

De-Training Effects Following Leucine-Enriched Whey Protein Supplementation and Resistance Training in Older Adults with Sarcopenia: A Randomized Controlled Trial with 24 Weeks of Follow-Up

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Abstract

OBJECTIVES: This study aimed to evaluate the effectiveness of de-training after a combined intervention of leucine-enriched whey protein supplementation and resistance training on skeletal muscle mass and strength in older adults with sarcopenia.

DESIGN: A randomized controlled trial.

SETTING: A community in Hyogo, Japan.

PARTICIPANTS: The study included older adults aged ≥ 65 years who were screened for sarcopenia at the Care Center from, a community in Hyogo, Japan. The participants were randomly allocated to one of three groups: whey protein supplementation after resistance training (RT + PRO; $n = 27$), resistance training only (RT; $n = 27$), and whey protein supplementation only (PRO; $n = 27$).

INTERVENTIONS: An intervention program was conducted over a period of 24 weeks. The program included RT and/or PRO supplementation twice a week for 24 weeks, followed by a de-training period for another 24 weeks. PRO supplementation included 11.0 g of protein and 2,300 mg of leucine. The total energy and protein intake amounts for the participants in all groups were controlled to achieve at least 30 kcal/kg ideal body weight (IBW)/day and 1.2 g/kg IBW/day, respectively, during the intervention and de-training periods.

MEASUREMENTS: The primary outcomes, such as mean change of appendicular skeletal muscle mass index (Δ ASMI), and secondary outcomes, such as handgrip strength (Δ HGS), were measured at baseline, the end of intervention, and at 12 and 24 weeks of de-training.

RESULTS: Compared to baseline, ASMI and HGS increased significantly at the end of the intervention period in the RT+PRO group (ASMI and HGS, $p < 0.01$); however, there were no significant differences in Δ ASMI and Δ HGS between each group. At 24 weeks of the de-training period, Δ ASMI and Δ HGS were higher in the RT + PRO group than in the RT group ($p < 0.05$ and $p < 0.01$, respectively).

CONCLUSION: We demonstrated that combined intervention of RT and PRO showed long-term maintenance in treating sarcopenia than RT only at 24 weeks after de-training. Therefore, PRO intake after RT may be useful in the treatment of sarcopenia in older adults.

Key words: Detraining, leucine, whey protein, resistance training, sarcopenia.

Introduction

Sarcopenia is characterized by progressive loss of skeletal muscle mass, muscle strength, and physical performance (1, 2). The development of sarcopenia in older adults has been reported to increase the risk of falls, impaired daily functioning, and death (1, 2). Causes of sarcopenia include aging, inadequate protein intake, and decreased physical activity (1, 2). Previous epidemiological studies have shown that sufficient total dietary protein intake is important to maintain skeletal muscle mass in older adults (3, 4).

Resistance training (RT) intervention programs improve skeletal muscle mass and strength in older adults (5). According to the PROT-AGE study and the European Society for Clinical Nutrition and Metabolism, whey protein (PRO) from milk is recommended to increase muscle protein synthesis in older adults (3, 4). Since muscle protein synthesis is highest 1–3 hours after the end of resistance exercise, rapid intake of milk-derived PRO, which is rapidly digested and absorbed, is believed to contribute efficiently to muscle protein accumulation in skeletal muscle (3, 6–9). Furthermore, PRO is rich in leucine, a branched-chain amino acid that enhances muscle protein synthesis, and an intake of approximately 2,500 mg of leucine per meal is required to increase skeletal muscle mass in older adults (3, 4). Leucine activates the mammalian target protein of the rapamycin signaling pathway and is associated with protein anabolism through mRNA translation regulation (6–9).

In 2014, diagnostic criteria for sarcopenia were developed for Asians by the Asian Working Group for Sarcopenia (AWGS) (1). There have been several reports of randomized controlled trials examining the effects of the intervention of RT or PRO intake in older adults with sarcopenia (10–15). However, older adults with frailty may have unanticipated events that involve absolute bed rest (e.g., femoral fractures, arthralgia, hospitalization, and infection) in daily life (1, 16). In these cases, treatments such as RT may be discontinued.

Moreover, the recurrence of sarcopenia status during the long-term follow-up period after the completion of the RT and/or leucine-enriched PRO intervention has been completed

has not been elucidated. It is unknown whether older adults who have increased skeletal muscle mass and strength through interventions such as leucine-rich PRO and RT will re-decrease skeletal muscle mass and strength when the interventions are discontinued.

De-training is defined not only as a reduction or cessation of physical exercise such as RT (including in combination with nutritional supplement intake such as PRO), but also as a partial or total loss of muscle strength and performance that were increased by previous physical exercise (17). Previous studies have reported that muscle strength decreases during the de-training period following the end of RT in middle-aged and older adults (18-21). However, the de-training effect of skeletal muscle mass and strength with combined RT and leucine-enriched PRO supplementation interventions is unknown.

The purpose of this study was to examine the 24-week follow-up of de-training effects after the combined intervention of a leucine-enriched PRO and RT on skeletal muscle mass, strength, and sarcopenia status in older adults with sarcopenia using a randomized controlled trial.

Materials and Methods

Participants

This intervention study was approved by the Medical Ethics Committee of Tokushima University (approval number: 3113-1). The clinical registry trial number was UMIN000043794. The study provided participants with verbal and written explanations of the purpose and potential risks of the intervention study according to the Helsinki Declaration. In this study, written informed consent was obtained from enrolled participants. The participants included in the study were independent. Participants were recruited from May to September 2019. Using the AWGS 2014 criteria (2), older adults aged ≥ 65 years ($n = 452$) were screened for sarcopenia at the Harima Care Center, a community in Hyogo, Japan. The AWGS cut-off values were as follows: low handgrip strength (HGS) (males: < 26.0 kg, females: < 18.0 kg) or slow usual walking speed (UWS, males and females: < 0.8 m/sec) and low appendicular skeletal muscle index (ASMI) (males: < 7.0 kg/m², females: < 5.7 kg/m²). ASMI was calculated by dividing the upper- and lower-limb skeletal muscle mass by height squared. Individuals who refused participation ($n = 33$), RT therapy ($n = 21$), did not have sarcopenia ($n = 290$), or had undertaken nutritional therapies for conditions such as type 2 diabetes ($n = 17$) or chronic kidney disease ($n = 10$) within 1-year pre-intervention were excluded. Finally, 81 participants with sarcopenia were randomly assigned to three intervention groups: RT group ($n = 27$), PRO group ($n = 27$), and RT and PRO (RT + PRO) group ($n = 27$). The 81 participants were randomized 1:1:1 to RT, PRO, or RT + PRO groups using three different randomization codes stratified by sex. The randomization sequence was computer-generated by a statistician blinded to the details of the assignment and not otherwise involved in the study. This open-label and exploratory randomized controlled trial used a parallel-group

comparison. Participants in the RT groups did not receive PRO placebo supplements. The CONSORT checklist is shown in Supplementary Table S1.

Intervention design

Participants in the RT + PRO group ingested leucine-enriched PRO supplements after the RT program. The effects observed in the RT + PRO group were evaluated against those in the RT group, who completed the same RT program but without PRO supplementation, and those in the PRO group, who ingested the same amount of PRO but without RT. The intervention lasted 24 weeks, with participants completing their assigned protocols twice weekly. This intervention study was conducted from June 2019 to April 2020, and the observation study (follow-up de-training period) was conducted from December 2019 to October 2020. All participants were instructed to record the days that supplementation and training were carried out in a book.

De-training design

The 24-week intervention period was followed by a 24-week follow-up de-training period without RT and/or leucine-enriched PRO supplementation. All participants discontinued RT and/or PRO intake during the de-training period. All participants were also instructed to maintain a lifestyle and physical activity equivalent to that at baseline during the de-training period.

RT program

The RT program included exercises using a resistance elastic band and bodyweight resistance exercises (22, 23) (Supplementary Table S2). The RT load was modified using a standardized method (50–70% of the 1 repetition maximum, 2–3 sets, session duration of 30–40 minutes) over the 24-week program, with two sessions per week.

PRO supplement

In the RT + PRO and PRO groups, the PRO test meal (HINEX RIHA DAYS, Otsuka Co., Inc., Japan) was provided to participants. The PRO supplement beverage contained 160 kcal of energy, 11.0 g of protein, 2.2 g of fat, 24.0 g of carbohydrate, and 2,300 mg of leucine per serving. Participants in the RT+PRO group ingested the protein supplement within 5 min after completion of the RT program. The PRO groups ingested the PRO supplement 3 h after lunch.

Food and nutritional management

The total amounts of energy and protein consumed by participants in all groups were controlled to achieve at least 30.0 kcal/kg ideal body weight (IBW)/day and 1.2 g/kg IBW/day in daily meals: breakfast, lunch, and dinner (without the amount of energy and protein of PRO supplement),

respectively, during the intervention and follow-up de-training period (3, 4). A registered dietician and care workers led the management of each food and nutrition. A survey on the participants' nutrition and diet was conducted at baseline, end of intervention, and at 12- and 24 weeks of de-training (24). The detailed food and nutritional management program is presented in Supplementary Table S3.

Outcome measures

The following outcomes were measured at baseline, the end of intervention, and 12 and 24 weeks of de-training by blinded research staff during each designated visit of a given participant. The mean change (Δ) of ASMI value was the primary outcome parameter from baseline to end of the intervention and 12 and 24 weeks of de-training. Δ HGS, Δ UWS, Δ knee extension strength (KES), and remission rate of sarcopenia were the secondary outcome parameters from pre to end of the intervention and 12 and 24 weeks of de-training.

Skeletal muscle mass

ASMI and body mass index (BMI) were measured using a direct segmental multi-frequency bioelectrical impedance machine (In Body Japan, Japan) and analysis (BIA). In accordance with the manufacturer's guidelines, participants wiped the bottom of their feet with a proprietary electrolyte tissue before standing on the electrodes embedded in the scale platform of the respective analyzers. The participants were instructed to stand upright and grasp the handles of the analyzer, thereby providing contact with a total of eight electrodes (two for each foot and hand). Direct segmental multi-frequency BIA was performed after urination and defecation in the morning after a 10-hour overnight fast. Excellent agreements were observed between dual-energy X-ray absorptiometry and direct segmental multi-frequency BIA techniques in whole muscle mass quantification (intra-class correlation coefficient [ICC] female = 0.95, ICC men = 0.96). In the segmental muscle mass quantification, excellent agreements between the methods were demonstrated for the upper limbs (ICC female $\geq$ 0.91, ICC men $\geq$ 0.87) and lower limbs (ICC female $\geq$ 0.83, ICC male $\geq$ 0.85), with good agreements shown among the trunk measurements (ICC female = 0.73, ICC male = 0.70) (25).

Muscle strength and physical performance

We measured the HGS for each hand using maximum isometric HGS (Digital Grip Strength Tester 5401; Takei Scientific Instruments, Japan) in a standing position; the value of the highest HGS was used as the participant's muscle strength (1, 2, 23). A hand-held dynamometer (μ Tas F1; Anima, Japan) was used to evaluate the maximum isometric KES, which represents lower extremity muscle strength (26). The participant sat on a bench, and the force sensor was securely fastened to a rigid bar with a belt at the distal end of the tibia. Maximal isometric KES was defined as the highest value of the

three trials. The μ Tas F1 can accurately measure isometric KES during knee joint extension with a force sensor. To identify the UWS, participants were instructed to walk 10 m at their normal speed, and the time taken to walk 4 m (3–7 m) was measured (1).

Total energy, macro-nutritional intake, and nutritional status

We conducted a nutritional survey to document the total daily energy/IBW (TEI), protein/IBW (TPI), fat (TFI), and carbohydrate intake/day (TCI). The IBW was calculated by multiplying an ideal BMI of 22.0 kg/m² with a person's actual height (m) squared. Nutrient and food intake were calculated using the fourth edition of the Standard Tables of Food Composition in Japan and a nutrition calculation database (Version 8 Excel eiyou, Kenpaku, Japan) (24).

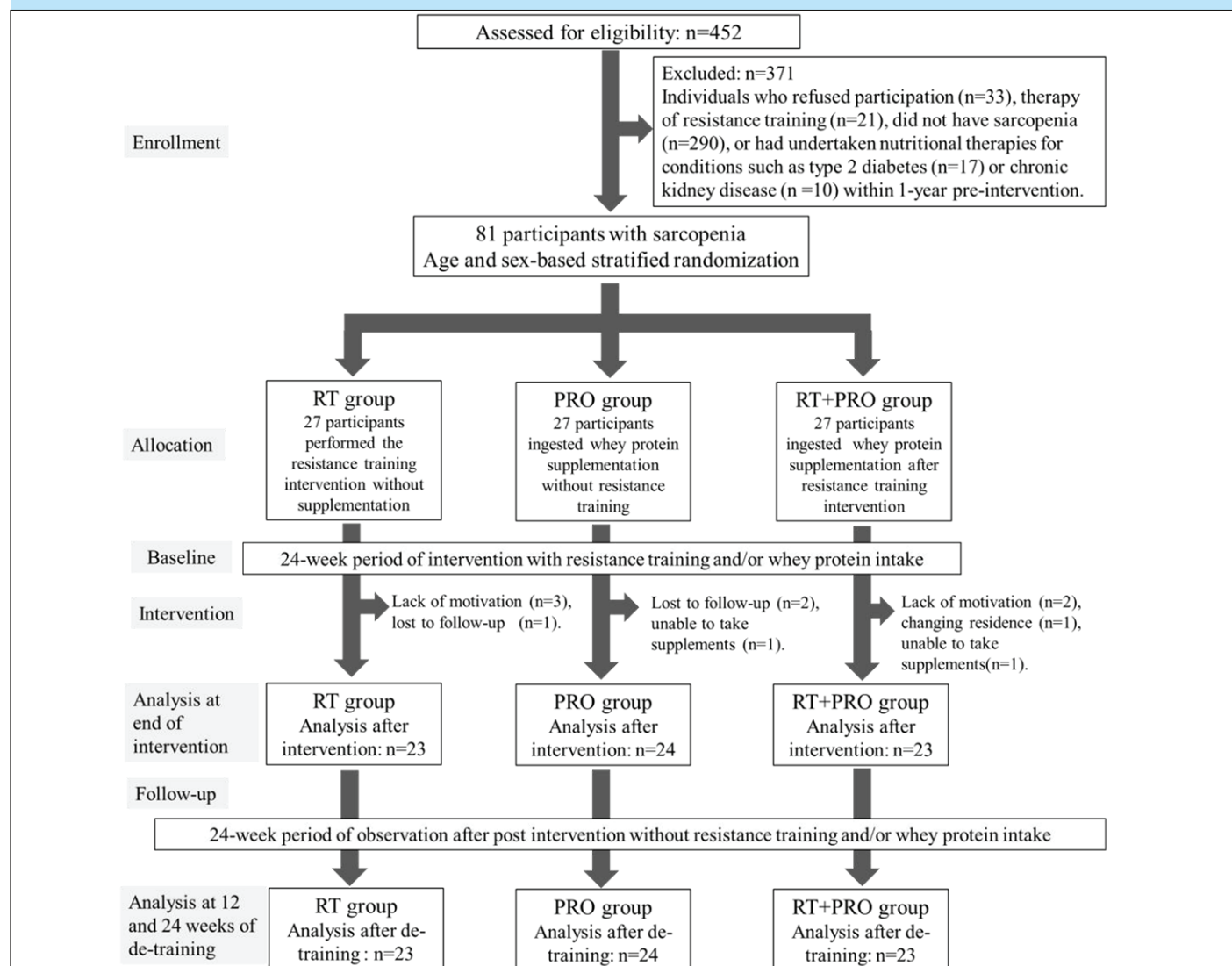
The nutritional survey included a food weighing method, in which food intake was documented for five consecutive days at baseline and end of intervention and at 12 and 24 weeks of de-training. Furthermore, in cases where no response was received, individual interviews were conducted regarding dietary intake status. Nutritional status was assessed using the Mini Nutritional Assessment®-Short Form (MNA-SF) (27). The MNA-SF scores were collected at baseline.

Total step number

All participants wore a tri-axial accelerometer-type wearable device (EZ-063 CALORIZM; Tanita, Tokyo, Japan) at baseline, except when bathing and sleeping. All participants recorded their daily activities for three consecutive days at baseline, the end of intervention, and 12 and 24 weeks of de-training. This information was used to estimate the normal total number of steps per day for each participant. Where information was missing, individual interviews were conducted.

Statistical analyses

All data were represented as mean $\pm$ standard deviation (%). We evaluated inter-group differences at baseline, post-intervention, and 12 and 24 weeks of de-training, and in clinical characteristics, primary and secondary outcome measures (ASMI, HGS, UWS, KES, and sarcopenia rate of remission), total energy, and macronutrients intake using an independent group one-way repeated-measures analysis of variance test or a chi-squared-test. A two-way repeated-measures analysis of variance was used to evaluate the effect of the intervention on the ASMI, HGS, UWS, and KES between groups. In the main results, the Δ of measured primary and secondary outcomes, baseline to end of the intervention, and 12 and 24 weeks of de-training were compared using the one-way repeated-measures analysis of variance test. The calculation was based on the effect size of 0.82 (absolute difference of 0.14 kg/m² ASMI with a standard deviation of 0.17 kg/m²) (23), α level of 0.05, and power (1- β) of 80%. We identified 25 individuals for each study group. We aimed to enroll at least 27 individuals per

Figure 1. Flow chart of study participants

group to be conservative and to allow for potential dropouts and missing data. We evaluated intra-group differences at baseline, end of intervention, 12 and 24 weeks of de-training, and in ASMI, HGS, UWS, KES, and sarcopenia rate of remission, using an intra-group one-way repeated-measures analysis or a McNemar's test. All statistical analyses were conducted using SPSS software (SPSS version 25, IBM, Japan), and the level of significance was set at $p < 0.05$.

Results

Eleven participants withdrew their consent and did not complete the 24-week post-randomization intervention period (RT: lack of motivation [n = 3], lost to follow-up [n = 1]; PRO: lost to follow-up [n = 2], unable to take supplements [n = 1]; RT + PRO: lack of motivation [n = 2], changed residence [n = 1], unable to take supplements [n = 1]; Figure 1). The final analyses for the RT, PRO, and RT+PRO groups consisted of 23, 24, and 23 participants, respectively. The mean compliance rates for RT and/or PRO supplementation over the 24-week intervention period were $73.2 \pm 7.1\%$ for the RT + PRO group,

$73.0 \pm 8.8\%$ for the PRO group, and $72.4 \pm 5.4\%$ for the RT group. The baseline clinical characteristics of the participants are presented in Table 1. There were no differences in the ASMI, HGS, UWS, and KES between the groups at baseline. There were no differences in total step number between the groups during the post-intervention, 12 weeks of de-training, and 24 weeks of de-training periods (post-intervention: RT group, 4079 ± 2050 ; PRO group, 3765 ± 1917 ; RT+PRO, 3999 ± 1943 ; 12 weeks of de-training: RT group, 4086 ± 1962 ; PRO group, 3805 ± 1825 ; RT+PRO, 4013 ± 1868 ; 24 weeks of de-training: RT group, 4099 ± 2025 ; PRO group, 3812 ± 1810 ; RT+PRO, 3906 ± 1902).

The primary and secondary outcomes at baseline, post-intervention, 12 weeks of de-training, and 24 weeks of de-training in older adults with sarcopenia in the RT, PRO, and RT + PRO groups are shown in Table 2. At the end of the intervention, the RT+PRO group showed an increase in SMI, HGS, and KES when compared to baseline. However, at the end of the intervention, the RT group and PRO group did not show an increase in HGS when compared to baseline. Furthermore, compared to baseline measures, ASMI, HGS, and

Table 1. Baseline clinical characteristics of PRO and RT + PRO groups

	RT (n=23)	PRO (n=24)	RT +PRO (n=23)	¹ p-value
Age (years)	77.6±5.2	77.8±4.5	77.7±3.3	0.990
Male sex (%)	17.4	16.7	13.0	0.910
BMI (kg/m ²)	20.3±2.9	20.1±3.1	20.3±2.4	0.962
MNA-SF (score)	10.2±1.6	10.4±1.8	10.7±1.3	0.654
TEI (kcal/day)	1426±136	1394±123	1438±146	0.521
TEI (kcal/kg IBW/day)	29.0±3.0	29.0±1.6	28.9±2.9	0.988
Total protein energy ratio (%)	14.5±1.7	14.4±1.6	14.8±1.2	0.608
TPI (g/kg IBW/day)	1.05±0.11	1.04±0.12	1.07±0.12	0.699
TPI (g/day)	51.6±6.3	50.0±6.5	53.2±5.9	0.236
TFI (g/day)	38.9±7.3	40.0±7.9	42.3±6.6	0.170
TCI (g/day)	222.0±29.3	208.6±17.0	211.1±21.2	0.114
ASMI (kg/m ²)	5.30±0.85	5.35±0.57	5.39±0.64	0.899
HGS (kg)	16.4±3.1	16.4±3.0	17.1±3.2	0.710
UWS (m/sec)	1.03±0.24	0.99±0.18	1.02±0.24	0.869
KES (kg)	14.1±5.8	14.1±3.3	13.9±2.8	0.989

Average± standard deviation, %; 1. The results of a non-paired one-way analysis of variance or χ^2 -test showed no differences in the baseline characteristics of the three groups; ASMI, appendicular skeletal muscle mass index; BMI, body mass index; HGS, handgrip strength; KES, knee extension strength; MNA-SF, Mini Nutritional Assessment Short Form; PRO, whey protein supplementation group; RT, resistance training group; RT+PRO, resistance training intervention with leucine enriched whey protein supplementation group; TCI, total carbohydrate intake; TEI, total energy intake; TFI, total fat intake; TPI, total protein intake; UWS, usual walking speed.

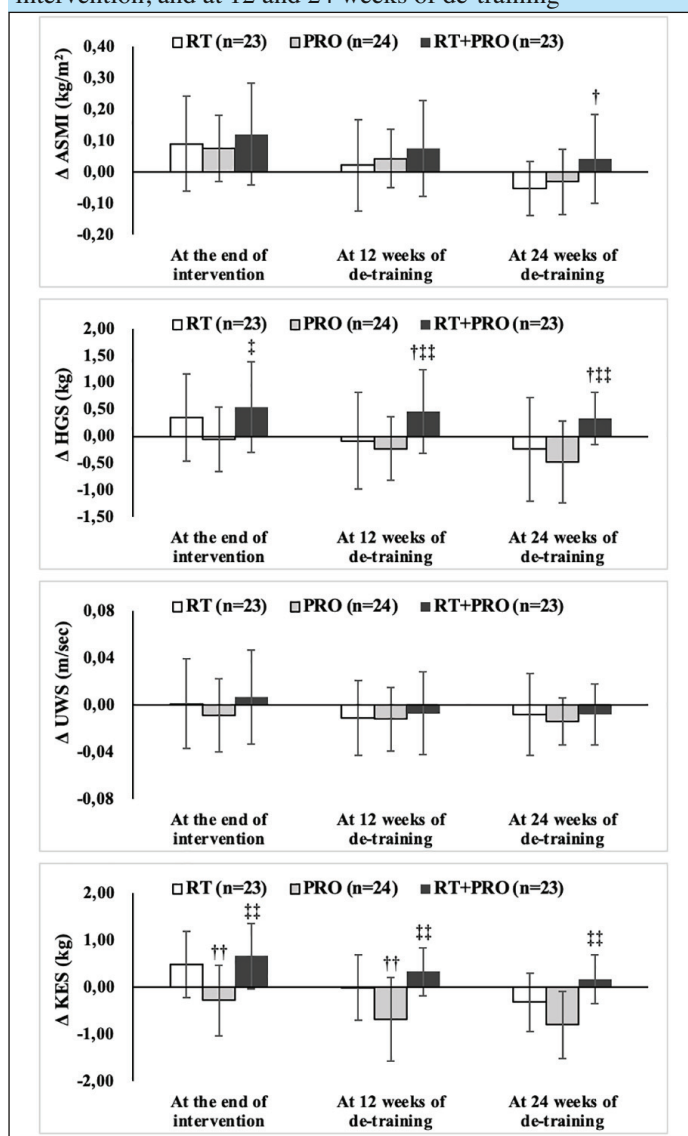
Table 2. Primary and secondary outcomes at baseline, post-intervention, and 12 weeks and 24 weeks of de-training in older adults with sarcopenia in the RT, PRO, and RT + PRO groups

At baseline				
Outcome	RT (n=23)	PRO (n=24)	RT+PRO (n=23)	
ASMI (kg/m ²)	5.30±0.85	5.35±0.57	5.39±0.64	
HGS (kg)	16.4±3.1	16.4±3.0	17.1±3.2	
UWS (m/sec)	1.03±0.24	0.99±0.18	1.02±0.24	
KES (kg)	14.1±5.8	14.1±3.3	13.9±2.8	
At the end of intervention				
Outcome	RT (n=23)	PRO (n=24)	RT+PRO (n=23)	¹ p-value
ASMI (kg/m ²)	5.39±0.92 ^{§§}	5.42±0.53 ^{§§§}	5.51±0.66 ^{§§}	0.545
HGS (kg)	16.8±3.0	16.3±3.0	17.6±3.4 ^{§§†}	0.026
UWS (m/sec)	1.03±0.27	0.98±0.21	1.03±0.24	0.795
KES (kg)	14.6±5.9 ^{§§}	13.8±3.2 ^{††}	14.6±3.0 ^{§§§††}	<0.001
At 12 weeks of de-training				
Outcome	RT (n=23)	PRO (n=24)	RT+PRO (n=23)	¹ p-value
ASMI (kg/m ²)	5.32±0.88	5.39±0.54	5.47±0.66 ^{§§}	0.432
HGS (kg)	16.3±2.9	16.2±2.9	17.5±3.4 ^{§§†††}	0.008
UWS (m/sec)	1.02±0.23	0.98±0.19	1.01±0.23	0.920
KES (kg)	14.1±5.8	13.4±3.2 ^{§§§††}	14.3±2.9 ^{§§†}	<0.001
At 24 weeks of de-training				
Outcome	RT (n=23)	PRO (n=24)	RT+PRO (n=23)	¹ p-value
ASMI (kg/m ²)	5.25±0.85	5.32±0.53	5.44±0.65 [†]	0.014
HGS (kg)	16.2±2.8	15.9±2.9 ^{§§}	17.4±3.2 ^{§†††}	0.002
UWS (m/sec)	1.02±0.24	0.98±0.19	1.01±0.24	0.735
KES (kg)	13.8±5.9 [§]	13.3±3.4 ^{§§§}	14.1±2.9 ^{††}	<0.001

Average± standard deviation; 1 Group × Time interaction; two-way repeated analysis of variance. [§]p<0.05, ^{§§}p<0.01, ^{§§§}p<0.001; paired one-way analysis of variance test. [†]p<0.05 and ^{††}p<0.01 indicate changes from baseline were significantly different compared to the RT group; [‡]p<0.05 and ^{‡‡}p<0.01 indicate changes from baseline were significantly different compared to the PRO group; a non-paired one-way analysis of variance test was performed. ASMI, appendicular skeletal muscle mass index; HGS, handgrip strength; KES, knee extension strength; PRO, whey protein group; RT, resistance training group; RT+PRO, resistance training and whey protein group; UWS, usual walking speed.

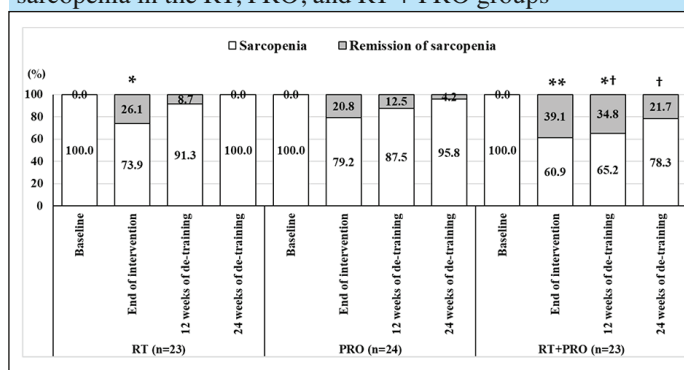
KES were higher in the RT + PRO group (ASMI and HGS, $p < 0.01$; KES, $p < 0.05$) at 12 weeks of de-training. At 24 weeks of de-training, HGS was higher in the RT + PRO group ($p < 0.05$) than at baseline, while KES was lower in the RT group ($p < 0.05$). Compared to baseline measures, HGS and KES were lower in the PRO group (HGS, $p < 0.01$, KES, $p < 0.001$) at 24 weeks of de-training. A significant group-by-time interaction was identified for ASMI at 24 weeks of de-training ($p = 0.014$). A significant group-by-time interaction was identified for HGS at the end of intervention ($p = 0.026$), at 12 weeks of de-training ($p = 0.008$), and at 24 weeks of de-training ($p = 0.002$). A significant group-by-time interaction was identified for KES at the end of the intervention period and at 12 and 24 weeks of de-training, ($p < 0.001$).

Figure 2. Mean change in ASMI, HGS, UWS, and KES post-intervention, and at 12 and 24 weeks of de-training



† $p < 0.05$ and †† $p < 0.01$ indicate changes from baseline were significantly different compared to the RT group; ‡ $p < 0.05$ and ‡‡ $p < 0.01$ indicate changes from baseline were significantly different compared to the PRO group; a non-paired one-way analysis of variance test was performed. ASMI, appendicular skeletal muscle mass index; HGS, handgrip strength; KES, knee extension strength; PRO, whey protein group; RT, resistance training group; RT+PRO, resistance training and whey protein group; UWS, usual walking speed.

Figure 3. Sarcopenia levels at baseline, post-intervention, and 12 weeks and 24 weeks of de-training in older adults with sarcopenia in the RT, PRO, and RT + PRO groups



*vs. baseline, χ^2 -test; †vs RT group, McNemar's test; PRO, whey protein group; RT, resistance training group; RT+PRO, resistance training and whey protein group.

The mean changes in ASMI, HGS, UWS, and KES at the end of the intervention period and 12 and 24 weeks of de-training are shown in Figure 2. At 24 weeks of de-training, Δ ASMI was higher in the RT + PRO group than in the RT group ($p < 0.05$). At 12 and 24 weeks of de-training, Δ HGS was higher in the RT + PRO group than in the RT and PRO groups ($p < 0.05$ and $p < 0.01$, respectively). After the end of the intervention period and at 12 weeks of de-training, Δ KES was lower in the PRO group than in the RT and RT + PRO groups.

Sarcopenia levels at baseline, post-intervention, 12 weeks of de-training, and 24 weeks of de-training are shown in Figure 3. The RT + PRO group had a 39.1% and 34.8% sarcopenia remission rate post-intervention ($p < 0.01$) and at 12 weeks of de-training ($p < 0.05$), respectively. The RT group had a 26.1% sarcopenia remission rate post-intervention ($p < 0.05$), but no difference was found in the sarcopenia remission rate at 12 weeks of de-training (8.7%) compared to baseline. The PRO group did not show a positive rate of sarcopenia remission post-intervention and at 12 and 24 weeks of de-training. At 12 and 24 weeks of de-training, the sarcopenia remission rate was higher in the RT + PRO group than in the RT group ($p < 0.05$).

Total energy and macronutrient intake post-intervention and at 12 and 24 weeks of de-training in the RT, PRO, and RT + PRO groups are shown in Supplementary Table S4. At the end of the intervention period and at 12 and 24 weeks of de-training, there were no differences in total energy and macronutrient intake among the groups.

Discussion

The results of this study showed that the combined de-training effects of leucine-enriched PRO intervention and RT were superior for the long-term maintenance of increased skeletal muscle mass and strength than RT alone for up to 24 weeks post-intervention. Moreover, the sarcopenia remission rate was higher in the RT and PRO supplementation than the RT alone for up to 24 weeks post-intervention. The combined intervention of RT and PRO showed long-term maintenance in treating sarcopenia than RT alone at 24 weeks after de-training. Therefore, it is of clinical importance in the treatment of

sarcopenia to examine the de-training effects of combined training after intervention with RT and PRO on skeletal muscle mass, strength, and sarcopenia status in older adults.

Recent systematic reviews and meta-analyses (28, 29) included several reports (10-15) that determined the effect of improved short-term (8–12 weeks of intervention periods) outcomes for leucine-rich protein supplements and exercise improving skeletal muscle mass and strength at the end of the intervention in older adults with sarcopenia. In this study, compared to baseline, ASMI, HGS, and KES were higher in the RT + PRO group than in the other groups at the end of the intervention. These results suggest that a combination of RT and PRO intake in older adults with sarcopenia improves multiple outcome measures, including skeletal muscle mass and upper- and lower-extremity muscle strength at the end of intervention. This finding is consistent with the view (10-15, 28, 29) that RT and PRO supplementation improve skeletal muscle mass and muscle strength at the end of the intervention in older adults with sarcopenia, representing a potential nutritional treatment for sarcopenia.

However, in older adults with frailty, this therapeutic intervention may be discontinued due to bed rest or hospitalization due to infection or unforeseen accidents. The long-term continuation of exercise interventions beyond 24 weeks in older adults with sarcopenia is difficult and may be discontinued. Furthermore, considering the frequent occurrence of temporary or continuous exercise interruptions in older adults, the sustained effects of RT on skeletal muscle and muscle strength are poorly reported and represent an important therapeutic challenge. Previous studies have shown that post-exercise de-training can significantly reduce bone density and muscle strength and increase the risk of fracture (18, 19). Unlike bone density and muscle strength, there are not as many de-training studies on skeletal muscle mass in older adults. Moreover, there have been no reports on the effects of de-training following a therapeutic intervention of RT and PRO supplementation for sarcopenia. Therefore, it is of clinical importance in the treatment of sarcopenia to examine the de-training effects of combined training after intervention with RT and PRO on skeletal muscle mass, strength, and sarcopenia status in older adults.

In this study, Δ ASMI and Δ HGS were higher in the RT + PRO group than in the RT group at 24 weeks of de-training. The findings of this study suggest that combined RT and PRO intake interventions can prevent significant long-term declines in skeletal muscle mass and strength in older adults with sarcopenia. Therefore, if sarcopenia treatment is discontinued, a combination of RT and PRO ingestion as an intervention method allows treatment to be resumed while maintaining skeletal muscle mass and strength. The present study showed that, compared to RT alone, the combined intervention of RT and PRO resulted in longer maintenance in treating sarcopenia at 24 weeks after de-training. In this study, we selected older adults diagnosed with sarcopenia, as defined by the AWGS 2014 criteria; the AWGS consensus update is that the desired outcome and indicator goals for interventions should be to achieve sarcopenia remission (2). It has also been recommended that the combination of exercise training and nutrition improves

skeletal muscle mass and strength in older adults compared to exercise training alone. Therefore, this randomized study is important as it provides evidence for the treatment and its follow-up of sarcopenia in Asia, using remission of sarcopenia as the outcome to analyze training and de-training effects.

In this study, the RT+PRO group experienced a greater increase in HGS than the other groups, and it remained significantly high at baseline for up to 24 weeks of de-training. Previous studies reported that neuromuscular effects, which contribute to muscle strength, are more maintained in de-training as compared to muscle hypertrophy effects, resulting in higher maintenance of muscle strength than muscle mass gain (30, 31). The results of this study indicate that combined RT and PRO interventions can contribute to the maintenance of upper extremity muscle strength over a long-term period of de-training.

On the other hand, compared to baseline, the RT + PRO group showed a significantly higher remission rate of sarcopenia at 12 weeks of de-training. The RT + PRO group had a higher sarcopenia remission rate than the RT group at both 12 and 24 weeks of de-training. However, the RT + PRO group showed no difference in the sarcopenia remission rate at 24 weeks of de-training compared to baseline. Therefore, combined RT and PRO intake interventions in older adults with sarcopenia should be re-trained after 24 weeks of de-training.

At the end of the intervention, the RT+PRO group showed an increase in multiple outcome measures such as SMI, HGS, and KES when compared to baseline. On the other hand, compared to baseline, the RT single interventions showed no difference in ASMI and HGS at 24 weeks of de-training. In addition, RT alone reduced the degree of sarcopenia at the end of the intervention (remission rate of 26.1%); however, 12 weeks into de-training, sarcopenia returned to baseline. Thus, it is clear that RT single interventions had little effect on the increased skeletal muscle mass and strength after de-training. To date, RT has been considered a standard treatment method for increasing skeletal muscle mass and strength in older adults. Yang et al.'s meta-analysis reported that muscle strength could be maintained up to 8–16 weeks of de-training by RT at medium-intensity loads for 24 weeks or more of the intervention period (18). However, RT alone may not be effective in treating sarcopenia after de-training.

Notably, the PRO group had a significant increase in ASMI post-intervention but no increase in muscle strength such as HGS or KES and no remission of sarcopenia. In addition, compared to baseline, the PRO group showed a decrease in muscle strength at week 24 of de-training. After the end of the intervention period and 12 weeks of de-training, Δ KES was lower in the PRO group than in the RT and RT + PRO groups. Thus, the PRO intake intervention without RT was not clinically effective in the treatment of sarcopenia. The effects of PRO intake on skeletal muscle and strength may be obtained by stimulating skeletal muscles with RT. It has been previously reported that grip strength loss is associated with a higher risk of recurrent falls than skeletal muscle mass loss (32). Previous studies have also reported that knee extension muscle weakness is associated with a higher risk of impaired daily functioning in older adults than skeletal muscle mass loss (33, 35-37).

In this study, all groups were nutritionally managed to meet the total energy and protein intake during the intervention and observation periods. Although total energy and total protein intake increased significantly during the intervention and observation periods compared to the pre-intervention period, there were no significant group differences before, during, or after the intervention.

This study has some limitations. This was a randomized controlled trial with a small sample size. It is necessary to increase the sample size and clarify the efficacy of RT and PRO supplementation in the future. In this study, it was not possible to collect biomarkers of muscle protein synthesis such as serum IGF-1 (38). Since neither the RT, PRO, or RT + PRO group showed improvement in walking speed in this study, intervention methods such as gait training should be considered. We measured skeletal muscle mass using direct segmental multi-frequency BIA machine. Although the AWGS 2019 consensus recommends measuring ASMI using the multi-frequency impedance method, future intervention trials should consider using dual-energy X-ray absorptiometry to assess body composition precisely (13).

This study showed that combined intervention of RT and PRO showed long-term maintenance in treating sarcopenia than RT only at 24 weeks after de-training. It can also prevent the declines in skeletal muscle mass and strength during de-training in older adults with sarcopenia. Therefore, PRO intake after RT may be useful in the treatment of sarcopenia in older adults.

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Ethical standards: This study complied with the current laws of Japan for research. The study was approved by the relevant Medical Ethics Committee of Tokushima University Hospital, and participants provided informed consent before data collection.

Data availability statement: All datasets generated or analyzed during the current study are not publicly available. However, datasets are available from the corresponding author upon reasonable request.

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