


ORIGINAL ARTICLE

EPIDEMIOLOGY, CLINICAL PRACTICE AND HEALTH

Effect of whey protein supplementation after resistance exercise on the muscle mass and physical function of healthy older women: A randomized controlled trial

Hiroyasu Mori¹  and Yasunobu Tokuda²

¹Diabetes Therapeutics and Research Center, University of Tokushima, Tokushima, Japan

²Faculty of Health Science Department, Hyogo University, Kakogawa, Japan

Correspondence

Registered Dietitian Hiroyasu Mori, Diabetes Therapeutics and Research Center, University of Tokushima, 3-18-15 Kuramoto, Tokushima, Tokushima 770-8503, Japan.
Email: hiroyam31@gmail.com

Received: 11 December 2017

Revised: 31 May 2018

Accepted: 2 July 2018

Aim: To evaluate the effectiveness of a 24-week program of nutritional supplementation using whey protein, ingested after resistance exercise, in increasing muscle mass and physical function among community-dwelling healthy older Japanese women.

Methods: We carried out a randomized controlled trial, with 81 healthy women, aged 65–80 years, allocated to three groups of 27 participants each: the exercise and protein supplementation group, the exercise only group, and the protein supplementation only group. A 24-week program of resistance exercise, carried out twice per week, was combined with whey protein supplementation, containing 22.3 g of protein. The total protein intake for participants in all three experimental groups was adjusted to a level of at least 1.2 g/kg bodyweight/day, and more during the intervention period. Between-group differences in the pre- to post-intervention change in skeletal muscle mass and physical function were evaluated using an analysis of variance.

Results: The pre- to post-intervention increase in the skeletal muscle mass index was significantly higher for the exercise only group than for the protein supplementation only group ($P = 0.008$), and significantly higher for the exercise and protein supplementation group than for either the exercise only ($P = 0.007$) or protein supplementation only ($P < 0.001$) groups. Similarly, the increase in grip strength and gait speed was significantly greater for the exercise and protein supplementation group than for the protein supplementation only group (grip strength $P = 0.014$, gait speed $P = 0.026$).

Conclusions: Whey protein supplementation, ingested after resistance exercise, could be effective for the prevention of sarcopenia among healthy community-dwelling older Japanese women. *Geriatr Gerontol Int* 2018; ••: ••–••.

Keywords: muscle mass, muscle strength, resistance exercise, total protein intake amount, whey protein.

Introduction

Sarcopenia is defined as an age-related decrease in skeletal muscle mass and physical function.¹ Among older Japanese individuals, the risk for falls is higher among women than men, with a decrease in physical function being a further risk factor for falls.²

The postprandial muscle protein synthesis response is modulated by the type and amount of total protein of the supplement.³ There is current evidence of a positive effect of a supplementation of approximately 20–25 g of whey protein or daily total protein intake of at least of 1.2 g/kg bodyweight/day during resistance exercise training in older individuals.^{4–8} Leucine contained in whey protein has been reported to stimulate muscle protein synthesis by activating the mammalian target of the rapamycin signaling pathway.^{4–8} As muscle protein synthesis peaks after exercise, followed by a gradual time-dependent decrease, providing whey protein after exercise in a supplement form that is quickly digested and absorbed could be important in optimizing muscle protein accumulation.^{9,10} However, previous studies have not identified a benefit of protein supplementation after resistance exercise in enhancing muscle mass among older individuals.¹¹ In these studies, the effect of combining resistance exercise to whey protein supplementation was not evaluated, using a meal intake in which the total daily protein intake amount was at least 1.2 g/kg bodyweight/day. Therefore, the aim of the present study was to specifically evaluate the effect of a whey protein supplement, ingested after resistance exercise, in a nutritional state in which total energy

and protein intake was maintained above the recommended amount, on the muscle mass and physical function among community-dwelling healthy older Japanese women, using a randomized controlled design.

Methods

Participants

Participants were 200 community-dwelling older women, aged 65–80 years, from the Higashi-harima community in Hyogo, Japan. This study was approved by the ethics committee of Hyogo University. The trial was registered at the University Hospital Medical Information Network (UMIN000023713).

Sample size was calculated to detect an increase in leg muscle strength of 3.3 kg (standard deviation 1.5 kg) during nutritional supplementation, combined with exercise, in older individuals compared with exercise alone.¹² Assuming a similar response to supplementation in the present study, identified using a repeated measures of analysis of variance (ANOVA), at a power of 80% with alpha set at 0.05 (2-tailed), 19 individuals were to be included in each group. To be conservative and to allow for a potential drop-out rate of 25%, we aimed to enroll 27 individuals per group.

Using the recommendation from the PROT-AGE study group of a total protein intake of at least of 1.2 g/kg bodyweight/day, with a higher concentration during resistance exercise training, as a baseline reference, participants completed a dietary survey to

screen for daily protein ingestion.³ Based on the results of this dietary survey, 28 participants were excluded due a daily protein intake <1.2 g/kg/day, with another 46 participants excluded due to an intake >1.3 g/kg/day. In addition, 45 participants with a history of sarcopenia, obesity, type 2 diabetes or kidney disease, within 1-year of the intervention, were excluded. Written informed consent was obtained from the remaining 81 participants enrolled in the trial.

The 81 healthy older participants were allocated to three groups, each with 27 participants: the exercise and protein (EX + PRO) group, the exercise only (EX) group, and the protein only (PRO) group. A stratified randomization strategy was used for group allocation to achieve a comparable age distribution among the groups, using a single blind method. None of the participants had taken part in a structured program of resistance exercise within 5 years before the study intervention.

Intervention design

The EX + PRO intervention consisted of ingestion of a whey protein supplement after the resistance exercise program. Effects of the EX + PRO intervention were evaluated against the EX group (control group 1), in which participants completed the same exercise program, but without protein supplementation, and the PRO group (control group 2), in which participants ingested the same protein supplement, but without prior exercise. The intervention period was 24 weeks, with participants completing their assigned protocol twice a week.

Measurement of body composition

The body mass index was calculated by dividing bodyweight (kg) by height squared (kg/m²). Body fat percentage and upper and lower limb muscle mass were evaluated using multifrequency bioelectrical impedance (In-Body bioelectrical impedance analyzer; Bio Space, Seoul, Korea).¹³

Measurement of physical functions

The following measures of physical function were evaluated: grip strength, knee extension strength and gait speed. Grip and knee extension strength were measured by handheld dynamometry (T.K.K5401; Takei Instruments, Tokyo, Japan; μ -tus F-100; ANIMA, Tokyo, Japan). Gait speed was measured in time units (0.01 s), using a stopwatch. Sarcopenia was calculated using the skeletal muscle mass index (SMI), physical function parameters, and the diagnostic algorithm and cut-off values defined by the Asian Working Group for Sarcopenia.¹⁴ Blood pressure was measured using an upper arm cuff with participants in a supine position.

Nutritional survey

A nutritional survey was carried out to document the total daily energy intake, the total intake of protein (adjusted by bodyweight, g/kg/day), fat and carbohydrates. The survey was complemented by weighing the daily food intake (Excel Eiyou version 5.0; Kenpakusha, Tokyo, Japan). The nutrition questionnaire and weighing of the daily food intake were completed on five consecutive days before the start of the intervention, and on a daily basis during the 24-week intervention period.¹⁵

Daily activity survey

Participants recorded their daily activity for three consecutive days, before the start of the program, with the information used to calculate each participant's regular physical activity level. Again, individual interviews were used to complete missing information. The estimated energy requirement to meet each individual's physical activity level was calculated as the basal metabolic rate \times bodyweight (kg) \times physical activity level.¹⁵

Resistance exercises

The exercise intervention program included both bodyweight resisted and resistance band exercises.^{16–18} Bodyweight resisted lower body exercises included rising and sitting from a chair, and leg extensions. Resistance elastic band exercises (REP BAND; Magister Corporation, Chattanooga, TN, USA) included upper and lower body exercises. The resistance load (50–70% of the 1 repetition maximum) was modified, in a standardized fashion, over the 24-week program. The detailed exercise regimen is presented in Appendix S1.

Protein supplementation

Protein supplementation was provided to participants in the EX + PRO and PRO groups. The protein supplement contained 92 kcal of energy, 22.3 g of protein, 0.3 g of fat, 0.1 g of carbohydrate, 1225 mg of valine, 2975 mg of leucine and 1175 mg of isoleucine per 25 g of one intake serving (Whey Protein; Ezaki Glico Company, Tokyo, Japan).

Nutritional management

Total protein intake for participants in all three experimental groups was adjusted to a level of at least 1.2 g/kg/day, and more during the intervention period.³ Nutritional management was provided by a nutritionist, and was based on the Japanese Dietary Reference Intakes and individual results on the nutrition survey carried out before the intervention.^{15,18} The detailed nutritional management regimen is presented in detail in Appendix S2.

Statistical analysis

Between-group differences in the distribution of physical characteristics were evaluated, pre-intervention, using an independent group one-way analysis of variance (ANOVA) analysis; between-group differences in SMI, grip strength and gait speed that were below the cut-off values were evaluated using a χ^2 -test analysis. Between-group differences in outcome variables, measured pre- and post-intervention, were evaluated using a two-way ANOVA. Changes in measured outcomes of bodyweight, limb muscle mass, SMI, grip strength, knee extension strength, gait speed and nutritional intake were evaluated between groups (EX + PRO, EX and PRO) and time (pre- and post-intervention) using a repeated measures ANOVA, with group as the independent factor and time as the repeated factor. For identified main effects and interactions, multiple comparisons were carried out using the Tukey post-hoc analysis, with the rate of change (Δ) in measured outcomes, pre- to post-intervention, compared using an unpaired one-way ANOVA. On multivariate regression analysis, physical characteristics were used as a predictive factor of the pre- to post-intervention change in lower limb muscle mass and knee extension.

All statistical analyses were carried out using IBM SPSS statistical software (IBM, Tokyo, Japan), with the level of significance defined as a *P*-value <0.05.

Results

Six participants, two in each of the three experimental groups, were unable to complete the intervention after randomization due to a lack of motivation ($n = 4$), knee pain ($n = 1$) and a change in residence ($n = 1$; Fig. 1). Baseline measures of physical characteristics are listed in Table 1, with no between-group differences identified. There was no difference in the physical activity level and estimated energy requirement between the groups in the pre-intervention period. The mean adherence rate to the program, over the 24-weeks of the intervention, was 90.1% in the EX + PRO group, 86.6% in the EX group and 86.5% in the PRO group.

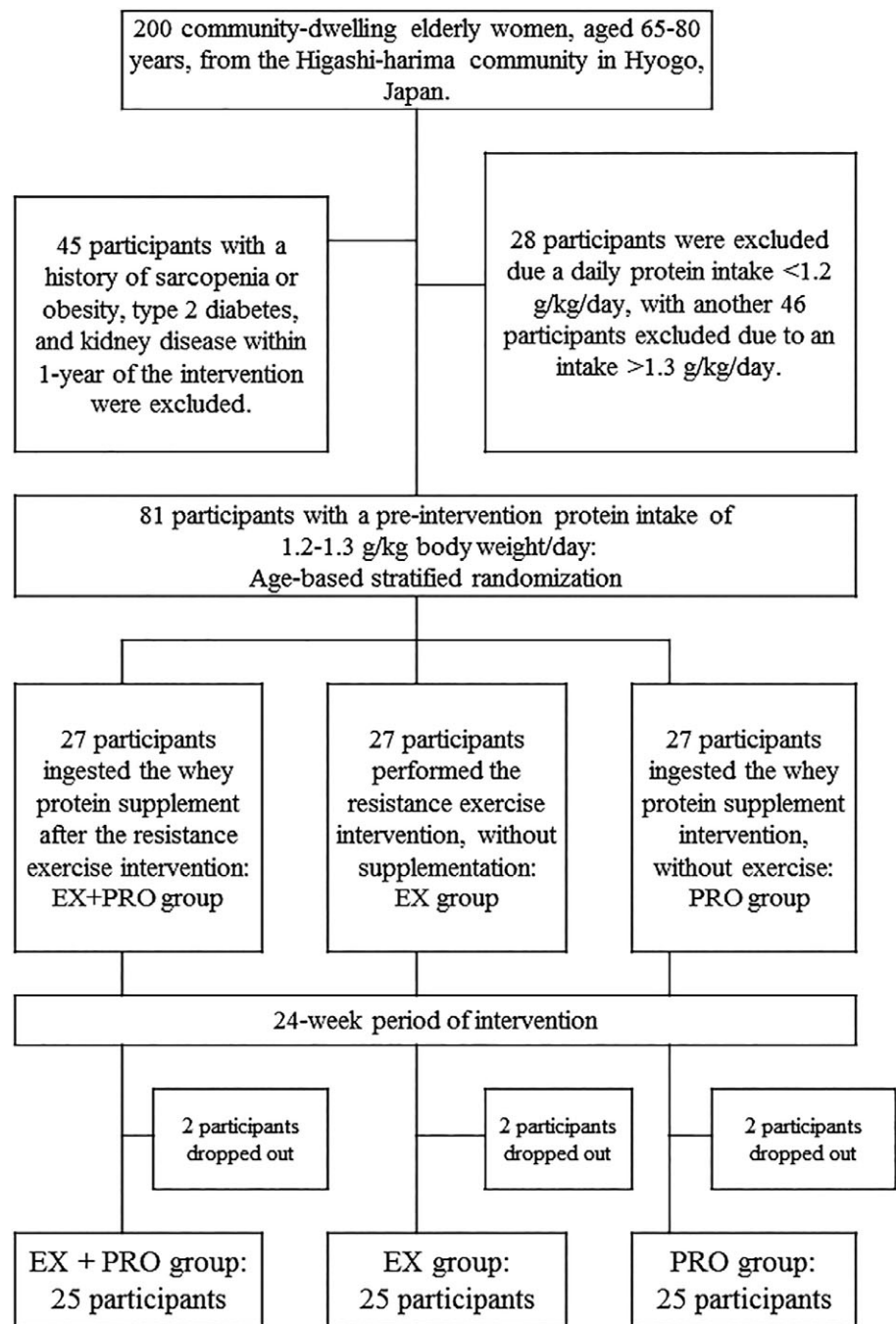


Figure 1 Flow chart of study participants. Participants with a pre-intervention protein ingestion of 1.2–1.3 g/kg bodyweight/day were randomly allocated to one of the three experimental groups: completing the exercise intervention with supplementation following (EX + PRO), completing only the exercise intervention (EX group) and ingestion of the supplementation only (PRO group).

The pre- to post-intervention changes in muscle mass and physical function are reported in Table 2. A significant group by time interaction was identified for lower limb muscle mass ($P < 0.001$), SMI ($P < 0.001$), grip strength ($P = 0.04$), knee extension strength ($P < 0.001$) and gait speed ($P = 0.019$). The pre- to post-intervention rate of change (Δ ; Fig. 2) in lower limb muscle mass, SMI and knee extension strength was significantly greater for the EX group than for the PRO group (lower limb muscle mass $P = 0.018$, SMI $P = 0.008$, knee extension strength $P = 0.027$), and significantly greater for the EX + PRO group than for both the EX (lower limb muscle mass $P = 0.038$, SMI $P = 0.007$, knee extension strength $P = 0.005$) and PRO (lower limb muscle mass, SMI, knee extension strength $P < 0.001$) groups. The change in upper limb muscle mass, grip strength and gait speed was also significantly greater for the EX + PRO group than for the PRO group (upper limb muscle mass $P = 0.029$, grip strength $P = 0.014$, gait speed $P = 0.026$).

Total energy and nutritional intake values, before and during the intervention, are reported in Table 2. All participants were able to ingest a total protein amount of at least 1.2 g/kg/day, and more

during the intervention period. The total energy intake during the intervention was never below the estimated energy requirement. There was no significant group by time interaction for total energy intake, total protein, protein/bodyweight, fat and carbohydrate intake.

Discussion

In the present study, we report the effectiveness of whey protein supplementation, ingested after resistance exercises, in increasing lower limb muscle mass, SMI and knee extension strength among community-dwelling healthy older Japanese women. Therefore, whey protein supplementation might be effective for the prevention of sarcopenia among healthy older women when ingested after resistance exercise in a nutritional state in which total energy and protein intake was maintained above the recommended amount.

Increases of 10–15% in muscle strength and 5% in muscle mass have been reported with high intensity ($\geq 80\%$ 1 maximum

Table 1 Physical characteristics of participants, pre-intervention

	EX + PRO group	EX group	PRO group	P-value		
	(n = 25)	(n = 25)	(n = 25)	EX + PRO vs EX	EX + PRO vs PRO	EX vs PRO
Age (years)	70.6 ± 4.2	70.6 ± 4.2	70.6 ± 4.6	0.837	1.000	1.000
SBP (mmHg)	116 ± 9	114 ± 10	118 ± 6	0.735	0.775	0.331
DBP (mmHg)	68 ± 9	69 ± 7	73 ± 12	0.921	0.113	0.235
Height (cm)	152.6 ± 5.7	155.3 ± 5.7	154.3 ± 5.1	0.194	0.503	0.806
Bodyweight (kg)	51.5 ± 6.1	55.4 ± 7.2	53.3 ± 5.9	0.081	0.59	0.457
BMI ² (kg/m ²)	22.1 ± 2.1	22.9 ± 2.9	22.3 ± 2.1	0.286	0.876	0.559
Body fat (%)	28.5 ± 6.1	29.7 ± 6.8	29.1 ± 5.5	0.774	0.937	0.936
Physical activity level	1.75 ± 0.04	1.76 ± 0.05	1.75 ± 0.05	0.884	0.991	0.821
Estimated energy requirement (kcal/day)	1865 ± 220	2008 ± 260	1929 ± 214	0.081	0.59	0.456
Upper limb muscle mass (kg)	3.5 ± 0.7	3.8 ± 0.7	3.4 ± 0.6	0.113	0.926	0.059
Lower limb muscle mass (kg)	10.7 ± 1.2	10.8 ± 1.4	11.0 ± 1.3	0.904	0.662	0.899
SMI (kg/m ²)	6.1 ± 0.5	6.1 ± 0.7	6.0 ± 0.6	0.999	0.984	0.974
Grip strength (kg)	22.4 ± 3.4	23.1 ± 5.3	23.0 ± 3.7	0.83	0.87	0.996
Knee extension strength (kg)	23.8 ± 6.3	26.7 ± 3.8	26.3 ± 5.9	0.159	0.262	0.958
Gait speed (m/s)	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	0.971	0.915	0.802
SMI ≤ cut-off value (%)	28.0	24.0	28.0	0.999	1.000	0.999
Grip strength ≤ cut-off value (%)	8.0	16.0	8.0	0.667	1.000	0.667
Gait speed ≤ cut-off value (%)	0.0	0.0	0.0	1.000	1.000	1.000

Mean value ± standard deviation. The results of a non-paired one-way ANOVA or χ^2 -test showed no differences in the characteristics of the three groups pre-intervention. BMI, body mass index; DBP, diastolic blood pressure; EX, exercise only group; EX + PRO, exercise and protein group; PRO, protein only group; SBP, systolic blood pressure; SMI, skeletal muscle mass index.

repetition) resistance exercise training among older individuals.^{19,20} Our resistance-training program produced an average increase of 2.9% in lower limb muscle mass and 4.0% in knee extension strength (EX group). Our use of a moderate intensity of resistance training (50–70% 1 maximum repetition) could explain the lower increase in muscle strength and mass than levels previously reported.²¹ Our combination of resistance training with whey protein supplementation increased lower limb muscle mass

by 5.3%, grip strength by 5.5% and knee extension strength by 14.1% (EX + PRO). Therefore, whey protein supplementation, provided after moderate-intensity whole-body movement resistance exercise, resulted in increases in lower and upper limb muscle strength among older women.

Previous studies have shown the effect of inflammatory cytokines in accelerating muscle protein degradation among older individuals with obesity and/or type 2 diabetes.^{22,23} Furthermore,

Table 2 Comparison of muscle mass, physical function and nutritional intake pre- and post-intervention, as well as during the intervention

	Pre-intervention			Post-intervention			P-value, group × timeinteraction
	EX + PRO group	EX group	PRO group	EX + PRO group	EX group	PRO group	
Bodyweight (kg)	51.5 ± 6.1	55.4 ± 7.2	53.3 ± 5.9	52.6 ± 6.2	55.9 ± 7.2	53.1 ± 5.9	<0.001
Upper limb muscle mass (kg)	3.5 ± 0.7	3.8 ± 0.7	3.4 ± 0.6	3.5 ± 0.7	3.8 ± 0.7	3.3 ± 0.6	0.060
Lower limb muscle mass (kg)	10.7 ± 1.2	10.8 ± 1.4	11.0 ± 1.3	11.2 ± 1.2	11.1 ± 1.4	11.1 ± 1.3	<0.001
SMI (kg/m ²)	6.1 ± 0.5	6.1 ± 0.6	6.0 ± 0.6	6.3 ± 0.5	6.2 ± 0.7	6.0 ± 0.6	<0.001
Grip strength (kg)	22.4 ± 3.4	23.1 ± 5.3	23.0 ± 3.7	23.6 ± 3.5	23.7 ± 5.6	23.6 ± 4.4	0.040
Knee extension strength (kg)	23.8 ± 6.4	26.7 ± 3.8	26.3 ± 5.9	26.4 ± 5.5	27.8 ± 4.3	25.1 ± 5.6	<0.001
Gait speed (m/s)	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.1	1.3 ± 0.2	1.3 ± 0.1	0.019
	Pre-intervention			During intervention			P value, group × timeinteraction
	EX + PRO group	EX group	PRO group	EX + PRO group	EX group	PRO group	
Total energy intake (kcal/day)	1806 ± 191	1840 ± 165	1813 ± 175	1847 ± 188	1889 ± 172	1850 ± 171	0.704
Protein (g/day)	64.1 ± 6.9	69.2 ± 8.2	67.4 ± 7.2	70.3 ± 7.9	75.6 ± 8.2	73.1 ± 7.3	0.425
Protein/bodyweight (g/kg/day)	1.3 ± 0.0	1.3 ± 0.0	1.3 ± 0.0	1.4 ± 0.0	1.4 ± 0.1	1.4 ± 0.1	0.660
Fat (g/day)	54.8 ± 9.0	53.9 ± 8.4	54.6 ± 6.7	54.0 ± 8.0	53.0 ± 5.7	55.1 ± 6.1	0.769
Carbohydrates (g/day)	263.9 ± 42.0	269.5 ± 34.3	262.2 ± 44.2	268.1 ± 42.6	277.3 ± 38.6	265.4 ± 34.2	0.411

Mean value ± standard deviation. Two-way ANOVA, group (the exercise and protein [EX + PRO] group, the exercise only [EX] group, and the protein only [PRO] group) × time (pre- and post- or during the intervention period) interaction.

the effects of exercise are modulated by an individual's nutritional status, with poor nutritional status (e.g. sarcopenia) lowering the benefits of exercise on muscle mass and strength.²⁴ Therefore, it is important to note that the benefits of combining a moderate-intensity resistance exercise program with whey protein supplementation reported in the present study are specific to healthy older women who had no history of obesity and/or type 2 diabetes, and a good nutritional status.

Over the duration of our 24-week intervention period, participants in all groups maintained a sufficient daily intake of protein ≥ 1.2 g/day. Furthermore, the total energy intake was never below the estimated energy requirement. The fact that protein intake was equivalent across the groups underlines the specific

effectiveness of our whey protein supplementation in increasing muscle mass and strength, with identified differences not likely to be due to between-group differences in total protein and energy intake. We propose that failure of previous studies to identify a benefit of protein supplementation and exercise on muscle mass and strength among older individuals is likely due to a lack of controlling for habitual total protein intake and, therefore, of the total daily intake of protein.^{11,25} There is a benefit of exercise alone in improving muscle mass and strength in the EX group, as long as the nutritional requirements for habitual total energy and protein are satisfied. We did not observe an increase in muscle mass and strength in the PRO group. However, the increase in SMI and knee extension strength was greater in the EX + PRO

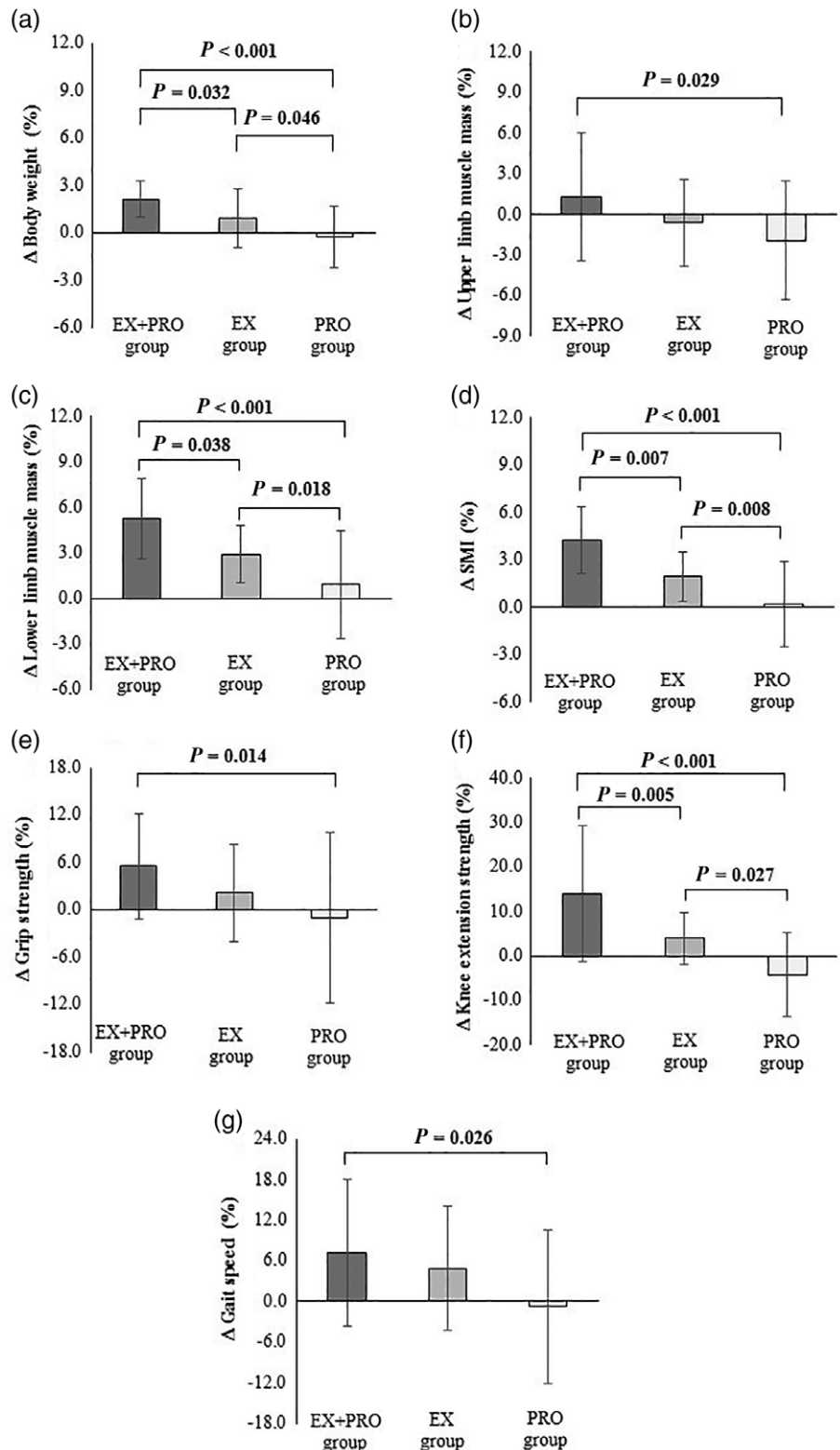


Figure 2 Mean (\pm SD) change in (a) bodyweight, (b) upper and (c) lower limb muscle mass, (d) skeletal muscle mass index (SMI), (e) grip strength, (f) knee extension strength, and (g) gait speed from baseline to the end of the 24-week intervention period, for the three experimental groups (completing the exercise intervention with supplementation following [EX + PRO], completing only the exercise intervention [EX group] and ingestion of the supplementation only [PRO group]). Between-group differences were evaluated using a Tukey post-hoc test; the *P*-value is shown.

than the EX group, indicating that the use of a whey protein supplement can enhance the effects of resistance exercise.

In the present study, we selected a protein supplement with previously demonstrated effectiveness in enhancing muscle protein synthesis among older individuals. The PROT-AGE study recommended a one-time daily ingestion of 20–25 g of protein as being effective,³ with whey protein having a high digestion and absorption rate,^{9,10} compared with other types of protein. Furthermore, there is evidence of a progressive age-associated attenuation of the sensitivity to leucine for high protein synthesis activity in muscles.^{4,5,7,8} A previous study reported that muscle protein synthesis in older individuals peaked approximately 1–3 h after resistance exercise, and 1–2 h after ingestion of a whey protein supplement.^{10,26} Although we identified a benefit of our whey protein supplementation in increasing muscle mass and strength, the physiological mechanisms by which a single dose of ingested whey protein, combined with resistance exercise, influences muscle protein synthesis will need to be clarified.

The present study had several limitations to be noted. Foremost, only women were included in our study group to control for known sex-specific differences in age-related muscle atrophy and sarcopenia.^{27,28} We could not evaluate biomarkers of serum vitamin D related to muscle strength, in our participants.²⁹ Future exercise and nutritional supplementation studies will need to include measurements of vitamin D status. There is current evidence of a positive effect of a supplementation of approximately 2500–2800 mg of leucine in increasing the rate of muscle protein synthesis in healthy older individuals.^{4–8} In the present study, we used a high dose of leucine supplementation of 2975 mg; however, a recent study reported an increase in muscle protein synthesis in healthy older women at a smaller amount of leucine supplementation.³⁰ In the present study, the EX + PRO group and EX group that should have a greater amount of exercise than the PRO group had greater bodyweight gain, even though the energy intakes were identical. Therefore, we could not evaluate accurate activity record/energy expenditure. This might be one of the limitations of the present study. Furthermore, we estimated muscle mass using BIA measurements. A strong positive correlation between dual-energy X-ray absorptiometry- and BIA-based measurements of muscle mass has previously been reported, confirming the validity of BIA measurements of muscle mass among older individuals.¹³ Therefore, the validity of the BIA data used in the present analysis was not deemed to have influenced the interpretation of our results. Finally, because our intervention period was relatively short at 24 weeks, future studies are required to clarify the benefits of a prolonged exercise and protein supplementation intervention in increasing muscle mass and strength.

The present results show a positive effect of whey protein supplementation, ingested after resistance exercise in a nutritional state in which total energy and protein intake was maintained above the recommended amount, in increasing muscle mass and strength among healthy community-dwelling older Japanese women.

Acknowledgements

We thank the participants in our study.

Disclosure statement

The authors declare no conflict of interest.

References

- Evans WJ. What is sarcopenia? *J Gerontol A Biol Sci Med Sci* 1995; **50A**: 5–8.
- Masumoto T, Yamada Y, Yamada M *et al*. Fall risk factors and sex differences among community-dwelling elderly individuals in Japan. *A Kameoka study. Nihon Koshu Eisei Zasshi* 2015; **62**: 390–401 [In Japanese].
- Bauer J, Biolo G, Cederholm T *et al*. Evidence-based recommendations for optimal dietary protein intake in older people: a position paper from the PROT-AGE Study Group. *J Am Med Dir Assoc* 2013; **14**: 542–559.
- Paddon-Jones D, Short KR, Campbell WW, Volpi E, Wolfe RR. Role of dietary protein in the sarcopenia of aging. *Am J Clin Nutr* 2008; **87**: 1562S–1566S.
- Paddon-Jones D, Rasmussen BB. Dietary protein recommendations and the prevention of sarcopenia. *Curr Opin Clin Nutr Metab Care* 2009; **12**: 86–90.
- Paddon-Jones D, Van LJ. Nutritional approaches to treating sarcopenia. In: Cruz-Jentoft AJ, Morley JE, eds. *Sarcopenia*. Chichester: Wiley-Blackwell, 2012; 275–295.
- Katsanos CS, Kobayashi H, Sheffield-Moore M, Aarsland A, Wolfe RR. A high proportion of leucine is required for optimal stimulation of the rate of muscle protein synthesis by essential amino acids in the elderly. *Am J Physiol Endocrinol Metab* 2006; **291**: E381–E387.
- Dillon EL, Sheffield-Moore M, Paddon-Jones D *et al*. Amino acid supplementation increases lean body mass, basal muscle protein synthesis, and insulin-like growth factor-1 expression in older women. *J Clin Endocrinol Metab* 2009; **94**: 1630–1637.
- Jordan LY, Melanson EL, Melby CL, Hickey MS, Miller BF. Nitrogen balance in older individuals in energy balance depends on timing of protein intake. *J Gerontol A Biol Sci Med Sci* 2010; **65**: 1068–1076.
- Kramer IF, Verdijk LB, Hamer HM *et al*. Impact of the macronutrient composition of a nutritional supplement on muscle protein synthesis rates in older men: a randomized, double blind, controlled trial. *J Clin Endocrinol Metab* 2015; **100**: 4124–4132.
- Thomas DK, Quinn MA, Saunders DH, Greig CA. Protein supplementation does not significantly augment the effects of resistance exercise training in older adults: a systematic review. *J Am Med Dir Assoc* 2016; **17**: 959.e1–959.e9.
- Devries MC, Phillips SM. Creatine supplementation during resistance training in older adults—a meta-analysis. *Med Sci Sports Exerc* 2014; **46**: 1194–1203.
- Ling CH, de Craen AJ, Slagboom PE *et al*. Accuracy of direct segmental multi-frequency bioimpedance analysis in the assessment of total body and segmental body composition in middle-aged adult population. *Clin Nutr* 2011; **30**: 610–615.
- Chen LK, Liu LK, Woo J *et al*. Sarcopenia in Asia: consensus report of the Asian Working Group for Sarcopenia. *J Am Med Dir Assoc* 2014; **15**: 95–101.
- National Institute of Health and Nutrition. 2010. Dietary reference intakes for Japanese -2010-: the summary report from the scientific committee of “dietary reference intakes for Japanese”. Ministry of Health, Labour and Welfare, Toyama Building Official Web Site. [Cited 5 July 2012.] Available from URL: http://www0.nih.gov.jp/eiken/english/research/pdf/dris2010_eng.pdf.
- Peterson MD, Rhea MR, Sen A, Gordon PM. Resistance exercise for muscular strength in older adults: a meta-analysis. *Ageing Res Rev* 2010; **9**: 226–237.
- Taaffe DR. Sarcopenia: exercise as a treatment strategy. *Aust Fam Physician* 2006; **35**: 130–134.
- Mori H, Tokuda Y. Effect of increased daily intake of protein, combined with a program of resistance exercises, on the muscle mass and physical function of community-dwelling elderly women. *J Aging Res Clin Practice* 2017; **6**: 56–61.
- Borst SE. Interventions for sarcopenia and muscle weakness in older people. *Age Ageing* 2004; **33**: 548–555.
- Roth SM, Ivey FM, Martel GF *et al*. Muscle size responses to strength training in young and older men and women. *J Am Geriatr Soc* 2001; **49**: 1428–1433.
- Liu CJ, Latham NK. Progressive resistance strength training for improving physical function in older adults. *Cochrane Database Syst Rev* 2009; **8**: CD002759.
- Bassil MS, Gougeon R. Muscle protein anabolism in type 2 diabetes. *Curr Opin Clin Nutr Metab Care* 2013; **16**: 83–88.
- Bano G, Trevisan C, Carraro S *et al*. Inflammation and sarcopenia: a systematic review and meta-analysis. *Maturitas* 2017; **96**: 10–15.
- Wakabayashi H, Sakuma K. Rehabilitation nutrition for sarcopenia with disability: a combination of both rehabilitation and nutrition care management. *J Cachexia Sarcopenia Muscle* 2014; **5**: 269–277.
- Fiatarone MA, O'Neill EF, Ryan ND *et al*. Exercise training and nutritional supplementation for physical frailty in very elderly people. *N Engl J Med* 1994; **330**: 1769–1775.
- Shad BJ, Thompson JL, Breen L. Does the muscle protein synthetic response to exercise and amino acid-based nutrition diminish with advancing age? A systematic review. *Am J Physiol Endocrinol Metab* 2014; **311**: E803–E817.
- Basaria S, Coviello AD, Travison TG. Adverse events associated with testosterone administration. *N Engl J Med* 2010; **363**: 109–122.
- Sanada K, Miyachi M, Yamamoto K *et al*. Prediction models of sarcopenia in Japanese adult men and women. *Jpn J Phys Fit Sport Med* 2010; **59**: 291–301 [in Japanese].

- 29 Granic A, Hill TR, Davies K *et al.* Vitamin D status, muscle strength and physical performance decline in very old adults: a prospective study. *Nutrients* 2017; **13**: 9 E379. <https://doi.org/10.3390/nu9040379>.
- 30 Wilkinson DJ, Bukhari SSI, Phillips BE *et al.* Effects of leucine-enriched essential amino acid and whey protein bolus dosing upon skeletal muscle protein synthesis at rest and after exercise in older women. *Clin Nutr* 2017; **23** [Epub ahead of print].

Supporting information

Additional supporting information may be found in the online version of this article at the publisher's website: .

Appendix S1. The detailed exercise regimen.

- (a) Bodyweight resisted exercises (1. rising and sitting from a chair; 2. leg extensions).
- (b) Resistance elastic band exercises (1. seated chest press; 2. seated row; 3. knee extensions; 4. squats; 5. knee-ups).
- (c) The exercise intervention program: elastic bands of five different resistance levels were used, with the level of resistance individually adjusted using a 1 maximum repetition (1 RM) test for the upper and lower limbs, before the intervention. Exercises were carried out under the supervision of an exercise instructor initially and then completed by participants at home.

Appendix S2. The detailed nutritional management regimen.

- (a) Protocol of protein supplementation in the exercise and protein (EX + PRO) group and protein only (PRO) group. The supplementation consisted of powdered whey protein dissolved in mineral water. Participants in the EX + PRO group ingested the protein supplement within 5 min after completion of the exercise program. The PRO groups ingested the protein supplement 3 h after lunch.

(b) Nutritional management.

Daily activity surveys were also reviewed to ensure sufficient total energy intake for all participants. The nutritionist instructed participants on the protein and energy contained within each serving of food and drink, and the care worker confirmed the intake of the protein supplementation at each meal during the intervention period at the participants' home. This facilitated guidance regarding desirable food selection and promoted autonomous dietary management. The participants used a food model to choose the appropriate amount of energy, nutrients and food products, and schedule for themselves and choose food compositions based on their objectives for their individual estimated energy requirement, and choose balanced meals divided into staple foods, main meals and side dishes.

Participants in the EX + PRO and exercise only (EX) groups carried out the resistance exercise program 3 h after lunch.

In this study, the EX group was instructed to ingest dairy, soy products, meat and fish, with the intake adjusted to the same amount of protein used in the protein supplement EX + PRO and PRO group.

How to cite this article: Mori H, Tokuda Y. Effect of whey protein supplementation after resistance exercise on the muscle mass and physical function of healthy older women: A randomized controlled trial. *Geriatr. Gerontol. Int.* 2018;1–7. <https://doi.org/10.1111/ggi.13499>