)
Fat percentage, pp	+0.7 (0.0, 1.5)	+0.6 (0.0, 1.2)	+0.7 (0.1, 1.2)	+0.5 (-0.4, 1.3)	-0.4 (-1.3, 0.5)

Values are presented as mean changes from 0-12 months with 95% confidence intervals (95% CI). Data were analyzed using a mixed model analysis with time (baseline and 12 months) and intervention group (three levels) as fixed predictors. If the time x group interaction term was

*significant (P < 0.05), between-group differences were further investigated using pairwise contrast analysis. *: Significantly different compared to CARB in pairwise analysis. 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; SF-36: short form 36; WHEY: whey protein supplementation.*