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

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Abstract: *Background:* In elderly women, significant loss of muscle mass due to declining levels of estrogen secretion is a health concern. Increasing the recommended dietary allowance of protein intake has been included as a general health guideline to prevent age-related sarcopenia. *Objectives:* To investigate effects of light-to-moderate resistance training combined with increased protein intake on the muscle mass, strength, and physical function of community-dwelling elderly women. *Design:* The 12-week training program combined weight-bearing and resistance band exercises, performed 3 times per week. *Setting:* Hyogo Prefecture, in either City K or Town H. *Practical Intervention:* Women were randomly allocated to three groups: exercise with protein intake adjusted to the recommended daily allowance (RDA) of 1.0–1.1 g/kg body weight/day (MP+EX group); exercise with protein intake adjusted above the RDA level at 1.2–1.3 g/kg body weight/day (HP+EX group); and a control group receiving classroom-based session on nutrition management, with protein intake adjusted to the RDA level (MP group). *Measurements:* Body weight and physical composition were measured by multi-frequency bioelectrical impedance analysis. *Results:* Exercise prevented decreases in muscle mass and strength and in performance of physical function tasks (p<0.05). Increasing dietary intake of protein above RDA level increased muscle mass (p<0.01), walking speed (p<0.01) and knee extensor strength (p<0.05). *Conclusion:* Adjusting protein intake to 1.2–1.3 g/kg body weight/day, in combination with light-to-moderate resistance training, can improve body composition and physical function in elderly women. The result of this study could be effective in lowering the incidence of age-related sarcopenia.

Key words: Community-dwelling elderly, exercise, muscle mass, protein intake, sarcopenia.

Introduction

Aging is associated with a decrease in skeletal muscle mass and strength which leads to limitations in physical activities, increased risk for falls and overall lowering of the quality of life of elderly people (1–7). A specific health concern is the significant loss of muscle mass in elderly women due to declining levels of estrogen secretion (8). The resulting loss of strength is one of the primary factors of the higher incidence of falls in older women, compared to older men. Increasing the recommended dietary allowance of protein intake has been included as a general health guideline to prevent age-related sarcopenia (9-11).

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The Japanese recommendations for dietary reference intakes (DRIs) indicate a daily protein intake of 1.0–1.1 g/kg body weight/day for people aged >65 years (12). These DRIs do not include protein intake levels necessary for elderly individuals performing resistance exercises for the purpose of increasing skeletal muscle mass and physical function, knowledge required to optimize outcomes of exercise (13).

According to the Long-Term Care Prevention Service, which aims to increase musculoskeletal system function in the elderly, few elderly individuals practice high-intensity resistance exercises using large-scale training equipment. Moderate-intensity resistance exercises performed using body weight and resistance bands provide an easy way for elderly people to exercise for the purposes of increasing skeletal muscle mass and physical function to prevent sarcopenia. Accordingly, the aim of our study was to evaluate the effectiveness of adjusting total protein intake to levels of 1.2–1.3 g/kg body weight/day, a level exceeding the DRIs, on skeletal muscle mass and physical function in elderly women

Figure 1
Flow chart of study participation
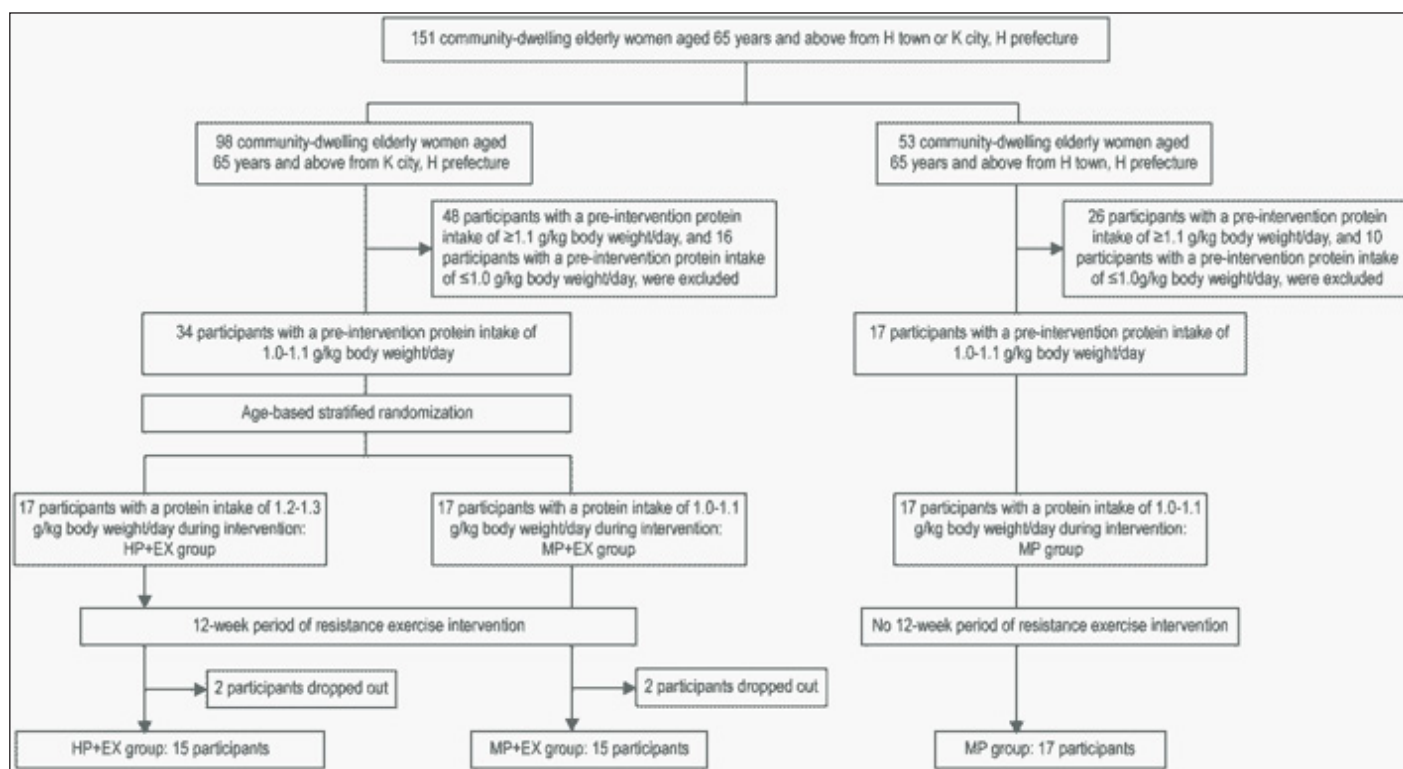
Using participants with a pre-intervention protein intake of 1.0-1.1g/kg body weight/day, we randomly divided the participants into a high protein intake group of 1.2-1.3g/kg body weight/day (HP+EX group) and a moderate protein intake group of the usual 1.0-1.1g/kg body weight/day (MP+EX group) during an intervention of light-to-moderate intensity resistance exercise. We also established a moderate protein intake group of the usual 1.0-1.1 g/kg body weight.

performing moderate intensity resistance exercises that used their own body weight and resistance bands, thereby preventing sarcopenia.

Methods

Participants

Participants were 151 Japanese women, ≥65 years old, living in Hyogo Prefecture, in either City K or Town H.

With the aim of using the DRI level of protein intake (1.0–1.1 g/kg/day12) as a baseline reference, a dietary survey was used to screen the daily protein intake of prospective participants. Based on this dietary survey, 26 participants were excluded as their total daily protein intake was <1.0 g/kg/day, and 74 participants for a daily protein intake >1.1 g/kg/day. Remaining participants were randomly allocated to three groups of 17 participants: an exercise intervention group with protein intake adjusted to the DRI of 1.0–1.1 g/kg/day (MP+EX group); an exercise intervention group with protein intake adjusted above the DRI at 1.2–1.3 g/kg/day (HP+EX group); and a control group who participated in classroom-based session on nutrition management, with their protein intake adjusted to the DRI of 1.0–1.1 g/kg/day (MP group). A stratified randomization strategy was used to achieve a comparable age distribution across groups. Over the study period, 4 participants withdrew due to family circumstances or poor physical conditioning, 2 participants from the HP+EX group and 2 participants from the MP+EX group. The information from these 4 participants was excluded from analysis, with the final study group formed of 15 participants in the HP+EX group, 15 participants in the MP+EX group, and 17 participants in the MP group (Figure 1).

Intervention design

A 12-week program was implemented. Groups HP+EX and MP+EX completed a standardized program of resistance exercise and a nutrition management, with MP group completed a classroom-based program on nutrition.

Measurement of body weight and physical composition

Body weight and physical composition were measured by multi-frequency bioelectrical impedance analysis (In Body 430, Bio Space, Seoul, Korea). The following parameters of body composition were obtained: body mass index (BMI), body fat percentage, lean body mass (LBM), and total limb muscle mass, which was calculated separately for the upper and lower limbs, and for the

trunk.

Measurement of physical functions

The following measures of physical function were evaluated: grip strength, knee extension strength, 5-m maximum walking speed, and timed up-and-go (TUG). Grip and knee extension strength were measured by hand held dynamometry (T.K.K5401; Takei Instruments, Tokyo, Japan. μ-tus F-100; ANIMA., Tokyo, Japan). Measures were taken twice, and the maximum value recorded for analysis.

Nutritional survey

A nutritional survey was conducted to document: total energy intake (TEI), total and adjusted protein intake (g/kg/day), total fat intake, and total carbohydrate intake. The survey was complemented by food weighing methods (Excel Eiyou version 5.0; Kenpakusha, Tokyo, Japan). The nutrition questionnaire and weighing were completed for 5 consecutive days before the start of the program, and daily during the 12-week intervention.

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 (PAL). Again, individual interviews were used to complete missing information. The estimated energy requirement (EER) to meet each individual's PAL was calculated as follows: EER = basal metabolic rate × body weight (kg) × PAL (12).

Exercise intervention

The exercise intervention consisted of a series of body weight resisted and resistance band exercises, performed 3 times per week for 12 weeks. Twice per week, exercises were performed under supervision of an exercise specialist, with participants completing the third session at home.

Resistance exercises

Body weight resisted exercises consisted of the following: abdominal crunches, rising and sitting from a chair; leg extensions; standing heel kicks, and calf raises. Five exercises were also performed with elastic bands (REP BAND; Magister Corporation, Chattanooga, TN): arm curls, pull-ups, leg extensions, squats, and sit-ups. Elastic bands of five difference resistance were used, with the level of resistance individually adjusted using 1 maximum repetition (1RM) testing for the upper and lower limbs prior to the intervention. The resistance load

was modified in standardized fashion over the 12-week program as follows: for weeks 1-4, participants performed 2 sets of 10 repetitions each of the body weight resisted exercises and 2 sets of 20 repetitions of the resistance band exercises, at a resistance load of 50% 1RM; for weeks 5-8, participants performed 2 sets of 15 repetitions each of the body weight resisted exercises and 2 sets of 15 repetitions each of the resistance band exercises at 60% 1RM; and for weeks 9-12, participants performed 3 sets of 15 repetitions each of the body weight resisted exercises and 3 sets of 12 repetitions each of the resistance band exercises at 70% 1RM.

Nutritional intervention

Total protein intake in the HP+EX group was adjusted to a daily level of 1.2–1.3 g/kg/day. Participants' were advised to increase their protein intake in their standard meals (breakfast, lunch, and dinner). The total protein intake for participants in the MP+EX and MP groups was adjusted to 1.0–1.1 g/kg/day.

Nutritional management

Nutritional management was provided by a nutritionist, based on the DRIs (14) and results of the nutrition survey conducted before the intervention. Nutritional management was provided according to the following schedule: once to all participants prior to the onset of the study; as part of the group nutrition guidance sessions in intervention weeks 1-2; and on an individual basis, twice per week, for intervention weeks 3-12. At these individual sessions, protein intake was verified to ensure compliance with group-specific levels. Daily activity surveys were also reviewed to ensure sufficient TEI for all participants.

Statistical analysis

Individual changes in EER and TEI, pre- and post-intervention, were evaluated using paired t-test analysis. Between-group differences in outcome variables, measured pre- and post-intervention, were evaluated using two-way analysis of variance (ANOVA). Changes in measured outcomes of muscle mass, physical function, and nutrient intake were evaluated between groups (HP+EX, MP+EX and MP) and time (pre- and post-intervention) using a repeated measures ANOVA with group as an independent factor and time as the repeated factor. For identified main effects and interactions, multiple comparisons were performed using the Tukey post hoc analysis, with specific change in pre- and post-intervention values compared using an unpaired one-way ANOVA. All statistical analyzes were performed using IBM SPSS statistical software (IBM, Tokyo, Japan), with the level of significance defined as a p value < 0.05.

Results

Baseline measures of body composition and physical function are listed in Table 1. Pre-intervention, measures were comparable between groups. Adherence to the schedule of resistance exercise of three sessions per week for 12 weeks was comparable for the two exercise groups, with a mean (SD) number of sessions completed of 34.3 ± 1.2, out of a possible maximum of 36, for the HP+EX group (95.3%) and 34.0 ± 1.0 for the MP+EX group (94.4%).

Table 1
Physical characteristics of subjects pre-intervention

	HP+EX group	MP+EX group	MP group
	(n=15)	(n=15)	(n=17)
Age	69.8±2.1	69.9±1.8	69.4±2.9
Height (cm)	152.6±3.9	151.3±4.3	151.9±3.3
Weight (kg)	48.2±3.8	48.2±3.6	48.1±4.0
BMI 2 (kg/m²)	20.7±1.5	21.1±1.4	20.9±2.0
Body fat (%)	24.9±2.9	24.4±3.1	23.8±2.8
LBM (kg)	36.1±2.4	36.4±2.5	36.6±3.1
Physical activity level	1.75±0.06	1.75±0.07	1.75±0.07
Estimated energy requirement	1,745±142	1,748±111	1,741±106

Mean value ± Standard deviation; The results of a non-paired one-way ANOVA showed no differences in the characteristics of the 3 groups pre-intervention; HP+EX group: Protein intake of 1.2-1.3 g/kg body weight/day during the 12-week period of exercise intervention; MP+EX group: Protein intake of 1.0-1.1 g/kg body weight/day during the 12-week period of exercise intervention; MP group: Protein intake of 1.0-1.1 g/kg body weight/day for the 12-week period; BMI: Body mass index; LBM: Lean body mass

Changes in measures of muscle mass pre- and post-intervention are reported in Table 2. A significant main effect of exercise on limb muscle mass was identified ($p<0.05$) with the largest change identified for the HP+EX group ($p<0.001$ compared to both the MP+EX and MP groups). A significant group X limb interaction was identified ($p<0.05$), with a significantly higher increase in muscle mass for the HP+EX group, compared to the MP group, for the upper limbs ($p<0.05$), trunk ($p<0.001$) and lower limbs ($p<0.001$). Comparing HP+EX and MP+EX groups, the magnitude of change in muscle mass was higher for the HP+EX group only for the lower limbs ($p<0.05$).

Effects of the intervention physical function are reported in Table 2. The ANOVA identified significant effects of exercise on knee extensor strength and 5-m maximum walking speed, as well as knee extensor strength x TUG interaction. Change in knee extensor strength was higher for the MP+EX group, compared to the MP group ($p<0.01$), with the highest magnitude of change obtained by the HP+EX group ($p<0.001$ compared to MP; $p<0.05$ compared to MP+EX group). Findings were similar for 5-m maximum walking speed, with greater improvement in walking speed in the MP+EX compared to MP groups ($p<0.05$), and largest increase

for the HP+EX group ($p<0.001$ compared to MP; $p<0.01$ compared to MP+EX group). The improvement in TUG was greater for the HP+EX compared to the MP group ($p<0.01$).

Nutritional intake, TEI, EER, and levels of total protein intake, adjusted protein intake/kg body weight, fat, and carbohydrates are listed in Table 3. Significant changes in TEI and protein intake (TEI, $p<0.05$; protein, $p<0.001$), as well as an interaction between adjusted protein intake and body weight ($p<0.001$) were identified. The magnitude of change was higher for the HP+EX group compared to the MP group (TEI, $p<0.05$; protein intake/kg, $p<0.001$) and the MP+EX group (TEI, $p<0.05$; protein intake/kg, $p<0.001$).

Discussion

In this study, we evaluated the effectiveness of providing an adjusted protein intake of 1.2-1.3 g/kg/day during a 12-week program of light-to-moderate intensity weight-bearing and resistance band exercises on the skeletal muscle mass and physical function of elderly women. The adjusted protein intake improved selected parameters of body composition and physical function, namely lower limb muscle mass, knee extension strength, and 5-m maximum walking speed. The mean protein intake for the HP+EX group was 11.4 ± 1.8 g/day, and increased by approximately 0.22 ± 0.02 g/kg/day over the 12-week program. Fat and carbohydrate intake were comparable between groups and TEI was never below EER.

Our current results support our a priori hypothesis of the necessity of increasing protein intake to optimize outcomes of exercise on muscle mass and physical function in the elderly. In fact, total limb muscle mass increased by 0.9 ± 0.3 kg when combining increased protein intake and exercise (HP+EX), compared to 0.2 ± 0.4 kg for exercise alone (MP+EX). Our measured effect of exercise alone is comparable to results reported by Kim et al. (15) of a 0.29 kg increase in total limb mass for elderly Japanese women completing a 12-week program. Kim et al. (15) combined resistance exercises with amino acid supplements, reporting an increase of approximately 0.34 kg in total limb muscle mass. Using an adjusted protein intake, our program surpassed these levels with a mean increase in total limb mass of 0.9 kg, with the most significant increase noted for the lower limbs. This lower limb bias may be explained by performance of a higher number of lower limb exercises. An important difference in our study, compared to Kim et al. (15), is that we graduated the workload of our program over the 12-weeks.

An important outcome of our study was the identification of a 0.2 ± 0.7 kg decrease in total limb muscle mass and 0.2 ± 1.0 kg decrease in knee extension strength for participants in the MP group over the study period. This emphasizes the need to adjust protein intake, at least

Table 2
Comparison of limb muscle mass and physical functions pre-/post-intervention

	Pre-intervention			Post-intervention			Main effect of time	Group ×time interaction	Δ (Post-intervention - Pre-intervention)		
	MP group	MP+EX group	HP+EX group	MP group	MP+EX group	HP+EX group			MP group	MP+EX group	HP+EX group
Total limb muscle mass (kg)	29.3±2.5	29.1±2.0	28.9±1.9	29.1±2.6	29.3±2.2	29.8±2.0	***	***	-0.2±0.7	0.2±0.4†	0.9±0.3†††,‡‡
Upper limb muscle mass (kg)	2.0±0.1	2.0±0.1	2.0±0.1	2.1±0.2	2.1±0.2	2.1±0.2	***	*	0.0±0.1	0.1±0.0	0.1±0.2†
Trunk muscle mass (kg)	14.7±1.2	14.6±1.0	14.5±1.0	14.5±1.3	14.6±1.1	14.7±1.0		*	-0.1±0.4	0.0±0.2	0.2±0.2††
Lower limb muscle mass (kg)	12.6±1.1	12.5±0.9	12.4±0.8	12.5±1.1	12.6±0.9	12.8±1.0	**	***	-0.1±0.3	0.1±0.2	0.4±0.3†††,‡
Grip strength (kg)	21.2±2.2	20.6±1.8	19.7±2.6	21.1±1.7	21.0±1.7	20.4±2.9	*		0.0±1.2	0.3±0.5	0.7±0.6
Knee extension strength (kg)	19.7±2.1	20.2±1.7	19.5±2.4	19.5±2.1	20.9±2.0	20.9±2.6	***	***	-0.2±1.0	0.7±0.6††	1.4±0.4†††,‡
5-m maximum walking speed (m/s)	1.80±0.17	1.80±0.12	1.82±0.20	1.81±0.17	1.84±0.12	1.92±0.19	***	***	0.01±0.05	0.04±0.04†	0.09±0.04†††,‡‡
Timed up & go (m/s)	0.91±0.05	0.89±0.07	0.91±0.07	0.91±0.06	0.90±0.07	0.93±0.07	***	**	0.00±0.02	0.01±0.01	0.02±0.01††

Mean value ± Standard deviation; Two-way ANOVA by time (Pre- and Post-intervation period) × group (HP+EX, MP+EX, MP group)* $p < 0.05$, ** $p < 0.01$,*** $p < 0.001$
One-way ANOVA and post-hoc by Tukey test, Significant difference with MP group: †$p < 0.05$, ††$p < 0.01$, †††$p < 0.001$, Significant difference with MP+EX group: ‡$p < 0.05$, ‡‡$p < 0.01$

to the minimum DRI of 1.0–1.1 g/kg/day, in combination with a light-to-moderate program of resistance training, to maintain muscle mass, with further adjustment in protein intake yielding more significant results.

According to Chevalier et al. (16), a dietary protein intake of 0.8 g/kg/day is insufficient for increasing the skeletal muscle mass of elderly persons and intake should be increased to levels of at least 1.2 g/kg/day. Several physiological reasons contribute to the increased requirement of protein intake in the elderly. Drummond et al. (17) reported a delay and/or decrease of the signaling responses to amino acids in skeletal muscle cells with age. Volpi et al. (18) further demonstrated that a higher proportion of the amino acids absorbed in the gastrointestinal tract of elderly persons is metabolized in the small intestine and liver. Although specific physiological measurements related to protein metabolism were not included in our study, our outcomes provide evidence of insufficient muscle protein synthesis during resistance exercise with a moderate adjustment of protein intake to levels of 1.0–1.2 g/kg/day and that a higher adjustment to levels of 1.2–1.3 g/kg/day are required to enhance effectiveness of resistance training on muscle mass and strength.

Our light-to-moderate level of resistance was sufficient to maintain muscle mass and strength in the MP+EX group. Levinger et al. (19) reported a 1.1-kg increase in skeletal muscle mass of elderly participants performing a 10-week program of high-intensity resistance exercises. In their review of research evidence for resistance training in elderly individuals, Miyachi et al. (20) indicated that an exercise intensity of 80% 1RM or above is required to increase the skeletal muscle mass with a single intervention of exercise. It is important to note that the participants in our study group had a relatively low PAL of 1.75; as none of our participants engaged in regular resistance training, prescription of a high-intensity program was not possible. Future research is required to

more clearly elucidate the effectiveness of supplemented protein intake and different levels of resistance training for enhancing gains in muscle mass and strength. However, we do emphasize that our program of light-to-moderate resistance was effective, with a practice rate of approximately 95%, and can be safely implemented as a home program for elderly individuals who are relatively deconditioned.

The benefits of combining a light-to-moderate resistance training program with nutritional supplementation in lowering the risk for sarcopenia in elderly individuals is supported by previous research. In their review comparing the transient response of muscles to high- and low-intensity resistance training regimes, Mallinson et al. (21) reported an increase in muscle protein synthesis (and increased muscle protein reserves) with high-intensity resistance exercise, performed at 90% 1RM, while low-intensity resistance exercises, performed at 30% 1RM, stimulated muscle protein synthesis. Therefore, light-to-moderate intensity weight-bearing and resistance band resistance exercise can have benefits for elderly women, who can easily perform these exercises.

In our study, we assumed that a training resistance of 50–70% 1RM would have little stimulus on protein synthesis in skeletal muscle. As a result, we did not include a control group of adjusted high protein intake alone, with no participation in the program of exercise. Therefore, we are unable to determine if the increase in muscle mass for the HP+EX group results from the increase in protein intake or from the combination of increased protein intake and exercise. Future research is required to clarify the pathway of muscle mass increase associated to a combination of supplemented protein intake and exercise. Another important limitation of our study is the inclusion only of women to control for known sex-specific differences in age-related muscle atrophy and sarcopenia (22, 23). Future research will need to verify effectiveness of protein supplementation in elderly

Table 3
Comparison pre-intervention and during intervention

	Pre-intervention			During intervention			Main effect of time	Group × time intervention	Δ (Post-intervention - Pre-intervention)		
	MP group	MP+EX group	HP+EX group	MP group	MP+EX group	HP+EX group			MP group	MP+EX group	HP+EX group
Total energy intake (kcal/day)	1,832±107	1,833±144	1,847±143	1,843±79	1,851±168	1,933±140	**	*	10±80	18±123	87±89†, ‡
Protein (g/day)	49.2±3.7	49.0±3.2	49.2±3.5	49.3±4.0	49.7±2.9	60.6±4.8	***	***	0.1±0.7	0.7±1.1	11.4±1.8†††, ‡‡‡
Protein/body weight (g/kg/day)	1.02±0.02	1.01±0.01	1.02±0.01	1.03±0.02	1.02±0.02	1.24±0.02	***	***	0.01±0.01	0.00±0.02	0.22±0.03†††, ‡‡‡
Fat (g/day)	53.5±9.0	52.7±5.4	52.7±10.2	54.5±8.9	53.6±5.5	56.7±5.3			1.0±11.1	0.9±4.5	4.0±11.5
Carbohydrates (g/day)	288.6±18.2	290.7±33.4	293.9±31.8	288.8±22.7	292.5±31.6	295.2±26.2			0.2±23.4	1.8±31.5	1.3±30.5

Mean value ± Standard deviation, Two-way ANOVA by time (Pre- and post-intervention period) × group (HP+EX, MP+EX, MP group), * $p < 0.05$, ** $p < 0.01$, ***$p < 0.001$; One-way ANOVA and post-hoc by Tukey test, significant difference with MP group: †$p < 0.05$, †††$p < 0.001$, Significant difference with MP+EX group: ‡$p < 0.05$, ‡‡‡$p < 0.001$

men. Moreover, we estimated muscle mass from BIA measurements. There are currently no population norms of BIA measurements for elderly women, and research is needed to establish norms compared to DEXA (24). Finally, the present study is that the sample size was too small, and the conclusion was not well supported by this data. For example, SD of total limb muscle mass is 1.9 and the change by intervention was 0.9kg in HP+EX group. Required sample size to make this change as significant is 37, which is more than twice of the actual number of sample.

Our study provides evidence of the benefit of increasing protein intake as one component of a resistance training program to improve muscle mass and strength in elderly women.

Conflict of Interest: All of the authors declare that they have no conflicts of interest regarding this paper.

Ethical standards: Ethics Committee of Hyogo University (approved # 12003).

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