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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). Secondary outcomes included measures of lower extremity strength and power, functional capabilities, and body composition.

Results: 184 participants completed the study. 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 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

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).

53 Lower intensity training modalities can be effective in enhancing muscle mass (41–43) and when
54 accounting for adherence, a home-based low intensity exercise program might therefore be an
55 equally (or more) effective long-term exercise intervention as heavy resistance exercise for older
56 adults.

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

60 1) Supplementation with higher quality whey protein will benefit muscle size and strength more
61 than supplementation with lower quality collagen protein in healthy older adults.

62 2) Adherence to home-based, light intensity resistance exercise is higher than adherence to
63 Center-based heavy resistance training, and thus exerts an equally beneficial long-term training
64 strategy for gaining/preserving muscle mass and strength when combined with whey protein
65 supplementation.

66 METHODS

67 The Counteracting Age-Related Loss of Muscle Mass (CALM) trial was conducted at Bispebjerg
68 Hospital, Copenhagen, Denmark between 2014 and 2018. The design of the trial and detailed
69 descriptions of methods and exclusion criteria has been published previously (44). The regional
70 ethics committee approved the trial protocol (H-4-2013-070), and the subjects gave their written
71 informed consent to participate. The trial was registered at Clinicaltrials.gov (Identifier:
72 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

140 used for assessing quadriceps cross-sectional area (qCSA). Using OsiriX v. 5.5.2 (OsiriX medical
141 imaging software, Geneva, Switzerland) each scan was analysed twice by the same blinded
142 investigator, showing a mean coefficient of variation between measurements of 0.7%. The mean
143 of the two measurements were used for further analysis.

144 *Secondary outcomes:*

145 To assess lower extremity strength, we measured dynamic peak torque (DPT) during concentric
146 contractions of the knee extensors at movement speeds of 60°/s in a knee joint range of motion
147 from 90° to 10° knee flexion (0° = full extension), as well as maximal voluntary isometric
148 contractions (MVIC) of at 70° knee flexion in an isokinetic dynamometer (Kinetic Communicator,
149 model 500-11, Chattanooga, TN, USA). From the isometric contractions, we also assessed rate of
150 force development (RFD). RFD was measured as the average force development from 0-200 ms
151 after onset of contraction in the MVIC measurements. Furthermore, leg extensor power was
152 measured in the Nottingham Power Rig (Queens Medical Center, Nottingham University, UK) (51).
153 The functional capabilities of the participants were assessed using the 400 m walk test (52).
154 Assessments of gait speed as well as measures of lower extremity strength and power were all
155 performed on the same day, typically the day after the MRI scan, and have been described in
156 detail elsewhere (53). Self-perceived quality of life was measured using the Danish version of the
157 36-item Short Form Health Survey (54). We report changes in the physical (PCS) and mental
158 component scores (MCS).

159 Body composition was assessed by dual-energy X-ray absorptiometry (Lunar iDXA, GE Medical
160 Systems, Pewaukee, WI, USA) using enCORE software (version 16). Study participants arrived
161 fasting from 21:00 the night before and refrained from strenuous activities for 48 hours prior to

162 the test. All scans were performed between 08:00 and 10:00 a week prior to the tests of strength
163 and function. From these scans we obtained lean tissue mass (LTM) as well as body fat
164 percentage. Regions of interest for the extremities and visceral body parts were set based on the
165 default definitions provided by the scanner software. The same examiner controlled the default
166 positioning of all regions, which were adjusted slightly when appropriate to take into account
167 inter-individual differences in body placement and body size.

168 Daily activity levels were measured by attaching an accelerometer-based activity monitor (activPal
169 3™, activPal 3c™, or activPal micro; PAL technologies, Glasgow, UK) on the anterior surface of the
170 thigh (55). The monitor was worn for 96 continuous hours covering a full weekend. Data are
171 represented as the average number of steps per day.

172 A detailed description of the dietary assessment can be found elsewhere (56). Briefly, participants
173 weighed their dietary intake for three consecutive days (Wednesday to Friday), and wrote down
174 the information in food logs. Trained staff then quantified nutrient intake using a dietary
175 assessment tool (VITAKOST™, MADLOG ApS, Kolding, Denmark). Dietary assessments were
176 performed prior to the intervention, and after 11 months of the intervention. Nutrient intake was
177 assessed for foods only. Total protein and energy intake from the supplement was manually
178 estimated by multiplying the compliance to the supplement with the dietary content of the
179 supplement. However, if the participants used other fluids than water for dissolving the
180 supplement, these fluids were registered in the food logs. For the participants who failed to report
181 their compliance to the supplement, but who were still receiving the supplement, we used the
182 median compliance rate from the respective groups.

183 Lastly, HbA1c, blood cholesterol and triglycerides, as well creatinine concentrations were
184 monitored to ensure that the supplementation did not cause the participants to develop insulin
185 resistance, impaired kidney function, or hyperlipidemia. Results from these measurements can be
186 found in **Supplementary Table 2.**

187 *Blinding:*

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

193 *Sample sizes:*

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

201 *Statistical analyses:*

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

205 during the intervention. Between-treatment effects are reported as mean difference in treatment
206 effect and associated 95% CI. The level of significance was set to <0.05. The effects of the
207 interventions were analysed using a modified intention-to-treat analytic strategy, including all
208 participants that completed at least one test at the 12-month timepoint, irrespective of adherence
209 to the interventions. As participants were not blinded to training interventions, we cannot exclude
210 that participants dropped out of the study due to their allocated study arm, and hence missing
211 data from participants who dropped out of the study was not imputed in the analysis. Effects of
212 the interventions were also analysed using per protocol analytic strategy, including participants
213 with >75% adherence to the supplements, as well as >65% and >75% adherence to training for
214 HRTW and LITW, respectively. Results from the per protocol analysis did not differ markedly from
215 the results of the modified intention-to-treat analysis and can be found in **Supplementary Table 1**
216 **and Supplementary Table 2.**

217 Changes from baseline to 12 months were investigated in two separate analyses; an analysis of the
218 effects of protein supplementation alone (CARB vs COLL vs WHEY), and an analysis of the effects of
219 training combined with whey protein supplementation (WHEY vs LITW vs HRTW). These analyses
220 were performed using a longitudinal mixed model with time (baseline and 12 month) and
221 intervention group (three levels) as fixed predictors, including their interaction, and person as
222 random term. Treatment inferences were based on significance test of the interaction term, and
223 further investigated by contrasts of intervention group changes from baseline to 12 months
224 between all pairs (CARB vs COLL vs WHEY, and WHEY vs LITW vs HRTW) of group combinations.
225 Analyses were not adjusted for covariates or multiple comparisons.

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

229 RESULTS

230 In total, we had 1285 contacts from potential participants of which 1148 were screened via
231 telephone. 280 participants were scheduled for an on-site screening visit of which 39 participants
232 declined to participate. 33 were excluded prior to enrollment in the study (30 due to medications
233 or diseases not discovered in the phone-screening, 2 due to performing >1 hour of heavy
234 resistance training weekly, and 1 due to excessive alcohol intake). Consort diagram is shown in
235 **Figure 1**. 208 participants were randomized and 184 completed the 12-month tests characteristics
236 of the included subjects are presented in **Table 1**. 24 participants dropped out during the study; 11
237 due to illness or injury unrelated to the intervention, 5 due to disliking the supplement, 3 due to
238 the testing being too extensive, and 5 due to personal reasons.

239 *Adherence*

240 Self-reported adherence to training was significantly higher for LITW compared to the staff-
241 registered adherence to training in HRTW ([Median [Interquartile range]], LITW: 89% [77%, 96%];
242 HRTW: 72% [62%, 78%]; P < 0.01) (see **Supplementary Table 1**). Supplement adherence did not
243 differ significantly between groups (CARB: 95% [77%, 97%]; COLL: 96% [86%, 99%]; WHEY: 88%
244 [82%, 93%], P=0.11), however, a total of 34 participants failed to report their intake of the
245 supplements throughout the intervention (supplemental table 1). These participants all came to
246 the research facilities to receive additional supplements as planned, but they are not included in
247 the adherence values due to their insufficient reporting of supplement intake.

248 In the supplementation-only analysis, changes in protein intake differed between groups
249 (Time*group interaction: $P < 10^{-5}$, **Table 2**). Compared to CARB, protein intake increased for COLL
250 ([mean between-group difference (95% CI)] +32.3 g/day (+19.7, +45.0), $P < 10^{-5}$) and WHEY (+27.2
251 g/day (+14.4, +39.9), $P < 10^{-4}$). In the combined training and supplementation analysis, changes in
252 protein intake did not differ between groups (Time*group interaction: P = 0.95). Changes in
253 energy intake did not differ significantly between groups in the supplementation-only analysis
254 (Time *group interaction: P = 0.49) or in the combined training and supplementation analysis
255 (Time*group interaction: P = 0.69).

256 *Quadriceps size*

257 In the supplementation-only analysis, we observed no between-group differences in changes in
258 qCSA, (Time*group interaction: P=0.17, **Figure 2A**). In the combined training and supplementation
259 analysis, the Time*group interaction term was significant (P=0.04). HRTW was associated with a
260 more positive change in qCSA compared to WHEY (Between-group difference [mean (95% CI)]:
261 +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).
262 Changes in qCSA were not significantly different for LITW compared to WHEY (+0.39 cm^2 (-0.88,
263 +1.66), P=0.82). Investigating within-group changes in qCSA, neither HRTW (0-12 month change:
264 +0.73 cm^2 (-0.32, +1.77)) nor LITW (-0.54 cm^2 (-1.70, +0.62)) exhibited marked changes, whereas a
265 decrease in qCSA was observed for WHEY (-0.93 cm^2 (-1.65, -0.21)).

266 *Lower body strength and power*

267 No between-group differences were observed in the supplementation-only analysis for neither
268 MVIC (Time*group interaction: P = 0.13, **Figure 2B**), DPT (P = 0.24, **Figure 2C**), RFD (P = 0.86, **Figure**
269 **2D**) or leg extensor power (P = 0.94, **Figure 2E**). In the combined training and supplementations

270 groups, changes in MVIC differed between groups (Time*group interaction: $P < 10^{-4}$), with HRTW
271 inducing greater gains in MVIC compared to LITW (Between-group difference: +16.8 Nm (+6.1,
272 +27.4), P = 0.01) and WHEY (+23.9 Nm (+14.2, +33.6), $P< 10^{-5}$). However, changes in MVIC for LITW
273 were not significantly different from WHEY (+7.1 Nm, (-2.8, 17.1), P = 0.34). Changes in DPT
274 differed between groups in the combined training and supplementation analysis (Time*Group
275 interaction: $P < 10^{-4}$). DPT increased in both HRTW (Between-group difference: +18.4 Nm (+10.1,
276 +26.6), $P <10^{-4}$) and LITW (+13.7 Nm (+5.3, +22.1), P = 0.01) compared to WHEY, but with no
277 significant difference between HRTW and LITW (+4.7 Nm (-4.4, +13.7), P = 0.57). No between-
278 group differences were observed in changes in RFD (Time*group interaction: P = 0.12) or leg
279 extensor power (P = 0.73) in the combined training and supplementation analysis. However, when
280 investigating within-group changes, HRTW increased RFD (0-12 month change: +73.5 Nm/s (+24.6,
281 +122.4)), with nominal increases in LITW (+52.1 Nm/s (-3.8, +108.0) and no apparent change in
282 WHEY (+12.2 Nm/s (-22.1, +46.5)).

283 *Functional capabilities*

284 In the supplementation-only analysis, no between-group differences were observed in changes in
285 400 m gait time (Time*group interaction: P = 0.99, **Figure 2F**), MCS (P = 0.36), or PCS (P = 0.38,
286 **Table 2**). In the combined training and supplementation analysis, changes in 400 m gait times
287 were not significantly different between groups (Time*group interaction: P = 0.14). However,
288 when investigating within-group changes, gait times decreased for HRTW (0-12 months change: -
289 7.8 s (-15.1, -0.45)) and decreased nominally for LITW (-4.7 s (-9.9, +0.6)), with no apparent change
290 in WHEY (+0.1 (-5.0, +5.2)). No between-group differences were observed in changes in MCS
291 (Time*group interaction: P = 0.83) or PCS (P = 0.49, **Table 2**) in the combined training and
292 supplementation analysis.

293 *Body composition*

294 In the supplementation-only analysis, changes in body weight (Time*group interaction: P = 0.46),
295 fat percentage (P = 0.95), and LTM (P = 0.29) did not differ between groups. However, when
296 investigating within-group changes, increases in fat percentage were observed in all
297 supplementation-only groups, with no marked changes in LTM or body weight (**Table 2**). In the
298 combined training and supplementation analysis, changes in LTM were not significantly different
299 between groups (Time*group interaction: P = 0.09). Investigating within-group changes in LTM,
300 nominal increases in LTM were observed in HRTW (0-12 month change: +0.39 kg (-0.01, +0.79)),
301 whereas no apparent change was observed for LITW (+0.10 kg (-0.33, +0.54)). Changes in fat
302 percentage (Time*group interaction: P = 0.10) as well as body weight (P = 0.57) did not differ
303 between groups in the combined training and supplementation analysis.

304 DISCUSSION

305 This study investigated the effect of two modifiable strategies to counteract age-related loss of
306 muscle mass in older adults; protein supplementation alone and or combined with resistance
307 exercise. Increasing daily protein intake from ~1.1 $g \cdot kg^{-1}$ to ~1.5 $g \cdot kg^{-1}$ by providing daily protein
308 supplements to healthy home-dwelling older individuals had no beneficial effects in any of the
309 performed measures. These results provide strong evidence that an increase in protein intake
310 alone does not add a benefit in preserving muscle mass or strength in healthy older adults living
311 independently and eating in accordance with current guidelines. Increasing protein content in an
312 iso-caloric diet has been shown to result in loss of fat mass (18), but in the present study
313 supplementation of any kind was associated with an increase in fat percentage, with no marked
314 change in LTM or body weight. Although this finding was not controlled against normal eating
315 behavior, increasing fat percentage could indicate that the older adults in the present study did

316 not adjust energy intake and/or expenditure sufficiently when supplemented with extra calories,
317 irrespective of the source of supplemented calories (protein/carbohydrate). As the regulation of
318 muscle protein synthesis mainly seem to be regulated by the essential amino acid (EAA) content of
319 the ingested protein (58), future studies could consider supplementing EAAs alone to avoid the
320 additional calories.

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

332 The impact of resistance exercise on top of whey supplementation was also investigated. The
333 effects of LITW were sparse and inferior to those of HRTW, despite the higher adherence to LITW.
334 While HRTW was effective in increasing muscle strength and the increments in MVIC and DTP
335 were comparable to what has been previously observed (37,60–62), the lack of change in muscle
336 mass was unexpected. Surprisingly, 1 year of supervised resistance training did not elicit significant
337 increases in qCSA, which have been shown in several studies reporting 5-10% increments in qCSA

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

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

356 LIMITATIONS

357 We recruited well-functioning home-dwelling healthy older adults with a rather active lifestyle. As
358 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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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 (LEP). 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					
HA1c, 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; HA1c: hemoglobin 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.

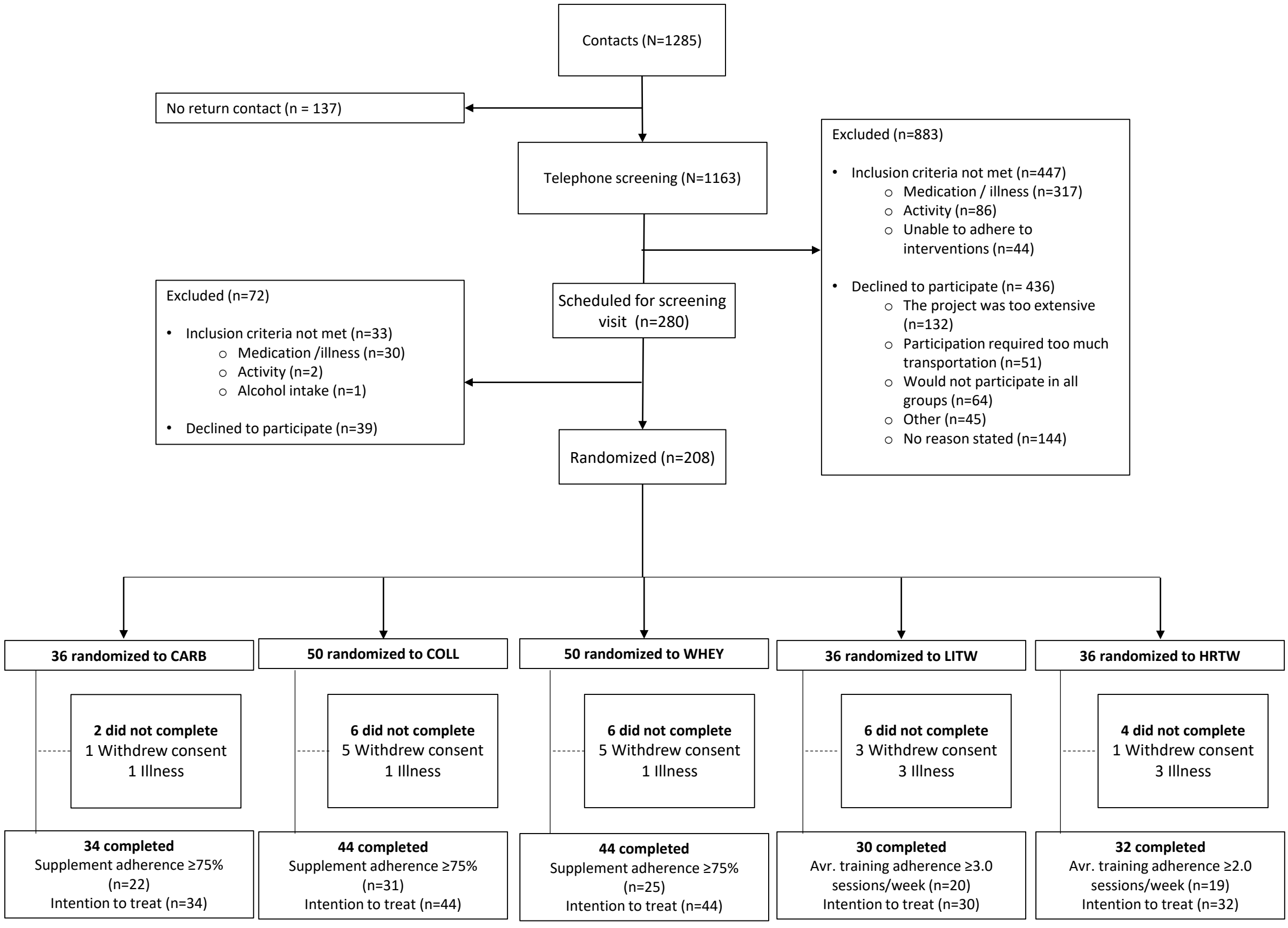
Table 2. Changes in dietary intake, activity level, self-perceived health, and body composition.

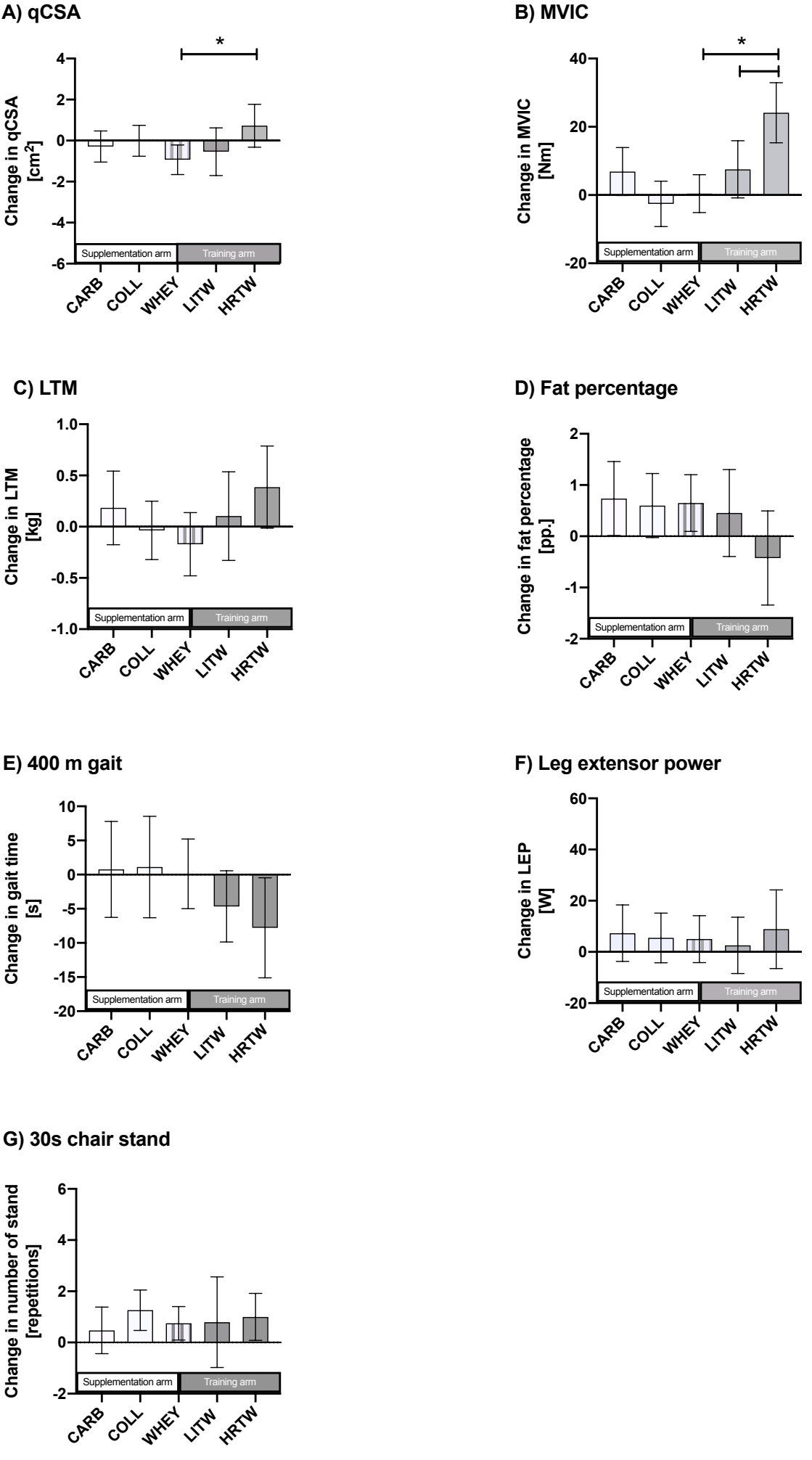
Changes from 0-12m [Mean (95% CI)]	CARB	COLL	WHEY	LITW	HRTW
	(n = 34)	(n = 44)	(n = 44)	(n = 30)	(n = 32)
Diet					
Estimated total protein intake, g/day	-4.9 (-15.8, 6.1)	+29.0 (+21.1, +36.8)*	+25.7 (+15.6, +35.8)*	+23.9 (+15.2, +32.5)	+26.7 (+18.9, +34.5)
Protein intake excluding supplement, g/day	-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 (-3505, +180)	+330 (-869, +1529)	-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.*


Contacts (N=1285)
No return contact (n = 137)
Telephone screening (N=1163)
Excluded (n=883)
• Inclusion criteria not met (n=447)
○ Medication / illness (n=317)
○ Activity (n=86)
○ Unable to adhere to interventions (n=44)
• Declined to participate (n= 436)
○ The project was too extensive (n=132)
○ Participation required too much transportation (n=51)
○ Would not participate in all groups (n=64)
○ Other (n=45)
○ No reason stated (n=144)
Scheduled for screening visit (n=280)
Excluded (n=72)
• Inclusion criteria not met (n=33)
○ Medication /illness (n=30)
○ Activity (n=2)
○ Alcohol intake (n=1)
• Declined to participate (n=39)
Randomized (n=208)
36 randomized to CARB
50 randomized to COLL
50 randomized to WHEY
36 randomized to LITW
36 randomized to HRTW
2 did not complete
1 Withdrew consent
1 Illness
6 did not complete
5 Withdrew consent
1 Illness
6 did not complete
5 Withdrew consent
1 Illness
6 did not complete
3 Withdrew consent
3 Illness
4 did not complete
1 Withdrew consent
3 Illness
34 completed
Supplement adherence ≥75% (n=22)
Intention to treat (n=34)
44 completed
Supplement adherence ≥75% (n=31)
Intention to treat (n=44)
44 completed
Supplement adherence ≥75% (n=25)
Intention to treat (n=44)
30 completed
Avr. training adherence ≥3.0 sessions/week (n=20)
Intention to treat (n=30)
32 completed
Avr. training adherence ≥2.0 sessions/week (n=19)
Intention to treat (n=32)


A) qCSA
Change in qCSA [cm²]
4
2
0
-2
-4
-6
*
Supplementation arm
Training arm
CARB
COLL
WHEY
LITW
HRTW
B) MVIC
Change in MVIC [Nm]
40
20
0
-20
*
Supplementation arm
Training arm
CARB
COLL
WHEY
LITW
HRTW
C) LTM
Change in LTM [kg]
1.0
0.5
0.0
-0.5
-1.0
Supplementation arm
Training arm
CARB
COLL
WHEY
LITW
HRTW
D) Fat percentage
Change in fat percentage [pp.]
2
1
0
-1
-2
Supplementation arm
Training arm
CARB
COLL
WHEY
LITW
HRTW
E) 400 m gait
Change in gait time [s]
10
5
0
-5
-10
-15
-20
Supplementation arm
Training arm
CARB
COLL
WHEY
LITW
HRTW
F) Leg extensor power
Change in LEP [W]
60
40
20
0
-20
Supplementation arm
Training arm
CARB
COLL
WHEY
LITW
HRTW
G) 30s chair stand
Change in number of stand [repetitions]
6
4
2
0
-2
Supplementation arm
Training arm
CARB
COLL
WHEY
LITW
HRTW