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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 ± 6 years (mean ± 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 $_{HR}$-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). $_{HR}$-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 $_{HR}$-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
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ORIGINAL ARTICLE

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)

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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 ± 6 years (mean ± 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 $_{HR}$-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). $_{HR}$-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 $_{HR}$-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 Exercise · Whey protein · Myoelectrical muscle fatigue · Quality of life

Abbreviations

BIA	Bioelectrical impedance analysis
BMI	Body mass index
C	Control
E	Exercise
EMG	Electromyography
EP	Exercise + Protein
ESPEN	European Society for Clinical Nutrition and Metabolism
HR-QOL	Health-related quality of life
LHU-SAT	Liverpool Hope University—Sarcopenia Ageing Trial
MVC	Maximal voluntary contraction
P	Protein
RCT	Randomised controlled trial
RDA	Recommended dietary allowance

Communicated by Guido Ferretti .

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SD Standard deviation
SMI Skeletal muscle index

Introduction

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

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

Despite these advancements in knowledge, a recent meta-analysis showed that there are inconsistent findings from randomised controlled trials (RCTs) regarding the benefits of protein intake alone or combined with resistive exercise on muscle and fat mass in healthy older adults (Ten Haaf et al. 2019). This is likely due to heterogeneity factors with most trials not achieving the upper per meal threshold of protein intake required to maximise muscle protein synthesis rates (Moore et al. 2015). In addition, several trials have failed to include a protein group alone which rules out the possible benefits of this nutrient for older adults who are not willing or able to exercise. It should also be noted that other RCTs (Norton et al. 2016) and cross-sectional studies (Houston et al. 2008) demonstrated that muscle mass still declines in 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 myoelectrical descriptors of fatigue, which is surprising considering 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 knowledge 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 supplementation 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 ~ 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 procedures 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 schematic of the trial design.

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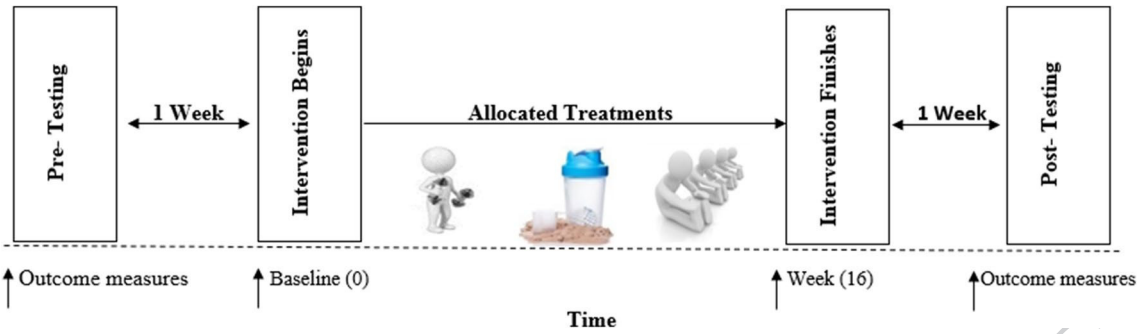

Fig. 1 Schematic of the trial design

Participants

The baseline characteristics of participants are presented in Table 1. Participants were ambulant, community-dwelling older adults (≥ 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
n = [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^2]	26.2 ± 4.5	28.1 ± 7.4	27.4 ± 4.9	27.1 ± 4.1
SMI [kg/m^2]	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

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Protein supplementation

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

Exercise history and dietary control

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

Outcome measures

Muscle and fat mass

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

Myoelectrical muscle fatigue

Muscle fatigue was measured using 16-channel electromyography (EMG) instrument following a validated technique by our laboratory (Alizadehkhaiyat et al. 2018; Hawkes et al. 2018). First, maximal voluntary contraction (MVC) of the dominant limbs was performed on the following exercises: *handgrip*, participants were seated upright in an armless chair (46–49 cm in height) with elbow flexed at 90° (verified by goniometer) and instructed to apply maximal pressure for 3 s to a handheld Jamar dynamometer (Biometrics Ltd, Wireless Dynamometer G200, Newport, UK); *leg flexion and extension* participants were seated upright in a heavy-duty chair mounted to the floor and attached to a portable strain gauge (Mecmesin 851–401 Multifunction Force/Torque Indicator, Mecmesin Limited, West Sussex, UK). The lower limbs were attached to the lever arm by a padded gauze strap placed above the malleoli. Straps were adjusted accordingly to ensure hip and knee angles were 85° and 90°, respectively, with the full extension being 0° (verified by goniometer). Participants performed six MVCs (3 × familiarisation, 3 × testing), with 30 s break between repetitions and 2 min between familiarisation and testing sets. Strong verbal encouragement was applied throughout. A pilot study carried out before data collection among ten younger adults (five males, five females) indicated the inter-day coefficient of variation for this procedure was < 1.5%.

EMG signals of the key agonist muscles during handgrip [flexor carpi radialis (FCR)], leg extension [rectus femoris (RF)] and leg flexion [bicep femoris (BF)] exercises at $MVC_{25\%max}$ were recorded for 70 s (the first and last 5 s were excluded from analysis) to provide an index of fatigue. To ensure $MVC_{25\%max}$ remained constant, visual feedback was provided by dynamometer (E-LINK version 14.02, Biometrics Ltd.) and myometer (Emperor Lite version 1.18–408, Mecmesin Ltd.) software. Participants' skin was prepared by shaving and cleaning with alcohol wipes before placement of self-adhesive Ag/AgCl bipolar surface electrodes with 10 mm diameter and 20 mm inter-electrode distance (Noraxon Inc.) (Kallenberg and Hermens 2008). To limit cross talk, electrodes were placed parallel to muscle fibres on the belly of the muscles following accepted anatomical criteria (Kallenberg and Hermens 2008). Signals were confirmed by manual muscle testing.

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

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Health-related quality of life

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

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

Statistical analysis

Statistical analyses were performed using SPSS Statistics 25 (IBM Corporation, New York, USA). Normality was assessed via Kolmogorov–Smirnov tests, which showed a skewed distribution for body composition, muscle fatigue and WHOQOL-BREF data. Logarithmic transformations were unsuccessful at normalising these variables, so non-parametric testing was used. Within-group comparisons of pre- and post-intervention were undertaken using Wilcoxon signed ranks tests. Between-group differences (*C* vs *E* vs EP vs *P*) were analysed via Kruskal–Wallis (*H*) test followed by Bonferroni-corrected Mann–Whitney (*U*) tests for post hoc comparisons. Cohen's *d* effect sizes (ES) were calculated with the magnitude of effects considered: small (0.20–0.49), medium (0.50–0.79) or large (> 0.80). ES were calculated by 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 consuming low (≤ 0.8 g/kg/day) or higher intake of protein (≥ 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 [± standard deviation (SD)] and differences 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 ± 10% and 78 ± 10% of their prescribed exercise sessions, respectively. Compliance with the protein supplement was 43 ± 14% and 74 ± 25% in EP and *P* trial groups, respectively. Taking into account habitual levels, protein intake increased from ~ 1.2 ± 0.4 at baseline to 1.5 ± 0.7 g/

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

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

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

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kg/day in EP during the trial, and from ~ 1.0 ± 0.2 at baseline to 1.9 ± 0.7 g/kg/day in *P* during the trial (Table 2).

Effect of intervention

Muscle and fat mass

No within- or between-group differences were observed for muscle or fat mass ($p > 0.05$, Table 3), and body weight (Δchange, *C*: 0.1 ± 0; *E*: − 0.8 ± 1.8; EP: − 0.8 ± 0.6; *P*: 1.0 ± 0.3 kg, $p = 0.61$), BMI (Δchange, *C*: 0 ± 0; *E*: − 0.3 ± 0.8; EP: − 0.1 ± 0.4; *P*: 0.3 ± 0.2 kg/m^2, $p = 0.72$) and SMI (Δchange, *C*: 0 ± 0.1; *E*: 0.1 ± 0; EP: 0.1 ± 0; *P*: − 0.1 ± 0.1 kg/m^2, $p = 0.66$) did not change.

Myoelectrical muscle fatigue

At baseline, muscle fatigue was successfully induced in the flexor carpi radialis, rectus femoris and bicep femoris, demonstrating the efficacy of this testing procedure (all $p < 0.001$). In response to the exercise intervention, the rectus femoris (*E*: − 3.8 ± 4.9 to 0.5 ± 6.4, $p = 0.028$; EP: − 6.3 ± 5.0 to − 0.9 ± 6.7, $p = 0.011$) and bicep femoris (*E*: − 5.4 ± 2.2 to − 0.9 ± 1.6, $p < 0.001$; EP: − 6.1 ± 2.7 to − 0.7 ± 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		*p* value
	Pre	Post	Pre	Post	Pre	Post	Pre	Post	
Muscle mass [kg]									
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
Fat mass [kg]									
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
Handgrip MVC [kg]									
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
Leg extension MVC [*n*], *n* = 99									
Men	343 ± 124	300 ± 105	284 ± 87	389 ± 103**	285 ± 56	373 ± 107**	276 ± 102	283 ± 103	**0.034`**
Women	180 ± 49	171 ± 44	233 ± 126	266 ± 61**	173 ± 46	279 ± 91**	190 ± 105	173 ± 56	**0.001**
Combined	249 ± 119	225 ± 98	258 ± 109	328 ± 104**	219 ± 75	318 ± 107**	243 ± 110	240 ± 102	**< 0.001**
Leg flexion MVC [n]									
Men	162 ± 79	148 ± 66	142 ± 44	188 ± 55**	146 ± 50	178 ± 60**	139 ± 48	160 ± 59	**0.039**
Women	103 ± 48	100 ± 39	148 ± 97	147 ± 35	97 ± 31	149 ± 44**	140 ± 142	101 ± 35	**0.014**
Combined	127 ± 68	120 ± 56	145 ± 74	168 ± 49**	117 ± 46	161 ± 52**	139 ± 91	138 ± 58	**0.001**
Fatigue FCR [slope %/min]									
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
Fatigue RF [slope %/min]									
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	**0.031**
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	**0.001**
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	**< 0.001**
Fatigue BF [slope %/min]									
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	**0.001**
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	**< 0.001**

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 ($p < 0.05$)

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

Health-related quality of life ($_{HR}$-QOL)

Post-intervention, significant improvements from baseline measures were found in $_{HR}$-QOL in the E group with respect to overall perception of health, psychological health, social relations and environmental health (Table 4). $_{HR}$-QOL levels were normal for age across all domains: of significance, higher age was associated with better perception of one's health; higher age was associated with better psychological health in *E*, and social relations were negatively related to age in the control group. There was no difference according to sex on any $_{HR}$-QOL measure.

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

Sub-group analysis

A separate analysis was performed to check for sex differences between groups relating to muscle and fat mass, and manifestations of myoelectrical muscle fatigue. Findings showed a similar outcome as to when sexes were combined in the analysis (Table 4).

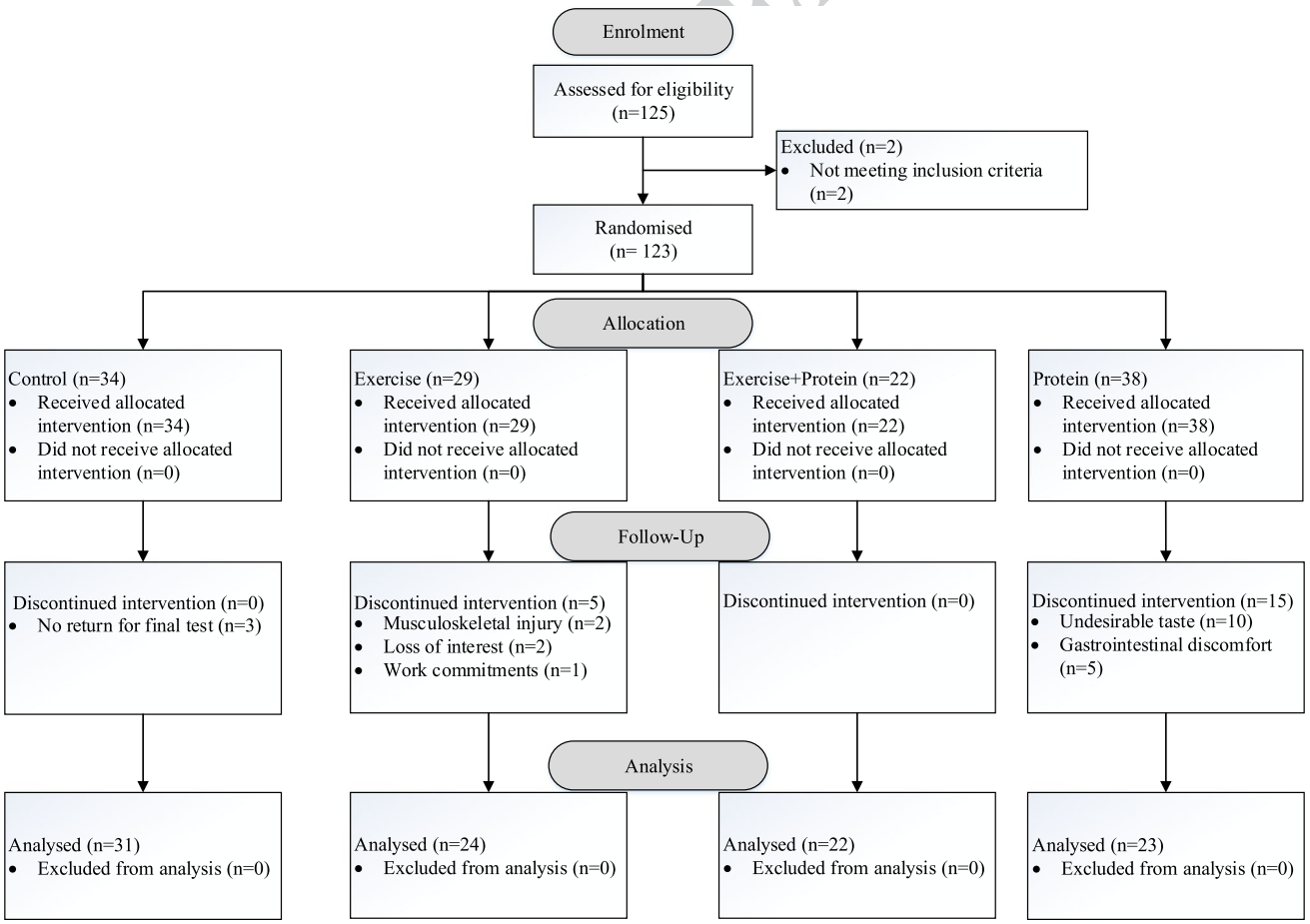

Fig. 2 Flowchart of RCT

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Table 4 WHOQOL-BREF means (±standard deviation) and Wilcoxon signed ranks test statistic (with exact *p* value*) 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 (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 (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 (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 (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 (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 (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

Discussion

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

In our trial, combined habitual intake of protein was ~ 1.1 ± 0.3 g/kg/day which is comparable to cross-sectional reports in older adults (ten Haaf et al. 2018a, b), although higher in community-dwelling older adults suffering from mobility limitations (Houston et al. 2008, 2017) (Fig. 3).

Effects on muscle and fat mass

Protein intake increased from ~ 1.2 to 1.5 g/kg/day in EP and ~ 1.0 to 1.9 g/kg/day in P groups, yet we observed no benefits on body composition. This finding supports our primary analysis of the LHU-SAT (Kirk et al. 2019) where protein supplementation did not augment the exercise-induced increases in muscle strength, physical functioning or aerobic capacity. A likely explanation for this is our population were already consuming sufficient quantities of protein at baseline. In support, three studies (Verdijk et al. 2009; Leenders et al. 2013; Holwerda et al. 2018) found no benefit of whey protein during resistance exercise on muscle mass in healthy older adults habitually consuming 1.1–1.2 g/kg/day of protein, whereas 12 weeks of milk protein during a walking exercise regimen increased muscle mass and decreased fat mass in healthy older adults with low levels of protein (~ 0.86 g/kg/day) at trial enrolment (ten Haaf et al. 2019). Another recent trial reported greater increases in muscle mass and reductions in fat mass, following 12 weeks of combined elastic band/body weight exercise and protein supplementation, although habitual protein intake was not assessed which limits comparisons with our trial (Krause et al. 2019). Taken together, these findings suggest that the current protein recommendations for well-nourished active older adults by the PROT-AGE and ESPEN study groups of at least 1.0–1.2 g/kg/day are sufficient.

During ageing, medical conditions may prohibit exercise participation and as such effective dietary supplements are warranted to curb muscle mass loss. However, we found no benefit of whey protein on muscle mass alone, which may be due to the above-mentioned explanations (i.e., adequate intake of protein at baseline). On the other hand, longer interventions (≥ 24 weeks) are recommended to observe detectable increases in muscle mass when prescribing protein alone in older adults (Tieland et al. 2017), and meta-analyses have reported small, but significant effects on muscle mass independent of exercise (Hanach

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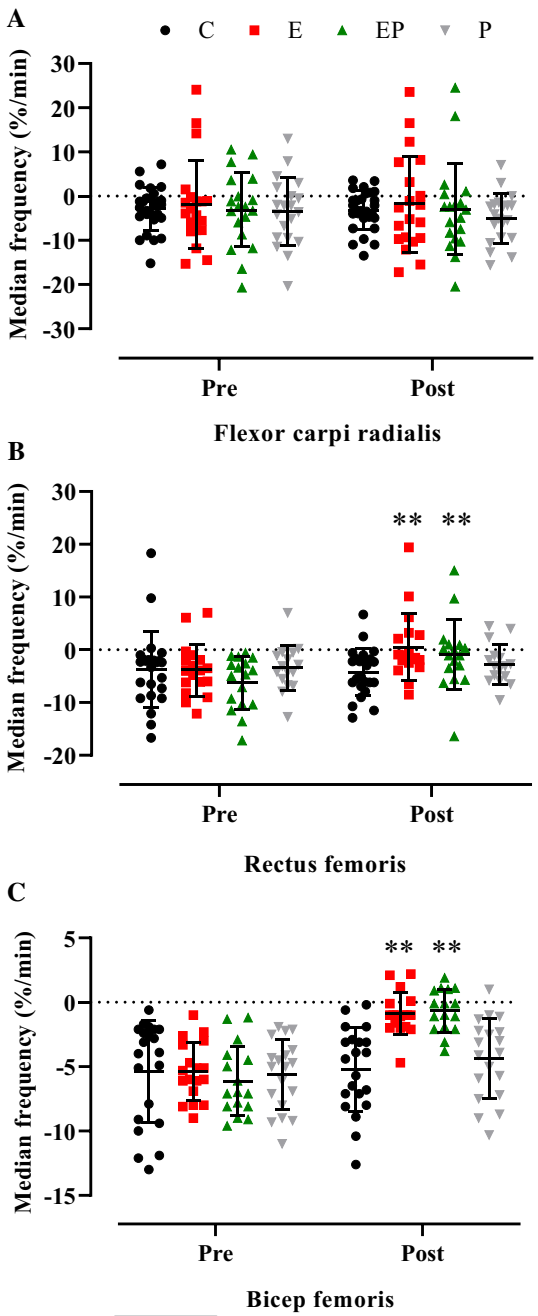

Fig. 3 Myoelectrical muscle fatigue values for **a** flexor carpi radialis, **b** rectus femoris and **c** bicep femoris in response to independent treatments (*C* Control, *E* Exercise, *EP* Exercise + Protein, *P* Protein). Values are mean ± SD with individual data points shown. **Indicates between-group 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 ~ 78 ± 10%).

Effects on $_{HR}$-QOL

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

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

Strength and limitations

To our knowledge, we have performed the largest RCT with four (Control; Exercise; Exercise + Protein; Protein) distinct treatment groups in community-dwelling older adults demonstrating valuable findings in terms of adaptations in myoelectrical fatigue and $_{HR}$-QOL. We are also the first group which has attempted to prescribe leucine and whey protein at recommended dosages, at each meal, and by individual body weight (Moore et al. 2015). However, some limitations should be noted. Firstly, we did not exclude those consuming protein above the RDA at trial enrolment, which we feel greatly hindered our findings. In this regard, future trials should investigate the effect in older adults habitually consuming the RDA of protein, to assess if a higher intake of protein (with or without exercise) is necessary to prevent, or at least delay, sarcopaenia. Secondly, the trial was not placebo controlled, which increases the risk of bias. Thirdly, with myoelectrical indices, signalling can be confounded by subcutaneous fat or cross talk between other muscles and thus this may have influenced our findings. Including a muscle activation measurement would have also enriched the findings and allowed the effect of protein intake on this parameter to be investigated. Finally, obtaining muscle tissue samples would have enabled the effect of protein timing on muscle protein synthesis rates to be explored. However, it should be noted that a recent trial found no effect of protein timing on resistance exercise-induced gains in muscle mass, strength and physical functioning in postmenopausal women (de Branco et al. 2019). Nevertheless, future RCTs should consider these aspects.

Conclusions

In conclusion, 16 weeks of multimodal exercise (resistance and functional) attenuated lower-limb myoelectrical fatigue 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.

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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.

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Compliance with ethical standards

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

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