

Dear Author,

Here are the proofs of your article.

- You can submit your corrections **online**, via **e-mail** or by **fax**.
- For **online** submission please insert your corrections in the online correction form. Always indicate the line number to which the correction refers.
- You can also insert your corrections in the proof PDF and **email** the annotated PDF.
- For fax submission, please ensure that your corrections are clearly legible. Use a fine black pen and write the correction in the margin, not too close to the edge of the page.
- Remember to note the **journal title**, **article number**, and **your name** when sending your response via e-mail or fax.
- **Check** the metadata sheet to make sure that the header information, especially author names and the corresponding affiliations are correctly shown.
- **Check** the questions that may have arisen during copy editing and insert your answers/ corrections.
- **Check** that the text is complete and that all figures, tables and their legends are included. Also check the accuracy of special characters, equations, and electronic supplementary material if applicable. If necessary refer to the *Edited manuscript*.
- The publication of inaccurate data such as dosages and units can have serious consequences. Please take particular care that all such details are correct.
- Please **do not** make changes that involve only matters of style. We have generally introduced forms that follow the journal's style. Substantial changes in content, e.g., new results, corrected values, title and authorship are not allowed without the approval of the responsible editor. In such a case, please contact the Editorial Office and return his/her consent together with the proof.
- If we do not receive your corrections **within 48 hours**, we will send you a reminder.
- Your article will be published **Online First** approximately one week after receipt of your corrected proofs. This is the **official first publication** citable with the DOI. **Further changes are, therefore, not possible.**
- The **printed version** will follow in a forthcoming issue.

### Please note

After online publication, subscribers (personal/institutional) to this journal will have access to the complete article via the DOI using the URL: [http://dx.doi.org/\[DOI\]](http://dx.doi.org/[DOI]).

If you would like to know when your article has been published online, take advantage of our free alert service. For registration and further information go to: <http://www.link.springer.com>.

Due to the electronic nature of the procedure, the manuscript and the original figures will only be returned to you on special request. When you return your corrections, please inform us if you would like to have these documents returned.

# Metadata of the article that will be visualized in OnlineFirst

|                      |  |   |
|----------------------|--|---|
| ArticleTitle         | Effects of exercise and whey protein on muscle mass, fat mass, myoelectrical muscle fatigue and health-related quality of life in older adults: a secondary analysis of the Liverpool Hope University, Sarcopenia Ageing Trial (LHU-SAT) |   |
| Article Sub-Title    |  |   |
| Article CopyRight    | Springer-Verlag GmbH Germany, part of Springer Nature<br>(This will be the copyright line in the final PDF)  |   |
| Journal Name         | European Journal of Applied Physiology   |   |
| Corresponding Author | Family Name  | <b>Kirk</b>   |
|                      | Particle   |   |
|                      | Given Name   | <b>Ben</b>  |
|                      | Suffix   |   |
|                      | Division   | School of Health Sciences   |
|                      | Organization   | Liverpool Hope University   |
|                      | Address  | Liverpool, UK   |
|                      | Division   | Department of Medicine, Western Health, Melbourne Medical School                        |
|                      | Organization   | University of Melbourne   |
|                      | Address  | 176 Furlong Road, St. Albans, Melbourne, VIC, 3121, Australia                           |
|                      | Division   | Australian Institute for Musculoskeletal Science (AIMSS)                                |
|                      | Organization   | University of Melbourne and Western Health  |
|                      | Address  | St Albans, Melbourne, VIC, Australia  |
|                      | Phone  |   |
|                      | Fax  |   |
|                      | Email  | ben.kirk@unimelb.edu.au   |
|                      | URL  |   |
|                      | ORCID  | <a href="http://orcid.org/0000-0002-0176-776X">http://orcid.org/0000-0002-0176-776X</a> |
| Author               | Family Name  | <b>Mooney</b>   |
|                      | Particle   |   |
|                      | Given Name   | <b>Kate</b>   |
|                      | Suffix   |   |
|                      | Division   | School of Health Sciences   |
|                      | Organization   | Liverpool Hope University   |
|                      | Address  | Liverpool, UK   |
|                      | Phone  |   |
|                      | Fax  |   |
|                      | Email  |   |
|                      | URL  |   |
|                      | ORCID  |   |
| Author               | Family Name  | <b>Cousins</b>  |
|                      | Particle   |   |
|                      | Given Name   | <b>Rosanna</b>  |
|                      | Suffix   |   |

Division School of Health Sciences  
Organization Liverpool Hope University  
Address Liverpool, UK  
Phone  
Fax  
Email  
URL  
ORCID

---

Author Family Name **Angell**  
Particle  
Given Name **Peter**  
Suffix  
Division School of Health Sciences  
Organization Liverpool Hope University  
Address Liverpool, UK  
Phone  
Fax  
Email  
URL  
ORCID

---

Author Family Name **Jackson**  
Particle  
Given Name **Matthew**  
Suffix  
Division School of Health Sciences  
Organization Liverpool Hope University  
Address Liverpool, UK  
Phone  
Fax  
Email  
URL  
ORCID

---

Author Family Name **Pugh**  
Particle  
Given Name **Jamie N.**  
Suffix  
Division Research Institute for Sport and Exercise Sciences  
Organization Liverpool John Moores University  
Address Liverpool, UK  
Phone  
Fax  
Email  
URL  
ORCID

---

Author Family Name **Coyles**

Particle  
Given Name **Ginny**  
Suffix  
Division School of Health Sciences  
Organization Liverpool Hope University  
Address Liverpool, UK  
Phone  
Fax  
Email  
URL  
ORCID

---

Author Family Name **Amirabdollahian**  
Particle  
Given Name **Farzad**  
Suffix  
Division School of Health Sciences  
Organization Liverpool Hope University  
Address Liverpool, UK  
Phone  
Fax  
Email  
URL  
ORCID

---

Author Family Name **Khaiyat**  
Particle  
Given Name **Omid**  
Suffix  
Division School of Health Sciences  
Organization Liverpool Hope University  
Address Liverpool, UK  
Phone  
Fax  
Email  
URL  
ORCID

---

Schedule Received 5 August 2019  
Revised  
Accepted 21 December 2019

---

Abstract

*Purpose:*

To investigate the effects of exercise in combination with, or without, a leucine-enriched whey protein supplement on muscle mass, fat mass, myoelectrical muscle fatigue and health-related quality of life (HR-QOL) in older adults.

*Methods:*

100 community-dwelling older adults [52% women, age:  $69 \pm 6$  years (mean  $\pm$  SD)] were randomised to four [Control (C); Exercise (E); Exercise + Protein (EP); Protein (P)] independent groups. E and EP groups completed 16 weeks of exercise [resistance (2 times/week) and functional (1 time/week)]. EP and P groups

were also administered a leucine-enriched whey protein supplement (3 times/day) based on body weight (1.5 g/kg/day). Muscle and fat mass (bioelectrical impedance analysis), myoelectrical muscle fatigue (surface electromyography) and <sub>HR</sub>-QOL (WHOQOL-BREF) were measured pre- and post-intervention.

*Results:*

At post-intervention, the rectus femoris (*E*: - 4.8%/min, *p* = 0.007, ES = 0.86; *EP*: - 3.3%/min, *p* = 0.045, ES = 0.58) and bicep femoris (*E*: - 3.9%/min, *p* < 0.001, ES = 1.46; *EP*: - 4.3%/min, *p* < 0.001, ES = 1.58) muscles became more resistant to fatigue in the E and EP groups, respectively (*p* < 0.05 versus C). <sub>HR</sub>-QOL improved in the E group only. Muscle and fat mass did not change (*p* > 0.05).

*Conclusion:*

Physical exercise is a potent method to improve myoelectrical muscle fatigue and <sub>HR</sub>-QOL in older adults. However, leucine-enriched whey protein did not augment this response in those already consuming sufficient quantities of protein at trial enrolment.

---

Keywords (separated by '-') Exercise - Whey protein - Myoelectrical muscle fatigue - Quality of life

---

Footnote Information Communicated by Guido Ferretti .

---



2 **Effects of exercise and whey protein on muscle mass, fat mass,**  
3 **myoelectrical muscle fatigue and health-related quality of life**  
4 **in older adults: a secondary analysis of the Liverpool Hope University,**  
5 **Sarcopenia Ageing Trial (LHU-SAT)**

6 Ben Kirk<sup>1,2,3</sup> · Kate Mooney<sup>1</sup> · Rosanna Cousins<sup>1</sup> · Peter Angell<sup>1</sup> · Matthew Jackson<sup>1</sup> · Jamie N. Pugh<sup>4</sup> ·  
7 Ginny Coyles<sup>1</sup> · Farzad Amirabdollahian<sup>1</sup> · Omid Khaiyat<sup>1</sup>

8 Received: 5 August 2019 / Accepted: 21 December 2019  
9 © Springer-Verlag GmbH Germany, part of Springer Nature 2019

10 **Abstract**

11 **Purpose** To investigate the effects of exercise in combination with, or without, a leucine-enriched whey protein supplement  
12 on muscle mass, fat mass, myoelectrical muscle fatigue and health-related quality of life (<sub>HR</sub>-QOL) in older adults.

13 **Methods** 100 community-dwelling older adults [52% women, age: 69 ± 6 years (mean ± SD)] were randomised to four [Con-  
14 trol (C); Exercise (E); Exercise + Protein (EP); Protein (P)] independent groups. E and EP groups completed 16 weeks of  
15 exercise [resistance (2 times/week) and functional (1 time/week)]. EP and P groups were also administered a leucine-enriched  
16 whey protein supplement (3 times/day) based on body weight (1.5 g/kg/day). Muscle and fat mass (bioelectrical impedance  
17 analysis), myoelectrical muscle fatigue (surface electromyography) and <sub>HR</sub>-QOL (WHOQOL-BREF) were measured pre-  
18 and post-intervention.

19 **Results** At post-intervention, the rectus femoris (*E*: -4.8%/min, *p* = 0.007, ES = 0.86; EP: -3.3%/min, *p* = 0.045, ES = 0.58)  
20 and bicep femoris (*E*: -3.9%/min, *p* < 0.001, ES = 1.46; EP: -4.3%/min, *p* < 0.001, ES = 1.58) muscles became more resist-  
21 ant to fatigue in the E and EP groups, respectively (*p* < 0.05 versus C). <sub>HR</sub>-QOL improved in the E group only. Muscle and  
22 fat mass did not change (*p* > 0.05).

23 **Conclusion** Physical exercise is a potent method to improve myoelectrical muscle fatigue and <sub>HR</sub>-QOL in older adults.  
24 However, leucine-enriched whey protein did not augment this response in those already consuming sufficient quantities of  
25 protein at trial enrolment.

26 **Keywords** Exercise · Whey protein · Myoelectrical muscle fatigue · Quality of life

27 **Abbreviations**

|         |   |          |
|---------|---|----------|
| BIA     | Bioelectrical impedance analysis                          | 28       |
| BMI     | Body mass index   | 29       |
| C       | Control   | 30       |
| E       | Exercise  | 31       |
| EMG     | Electromyography  | 32       |
| EP      | Exercise + Protein  | 33       |
| ESPEN   | European Society for Clinical Nutrition and<br>Metabolism | 34<br>35 |
| HR-QOL  | Health-related quality of life                            | 36       |
| LHU-SAT | Liverpool Hope University—Sarcopenia<br>Ageing Trial      | 37<br>38 |
| MVC     | Maximal voluntary contraction                             | 39       |
| P       | Protein   | 40       |
| RCT     | Randomised controlled trial                               | 41       |
| RDA     | Recommended dietary allowance                             | 42       |

A1 Communicated by Guido Ferretti .

A2 ✉ Ben Kirk  
A3 ben.kirk@unimelb.edu.au

A4 <sup>1</sup> School of Health Sciences, Liverpool Hope University,  
A5 Liverpool, UK

A6 <sup>2</sup> Department of Medicine, Western Health, Melbourne  
A7 Medical School, University of Melbourne, 176 Furlong  
A8 Road, St. Albans, Melbourne, VIC 3121, Australia

A9 <sup>3</sup> Australian Institute for Musculoskeletal Science  
A10 (AIMSS), University of Melbourne and Western Health,  
A11 St Albans, Melbourne, VIC, Australia

A12 <sup>4</sup> Research Institute for Sport and Exercise Sciences, Liverpool  
A13 John Moores University, Liverpool, UK

43 SD Standard deviation  
44 SMI Skeletal muscle index

## 45 Introduction

46 Age-related decreases in muscle mass and strength, and  
47 increases in fat mass, are hallmarks of ageing (Zamboni  
48 et al. 2008). When occurring simultaneously, these changes  
49 can be described as a hazardous duet, elevating the risk of  
50 falls and fractures (Scott et al. 2014). A proxy of muscle  
51 function, known as muscle fatigue (defined as the temporary  
52 decline in muscle force/power), is also linked to a reduction  
53 in balance and walking performance (Senefeld et al. 2017),  
54 and an increase in fall risk when the myoelectrical properties  
55 of an aged muscle are examined (Schwendner et al. 1997).  
56 As such, strategies to maximise musculoskeletal health  
57 whilst limiting adipose tissue accumulation are an urgent  
58 socioeconomic need.

59 Physical activity, particularly strength- and functional-  
60 based movements, are recommended to support gains in  
61 muscle mass and strength (Morton et al. 2017), as well as  
62 neuromuscular qualities such as balance, flexibility and  
63 endurance (Liu et al. 2014). These benefits also translate  
64 into enhancements in health-related quality of life ( $_{HR}$ -QOL)  
65 (Hart and Buck 2019). Moreover, the PROT-AGE (Bauer  
66 et al. 2013) and ESPEN (Deutz et al. 2014) consensus groups  
67 advocate a higher intake of protein (1.0–1.2 and  $\geq$  1.2 g/kg/  
68 day, respectively) including leucine (2.5–3 g per meal) to  
69 increase muscle mass and function (strength or performance)  
70 in healthy older adults undergoing exercise. Furthermore,  
71 protein metabolism studies comparing young and old show  
72 that to maximise muscle protein turnover, an intake of 1.5 g/  
73 kg/day should be prescribed in the latter cohort (Moore et al.  
74 2015). Remaining physically active and consuming a higher  
75 protein diet are also connected to a healthier body composi-  
76 tional status (Houston et al. 2008), although less is known  
77 regarding the effects of protein alone on  $_{HR}$ -QOL.

78 Despite these advancements in knowledge, a recent meta-  
79 analysis showed that there are inconsistent findings from  
80 randomised controlled trials (RCTs) regarding the benefits of  
81 protein intake alone or combined with resistive exercise on  
82 muscle and fat mass in healthy older adults (Ten Haaf et al.  
83 2019). This is likely due to heterogeneity factors with most  
84 trials not achieving the upper per meal threshold of protein  
85 intake required to maximise muscle protein synthesis rates  
86 (Moore et al. 2015). In addition, several trials have failed to  
87 include a protein group alone which rules out the possible  
88 benefits of this nutrient for older adults who are not willing  
89 or able to exercise. It should also be noted that other RCTs  
90 (Norton et al. 2016) and cross-sectional studies (Houston  
91 et al. 2008) demonstrated that muscle mass still declines in  
92 healthy older adults with a protein intake of 1–1.2 g/kg/day,

which supports the upper protein recommendation of 1.5 g/  
kg/day by Moore et al. 2015 to maximise the accretion of  
muscle proteins.

There is also a complete lack of data investigating the  
effect of protein intake (with or without exercise) on myoe-  
lectrical descriptors of fatigue, which is surprising consid-  
ering that neural adaptations to exercise are suggested to  
play a more significant role with advancing age (Sale 1988).  
In addition, increases in muscle fatigue results in impaired  
balance and walking performance (Senefeld et al. 2017) and  
increases the risk of falling (Schwendner et al. 1997). As  
such, further RCTs are warranted to address these knowl-  
edge gaps.

We previously reported [Liverpool Hope University—  
Sarcopenia Ageing Trial (LHU-SAT)] on adaptations in  
muscle strength, physical functioning, aerobic capacity and  
cardiometabolic health, following a 16-week RCT which  
investigated the effects of exercise and protein supplemen-  
tation in older adults (Kirk et al. 2019). We also reported  
on physical activity levels 6 months post-completion of this  
trial (Kirk et al. 2019).

Here, we conducted a secondary analysis of the LHU-  
SAT to examine the effects on (1) muscle mass, (2) fat  
mass, (3) myoelectrical muscle fatigue and (4)  $_{HR}$ -QOL, in  
older adults. We hypothesised that increasing protein intake  
to  $\sim$  1.5 g/kg/day with sufficient quantities of leucine ( $>$  3 g  
per serving) would increase muscle mass, decrease fat mass  
and attenuate myoelectrical manifestations of fatigue, and  
these benefits would translate into enhancements of  $_{HR}$ -QOL.

## Methods

### Trial design

The LHU-SAT was a randomised, single-blind, four-group  
[Control (C); Exercise (E); Exercise + Protein (EP); Protein  
(P)], trial conducted in the UK between September 2016 and  
March 2018 (Trial Registration: Clinicaltrials.gov; Identifier:  
NCT02912130). Recruitment, randomisation, study proced-  
ures and inclusion and exclusion criteria have previously  
been described in detail elsewhere (Kirk et al. 2019). Prior  
to study commencement, all participants provided written  
informed consent and ethical approval was granted from the  
North-West of England NHS Research Ethics Committee  
UK (REC Number: 16/NW/0480). Primary and secondary  
outcomes of LHU-SAT can be viewed at: <https://clinicaltrials.gov/ct2/show/NCT02912130>. Figure 1 provides a sche-  
matic of the trial design.



Fig. 1 Schematic of the trial design

**Participants**

The baseline characteristics of participants are presented in Table 1. Participants were ambulant, community-dwelling older adults ( $\geq 60$  years) free of pre-existing medical conditions and largely British Caucasian (98%). Recruitment was conducted via poster advertisements (at local community centres, ageing charity shops, GP surgeries) and those who expressed an interest contacted the researchers (BK and KM) either via telephone or by enquiring at Liverpool Hope University. Eligibility was confirmed by inclusion/exclusion criteria which can be viewed at: <https://clinicaltrials.gov/ct2/show/NCT02912130>.

If eligible, participants attended the clinical laboratories in the fasted state where outcome measures (muscle mass, fat mass, myoelectrical muscle fatigue, health-related quality of life surveys) were performed within 7 days of commencement, and completion, of the trial. To minimise diurnal variation, the outcome measures were carried out in the morning period before and after the intervention. Participants were then block randomised to one of four independent groups by an external member not part of the research team.

**Exercise intervention**

E and EP trial groups completed 16 weeks of exercise [resistance (2 times/week) and functional (1 time/week)] on non-consecutive days. All exercise sessions were carried out and supervised by the researchers [BK and KM (degree qualified sport and exercise scientists)], and attendance was recorded by administrative staff at the gymnasium reception. Briefly, progressive resistance exercise comprised eight exercises, including leg press, chest press, calf press, shoulder press, seated row, back extension and bicep curl. Participants completed two sets to fatigue of each exercise with 3-min breaks between sets. Over the 16 weeks, weight was increased by 2.5 and 5 kg for upper and lower body exercises (respectively) when 12 or more repetitions could be completed in two consecutive sets. Functional exercise was employed to improve mobility, balance and endurance, as well as to practise functional-based movements of daily living. The functional exercise circuit consisted of 12 bases with 1 min of exercise performed at each base. The star exercise was performed first, followed by wall pushup, battle ropes, Superman, hip thrust, single leg balance, hip hinge, ball throw, lunge, knee plank and box squat and finished with a mini obstacle course. For further details and schematic, see Kirk et al. (2019).

**Table 1** Baseline characteristics of participants

| Parameter                | Control     | Exercise    | Exercise + Protein | Protein     |
|--------------------------|-------------|-------------|--------------------|-------------|
| <i>n</i> = [number]      | 31          | 24          | 22                 | 23          |
| Sex [men/women]          | 13/18       | 12/12       | 9/13               | 14/9        |
| Age [yrs]                | 68 ± 6      | 66 ± 4      | 69 ± 6             | 72 ± 6      |
| Height [m]               | 1.66 ± 0.9  | 1.68 ± 0.1  | 1.64 ± 0.1         | 1.68 ± 0.1  |
| Weight [kg]              | 72.6 ± 13.4 | 79.5 ± 21.6 | 74.2 ± 18.1        | 76.3 ± 12.7 |
| BMI [kg/m <sup>2</sup> ] | 26.2 ± 4.5  | 28.1 ± 7.4  | 27.4 ± 4.9         | 27.1 ± 4.1  |
| SMI [kg/m <sup>2</sup> ] | 8.8 ± 0.8   | 9.0 ± 1.1   | 8.9 ± 1.0          | 8.9 ± 0.9   |

Data are means ± SD. No significant difference between groups at baseline ( $p > 0.05$ )  
*BMI* body mass index, *SMI* skeletal muscle index



## 185 Protein supplementation

186 The EP and P trial groups were prescribed a leucine-enriched  
187 whey protein isolate supplement (MyProtein, Northwich,  
188 Cheshire, UK) mixed with 250 ml of water three times/day  
189 (at meal times) for 16 weeks. The supplement was vanilla  
190 flavoured and prescribed by individual body weight (1.5 g/  
191 kg/day; 0.5 g/kg/meal). Each supplement contained at least  
192 3 g of leucine. For further details see (Kirk et al. 2019).

## 193 Exercise history and dietary control

194 Previous exercise history was based on self-report during  
195 initial telephone consultation. Participants who took part in  
196 any scheduled exercise (physical or cardiovascular based)  
197 over the previous 12 months were excluded at baseline. Dur-  
198 ing the trial, E and EP trial participants were instructed to  
199 refrain from exercise participation other than that adminis-  
200 tered by the researchers. Dietary compliance with the protein  
201 supplement was evaluated by means of self-report logs and  
202 counting unused sachets returned on a monthly basis. EP  
203 and P trial participants were instructed to refrain from any  
204 nutritional supplements other than that administered by the  
205 research team. Four-day food diaries were completed by all  
206 trial participants to ensure that habitual dietary intake did  
207 not influence the findings.

## 208 Outcome measures

### 209 Muscle and fat mass

210 Participants removed shoes, socks, watches, jewellery and  
211 any heavy clothing, prior to height (nearest 0.1 cm; SECA  
212 213 Stadiometer) and weight (nearest 0.1 kg; TANITA  
213 MC-180MA) measurements. Body mass index (BMI) was  
214 calculated using standard procedures ( $\text{kg}/\text{cm}^2$ ) (Gallagher  
215 et al. 1996). Muscle and fat mass were evaluated using  
216 multi-frequency bioelectrical impedance analysis (BIA)  
217 (Maltron; BioScan 920-II) with participants positioned  
218 supine on a medical bed in the fasted state. Muscle mass  
219 was calculated using the BIA equation from Janssen and col-  
220 leagues (2000a). This method has been cross-validated with  
221 magnetic resonance imaging of muscle mass in older adults  
222 (Janssen et al. 2000a). Finally, skeletal muscle index (SMI)  
223 was calculated using the following formula: total muscle  
224 mass divided by height squared ( $\text{kg}/\text{m}^2$ ).

### 225 Myoelectrical muscle fatigue

226 Muscle fatigue was measured using 16-channel electromyo-  
227 graphy (EMG) instrument following a validated technique  
228 by our laboratory (Alizadehkhayat et al. 2018; Hawkes et al.  
229 2018). First, maximal voluntary contraction (MVC) of the

230 dominant limbs was performed on the following exercises: 230  
231 *handgrip*, participants were seated upright in an armless 231  
232 chair (46–49 cm in height) with elbow flexed at  $90^\circ$  (verified 232  
233 by goniometer) and instructed to apply maximal pressure 233  
234 for 3 s to a handheld Jamar dynamometer (Biometrics Ltd, 234  
235 Wireless Dynamometer G200, Newport, UK); *leg flexion* 235  
236 *and extension* participants were seated upright in a heavy- 236  
237 duty chair mounted to the floor and attached to a portable 237  
238 strain gauge (Mecmesin 851–401 Multifunction Force/ 238  
239 Torque Indicator, Mecmesin Limited, West Sussex, UK). 239  
240 The lower limbs were attached to the lever arm by a padded 240  
241 gauze strap placed above the malleoli. Straps were adjusted 241  
242 accordingly to ensure hip and knee angles were  $85^\circ$  and  $90^\circ$ , 242  
243 respectively, with the full extension being  $0^\circ$  (verified by 243  
244 goniometer). Participants performed six MVCs ( $3 \times$  famil- 244  
245 iarisation,  $3 \times$  testing), with 30 s break between repetitions 245  
246 and 2 min between familiarisation and testing sets. Strong 246  
247 verbal encouragement was applied throughout. A pilot study 247  
248 carried out before data collection among ten younger adults 248  
249 (five males, five females) indicated the inter-day coefficient 249  
250 of variation for this procedure was  $< 1.5\%$ . 250

251 EMG signals of the key agonist muscles during handgrip 251  
252 [flexor carpi radialis (FCR)], leg extension [rectus femo- 252  
253 ris (RF)] and leg flexion [bicep femoris (BF)] exercises at 253  
254  $\text{MVC}_{25\% \text{max}}$  were recorded for 70 s (the first and last 5 s were 254  
255 excluded from analysis) to provide an index of fatigue. To 255  
256 ensure  $\text{MVC}_{25\% \text{max}}$  remained constant, visual feedback was 256  
257 provided by dynamometer (E-LINK version 14.02, Biom- 257  
258 etrics Ltd.) and myometer (Emperor Lite version 1.18–408, 258  
259 Mecmesin Ltd.) software. Participants' skin was prepared 259  
260 by shaving and cleaning with alcohol wipes before place- 260  
261 ment of self-adhesive Ag/AgCl bipolar surface electrodes 261  
262 with 10 mm diameter and 20 mm inter-electrode distance 262  
263 (Noraxon Inc.) (Kallenberg and Hermens 2008). To limit 263  
264 cross talk, electrodes were placed parallel to muscle fibres 264  
265 on the belly of the muscles following accepted anatomical 265  
266 criteria (Kallenberg and Hermens 2008). Signals were con- 266  
267 firmed by manual muscle testing. 267

268 A Telemyo DTS system (Noraxon Inc., Scottsdale, 268  
269 Arizona, USA) and MyoResearch software (Version 3.8, 269  
270 Noraxon Inc.) were used for signal acquisition and data 270  
271 analysis, respectively. Signals were differentially ampli- 271  
272 fied (CMRR  $> 100$  dB; input impedance  $> 100$  Mohm; 272  
273 gain 500 dB), digitised at a sampling rate of 1500 Hz and 273  
274 band-pass filtered at 20–500 Hz. Poor quality signals were 274  
275 excluded based on the signal to noise ratio (Hawkes et al. 275  
276 2018). Fatigability of each muscle was quantified by calcu- 276  
277 lating the median frequency in 1-s intervals across the 60 s 277  
278 of sustained  $\text{MVC}_{25\% \text{max}}$ . A fast Fourier transformation was 278  
279 performed to allow analysis of the EMG power spectrum. 279  
280 Median frequency was normalised relative to starting value 280  
281 and the mean rate of change, assessed by linear regression, 281  
282 was used as an indicator muscle fatigue (%/min). 282

283 **Health-related quality of life**

284 Health-related quality of life ( $_{HR}$ -QoL) was measured using  
 285 the WHOQOL-BREF (World Health Organisation 1996).  
 286 This is a 26-item questionnaire comprising two individual  
 287 items which ask participants to rate their overall QoL, and  
 288 to estimate satisfaction with their health, and four domains  
 289 assessing physical health (seven items), psychological health  
 290 (six items), social relationships (three items) and environ-  
 291 mental health (eight items), all referring to the past 4 weeks.  
 292 All domains were scaled in a positive direction, and follow-  
 293 ing the guidance, domain totals were transformed to a 0–100  
 294 scale, which allows comparison across domains.

295 The WHOQOL-BREF was self-administered in a quiet  
 296 room twice: the first time at baseline, after collecting  
 297 informed consent and confirming demographic information,  
 298 and a second time, after the intervention was completed. Ten  
 299 participants had more than 20% missing data, so following  
 300 WHOQOL-BREF guidance these participants were with-  
 301 drawn from this part of the study.

302 **Statistical analysis**

303 Statistical analyses were performed using SPSS Statistics  
 304 25 (IBM Corporation, New York, USA). Normality was  
 305 assessed via Kolmogorov–Smirnov tests, which showed a  
 306 skewed distribution for body composition, muscle fatigue  
 307 and WHOQOL-BREF data. Logarithmic transformations  
 308 were unsuccessful at normalising these variables, so non-  
 309 parametric testing was used. Within-group comparisons of  
 310 pre- and post-intervention were undertaken using Wilcoxon  
 311 signed ranks tests. Between-group differences (*C* vs *E* vs *EP*  
 312 vs *P*) were analysed via Kruskal–Wallis (*H*) test followed by  
 313 Bonferroni-corrected Mann–Whitney (*U*) tests for post hoc  
 314 comparisons. Cohen's *d* effect sizes (ES) were calculated  
 315 with the magnitude of effects considered: small (0.20–0.49),  
 316 medium (0.50–0.79) or large (> 0.80). ES were calculated by  
 317 dividing the test statistic (*Z* score) by the square root of total

observations. Sub-groups analyses were performed to check  
 for differences between sexes and between groups consum-  
 ing low ( $\leq 0.8$  g/kg/day) or higher intake of protein ( $\geq 0.8$  g/  
 kg/day) at baseline. Participants' food diaries were analysed  
 for energy and macro- and micro nutrient content through  
 dietary analysis software (Nutritics LTD, Ireland). Data are  
 expressed as mean [ $\pm$  standard deviation (SD)] and differ-  
 ences between values are displayed throughout. The alpha  
 level for statistical significance was set at  $p < 0.05$  a priori.

**Results****Baseline characteristics**

In total, 125 community-dwelling older adults were screened  
 for eligibility, with 123 enrolled, and 100 completing the  
 trial (Fig. 1). Nearly all participants were British Caucasians,  
 except for one Asian participant in E and one in P. In C, 3  
 participants failed to return for follow-up testing, while there  
 were 5 dropouts in E due to musculoskeletal injuries ( $n = 3$ ),  
 disinterest ( $n = 1$ ) and return to work commitments ( $n = 1$ ),  
 and 15 dropouts in P owing to undesirable taste ( $n = 10$ ) and  
 gastrointestinal discomfort ( $n = 5$ ) with the supplement.

Trial groups did not differ in baseline characteristics,  
 energy or macronutrient intake ( $p > 0.05$ ; Tables 1 and 2).  
 In addition, estimates of Vitamin D and Omega-3 (capable  
 of influencing muscle anabolism) did not differ between  
 groups.

**Exercise and protein compliance**

As previously reported, participants in E and EP trial groups  
 attended  $77 \pm 10\%$  and  $78 \pm 10\%$  of their prescribed exercise  
 sessions, respectively. Compliance with the protein supple-  
 ment was  $43 \pm 14\%$  and  $74 \pm 25\%$  in EP and *P* trial groups,  
 respectively. Taking into account habitual levels, protein  
 intake increased from  $\sim 1.2 \pm 0.4$  at baseline to  $1.5 \pm 0.7$  g/

**Table 2** Estimates of energy intake from 4-day food diaries

| Parameter                   | Control         |                 | Exercise        |                 | Exercise + Protein |                  | Protein         |                 | <i>p</i> value |
|-----------------------------|-----------------|-----------------|-----------------|-----------------|--------------------|------------------|-----------------|-----------------|----------------|
|                             | Pre             | Post            | Pre             | Post            | Pre                | Post             | Pre             | Post            |                |
| Energy intake [kcal/day]    | 1711 $\pm$ 330  | 1673 $\pm$ 272  | 1811 $\pm$ 386  | 1944 $\pm$ 568  | 1728 $\pm$ 360     | 1969 $\pm$ 430   | 1759 $\pm$ 348  | 1827 $\pm$ 443  | 0.11           |
| Protein intake[g/day]       | 72 $\pm$ 17     | 74 $\pm$ 14     | 82 $\pm$ 27     | 78 $\pm$ 21     | 77 $\pm$ 22        | 110 $\pm$ 31**   | 73 $\pm$ 12     | 79 $\pm$ 21     | < 0.001        |
| Protein intake [g/kg/day]   | 0.98 $\pm$ 0.3  | 1.01 $\pm$ 0.3  | 1.10 $\pm$ 0.4  | 1.04 $\pm$ 0.3  | 1.16 $\pm$ 0.4     | 1.63 $\pm$ 0.5** | 0.99 $\pm$ 0.2  | 1.08 $\pm$ 0.4  | < 0.001        |
| Carbohydrate intake [g/day] | 178 $\pm$ 52    | 178 $\pm$ 44    | 192 $\pm$ 40    | 211 $\pm$ 68    | 169 $\pm$ 42       | 188 $\pm$ 60     | 199 $\pm$ 54    | 193 $\pm$ 44    | 0.29           |
| Fat intake[g/day]           | 64 $\pm$ 19     | 61 $\pm$ 13**   | 70 $\pm$ 18     | 73 $\pm$ 22     | 70 $\pm$ 23        | 75 $\pm$ 24      | 64 $\pm$ 19     | 72 $\pm$ 26     | < 0.001        |
| Vitamin D [ $\mu$ g/day]    | 4.4 $\pm$ 3.7   | 4.5 $\pm$ 4.1   | 8.3 $\pm$ 8.8   | 8.2 $\pm$ 9.1   | 5.4 $\pm$ 5.1      | 6.0 $\pm$ 5.3    | 6.5 $\pm$ 5.9   | 8.7 $\pm$ 7.9   | 0.47           |
| Omega-3 [g/day]             | 1.09 $\pm$ 0.86 | 1.24 $\pm$ 0.77 | 1.86 $\pm$ 1.32 | 1.28 $\pm$ 0.93 | 1.48 $\pm$ 1.61    | 1.61 $\pm$ 1.45  | 1.67 $\pm$ 1.26 | 1.61 $\pm$ 1.49 | 0.41           |

Values are means  $\pm$  SD. No significant difference between groups at baseline ( $p > 0.05$ ). \*\*Indicates between-group difference at post-interven-  
 tion ( $p < 0.05$ )

350 kg/day in EP during the trial, and from  $\sim 1.0 \pm 0.2$  at baseline and SMI ( $\Delta$ change, C:  $0 \pm 0.1$ ; E:  $0.1 \pm 0$ ; EP:  $0.1 \pm 0$ ; P: 359  
351 to  $1.9 \pm 0.7$  g/kg/day in P during the trial (Table 2).  $-0.1 \pm 0.1$  kg/m<sup>2</sup>,  $p = 0.66$ ) did not change. 360

## 352 Effect of intervention

### 353 Muscle and fat mass

354 No within- or between-group differences were observed  
355 for muscle or fat mass ( $p > 0.05$ , Table 3), and body weight  
356 ( $\Delta$ change, C:  $0.1 \pm 0$ ; E:  $-0.8 \pm 1.8$ ; EP:  $-0.8 \pm 0.6$ ;  
357 P:  $1.0 \pm 0.3$  kg,  $p = 0.61$ ), BMI ( $\Delta$ change, C:  $0 \pm 0$ ; E:  
358  $-0.3 \pm 0.8$ ; EP:  $-0.1 \pm 0.4$ ; P:  $0.3 \pm 0.2$  kg/m<sup>2</sup>,  $p = 0.72$ )

### Myoelectrical muscle fatigue

362 At baseline, muscle fatigue was successfully induced in  
363 the flexor carpi radialis, rectus femoris and bicep femo-  
364 ris, demonstrating the efficacy of this testing procedure  
365 (all  $p < 0.001$ ). In response to the exercise intervention,  
366 the rectus femoris (E:  $-3.8 \pm 4.9$  to  $0.5 \pm 6.4$ ,  $p = 0.028$ ;  
367 EP:  $-6.3 \pm 5.0$  to  $-0.9 \pm 6.7$ ,  $p = 0.011$ ) and bicep femoris  
368 (E:  $-5.4 \pm 2.2$  to  $-0.9 \pm 1.6$ ,  $p < 0.001$ ; EP:  $-6.1 \pm 2.7$  to  
369  $-0.7 \pm 1.6$ ,  $p < 0.001$ ) muscles became less fatigable, with

**Table 3** Effect of intervention on muscle and fat mass, and myoelectrical muscle fatigue

| Parameter                            | Control     |             | Exercise    |              | Exercise + Protein |               | Protein     |             | <i>p</i> value    |
|--------------------------------------|-------------|-------------|-------------|--------------|--------------------|---------------|-------------|-------------|-------------------|
|                                      | Pre         | Post        | Pre         | Post         | Pre                | Post          | Pre         | Post        |                   |
| <b>Muscle mass [kg]</b>              |             |             |             |              |                    |               |             |             |                   |
| Men                                  | 27.9 ± 3.0  | 27.7 ± 3.3  | 29.9 ± 3.6  | 30.3 ± 3.5   | 28.3 ± 5.2         | 28.9 ± 5.5    | 27.6 ± 3.6  | 27.5 ± 3.5  | 0.38              |
| Women                                | 22.1 ± 2.4  | 22.3 ± 2.4  | 21.5 ± 2.5  | 21.8 ± 2.1   | 21.3 ± 2.7         | 21.5 ± 2.8    | 21.1 ± 2.9  | 20.8 ± 2.2  | 0.53              |
| Combined                             | 24.4 ± 3.9  | 24.5 ± 3.8  | 25.5 ± 5.2  | 25.9 ± 5.2   | 24.0 ± 5.1         | 24.3 ± 5.4    | 25.2 ± 4.6  | 25.0 ± 4.5  | 0.68              |
| <b>Fat mass [kg]</b>                 |             |             |             |              |                    |               |             |             |                   |
| Men                                  | 20.8 ± 5.3  | 20.9 ± 4.6  | 25.1 ± 14.1 | 25.9 ± 10.9  | 25.1 ± 5.8         | 25.7 ± 5.4    | 25.3 ± 8.4  | 24.2 ± 10.5 | 0.18              |
| Women                                | 25.5 ± 11.9 | 25.3 ± 12.5 | 28.2 ± 17.6 | 27.7 ± 15.9  | 22.8 ± 10.5        | 22.6 ± 10.2   | 27.6 ± 8.5  | 28.6 ± 10.1 | 0.72              |
| Combined                             | 23.6 ± 10.0 | 23.5 ± 10.2 | 26.7 ± 15.8 | 26.9 ± 13.5  | 23.7 ± 8.9         | 23.8 ± 8.7    | 26.1 ± 8.3  | 25.8 ± 10.3 | 0.75              |
| <b>Handgrip MVC [kg]</b>             |             |             |             |              |                    |               |             |             |                   |
| Men                                  | 34.6 ± 10.6 | 35.7 ± 11.6 | 38.5 ± 7.1  | 41.6 ± 7.0   | 32.9 ± 6.9         | 37.2 ± 8.4    | 32.7 ± 7.9  | 35.3 ± 10.0 | 0.32              |
| Women                                | 23.9 ± 4.1  | 22.4 ± 4.4  | 21.7 ± 4.8  | 24.4 ± 3.5   | 23.2 ± 5.5         | 27.0 ± 9.2    | 22.4 ± 4.4  | 22.8 ± 4.5  | 0.41              |
| Combined                             | 28.4 ± 9.1  | 28.0 ± 10.5 | 30.1 ± 10.5 | 33.0 ± 10.3  | 27.4 ± 7.3         | 31.3 ± 10.1   | 28.7 ± 8.4  | 30.4 ± 10.2 | 0.25              |
| <b>Leg extension MVC [n], n = 99</b> |             |             |             |              |                    |               |             |             |                   |
| Men                                  | 343 ± 124   | 300 ± 105   | 284 ± 87    | 389 ± 103**  | 285 ± 56           | 373 ± 107**   | 276 ± 102   | 283 ± 103   | <b>0.034</b>      |
| Women                                | 180 ± 49    | 171 ± 44    | 233 ± 126   | 266 ± 61**   | 173 ± 46           | 279 ± 91**    | 190 ± 105   | 173 ± 56    | <b>0.001</b>      |
| Combined                             | 249 ± 119   | 225 ± 98    | 258 ± 109   | 328 ± 104**  | 219 ± 75           | 318 ± 107**   | 243 ± 110   | 240 ± 102   | <b>&lt; 0.001</b> |
| <b>Leg flexion MVC [n]</b>           |             |             |             |              |                    |               |             |             |                   |
| Men                                  | 162 ± 79    | 148 ± 66    | 142 ± 44    | 188 ± 55**   | 146 ± 50           | 178 ± 60**    | 139 ± 48    | 160 ± 59    | <b>0.039</b>      |
| Women                                | 103 ± 48    | 100 ± 39    | 148 ± 97    | 147 ± 35     | 97 ± 31            | 149 ± 44**    | 140 ± 142   | 101 ± 35    | <b>0.014</b>      |
| Combined                             | 127 ± 68    | 120 ± 56    | 145 ± 74    | 168 ± 49**   | 117 ± 46           | 161 ± 52**    | 139 ± 91    | 138 ± 58    | <b>0.001</b>      |
| <b>Fatigue FCR [slope %/min]</b>     |             |             |             |              |                    |               |             |             |                   |
| Men                                  | -4.4 ± 6.3  | -4.3 ± 5.8  | -5.1 ± 10.9 | -4.8 ± 8.4   | -7.9 ± 8.7         | -4.6 ± 5.6    | -3.6 ± 8.9  | -6.0 ± 5.4  | 0.63              |
| Women                                | -0.3 ± 9.4  | 0.6 ± 12.0  | -2.5 ± 14.7 | 0.5 ± 13.7   | 0.4 ± 6.5          | -1.8 ± 12.9   | -6.8 ± 13.0 | -1.6 ± 4.8  | 0.77              |
| Combined                             | -1.9 ± 8.4  | -1.3 ± 10.2 | -3.9 ± 12.5 | -2.4 ± 11.1  | -3.1 ± 8.4         | -3.0 ± 10.3   | -4.7 ± 10.3 | -5.0 ± 5.7  | 0.54              |
| <b>Fatigue RF [slope %/min]</b>      |             |             |             |              |                    |               |             |             |                   |
| Men                                  | -4.4 ± 8.3  | -5.1 ± 4.2  | -1.4 ± 5.1  | -3.7 ± 5.8   | -7.3 ± 6.1         | -0.9 ± 6.9**  | -2.2 ± 16.7 | -3.2 ± 12.7 | <b>0.031</b>      |
| Women                                | -3.9 ± 16.0 | -3.4 ± 5.3  | -7.1 ± 9.2  | 0.1 ± 10.9** | -6.5 ± 9.4         | -7.1 ± 10.9   | 2.3 ± 10.1  | -1.3 ± 3.4  | <b>0.001</b>      |
| Combined                             | -4.1 ± 13.3 | -4.1 ± 4.8  | -4.2 ± 7.8  | -1.8 ± 8.7** | -6.9 ± 7.9         | -4.3 ± 9.6**  | -0.7 ± 14.7 | -2.5 ± 10.4 | <b>&lt; 0.001</b> |
| <b>Fatigue BF [slope %/min]</b>      |             |             |             |              |                    |               |             |             |                   |
| Men                                  | -1.7 ± 22.3 | -1.9 ± 5.2  | 0.3 ± 6.9   | 0.4 ± 16.7   | -5.1 ± 15.9        | -5.4 ± 13.2   | 0.4 ± 15.6  | 2.4 ± 14.8  | 0.68              |
| Women                                | 4.6 ± 21.8  | 6.2 ± 17.8  | -10.9 ± 9.6 | 2.5 ± 25.6** | -5.1 ± 12.7        | -1.9 ± 12.6** | 2.9 ± 14.9  | -2.9 ± 12.7 | <b>0.001</b>      |
| Combined                             | 2.1 ± 21.8  | 2.9 ± 14.5  | -4.7 ± 9.8  | 1.3 ± 20.4** | -5.1 ± 13.8        | -3.5 ± 12.6** | 1.4 ± 15.0  | 0.4 ± 13.9  | <b>&lt; 0.001</b> |

Data are means ± SD. C: flexor carpi radialis (FCR); rectus femoris (RF); bicep femoris (BF). Maximal voluntary contraction (MVC). \*\*Indicates between-group difference at post-intervention ( $p < 0.05$ )

370 no change in *C* or *P* groups. At post-intervention, between-  
 371 group comparisons showed the rectus femoris (*E*:  $-4.8\%/$   
 372 min,  $ES = 0.86$ ,  $p = 0.007$ ; *EP*:  $-3.3\%/$ min,  $ES = 0.58$ ,  
 373  $p = 0.045$ ) and bicep femoris (*E*:  $-3.9\%/$ min,  $ES = 1.46$ ,  
 374  $p < 0.001$ ; *EP*:  $-4.3\%/$ min,  $ES = 1.58$ ,  $p < 0.001$ ) muscles  
 375 were more resistant to fatigue in exercising (*E* and *EP*)  
 376 groups ( $p > 0.05$  versus *C*). No other changes were observed  
 377 (Fig. 2).

### 378 Health-related quality of life (HR-QOL)

379 Post-intervention, significant improvements from baseline  
 380 measures were found in HR-QOL in the *E* group with respect  
 381 to overall perception of health, psychological health, social  
 382 relations and environmental health (Table 4). HR-QOL lev-  
 383 els were normal for age across all domains: of significance,  
 384 higher age was associated with better perception of one's  
 385 health; higher age was associated with better psychological  
 386 health in *E*, and social relations were negatively related to  
 387 age in the control group. There was no difference according  
 388 to sex on any HR-QOL measure.

389 There was a difference between groups at baseline on  
 390 some aspects of HR-QOL: Overall Health was better in  
 391 *C* than *E* ( $p = 0.001$ ); Social Relations better in *C* than  
 392 *P* ( $p = 0.005$ ), and *EP* better than *E* for Environmental  
 393 Health. Repeated measures multivariate analyses of all  
 394 domains and interventions indicated that between-group  
 395 contrasts were significant for Social Relations ( $F = 4.22$ ,  
 396  $p < 0.01$ , partial  $\eta^2 = 0.128$ ). Games-Howell group con-  
 397 trasts indicated a difference in *C* and *P* means ( $p = 0.02$ )  
 398 and *EP* and *P* means ( $p = 0.04$ ).

### Sub-group analysis

399 A separate analysis was performed to check for sex differ-  
 400 ences between groups relating to muscle and fat mass, and  
 401 manifestations of myoelectrical muscle fatigue. Findings  
 402 showed a similar outcome as to when sexes were combined  
 403 in the analysis (Table 4).  
 404

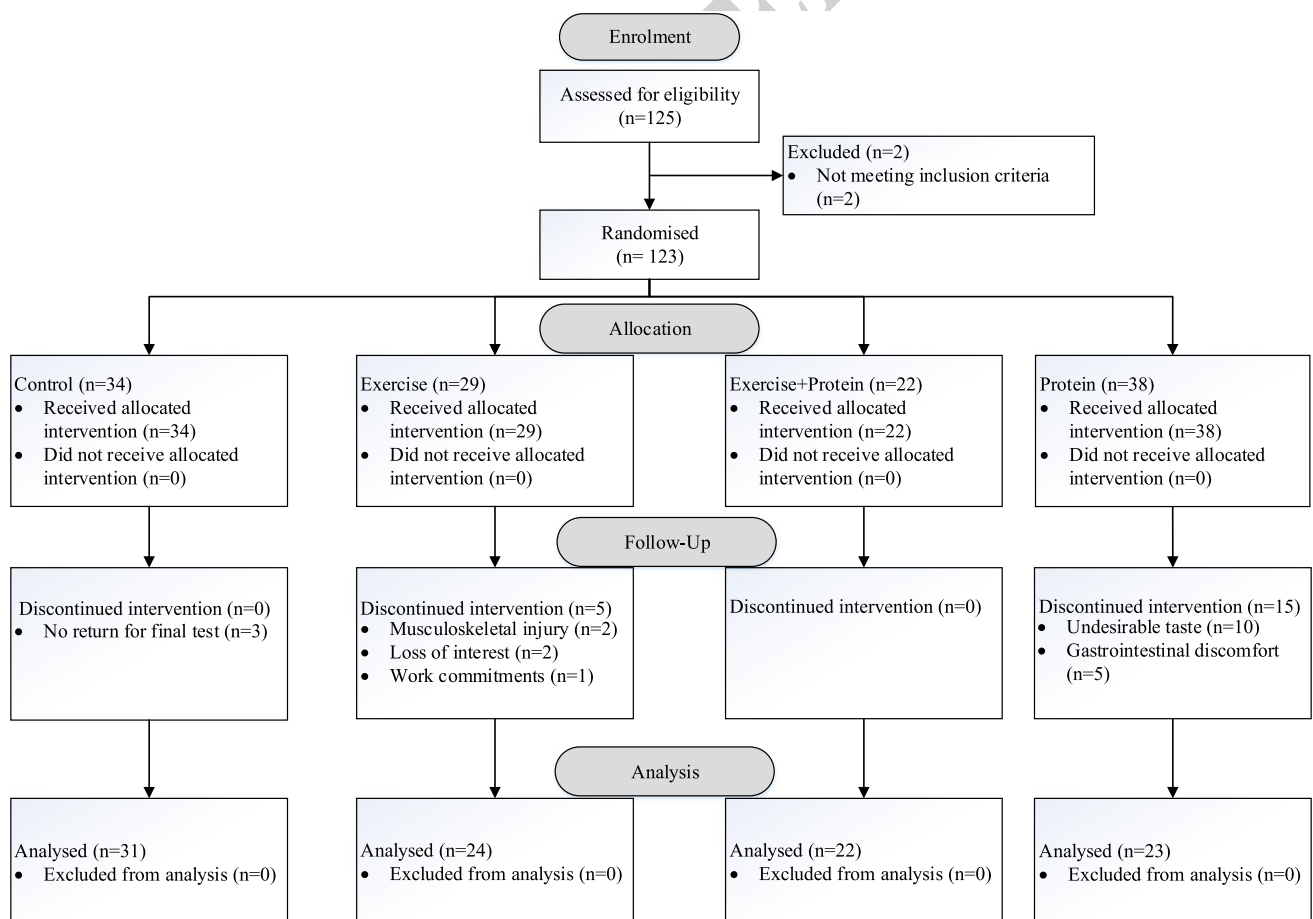


Fig. 2 Flowchart of RCT

**Table 4** WHOQOL-BREF means ( $\pm$  standard deviation) and Wilcoxon signed ranks test statistic (with exact  $p$  value<sup>\*</sup>) according to the intervention group and domain

| Domain                                      | Time test | Control ( $n=30$ ) | Exercise ( $n=21$ ) | Exercise + Protein ( $n=18$ ) | Protein ( $n=21$ ) |
|---|-----------|--------------------|---------------------|-------------------------------|--------------------|
| Overall quality of life<br>(range 0–5)      | Pre       | 4.60 (0.50)        | 4.19 (0.93)         | 4.61 (0.50)                   | 4.38 (0.59)        |
|   | Post      | 4.60 (0.56)        | 4.33 (0.80)         | 4.72 (0.46)                   | 4.57 (0.60)        |
|   | Z / p     | 0 (1)              | –1.00 (.25)         | –1.41 (.25)                   | –1.63 (.11)        |
| Overall perception of health<br>(range 0–5) | Pre       | 4.20 (0.61)        | 3.38 (1.02)         | 4.11 (0.68)                   | 3.81 (0.68)        |
|   | Post      | 4.03 (0.89)        | 3.90 (1.04)         | 4.33 (0.49)                   | 3.81 (0.87)        |
|   | Z / p     | –1.52 (0.25)       | –2.64 (<0.01)       | –1.63 (0.11)                  | –0.09 (0.50)       |
| Physical health<br>(range 0–100)            | Pre       | 61.97 (6.20)       | 59.67 (8.85)        | 63.44 (6.83)                  | 61.24 (7.71)       |
|   | Post      | 62.57 (6.75)       | 62.43 (6.52)        | 66.22 (5.74)                  | 61.48 (6.21)       |
|   | Z / p     | –.48 (0.66)        | –1.47 (0.08)        | –1.44 (0.10)                  | 0 (0.52)           |
| Psychological health<br>(range 0–100)       | Pre       | 69.03 (9.79)       | 60.24 (14.11)       | 67.50 (8.14)                  | 66.86 (6.89)       |
|   | Post      | 67.87 (7.78)       | 65.05 (13.13)       | 71.00 (6.83)                  | 65.00 (9.38)       |
|   | Z / p     | –.89 (0.39)        | –2.51 (<0.01)       | –1.70 (0.055)                 | –0.85 (0.21)       |
| Social relationship<br>(range 0–100)        | Pre       | 82.13 (16.21)      | 69.05 (19.35)       | 77.83 (17.53)                 | 66.38 (19.95)      |
|   | Post      | 79.80 (15.39)      | 75.29 (17.60)       | 81.78 (13.83)                 | 67.86 (18.18)      |
|   | Z / p     | –.87 (0.41)        | –1.69 (<0.05)       | –0.28 (0.40)                  | –0.20 (0.44)       |
| Environmental health<br>(range 0–100)       | Pre       | 87.27 (10.36)      | 81.57 (8.76)        | 89.79 (8.59)                  | 87.43 (10.51)      |
|   | Post      | 83.83 (11.88)      | 86.43 (15.49)       | 92.44 (8.47)                  | 87.38 (11.06)      |
|   | Z / p     | –2.46 (0.014)      | –2.53 (<0.01)       | –1.12 (0.14)                  | –0.14 (0.45)       |

$p$  values from two-tailed tests for Control group and one-tailed tests for three intervention groups

## 405 Discussion

406 We conducted a secondary analysis of the LHU-SAT and  
407 found significant improvements in myoelectrical muscle  
408 fatigue and  $HR$ -QOL in response to 16 weeks of exercise;  
409 however, leucine-enriched whey protein supplementation did  
410 not augment this response. In addition, we found no changes  
411 in muscle or fat mass.

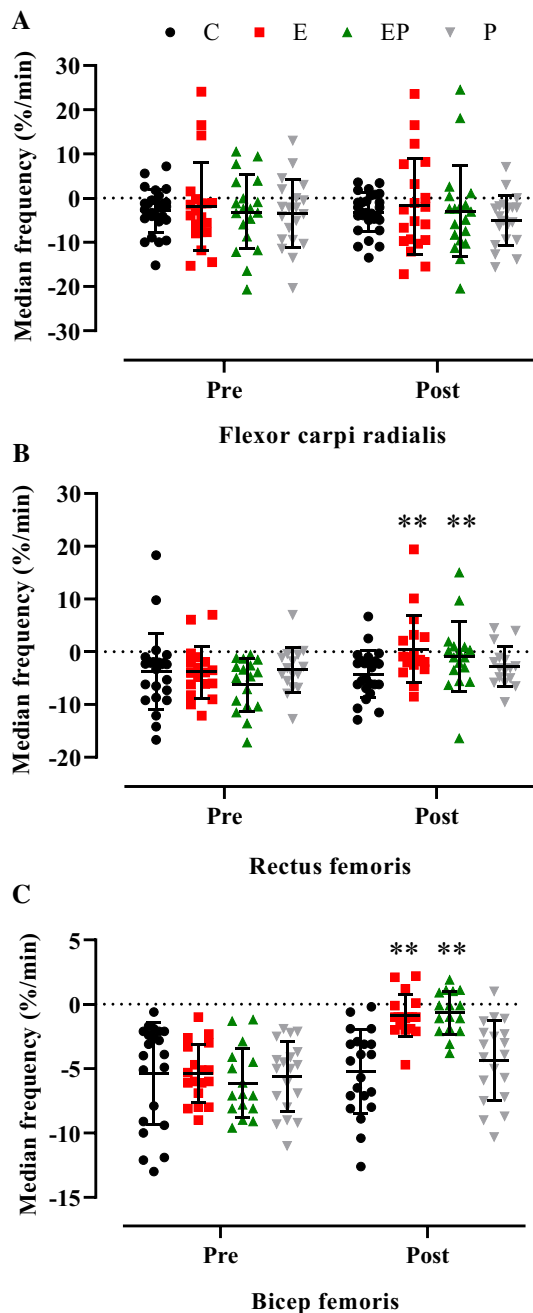
412 In our trial, combined habitual intake of protein  
413 was  $\sim 1.1 \pm 0.3$  g/kg/day which is comparable to cross-  
414 sectional reports in older adults (ten Haaf et al. 2018a, b),  
415 although higher in community-dwelling older adults suffer-  
416 ing from mobility limitations (Houston et al. 2008, 2017)  
417 (Fig. 3).

### 418 Effects on muscle and fat mass

419 Protein intake increased from  $\sim 1.2$  to  $1.5$  g/kg/day in EP  
420 and  $\sim 1.0$  to  $1.9$  g/kg/day in P groups, yet we observed no  
421 benefits on body composition. This finding supports our pri-  
422 mary analysis of the LHU-SAT (Kirk et al. 2019) where pro-  
423 tein supplementation did not augment the exercise-induced  
424 increases in muscle strength, physical functioning or aerobic  
425 capacity. A likely explanation for this is our population were  
426 already consuming sufficient quantities of protein at base-  
427 line. In support, three studies (Verdijk et al. 2009; Leend-  
428 ers et al. 2013; Holwerda et al. 2018) found no benefit of

429 whey protein during resistance exercise on muscle mass in  
430 healthy older adults habitually consuming  $1.1$ – $1.2$  g/kg/day  
431 of protein, whereas 12 weeks of milk protein during a walk-  
432 ing exercise regimen increased muscle mass and decreased  
433 fat mass in healthy older adults with low levels of protein  
434 ( $\sim 0.86$  g/kg/day) at trial enrolment (ten Haaf et al. 2019).  
435 Another recent trial reported greater increases in muscle  
436 mass and reductions in fat mass, following 12 weeks of  
437 combined elastic band/body weight exercise and protein  
438 supplementation, although habitual protein intake was not  
439 assessed which limits comparisons with our trial (Krause  
440 et al. 2019). Taken together, these findings suggest that the  
441 current protein recommendations for well-nourished active  
442 older adults by the PROT-AGE and ESPEN study groups of  
443 at least  $1.0$ – $1.2$  g/kg/day are sufficient.

444 During ageing, medical conditions may prohibit exer-  
445 cise participation and as such effective dietary suppl-  
446 ements are warranted to curb muscle mass loss. However,  
447 we found no benefit of whey protein on muscle mass alone,  
448 which may be due to the above-mentioned explanations  
449 (i.e., adequate intake of protein at baseline). On the other  
450 hand, longer interventions ( $\geq 24$  weeks) are recommended  
451 to observe detectable increases in muscle mass when pre-  
452 scribing protein alone in older adults (Tieland et al. 2017),  
453 and meta-analyses have reported small, but significant  
454 effects on muscle mass independent of exercise (Hanach



**Fig. 3** Myoelectrical muscle fatigue values for **a** flexor carpi radialis, **b** rectus femoris and **c** bicep femoris in response to independent treatments (*C* Control, *E* Exercise, *EP* Exercise + Protein, *P* Protein). Values are mean  $\pm$  SD with individual data points shown. \*\* Indicates between-group difference at post-intervention ( $p < 0.05$ )

et al. 2019). As our trial was powered to determine the effects of concomitant exercise and protein supplementation, we may have been underpowered to unearth the effects of protein alone.

## Effects on myoelectrical muscle fatigue

We are one of few trials which investigated the effects of exercise and whey protein on manifestations of peripheral fatigue in older adults. We chose to employ a low intensity contraction ( $MVC_{25\%max}$ ) as older adults typically carry out daily tasks during repeated, sustained contractions. To date, only two trials have examined adaptations in fatigability following an exercise or nutritional intervention, and both utilised moderate–high intensity contractions. Gryson et al. (2014) reported improvements in time to failure during a sustained  $MVC_{75\%max}$  contraction, following 16 weeks of aerobic/resistance exercise combined with a leucine-enriched protein beverage. In contrast, Negro et al. (2019) investigated the effects of a single mixed nutritional supplement (essential amino acids, creatine and vitamin D) on central and peripheral fatigue at  $MVC_{60\%max}$  in older adults with null findings (Negro et al. 2019), despite observing increases in muscle mass and strength. The authors interpreted this finding as indirect confirmation of type II fibre hypertrophy, outlining the supplement likely failed to enhance the fatigue-resistant type I fibres.

In our trial, the exercise-induced adaptations in fatigability were specific to the lower limbs, despite employing a whole-body exercise intervention. As our testing protocol successfully induced fatigue in both limbs, and we previously reported improvements in aerobic capacity (via the six minute walk test) (Kirk et al. 2019), we believe this finding is due to a greater atrophy/weakness of the lower compared to upper limb muscles observed in ageing (Janssen et al. 2000b). Indeed, muscle fatigue was measured relative to MVC. Thus, it is conceivable the loss of strength was higher in the lower limbs at trial enrolment, rendering greater scope for improvement with the exercise intervention.

Irrespective of the mechanism, this finding has clinical relevance considering increased fatigability of the lower limbs is linked to decrements in balance and walking performance (Senefeld et al. 2017), and older adults with a history of falls compared to non-fallers have a shorter endurance time and longer time to recover from lower-limb fatiguing contractions as evidenced by the electromyogram (Schwendner et al. 1997). Moreover, a recent Cochrane review (Sherrington et al. 2019) found that combining multiple exercises (muscle strengthening, functional and balance) offsets falls in community-dwelling older adults by 34%. Our data strengthen these findings and highlight the benefits of multimodal exercise in ageing, with a well-tolerated regimen (combined exercise adherence  $\sim 78 \pm 10\%$ ).

## Effects on $HR_{R-QOL}$

Using the robust WHOQOL-BREF, we found an increase in exercise activity in previously sedentary adults improved

509  $HR$ -QOL, which corroborates previous findings (Hart and Buck  
510 2019). However, the benefit was exclusive to the exercise  
511 group alone, with no change in the combined (Exercise + Pro-  
512 tein) group. Considering the complete absence of benefit for  
513 the surviving protein group, and feedback from participants  
514 in the protein group (and endorsed particularly by some of  
515 those who withdrew from the trial), told us that the supplement  
516 was difficult to consume. That is, whereas the exercise was  
517 perceived as 'good' for participants, even when stretched, the  
518 protein beverage was not. Our findings suggest that exercise  
519 should be the primary vehicle for ameliorating potentials for  
520 sarcopaenia in sedentary older adults who are generally well  
521 nourished, and that a regular intervention at the right level  
522 could be sustained because of the demonstrated perceived  
523 improvement in  $HR$ -QOL.

## 524 Strength and limitations

525 To our knowledge, we have performed the largest RCT with  
526 four (Control; Exercise; Exercise + Protein; Protein) distinct  
527 treatment groups in community-dwelling older adults demon-  
528 strating valuable findings in terms of adaptations in myoelec-  
529 trical fatigue and  $HR$ -QOL. We are also the first group which  
530 has attempted to prescribe leucine and whey protein at recom-  
531 mended dosages, at each meal, and by individual body weight  
532 (Moore et al. 2015). However, some limitations should be  
533 noted. Firstly, we did not exclude those consuming protein  
534 above the RDA at trial enrolment, which we feel greatly hin-  
535 dered our findings. In this regard, future trials should investi-  
536 gate the effect in older adults habitually consuming the RDA of  
537 protein, to assess if a higher intake of protein (with or without  
538 exercise) is necessary to prevent, or at least delay, sarcopaenia.  
539 Secondly, the trial was not placebo controlled, which increases  
540 the risk of bias. Thirdly, with myoelectrical indices, signalling  
541 can be confounded by subcutaneous fat or cross talk between  
542 other muscles and thus this may have influenced our findings.  
543 Including a muscle activation measurement would have also  
544 enriched the findings and allowed the effect of protein intake  
545 on this parameter to be investigated. Finally, obtaining muscle  
546 tissue samples would have enabled the effect of protein timing  
547 on muscle protein synthesis rates to be explored. However, it  
548 should be noted that a recent trial found no effect of protein  
549 timing on resistance exercise-induced gains in muscle mass,  
550 strength and physical functioning in postmenopausal women  
551 (de Branco et al. 2019). Nevertheless, future RCTs should con-  
552 sider these aspects.

## 553 Conclusions

554 In conclusion, 16 weeks of multimodal exercise (resistance  
555 and functional) attenuated lower-limb myoelectrical fatigue  
556 and enhanced  $HR$ -QOL. However, leucine-enriched whey

protein did not augment this response in older adults already  
consuming sufficient quantities of protein at trial enrolment.

**Acknowledgements** The authors would like to thank the participants  
for their dedication and continuous contribution throughout the trial.

**Author contributions** BK, KM, RC, PA, GC, FA and OK conceived  
and designed the trial. BK and KM performed the laboratory tests and  
ran the exercise and protein interventions, while MJ and JP provided  
support when needed. BK, KM and RC processed and analysed the  
data, overseen by FA and OK. BK wrote the manuscript with assistance  
from RC. All authors edited and approved the final version.

**Funding** No external funding was received for this project.

**Compliance with ethical standards**

**Conflict of interest** The authors declare that they have no conflict of  
interest.

## References

- Alizadehkhayat O, Roebuck MM, Makki AT, Frostick SP (2018) Sub-  
acromial impingement syndrome: an electromyographic study of  
shoulder girdle muscle fatigue. *J Electromyogr Kinesiol* 38:136–  
142. <https://doi.org/10.1016/j.jelekin.2017.12.001>
- Bauer J, Biolo G, Cederholm T et al (2013) Evidence-based recommen-  
dations for optimal dietary protein intake in older people: a posi-  
tion paper from the PROT-AGE study group. *J Am Med Dir Assoc*  
14:542–559. <https://doi.org/10.1016/J.JAMDA.2013.05.021>
- de Branco FMS, Carneiro MAS, Rossato LT et al (2019) Protein tim-  
ing has no effect on lean mass, strength and functional capacity  
gains induced by resistance exercise in postmenopausal women:  
a randomized clinical trial. *Clin Nutr*. <https://doi.org/10.1016/j.clnu.2019.01.008>
- Deutz NEP, Bauer JM, Barazzoni R et al (2014) Protein intake and  
exercise for optimal muscle function with aging: recommen-  
dations from the ESPEN Expert Group. *Clin Nutr*. <https://doi.org/10.1016/j.clnu.2014.04.007>
- Gallagher D, Visser M, Sepulveda D et al (1996) How useful is body  
mass index for comparison of body fatness across age, sex,  
and ethnic groups? *Am J Epidemiol* 143:228–239. <https://doi.org/10.1093/oxfordjournals.aje.a008733>
- Gryson C, Ratel S, Rance M et al (2014) Four-month course of soluble  
milk proteins interacts with exercise to improve muscle strength  
and delay fatigue in elderly participants. *J Am Med Dir Assoc*  
15:958.e1–958.e9. <https://doi.org/10.1016/j.jamda.2014.09.011>
- Hanach NI, McCullough F, Avery A (2019) The impact of dairy protein  
intake on muscle mass, muscle strength, and physical performance  
in middle-aged to older adults with or without existing sarcopa-  
enia: a systematic review and meta-analysis. *Adv Nutr*. <https://doi.org/10.1093/advances/nmy065>
- Hart PD, Buck DJ (2019) The effect of resistance training on health-  
related quality of life in older adults: systematic review and meta-  
analysis. *Heal Promot Perspect* 9:1–12. <https://doi.org/10.15171/hpp.2019.01>
- Hawkes D, Grant M, McMahon J et al (2018) Can grip strength be used  
as a surrogate marker to monitor recovery from shoulder fatigue?  
*J Electromyogr Kinesiol* 41:139–146. <https://doi.org/10.1016/j.jelekin.2018.06.002>
- Holwerda AM, Overkamp M, Paulussen KJM et al (2018) Protein  
supplementation after exercise and before sleep does not further

- 612 augment muscle mass and strength gains during resistance exercise training in active older men. *J Nutr* 148:1723–1732. <https://doi.org/10.1093/jn/nxy169>
- 613
- 614 Houston DK, Nicklas BJ, Ding J, et al (2008) Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the health, aging, and body composition (Health ABC) Study. 150–155
- 615
- 616 Houston DK, Toozé JA, Garcia K et al (2017) Protein intake and mobility limitation in community-dwelling older adults: the health ABC study. *J Am Geriatr Soc* 65:1705–1711. <https://doi.org/10.1111/jgs.14856>
- 617
- 618
- 619 Janssen I, Heymsfield SB, Baumgartner RN, Ross R (2000a) Estimation of skeletal muscle mass by bioelectrical impedance analysis. *J Appl Physiol* 89:465–471. <https://doi.org/10.1152/jappl.2000.89.2.465>
- 620
- 621
- 622 Janssen I, Heymsfield SB, Wang Z, Ross R (2000b) Skeletal muscle mass and distribution in 468 men and women aged 18–88 yr. *J Appl Physiol* 89:81–88
- 623
- 624 Kallenberg LAC, Hermens HJ (2008) Behaviour of a surface EMG based measure for motor control: Motor unit action potential rate in relation to force and muscle fatigue. *J Electromyogr Kinesiol* 18:780–788. <https://doi.org/10.1016/J.JELEKIN.2007.02.011>
- 625
- 626 Kirk B, Mooney K, Amirabdollahian F, Khaiyat O (2019) Exercise and dietary-protein as a countermeasure to skeletal muscle weakness: Liverpool Hope University—Sarcopenia Aging Trial (LHU-SAT). *Front Physiol* 10:445. <https://doi.org/10.3389/fphys.2019.00445>
- 627
- 628 Krause M, Crognale D, Cogan K et al (2019) The effects of a combined bodyweight-based and elastic bands resistance training, with or without protein supplementation, on muscle mass, signaling and heat shock response in healthy older people. *Exp Gerontol* 115:104–113. <https://doi.org/10.1016/j.exger.2018.12.004>
- 629
- 630 Leenders M, Verdijk LB, Van Der Hoeven L et al (2013) Protein supplementation during resistance-type exercise training in the elderly. *Med Sci Sports Exerc* 45:542–552. <https://doi.org/10.1249/MSS.0b013e318272fcd8>
- 631
- 632 Liu C, Shiroy DM, Jones LY, Clark DO (2014) Systematic review of functional training on muscle strength, physical functioning, and activities of daily living in older adults. *Eur Rev Aging Phys Act* 11:95–106. <https://doi.org/10.1007/s11556-014-0144-1>
- 633
- 634 Moore DR, Churchward-Venne TA, Witard O et al (2015) Protein ingestion to stimulate myofibrillar protein synthesis requires greater relative protein intakes in healthy older versus younger men. *J Gerontol Ser A Biol Sci Med Sci* 70:57–62. <https://doi.org/10.1093/gerona/glu103>
- 635
- 636 Morton RW, Murphy KT, McKellar SR et al (2017) A systematic review, meta-analysis and meta-regression of the effect of protein supplementation on resistance training-induced gains in muscle mass and strength in healthy adults. *Br J Sports Med* 52:376–384. <https://doi.org/10.1136/bjsports-2017-097608>
- 637
- 638 Negro M, Perna S, Spadaccini D et al (2019) Effects of 12 weeks of essential amino acids (EAA)-based multi-ingredient nutritional supplementation on muscle mass, muscle strength, muscle power and fatigue in healthy elderly subjects: a randomized controlled double-blind study. *J Nutr Heal Aging*. <https://doi.org/10.1007/s12603-019-1163-4>
- 639
- 640 Norton C, Toomey C, McCormack WG et al (2016) Protein supplementation at breakfast and lunch for 24 weeks beyond habitual intakes increases whole-body lean tissue mass in healthy older adults. *J Nutr* 146:65–69. <https://doi.org/10.3945/jn.115.219022>
- 641
- 642 Sale DG (1988) Neural adaptation to resistance training. *Med Sci Sports Exerc* 20:S135–S145
- 643
- 644 Schwendner KI, Mikesky AE, Holt WS et al (1997) Differences in muscle endurance and recovery between fallers and nonfallers, and between young and older women. *J Gerontol Ser A Biol Sci Med Sci* 52A:M155–M160. <https://doi.org/10.1093/geron/a/52A.3.M155>
- 645
- 646 Scott D, Sanders KM, Aitken D et al (2014) Sarcopenic obesity and dynapenic obesity: 5-year associations with falls risk in middle-aged and older adults. *Obesity*. <https://doi.org/10.1002/oby.20734>
- 647
- 648 Senefeld J, Yoon T, Hunter SK (2017) Age differences in dynamic fatigability and variability of arm and leg muscles: associations with physical function. *Exp Gerontol* 87:74–83. <https://doi.org/10.1016/J.EXGER.2016.10.008>
- 649
- 650 Sherrington C, Fairhall NJ, Wallbank GK et al (2019) Exercise for preventing falls in older people living in the community. *Cochrane Database Syst Rev*. <https://doi.org/10.1002/14651858.CD012424.pub2>
- 651
- 652 ten Haaf DSM, de Regt MF, Visser M et al (2018a) Insufficient protein intake is highly prevalent among physically active elderly. *J Nutr Health Aging* 22:1112–1114. <https://doi.org/10.1007/s12603-018-1075-8>
- 653
- 654 ten Haaf DSM, Nuijten MAH, Maessen MFH et al (2018b) Effects of protein supplementation on lean body mass, muscle strength, and physical performance in nonfrail community-dwelling older adults: a systematic review and meta-analysis. *Am J Clin Nutr* 108:1043–1059. <https://doi.org/10.1093/ajcn/nqy192>
- 655
- 656 ten Haaf DSM, Eijsvogels TMH, Bongers CCWG et al (2019) Protein supplementation improves lean body mass in physically active older adults: a randomized placebo-controlled trial. *J Cachexia Sarcopenia Muscle*. <https://doi.org/10.1002/jcsm.12394>
- 657
- 658 Tieland M, Franssen R, Dullemeijer C et al (2017) The impact of dietary protein or amino acid supplementation on muscle mass and strength in elderly people: individual participant data and meta-analysis of RCT's. *J Nutr Heal Aging* 21:994–1001. <https://doi.org/10.1007/s12603-017-0896-1>
- 659
- 660 Verdijk L, Jonkers RA, Gleeson BG et al (2009) Protein supplementation before and after exercise does not further augment skeletal muscle hypertrophy after resistance training in elderly men. *Am J Clin Nutr* 89:608–616. <https://doi.org/10.3945/ajcn.2008.26626>
- 661
- 662 World Health Organisation (1996) WHOQOL-BREF: introduction, administration, scoring and generic version of the assessment: field trial version, December 1996
- 663
- 664 Zamboni M, Mazzali G, Fantin F et al (2008) Sarcopenic obesity: a new category of obesity in the elderly. *Nutr Metab Cardiovasc Dis* 18:388–395
- 665
- 666
- 667
- 668
- 669
- 670
- 671
- 672
- 673
- 674
- 675
- 676
- 677
- 678
- 679
- 680
- 681
- 682
- 683
- 684
- 685
- 686
- 687
- 688
- 689
- 690
- 691
- 692
- 693
- 694
- 695
- 696
- 697
- 698
- 699
- 700
- 701
- 702
- 703
- 704
- 705
- 706
- 707
- 708
- 709
- 710
- 711
- 712
- 713
- 714
- 715
- 716
- 717
- 718
- 719



|          |             |
|----------|-------------|
| Journal: | <b>421</b>  |
| Article: | <b>4293</b> |

## Author Query Form

**Please ensure you fill out your response to the queries raised below and return this form along with your corrections**

Dear Author

During the process of typesetting your article, the following queries have arisen. Please check your typeset proof carefully against the queries listed below and mark the necessary changes either directly on the proof/online grid or in the 'Author's response' area provided below

| Query | Details Required   | Author's Response |
|-------|--|-------------------|
| AQ1   | Please check and confirm the inserted citation of Fig. 3 is correct. If not, please suggest an alternative citation. Please note that figures should be cited in sequential order in the text. |                   |
| AQ2   | Author: Kindly check the clarity of the sentence 'Considering the complete absence of benefit for the...'.<br>'  |                   |

Author Proof