ORIGINAL

A combined health promotion program of exercise with protein and vitamin D-enriched menu enhances skeletal muscle mass and strength in Japanese elderly men

Ge Li MS[1], Yuka Kawakami-Shinoda PhD[1,†], ALIMA MS[1], Masaki Takiwaki PhD[2], Kentaro Abe BS[2], Toshio Hosaka MD, PhD[3], Toshinao Goda PhD[1], and Hidekazu Arai PhD[1,†]

[1]*Laboratory of Clinical Nutrition and Management, Graduate Division of Nutritional and Environmental Sciences, and Graduate School of Integrated Pharmaceutical and Nutritional Sciences, The University of Shizuoka, Shizuoka, Japan,* [2]*Medical Equipment Business Operations, JEOL Ltd., Tokyo, Japan,* [3]*Laboratory of Clinical Nutrition, Graduate Division of Nutritional and Environmental Sciences, and Graduate School of Integrated Pharmaceutical and Nutritional Sciences, The University of Shizuoka, Shizuoka, Japan*

[†]*Y. Kawakami-Shinoda and H. Arai contributed equally to this work.*

Abstract : The study aimed to investigate the effectiveness of combining a protein and vitamin D-enriched menu with an exercise program to reduce frailty risk and enhance muscle performance. However, current evidence lacks accurate dosage and effectiveness information for this combination. This study involved Japanese men in their 60s who were randomly assigned to two groups : the ExN group, which received a 10-day exercise program along with enriched diet, and the Ex group, which underwent the exercise program alone. The effects of these interventions on muscle mass, strength, and serum vitamin D metabolite levels were assessed. The ExN intervention resulted in a significant increase in skeletal muscle mass and serum 25-hydroxyvitamin D_3 (25(OH)D_3) levels, while the Ex intervention did not yield the same effects. These results indicate that a combined program of exercise with protein and vitamin D-enriched meal improves serum 25(OH)D_3 levels and skeletal muscle mass among older Japanese men. J. Med. Invest. 72 : 76-84, February, 2025

Keywords **:** *meal intervention, exercise, vitamin D, protein, skeletal muscle mass*

INTRODUCTION

Undernutrition is characterized by weight loss (1) and low muscle mass (2-5), which can eventually lead to frailty (6, 7). Nutrition and physical activity are two of the three key factors in preventing frailty (8). These factors have the potential to delay the onset and progression of age-related pathologies (9).

To minimize the risk of falls and fractures and maintain a high quality of life for older individuals, it is recommended to consume sufficient amounts of vitamin D and protein (10-12). Studies have shown that protein intake between 1.2 and 1.5 g/kg/day, which exceeds the American recommended dietary allowance (RDA), promotes an increase in lean body mass (LBM) (13) and grip strength (14). In fact, research comparing the muscle protein synthesis response to protein consumption in young and older adults suggests that older individuals require a relatively higher protein intake to stimulate skeletal muscle protein synthesis to its maximum extent (15, 16). The protein requirements for older adults with a risk of frailty are likely higher than the current RDA (17), as they have a greater protein need compared to younger, healthy adults (4). Additionally, a significant number of elderly individuals have a serum 25-hydroxyvitamin D (25(OH)D) level below 75 nmol/L (18). Furthermore, the median vitamin D intake among Japanese elderly individuals is insufficient, falling below the recommended adequate intake of 340 IU (19), regardless of gender (20). It is worth noting that vitamin D plays a role in muscle growth, development, and contraction (21), and studies have found a positive association between vitamin D levels and muscle strength and muscle mass (22).

The importance of exercise in maintaining good health is widely acknowledged. Previous studies have demonstrated that older individuals who follow an exercise regimen experience notable improvements in muscle mass, muscle quality, physical performance, and muscle strength (23-26). Therefore, it is recommended that elderly individuals engage in at least 40 minutes of physical activity each day, regardless of the intensity, to prevent frailty (27). However, less than 40% of Japanese elderly individuals have incorporated regular exercise into their routines. Additionally, on average, half of the elderly population in Japan spends less than 1 hour per day engaging in physical activity (28).

Currently, there is a lack of research examining the impacts of a comprehensive program that combines a nutritious diet and exercise on the body (29). Most existing studies have focused on nutritional interventions involving supplements (30-32). Understanding the extent and duration of the effects of healthy eating and exercise on various health indicators would be valuable in developing a practical program for preventing frailty.

In light of this, our study aimed to evaluate the effectiveness of a combined health promotion program that incorporates exercise along with a menu enriched with protein and vitamin D. The goal was to determine how this program contributes to the prevention of frailty.

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Address correspondence and reprint requests to Hidekazu Arai, Laboratory of Clinical Nutrition and Management, Graduate Division of Nutritional and Environmental Sciences, and Graduate School of Integrated Pharmaceutical and Nutritional Sciences, The University of Shizuoka, 52-1 Yada, Suruga-ku, Shizuoka 422-8526, Japan and Fax : +81-54-264-5511. E-mail : arai@u-shizuoka-ken.ac.jp

MATERIALS AND METHODS

Participants

A total of seven Japanese men between the ages of 60 and 69 were recruited for this study. These individuals were considered to be in good health, with no known allergies to the ingredients used in the food provided. Moreover, they were not currently taking any medications for kidney or heart conditions, nor did they have any neurological diseases (e.g., stroke or Parkinson's disease), bone diseases, muscular diseases (e.g., muscular dystrophy), or inflammatory conditions. Before participating, the participants provided written informed consent, and the study protocol received approval from the ethics committee at the University of Shizuoka (approval number : 3-34) and registered with the University Hospital Medical Information Network (registered number : UMIN000051514). The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

Study Protocol

The study protocol can be found in Supplemental Figure 1. We employed a randomized crossover study design. Initially, the participants were divided into two intervention groups : the ExN group, which followed an exercise program with a nutritious menu enriched with protein and vitamin D, and the Ex group, which only followed the exercise program alone. Each intervention period lasted for 10 consecutive days. After a 10-day washout period, the participants were then assigned to the opposite intervention group for another 10-day period. Fasting blood samples were collected at 8:30 on both Day 1 (pre-intervention) and Day 11 (post-intervention). To measure urinary excretion, 24-hour urine samples were collected strictly from 8:00 on Day 0 to 8:00 on Day 1 and from 8:00 of Day 10 to 8:00 of Day 11. Anthropometric measurements and assessments of muscle strength were conducted in the morning on both Day 1 and Day 11. On the day of the 24-hour urine collection, participants were instructed to keep a record of the amounts of food consumed during their meals. This study was conducted from late January to late February in the northern hemisphere, where daylight hours are relatively short. The subjects were then checked to ensure that they were not engaging in any behavior that would expose them to prolonged exposure to sunlight.

Diets

Participants in the Ex group were instructed to maintain their normal lifestyle and eating habits throughout the intervention period. They did not receive any meals as part of the study.

In contrast, participants in the ExN group were provided with a set of meals that followed a cycle menu and were designed to supplement their protein and vitamin D intake. The provided meals included fermented soybeans and milk for breakfast, a lunch box for lunch, grilled fish with seasoning and furikake (rice seasoning) for dinner, and yogurt as a snack. The nutritional information for all the meals provided to the ExN can be found in Table 1. Participants were instructed to consume all the meals provided to them. However, it is important to note that while these meals were designed to supplement protein and vitamin D, participants were not restricted from consuming any additional food outside of what was provided to them.

Exercise Program

Both groups of participants engaged in the "Muscle Genki Gymnastics", which were developed by the Japanese Ministry of the Environment. These exercises were designed to be easily performed at home once a day. A video demonstration of the exercises can be found at this link : https://www.youtube.com/watch?v=KhhLVEtVh-E. The exercise routine consisted of a 9-minuted warm-up session followed by a 14-minute muscle training session. During the muscle training portion, various exercises were included, such as squats, push-ups, leg raises (leg-up sit-ups), and exercises targeting the back and legs. The aim of these exercises was to enhance the strength and function of major muscle groups, including the quadriceps, chest, rectus abdominis, iliopsoas, erector spinae, and latissimus dorsi. These exercises targeted a wide range of large muscles in the body.

Blood and Urine Analysis Methods

Blood samples were centrifuged at 940 g for 10 minutes at 4°C. Following centrifugation, the samples were separated into serum, which was then stored at −80°C until further analysis. Urine collected for 24 hours was used to measure urine pH, urinary creatinine, urinary 3-methylhistidine, and urea nitrogen concentrations. Serum creatinine (S-Cr) levels and urine parameters were determined using enzymatic methods (SRL Inc.,

Table 1. Nutritional values of the provided menus in the ExN group

			Menu 1	Menu 2	Menu 3
Breakfast			Fermented soybeans	Fermented soybeans	Fermented soybeans
			Milk	Milk	Milk
Lunch			Lunch Box No 1	Lunch Box No 2	Lunch Box No 3
Dinner (main dish)			Chum salmon	Bonito	Mackerel pike
Snack			Yogurt	Yogurt	Yogurt
Total nutrition values	Energy	(kJ/day)	4,766	4,862	5,121
	Protein	(g/day)	63.1	78.9	69.3
	Fat	(g/day)	51.4	41.0	53.7
	Carbohydrate	(g/day)	101.5	120.9	115.9
	NaCl	(g/day)	3.1	4.0	4.2
	Calcium	(mg/day)	834	829	762
	Vitamin D	(IU/day)	1,060	1,204	1,068

ExN, exercise program with protein and vitamin D-enriched diet ; NaCl, sodium chloride

Tokyo, Japan), except for the analysis of urinary pH. The pH was measured using a portable pH meter (LAQUA act, D-71 ; Horiba Scientific, Kyoto, Japan).

To analyze the serum vitamin D metabolites, such as 25-hydroxyvitamin D2 (25(OH)D_2), 25-hydroxyvitamin D_3 (25(OH)D_3), 24,25-dihydroxyvitamin D_3 (24,25(OH)$_2D_3$), and 3-epimer of 25(OH)D_3 (3-epi-25(OH)D_3), liquid chromatography/tandem mass spectrometry (LC-MS/MS) was employed. The analysis was performed using the Xevo TQ-XS (Waters, Milford, MA, USA). An in-house method, along with the JeoQuant™ Kit for VD Metabolites (JEOL, Tokyo, Japan), was utilized for this purpose.

Anthropometric Measurements and Muscle Strength Assessments

Body weight and body composition were determined using the bioelectrical impedance method with the InBody 770 (InBody Japan Inc., Tokyo, Japan). Height measurements were taken using a YL-65S stadiometer (Yagami, Aichi, Japan). Calf circumference, which serves as a skeletal muscle marker for diagnosing sarcopenia, was used to estimate skeletal muscle mass. This was measured using an insert tape (Abbott Japan Co., Ltd., Tokyo, Japan). Muscle strength assessments were performed using two methods. The "timed up & go (TUG) test" was utilized to evaluate mobility and agility. It involved measuring the time taken for a person to rise from a chair, walk a distance of 3 meters, turn around, walk back to the chair, and sit down again. On the other hand, grip strength was measured using the TKK 5401 Grip-D (Takei Scientific Instruments Co., Ltd., Niigata, Japan).

To assess the participants' physical activity levels, they were provided with a wearable activity tracker Fitbit Charge 4 (Fitbit Inc., San Francisco, CA, USA), during the intervention period. This allowed for the recording of daily energy expenditure and the number of activities performed.

Dietary Assessments

During the 24-hour urine collection, participants were required to meticulously record all the foods and beverages they consumed, including the names of the dishes and types and quantities of ingredients. Additionally, they were instructed to take pictures of their meals. To ensure the accuracy of the dietary assessment, participants had the opportunity to clarify any uncertainties in their dietary records through consultations with dietitians. The collected data was analyzed using Eiyo-Plus (Kenpakusha Co., Tokyo, Japan).

Statistical Analysis

The statistical analyses were conducted using SPSS for Windows version 26.0 (SPSS, Chicago, IL, USA). All results were expressed as mean ± standard deviation (SD) or medians (25th to 75th interquartile range). To assess data normality, the Shapiro-Wilk test was utilized. For normally distributed data, differences in serum, and urine parameters between pre- and post-intervention, as well as between the ExN and Ex groups, were examined using a paired *t*-test. In the case of non-normally distributed data, the Wilcoxon signed-rank test was employed. In all statistical analyses, *p*-values <0.05 were considered statistically significant.

RESULTS

Characteristics of the Participants and Physical Activity Level

A total of seven participants (age, 64.0 ± 2.8 years ; body mass index (BMI), 24.4 ± 3.0 kg/m^2 ; skeletal muscle mass, 28.7 ± 1.5 kg) were enrolled in the study. Prior to the intervention, we confirmed that the participants had normal renal function based on their S-Cr levels and estimated glomerular filtration rate (eGFR) (Table 2).

There were no significant differences observed between the ExN and Ex groups in terms of daily energy expenditure and the number of steps taken, as indicated in Supplemental Table 1.

Dietary Intake

In the ExN group, there was a significant increase in the dietary intake of energy, protein, fat, calcium, and vitamin D at post-intervention than at pre-intervention ($p < 0.05$). Furthermore, the ExN group had significantly higher dietary intake of protein, calcium, and vitamin D at post-intervention compared to the Ex group ($p < 0.05$) (Table 3).

Biological Parameters

The serum 25(OH)D_3 levels at pre-intervention in the ExN group were significantly lower compared to the Ex group ($p < 0.05$). The serum 25(OH)D_3 levels at post-intervention in the ExN group were significantly higher than those at pre-intervention ($p = 0.003$). The difference in Δserum 25(OH)D_3 levels at post-intervention was higher in the ExN group than in the Ex group ($p < 0.05$). Additionally, in the ExN group, the serum 24,25(OH)$_2D_3$ and 3-epi-25(OH)D_3 levels at post-intervention were significantly higher than those at pre-intervention ($p = 0.046$, $p = 0.003$). However, the serum 25(OH)D_2 levels remain unchanged by the intervention in both the Ex and ExN groups (Table 4).

Table 2. Participants' baseline characteristics.

n=7		Baseline
Age	(years)	64.0 ± 2.8
Height	(cm)	167.4 ± 2.8
BW	(kg)	68.3 ± 9.0
BMI	(kg/m^2)	24.4 ± 3.0
LBM	(kg)	48.8 ± 2.4
Skeletal muscle mass	(kg)	28.7 ± 1.5
Calf circumference	(cm)	37.8 ± 2.4
Walking speed	(s)	7.9 ± 1.1
Grip strength	(kg)	34.5 ± 6.1
S-25(OH)D_3	(mg/dL)	21.6 ± 8.2
S-24,25(OH)$_2D_3$	(mg/dL)	1.2 ± 0.6
S-3epi-25(OH)D_3	(mg/dL)	0.7 ± 0.2
S-25(OH)D_2	(mg/dL)	0.6 ± 0.5
HbA1c	(%)	6.2 ± 2.2
TG	(mg/dL)	154.9 ± 101.7
S-Cr	(mg/dL)	0.8 ± 0.1
eGFR	(mL/min/1.73 m^2)	72.6 ± 12.2
Energy intake	(kcal/day)	2,093 ± 286
Protein intake	(g/day)	84.6 ± 18.1
Fat intake	(g/day)	62.5 ± 11.2
Carbohydrate intake	(g/day)	279.1 ± 72.0
Vitamin D intake	(IU/day)	184 ± 148

Values are presented as the mean ± SD.
BW : body weight ; BMI : body mass index ; LBM : lean body mass ; HbA1c : Hemoglobin A1c ; TG: triglyceride ; S-Cr : serum creatinine ; eGFR : estimated glomerular filtration rate

Table 3. Dietary intake of the Ex and ExN groups

		Ex			ExN		
		Pre-intervention	Post-intervention	*p*-value	Pre-intervention	Post-intervention	*p*-value
Energy	(kJ/day)	8,489 ± 1,238	9,489 ± 1,912	0.262	9,188 ± 1,498	11,088 ± 1,540	**0.009**
Protein	(g/day)	77.4 ± 17.6	90.5 ± 13.1	0.247	84.0 ± 19.7	114.1 ± 20.0*	**0.014**
Fat	(g/day)	60.0 ± 10.7	70.5 ± 28.5	0.365	66.5 ± 16.0	105.2 ± 21.7	**<0.001**
Carbohydrate	(g/day)	297.7 ± 56.9	300.5 ± 105.9	0.956	289.7 ± 86.0	303.0 ± 50.5	**0.667**
NaCl	(g/day)	6.9 (6.2, 12.4)	10.6 (8.1, 14.4)	0.310	8.5 (6.8, 12.1)	8.2 (7.3, 12.8)	**0.710**
Calcium	(mg/day)	695 ± 394	701 ± 284	0.973	867 ± 658	1,394 ± 367**	**0.034**
Vitamin D	(IU/day)	248 (20, 308)	324 (164, 1,244)	0.128	300 (52, 432)	1,156 (1,092, 1,424)$	**<0.001**

Values are presented as the mean ± SD or medians (25th to 75th interquartile range) (n = 7)
Ex, exercise program alone ; ExN, exercise program with protein and vitamin D-enriched diet; NaCl, sodium chloride
* $p < 0.05$, Ex vs. ExN, paired *t*-test
** $p < 0.01$, Ex vs. ExN, paired *t*-test
$ $p < 0.05$, Ex vs. ExN, Wilcoxon signed-rank test
p-value between pre-intervention and post-intervention, paired *t*-test

Table 4. Effects of intervention program of exercise with and without protein and vitamin D-enriched menu on serum vitamin D metabolite levels

		Ex				ExN			
		Pre-intervention	Post-intervention	Change	*p*-value	Pre-intervention	Post-intervention	Change	*p*-value
25(OH)D_3	(nmol/L)	56.8 ± 18.8	55.5 ± 20.5	−1.3 ± 5.3	0.546	52.8 ± 19.5*	57.5 ± 17.5	4.8 ± 2.8*	**0.003**
24,25(OH)2D_3	(nmol/L)	3.1 ± 1.2	2.9 ± 1.4	−0.2 ± 0.2	0.319	2.6 ± 1.2**	3.1 ± 1.2	0.5 ± 0.5**	**0.046**
3-epi-25(OH)D_3	(nmol/L)	2.0 ± 0.5	2.0 ± 0.5	0.0 ± 0.3	0.468	1.8 ± 0.5*	2.0 ± 0.5**	0.3 ± 0.3**	**0.003**
25(OH)D_2	(nmol/L)	1.0 (0.7, 1.2)	1.0 (0.7, 1.5)	−0.0 (−0.2, 0.0)	0.063	1.2 (0.7, 1.5)	1.2 (0.7, 1.7)	0.0 (−0.0, 0.2)*	0.176

Values are presented as the mean ± SD or medians (25th to 75th interquartile range) (n = 7)
Ex, exercise program alone ; ExN, exercise program with protein and vitamin D-enriched diet
* $p < 0.05$, Ex vs. ExN, paired *t*-test
** $p < 0.01$, Ex vs. ExN, paired *t*-test
p-value between pre-intervention and post-intervention, paired *t*-test

The results of the 24-hour urine collection were shown in Supplement Table 2. Urinary 3-methylhistidine excretion in the ExN group was higher than in the Ex group, but no significant differences were observed before or after the intervention. No significant differences were found in the other parameters.

Muscle Strength

In the Ex group, the calf circumference at post-intervention was significantly greater than that at pre-intervention (p = 0.012). There was no change in the TUG test during the intervention period in the Ex group, whereas it was significantly reduced during the intervention period in the ExN group (p = 0.037). The TUG test at post-intervention was significantly shorter in the ExN group than in the Ex group ($p < 0.01$). Furthermore, in the ExN group, grip strength at post-intervention was significantly greater than that at pre-intervention (p = 0.048) (Table 5).

Body Composition

In the ExN group, the LBM at post-intervention was significantly higher than that at pre-intervention (p = 0.045). The ΔLBM at post-intervention was significantly higher in the ExN group than in the Ex group ($p < 0.01$). Similarly, the intervention lead to an increase in skeletal muscle mass in the ExN group, but not in the Ex group (Table 5). There were no significant differences in body fat between the ExN and Ex groups, both before, and after intervention.

DISCUSSION

The objective of this study was to evaluate the effectiveness of a combined program consisting of a protein and vitamin D-enriched menu along with an exercise program for preventing frailty. The rationale behind conducting this study was to demonstrate visible changes in a short duration, with the aim of raising participants' awareness on their ongoing health.

The findings of this study showed significant improvements in skeletal muscle mass, walking speed, and grip strength in the ExN group. Notably, there were no significant differences in energy intake and expenditure between the ExN and Ex groups, as estimated by a wearable active tracker. These favorable results indicate that a nutritious diet, in addition to the exercise program, played a crucial role in the observed outcomes. Previous research by Yamada *et al.* demonstrated that older adults who received a combined program of resistance exercise and dietary supplementation with protein (10 g/day) and vitamin D (800 IU/day) for 12 weeks achieved the greatest improvement

Table 5. Effects of intervention program of exercise with and without protein and vitamin D-enriched menu on the parameters related with skeletal muscle mass and strength

		Ex				ExN			
		Pre-intervention	Post-intervention	Change	*p*-value	Pre-intervention	Post-intervention	Change	*p*-value
Calf circumference	(cm)	35.7 (37.5, 38.8)	37.9 (36.0, 39.2)	0.3 (0.0, 0.4)	**0.012**	36.8 (36.7, 39.2)	37.6 (36.3, 39.4)	0.2 (0.1, 0.4)	0.169
TUG test	(s)	7.8 ± 0.8	7.6 ± 0.8	−0.2 ± 0.4	0.212	7.8 ± 1.1	7.2 ± 0.7**	−0.5 ± 0.5	**0.037**
Grip strength	(kg)	35.4 ± 5.9	36.0 ± 5.5	0.6 ± 2.4	0.529	35.0 ± 5.2	37.1 ± 4.3	2.1 ± 2.3	**0.048**
Body Weight	(kg)	68.5 ± 9.0	68.5 ± 9.0	0.1 ± 0.5	0.700	68.6 ± 8.9	69.2 ± 9.1	0.6 ± 1.0	0.209
Lean body mass	(kg)	48.9 ± 2.8	48.6 ± 2.9	−0.3 ± 0.3	0.063	48.8 ± 2.3	49.4 ± 2.7	0.6 ± 0.6**	**0.045**
Skeletal muscle mass	(kg)	28.8 ± 1.7	28.6 ± 1.8	−0.2 ± 0.2	0.139	28.8 ± 1.5	29.1 ± 1.6*	0.3 ± 0.3*	**0.025**
Body fat	(kg)	14.5 (13.0, 21.7)	16.1 (13.1, 21.5)	0.1 (0.0, 0.5)	0.179	15.6 (13.0, 21.9)	14.9 (12.5, 23.2)	−0.5 (−0.7, 0.8)	0.931

Values are presented as the mean ± SD or medians (25th to 75th interquartile range) (n = 7)
Ex, exercise program alone ; ExN, exercise program with protein and vitamin D-enriched diet
TUG : "timed up and go" test
* $p < 0.05$, Ex vs. ExN, paired *t*-test
** $p < 0.01$, Ex vs. ExN, paired *t*-test
p-value between pre-intervention and post-intervention, paired *t*-test

in grip strength (30). This study suggests that fortifying protein and vitamin D intake may have a positive impact on muscle strength gains. On the other hand, Molnár *et al.* showed that grip strength and muscle mass improved after a 3-month vitamin D supplementation (33). However, the improvements were relatively modest compared to the control group. It is worth noting that their study did not provide clear information on the total protein intake, with only an additional 30 g of protein mentioned in the daily intake. In contrast, in our study, the participants' daily protein intake was about 70–80 g, and we provided an additional 30–40 g of protein. Consequently, we estimate that a daily protein intake of about 100 g (1.4 g/kg BW) may be necessary to enhance muscle strength. Thus, our results suggest that the combination of exercise and a nutritious diet, characterized by adequate intake of protein and vitamin D intake, effectively improves muscle mass, and strength.

A previous meta-analysis reported that if subjects had adequate dietary protein intake (1.1-1.2 g/kg BW/day), additional dietary protein intake did not increase muscle mass (34). Dietary protein intake and resistance training intensity in our study were comparable to those in the meta-analysis. On the other hand, few reports in this meta-analysis reported studies with the addition of vitamin D. Therefore, the addition of adequate vitamin D, as well as adequate protein intake and resistance exercise, was considered important as one of the factors that increase muscle mass.

In this study, our focus was on vitamin D due to its association with frailty, particularly in individuals with serum levels below 50 nmol/L. To address this, we implemented a nutritional intervention using a fish-based menu, known for its high vitamin D content. The ExN group demonstrated an average increase in serum 25(OH)D_3 levels of 4.8 ± 2.8 nmol/L. A previous study conducted on institutionalized women aged over 60 reported an increase of 25.3 ± 1.8 nmol/L in serum 25(OH)D levels after consumption of a vitamin D_3 (400 IU/day) and calcium-fortified (800 mg/day) yogurt over 56 days (35). Our study shows a similar increasing trend in serum 25(OH)D_3 levels. However, it remains unclear whether a regular intake exceeding 400 IU/day of vitamin D is required. Based on our findings, a daily intake of 1000 IU/day of vitamin D appears to be sufficient. it is noteworthy to highlight that our study used a vitamin D-enriched menu to enhance dietary vitamin D intake, in contrast to most previous studies that relied on vitamin D supplements. We speculated that ensuring an adequate supply of vitamin D through dietary sources would effectively increase serum 25(OH)D_3 levels, even within a short 10-day period.

In this study, the test meal provided for the ExN group met the RDA for protein and calcium and the adequate intake for vitamin D of men in their 60s (14). The protein intake range of 1.2–1.5 g/kg/day exceeded the RDA and has been reported to be beneficial for increasing LBM (17). Vitamin D supplementation has also demonstrated positive effects on muscle strength and muscle mass (36), effectively reducing the risk of falls and fractures (37, 38). A previous study reported that the consumption of a whey protein supplement enriched with vitamin D, calcium, and leucine for 13 weeks improved serum 25(OH)D levels in non-malnourished older adults with sarcopenia (31). Thus, the addition of protein, and vitamin D to the intervention may be effective in maintaining muscle function.

It is important to note that the participants in the ExN group were allowed to consume additional foods beyond the provided menu, and the average daily protein intake for this group was 114 g, equivalent to 1.44 g/kg of their usual body weight per day. While a high protein diet can be detrimental to individuals with kidney dysfunction (39-41), there is limited evidence suggesting that it poses risks to healthy individuals (42, 43). In our study, we ensured that all participants had normal renal function. However, the optimal protein intake for the elderly population remains unclear and warrants further investigation. Further studies should examine whether there are upper limits for protein intake in this population.

This study has certain limitations that should be acknowledged. First, the sample size was relatively small, which limits the generalizability of the findings to a larger population. Second, the nutritional values of the food consumed were estimated using a food recording method, which may introduce inaccuracies in the quantification of nutrient amounts. Third, the involvement of energy as a factor in the increase in muscle mass cannot be ruled out, since the increase in protein leads to an increase in energy intake. Improvements and testing of protocols that can resolve this point are needed. Lastly, it is undeniable that the study was conducted based on elderly people who were relatively healthy and actively able to exercise and eat, and therefore, the biased population had an effect on the results of the study. As the super-aging population continues to advance, people in their 60s are an important age group for preventing frailty, and there is a need for conscious efforts to address this issue from an early age. We believe that this study will provide the basis for such efforts.

Despite these limitations, the results of this study demonstrated promising outcomes. The combined program involving exercise and a protein and vitamin D-enriched menu resulted in notable improvements, including increased serum 25(OH)D_3 levels, enhanced skeletal muscle mass, improved walking speed, and increased grip strength among elderly individuals. These results suggest that the combination of regular exercise and a nutritious diet can be effective in mitigating the risk of frailty, even within a relatively short intervention period of 10 days.

CONFLICT OF INTERESTS-DISCLOSURE

The authors reported no conflicts of interest. This work was supported by the University of Shizuoka Grant for Scientific and Educational Research (to HA and YK) as well as the Food and Healthcare Open Innovation Project of Shizuoka Prefecture (to TG). The funders did not exert any influence on study design, data collection, data analysis and manuscript preparation.

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Author Contributions

The authors made the following contributions to the manuscript : YK-S and HA conceptualized the research idea and designed the study. GL, YK-S, and A were responsible for data collection, analysis, and interpretation. GL drafted the manuscript. MT and KA analyzed vitamin D metabolites. TH and TG provided valuable guidance and advice. YK-S and HA reviewed and edited the manuscript. All authors participated in the manuscript revisions and reviewed the final version.

Disclosure Statement

The authors declare that they have no competing interests.

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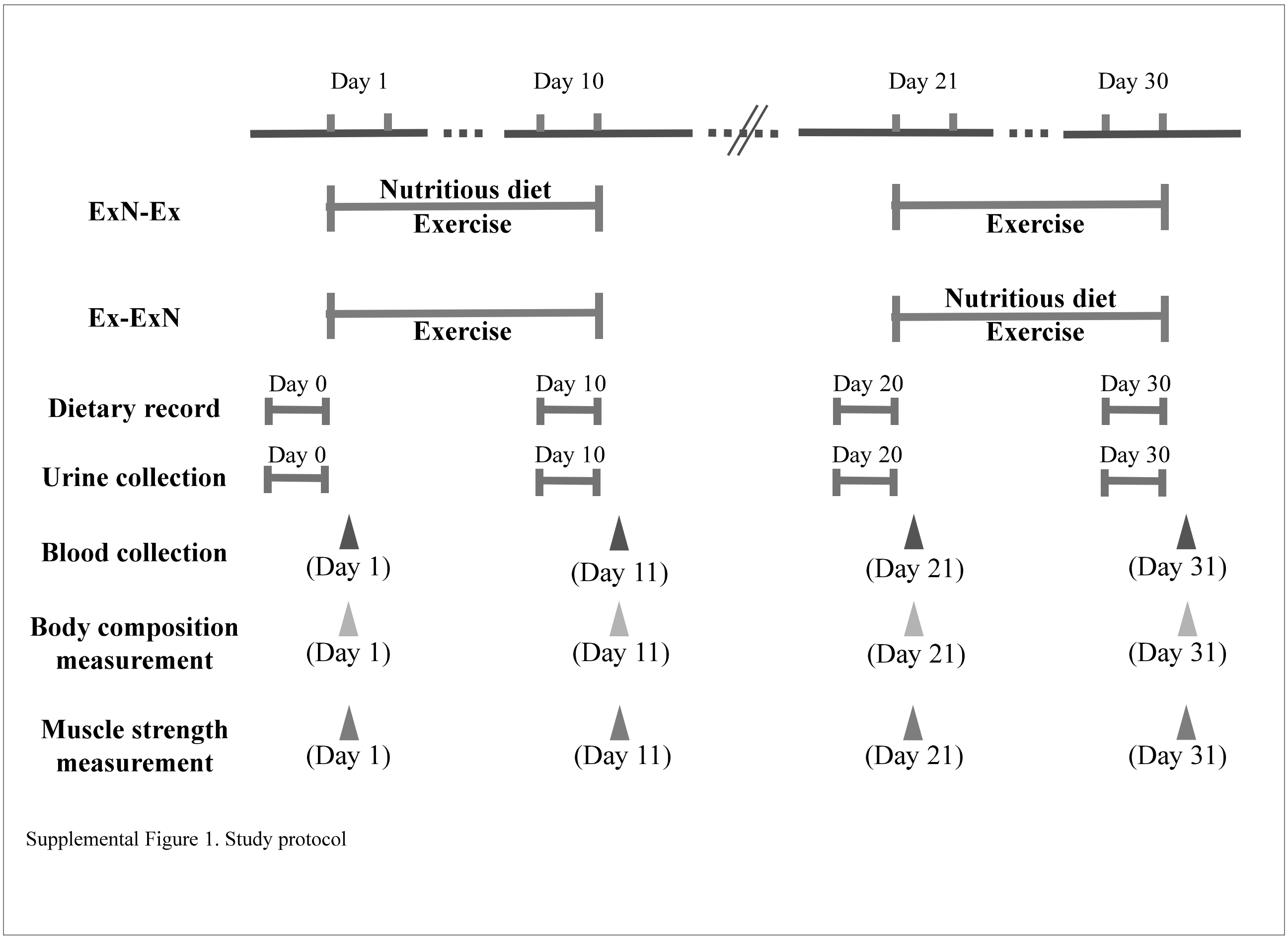

Supplemental Figure 1. Study protocol

Supplemental Table 1. Physical activity during the intervention period of the ExN and Ex groups estimated using an activity tracker

		Ex	ExN
Physical activity	(kJ)	5,523 ± 2,515	5,284 ± 2,715
Steps	(steps)	11,730 ± 4,678	10,844 ± 5,349

Values are presented as the mean ± SD
Ex, exercise program alone ; ExN, exercise program with protein and vitamin D-enriched diet
*$p< 0.05$, Ex vs. ExN, paired *t*-test

Supplemental Table 2. Effects of intervention program of exercise with and without protein and vitamin D-enriched menu on urinary metabolite levels

		Ex				ExN			
		Pre-intervention	Post-intervention	Change	*p*-value	Pre-intervention	Post-intervention	Change	*p*-value
pH		6.0 ± 0.1	6.3 ± 0.2	0.3 ± 0.2	**0.009**	6.1 ± 0.3	6.2 ± 0.3	0.1 ± 0.3	0.513
urine volume	(g/day)	1,889.2 ± 457.4	1,741.7 ± 553.8	−147.5 ± 357.8	0.317	1,907.7 ± 345.0	2,102.8 ± 526.0	195.1 ± 536.4	0.373
U-Cr	(mg/dL)	73.3 ± 21.1	82.6 ± 25.7	9.3 ± 9.7	**0.043**	68.9 ± 22.1	65.9 ± 16.2	−3.0 ± 16.9	0.656
U-3MH	(µmol/day)	279.2 ± 80.2	251.8 ± 57.2*	−27.4 ± 67.6	0.325	291.9 ± 107.3	310.5 ± 76.6	18.6 ± 87.7	0.594
UN	(g/day)	615.2 ± 107.8	646.9 ± 111.6	31.7 ± 104.8	0.454	581.5 ± 158.4	653.2 ± 134.8	71.7 ± 86.5	0.071

Values are presented as the mean ± SD or medians (25th to 75th interquartile range) (n = 7)
Ex, exercise program alone ; ExN, exercise program with protein and vitamin D-enriched diet
* $p < 0.05$, Ex vs. ExN, paired *t*-test
p-value between pre-intervention and post-intervention, paired *t*-test