

Effects of High-protein, Leucine-enriched Oral Nutritional Supplement and Resistance Exercise on Physical Performance among Malnourished Older Adults with Sarcopenia

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Abstract Background: Malnutrition combined with sarcopenia among older adults has resulted in poor outcomes, diminished quality of life (QoL), and increased mortality rates. Inadequate calorie and protein intake are factors contributing to sarcopenia. Specific nutritional interventions and exercise regimens may improve muscle strength and health outcomes. **Aim:** To determine the effects of a high-protein, leucine-enriched oral nutritional supplement (ONS) and resistance training (RT) on physical performance, muscle strength, QoL, and other outcomes among adults with sarcopenia. **Methods:** This was a single arm, open-label, 12-week prospective, multicenter study that involved outpatient malnourished older adults with sarcopenia from four hospitals in Thailand. During the 12-week period, subjects were given two servings of high-protein, leucine-enriched ONS per day with RT four times/week. Accordingly, short physical performance battery (SPPB), hand grip strength, muscle mass by dual-energy x-ray absorptiometry (DXA), QoL, frailty, and functional status of all subjects were assessed. **Results:** A total of 47 participants were enrolled in this study. After 12 weeks, 5-time chair stand results decreased from 11.59 ± 3.31 to 8.97 ± 1.85 seconds ($p < 0.001$), SPPB scores increased from 10.64 ± 1.51 to 11.84 ± 0.43 ($p < 0.001$), hand grip strength increased from 20.24 ± 5.69 to 21.96 ± 5.75 kg ($p < 0.001$), and appendicular lean mass index values increased from 5.15 ± 0.67 to 5.41 ± 0.93 kg/m² ($p = 0.004$). Recovery from sarcopenia was observed in 80% of the subjects. QoL and frailty improved significantly. Moreover, MNA scores changed from 21.63 ± 2.10 to 25.53 ± 2.01 ($p < 0.001$). Caloric and protein intake values changed from 26.25 ± 8.77 to 35.21 ± 8.23 kcal/kg/day and 1.10 ± 0.51 to 1.60 ± 0.35 g/kg/day, respectively ($p < 0.001$ both). **Conclusions:** High-protein, leucine-enriched ONS and RT have benefits for malnourished sarcopenic older adults through improvements in physical performance, muscle strength, muscle mass, recovery rates from malnutrition, frailty, and QoL.

Keywords: sarcopenia, malnutrition, leucine-enriched formula, older adults, oral nutritional supplement

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1. Introduction

Sarcopenia is a progressive and generalized skeletal muscle disorder that involves the accelerated loss of

muscle mass and muscle function, which are known to be associated with increased adverse outcomes that involve falls, functional decline, frailty, and increased mortality [1]. It occurs commonly as an age-related process among older people. It is not only influenced by concurrent risk factors but also by various genetic and lifestyle factors [2].

The prevalence of age-related sarcopenia in older adults over the age of 60 ranges from 8 to 13% of the healthy population [3], 37 to 41.1% of hospitalized patients [4,5], and in Thailand, it was observed that 30.5% of community-dwelling older adults had sarcopenia [6]. Older adults who are malnourished due to reduced food intake can exhibit impaired nutrient absorption or an increase in disease burden/inflammation. These adults were found to be more susceptible to sarcopenia [7,8]. Mortality rates were over four times higher in patients diagnosed with malnutrition-sarcopenia syndrome than those without [2].

Exercise increases blood flow to the muscles resulting in muscle mass synthesis [9]. The Asian Working Group on Sarcopenia (AWGS) recommends multimodal treatment for older patients diagnosed with sarcopenia that includes dietary support and exercise [1]. Resistance training has been found to be effective for maintaining muscle mass and gait speed, while it has the potential to increase balance [10,11,12]. Therefore, consumption of adequate nutrients, particularly those obtained from protein, combined with exercise can boost the advantages of maintaining or improving muscle functions in older adults [13,14].

To preserve muscle mass in older adults, the European Society for Clinical Nutrition and Metabolism (ESPEN) has recommended a protein intake of 1.0–1.2 g/kg per day for healthy older adults, and 1.2–1.5 g/kg per day for those diagnosed with acute or chronic illnesses [13]. However, studies show that fewer than 15% of older adults tend to follow these recommendations [15] and 71.8% of Thai elderly outpatients consume less than 0.8g/kg/day of protein [16]. Notably, the quality of the protein consumed is just as important as the quantity. To be more specific, 25–30 g of high-quality protein and up to 2.8–3 g of leucine should be consumed at least twice daily (minimum suggested intake of leucine, 78.5 mg/kg/day) to overcome the anabolic resistance of aging muscles and to maximize muscle protein synthesis throughout the day [17]. Because of low levels of food security combined with age-related changes in gastrointestinal function in older adults, it is worrisome that they may not receive enough protein. For malnourished older adults, a protein-enriched formula provides an attractive option as a form of protein supplementation. Few clinical studies have been conducted on how high-protein diets affect older people diagnosed with sarcopenia who are suffering from malnutrition.

The aim of the study is to evaluate the effect of a high-protein with leucine-enriched oral nutrition supplement and the influence of resistance training on physical performance in malnourished older adults diagnosed with sarcopenia. The primary outcome of this study was a change in the physical performance of subjects undergoing the 5-time chair stand test. The secondary outcomes were to assess any changes in Short Physical Performance Battery (SPPB), which included the 4 meter-gait speed and balance test, muscle strength using a hand grip dynamometer, the assessment of muscle mass and fat mass using dual-energy x-ray absorptiometry (DXA), an analysis of nutritional status consisting of an MNA score, serum albumin, and total lymphocyte count (TLC), along with calorie and protein intake after 12 weeks. Moreover,

an evaluation of any changes in the quality of life and frailty scores using SarQoL, frail scale, and Thai Barthel assessment of daily activities was recorded at the end of study. Side effects were monitored for the safety of all subjects during each visit.

2. Method

2.1. Study Design

This was a single arm, open-label, 12-week pre-and post-test, multicenter study that included 47 malnourished older adults diagnosed with sarcopenia who were admitted to the outpatient departments of four hospitals located in Thailand. The study protocol was approved by the Institutional Review Board, Royal Thai Army Medical Department, Institutional Review Board, Faculty of Medicine, Chulalongkorn University, Khon Kaen Hospital Institute Review Board in Human Research and Research Ethics Committee, Faculty of Medicine, Chiang Mai University. Informed consent was signed by all participants before the study was initiated. Furthermore, the study was registered at the Thai Clinical Trials Registry (TCTR20210421002).

2.1.1. Sample Size Estimation

$$N = \frac{(Z_{\alpha} + Z_{\beta})^2 Sp^2}{D^2}$$

N, sample size; Z_{α} , Z value for alpha error; Z_{β} , Z value for beta error; Sp^2 , pooled variance; D^2 , mean difference.

The required sample size was calculated according to the above formula, which was based on changes in the amount of time recorded for the 5-time chair stand test administered by Park et al [18]. With a pooled variance value of 29.6, a 95% confidence level, a 95% power of study, and a 5% drop-out rate, changes were detected in 35 subjects with regard to the amount time needed to implement the 5-time chair stand.

2.2. Participants

Subjects aged 60 and over who were at risk of suffering from malnutrition or who were malnourished according to the Mini Nutritional Assessment (MNA) with a score of less than 24, as well as those who were evaluated for sarcopenia using the criteria established by the Asian Working Group for Sarcopenia (AWGS). AWGS defines sarcopenia as “low muscle mass” as indicated by bioelectrical impedance (BIA) and “low muscle strength”. This was determined by measuring hand grip strength (HGS) with a dynamometer (JAMAR®) or “low physical performance” based on the 5-time chair stand test. All subjects were able to stand, sit, and walk without assistance. The following factors resulted in exclusion from the study. Subjects who were diagnosed with any diseases associated with consequential disturbing outcomes, such as Alzheimer's disease, thyroid disease, severe liver disease (Class C of Child Pugh Score assessment), chronic kidney disease with $GFR < 30 \text{ ml/min/1.73m}^2$, recent myocardial infarction < 6

months before participation, unstable angina, and those with compromised musculoskeletal disorders who needed to participate in restricted resistance exercise, were all excluded from the study. The exclusion criteria also eliminated any subjects having potentially high sensitivity C-Reactive Protein (hs-CRP) >10 mg/L, those who had received radiation therapy or chemotherapy treatment within three months, and particularly those being given bone marrow suppressants or immunosuppressants (including prednisolone at doses of more than 10 mg/day). Other subjects were excluded from taking part in the study if they were diagnosed at the end stage of a disease, had a history of immunity problems, such as HIV and autoimmune disease, were allergic to any other components of the study formula, those who were taking any investigated product or medical food indicated in any other study within 30 days before joining this study, those whose body composition could not be determined by BIA (i.e., having had a pacemaker implanted), or those who were taking vitamin D supplements of more than 10,000 IU/day.

2.2. Intervention

During the 12-week trial period, subjects were given two servings (sachets) of a high-protein, leucine-enriched oral nutritional supplement (ONCE SARCOPENIA) per day and participated in resistance training four times per week. ONCE SARCOPENIA is a high-protein, leucine-enhanced formula with a carbohydrate: protein: fat ratio of 40: 23: 37. Each serving (250 ml) contains 17 grams of protein, 3 grams of leucine, and 400 IUs of vitamin D. Detailed formula components are listed in Table 1. In order to assess subject compliance, subjects brought any remaining sachets back to investigators and were given new ones on these visits over weeks 4 and 8. Any side effects observed from the investigational product administered during this study were recorded.

With regard to resistance exercise training, before enrollment, all physical therapists taking part in the study attended a meeting to standardize the resistance exercise protocol (Supplementary Figure 1). On the first day, all subjects were trained on how to do resistance exercises and how to select the appropriate resistance exercise band (Theraband®). The intensity of the exercise was stepped up according to each individual subject's level of fitness.

Exercise guidebooks, exercise demonstration VDO clips, and record books were created for each subject. The guidebooks presented specific details of posture, the recommended number of sets, and explanations for proper elastic exercise band use. Additionally, the subjects kept track of how often they worked out and gave the investigators a record form at each visit that enabled the investigators to assess their degree of exercise compliance.

Throughout the research project, subjects were coached on how to properly do resistance exercises. This was done to ensure optimal correctness and safety over the course of the study. Besides hospital visits, home visits were made on weeks 2, 6, and 10, and were followed up with phone calls on weeks 3, 5, 7, 9, and 11.

Table 1. Composition of ONCE SARCOPENIA

Ingredients	Content (g) per serving (250 ml/300 kcal)	Caloric distribution
Protein (Whey protein isolate, Leucine, Casein)	17 (leucine 3 g)	23
Carbohydrate (Maltodextrin, Isomaltulose, Maltitol, Fibersol-2, FOS)	35	40
Fat (MCT oil, High oleic safflower oil, Rice bran oil, Canola oil)	12	37
Highlight Nutrient		
Vitamin D	400 IU	
Vitamin B12	1.29 mcg	
Folic acid	105 mcg	
Calcium	360 mg	
Sodium	220 mg	
Carnitine	48 mg	
Inositol	227 mg	

3. Measurements and Outcomes

3.1. Physical Performance, Muscle Mass and Muscle Strength Measurements

Physical performance was evaluated using the short physical performance battery (SPPB), the assessment of muscle strength with a hand grip dynamometer, and by determining muscle mass using dual-energy x-ray absorptiometry (DXA) (Hologic®) on weeks 0 and 12.

3.2. Anthropometric and Nutritional Measurement

The weight, height, mid-arm and calf circumferences (MAC, CC), and nutritional status of all subjects were determined by MNA on every visit.

3.3. Dietary Data

A 3-day food record on daily food consumption that included two weekdays and one weekend day was taken at the baseline and on every four weeks throughout the course of the study period. The dietitians contacted subjects weekly to assess their nutritional intake during the trial. At week 0 (baseline), and on weeks 4, 8, and 12, subjects submitted their records to the dietitian and underwent an extra food history evaluation.

3.4. Functional status, Quality of Life and frailty Assessment

The Sarcopenia and Quality of Life (SarQoL®) questionnaire were used to measure the subjects' quality of life on week 0 (baseline) and at the end of the study on week 12. The FRAIL scale was used to measure frailty, while a Thai version of the Barthel index of daily living activities was used to measure the patient's ability to perform daily activities.

3.5. Blood Samplings

Biochemical blood tests (assessing complete blood count (CBC), Creatinine (Cr), glomerular filtration rate (GFR), aspartate aminotransferase (AST), alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transferase (GGT), albumin, prothrombin time (PT), international normalized ratio (INR), serum electrolyte, vitamin B12, vitamin D, homocysteine, Parathyroid Hormone (PTH), total cholesterol, triglycerides, low-density lipoprotein (LDL), high-density lipoprotein (HDL) levels, and fasting blood glucose) were administered on week 0 (baseline) and on week 12.

4. Statistical Analysis

All statistical tests were performed using SPSS software version 18.0 for windows (SPSS Inc., Chicago, IL). The data were presented as mean $\pm$ standard deviations of mean values. Paired sample t-test or the Wilcoxon Signed rank test was applied to compare parameters between two time points. A p-value of 0.05 or lower was considered statistically significant.

5. Results

5.1. Baseline Characteristics

Out of the 47 subjects, there were 35 women and 12 men. Their average age was 71.78 ± 8.18 years. Their mean body mass index (BMI) was 20.12 ± 2.73 kg/m², while their mean weight was 46.54 ± 6.42 kg. The average MNA score was 21.63 ± 2.10 , while all subjects were

either characterized as suffering from malnutrition or were at risk of becoming malnourished. The demographic and clinical characteristics of all subjects at the baseline are presented in Table 2.

Table 2. Baseline characteristics

Parameter	Value (n = 47)
Age (year)	71.78 $\pm$ 8.18
Gender (N (%), female)	35 (74.47%)
Body Mass Index (kg/m ²)	20.12 $\pm$ 2.73
Body weight (kg)	46.54 $\pm$ 6.42
Mini Nutritional Assessment (MNA)	21.63 $\pm$ 2.10
Illness and disease history, N (%)	
Dyslipidemia	26 (55.32%)
Hypertension	21 (44.68%)
Diabetes Mellitus type 2	12 (25.53%)
Gastrointestinal	
- Gastroesophageal Reflux Disease (3)	4 (8.51%)
- Dyspepsia (1)	
Cardiovascular disease	3 (6.38%)
Liver disease	
- Fatty liver	1 (2.13%)

Plus-minus values are means $\pm$ SD.

5.2. Physical Performance and Muscle Strength

Short physical performance battery (SPPB) values increased significantly from week 4 to week 12 when compared with baseline values (Figure 1A). The amount of time needed to complete the 5-time chair stand test decreased from week 0 to week 4 (Figure 1B) and significantly declined on week 12 when compared with the baseline values ($p < 0.001$). These 12-week findings were in accordance with the results of the 4-meter gait speed ($p < 0.001$) test, as is shown in Table 3.

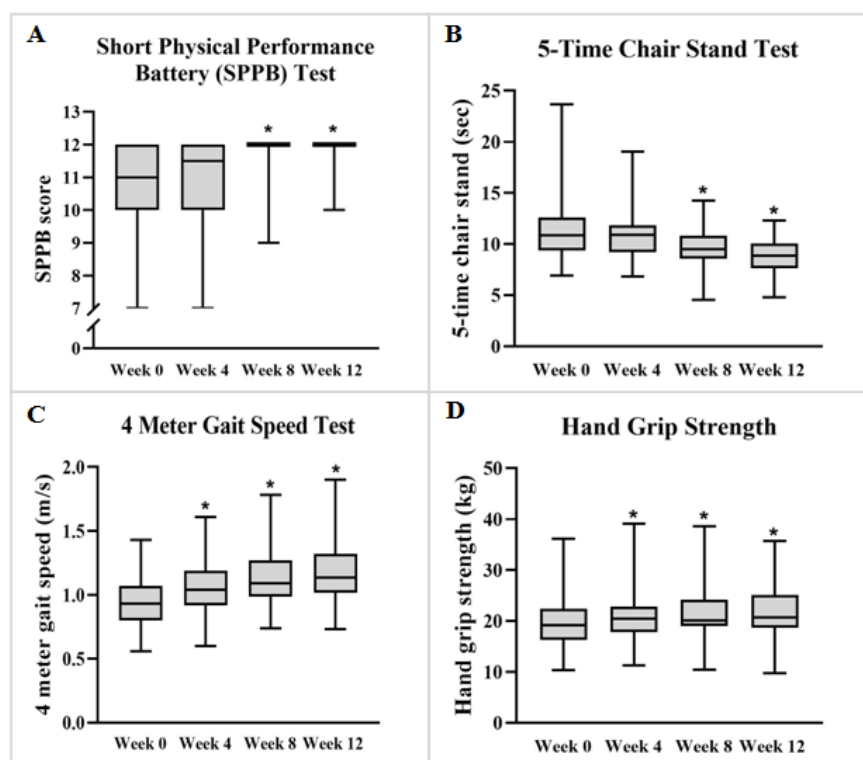


Figure 1. Comparison of physical performance assessment (A, B and C) and muscle strength test (D) between week 0 (baseline) with weeks 4, 8 and 12. * Significant difference between baseline (week 0) with weeks 4, 8 and 12. ($p < 0.05$)

Hand grip strength increased significantly from week 4 to week 12 when compared with the baseline values ($p<0.001$ and $p<0.001$, respectively), as is shown in Figure 1D.

5.3. Muscle Mass and Fat Mass Analysis

After 12 weeks of taking the supplement, muscle mass rose significantly in the arms and legs of all subjects. Appendicular lean soft tissue increased by 4.4 % ($p<0.001$) and appendicular lean mass index increased by 4.90 % ($p=0.004$), whereas total fat mass increased in both arms and legs. However, these increases were not determined to be significant (Table 3). Notably, 80% of subjects were found to have recovered from sarcopenia at the end of study.

5.4. Nutritional Status

The MNA nutritional assessment showed that between week 4 and week 12, the subjects' nutritional status had greatly improved since first visit of the study (Figure 2E). All subjects with malnutrition or at risk of malnutrition recovered after the end of the study (mean score was 25.53 ± 2.01 , $p<0.001$) as shown in Table 4. The mean weight increased significantly in week 12 ($p<0.001$). For dietary assessment, at 12 weeks, the mean daily calorie intake rose from 26.25 ± 8.77 kcal/kg/day to 35.21 ± 8.23

kcal/kg/day ($p<0.001$) and consumed an average protein amount of 1.60 ± 0.35 g/kg/day ($p<0.001$) as presented in Table 4. Serum vitamin B12 and vitamin D levels rose substantially ($p<0.001$ and $p=0.011$, respectively), whereas homocysteine and parathyroid hormone levels declined significantly ($p<0.001$ and $p<0.001$, respectively) as shown in Table 4.

5.5. Quality of Life and Frailty

The total score of SarQoL was found to be significantly different from the baseline value ($p<0.001$) including for all parameters of quality of life, such as physical and mental health, locomotion, and functionality, whereas the fear score in older adults decreased significantly when compared with the baseline values ($p=0.030$). The frail scale on week 12 was significantly lower than the baseline value ($p=0.001$). For the Thai Barthel assessment of daily living activities, the mean score increased significantly on week 12 ($p=0.005$), as is shown in Table 5.

5.6. Subject Compliance and Side Effects

The degree of compliance for ONCE SARCOPENIA consumption was determined to be 93%. The percentage of subjects who complied with their fitness routine was 78%. Side effects, such as flatulence, nausea, and mild abdominal distension, were less than 5%.

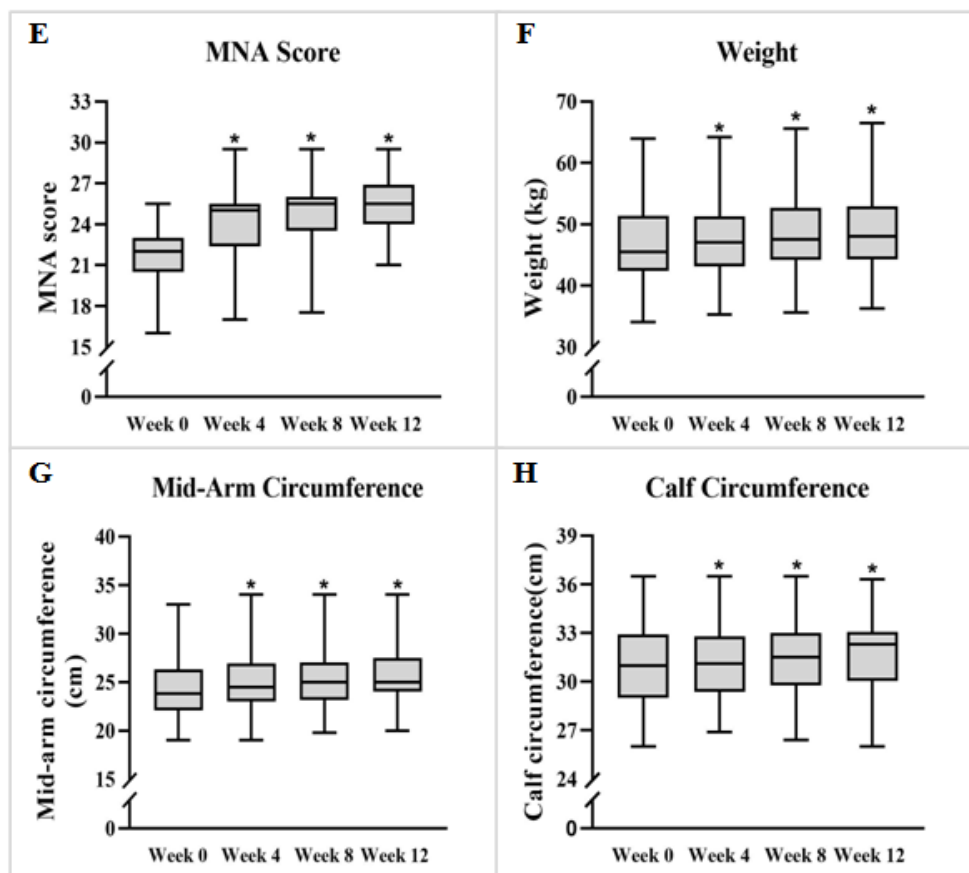


Figure 2. Comparison of nutritional assessment between week 0 (baseline) with weeks 4, 8 and 12. * Significant difference between week 0 (baseline) with weeks 4, 8 and 12. ($p \leq 0.05$)

Table 3. Comparison of sarcopenic parameters between week 0 (baseline) and week 12

	week0	week12	p-value	% Change
Physical performance				
SPPB total score	10.64 ± 1.51	11.84 ± 0.43	<0.001 ^a	12.14 ± 15.63
5-time chair stand (sec)	11.59 ± 3.31	8.97 ± 1.85	<0.001 ^a	-19.60 ± 19.31
4-meter gait speed (m/s)	0.92 ± 0.19	1.19 ± 0.24	<0.001 ^a	30.50 ± 30.23
Muscle strength				
Hand grip strength (kg)	20.24 ± 5.69	21.96 ± 5.75	<0.001 ^a	10.22 ± 16.70
Anthropometry				
*Muscle mass				
Left arm (kg)	1.37 ± 0.35	1.46 ± 0.49	0.051	3.97 ± 12.66
Right arm (kg)	1.55 ± 0.38	1.64 ± 0.49	0.006 ^a	4.36 ± 11.33
Left leg (kg)	4.47 ± 0.82	4.73 ± 1.01	<0.001 ^a	4.88 ± 10.35
Right leg (kg)	4.68 ± 0.81	4.91 ± 1.03	0.003 ^a	4.31 ± 12.10
Appendicular lean soft tissue (kg)	12.07 ± 2.25	12.74 ± 2.93	<0.001 ^a	4.43 ± 10.45
Appendicular Lean Mass Index (kg/m ²)	5.15 ± 0.67	5.41 ± 0.93	0.004 ^a	4.87 ± 11.03
Fat mass				
Total fat mass (kg)	14.90 ± 5.15	15.42 ± 5.61	0.236 ^a	5.25 ± 18.82

Plus-minus values are means ± SD.

* Presented as lean mass determined from dual-energy x-ray absorptiometry (DXA)

^a Compared with baseline value determined by Wilcoxon Signed Ranks test

^b Compared with baseline value determined by Paired-Samples t-test.

Table 4. Comparison of nutritional and relevant blood chemistry parameters between week 0 (baseline) and week 12

	week0	week12	p-value	% Change
Mini Nutritional Assessment (MNA)	21.63 ± 2.10	25.53 ± 2.01	<0.001 ^b	18.23 ± 11.61
Weight (kg)	46.56 ± 6.54	48.53 ± 6.77	<0.001 ^b	4.05 ± 2.69
Calorie intake (kcal/kg/day)	26.25 ± 8.77	35.21 ± 8.23	<0.001 ^a	38.27 ± 30.99
Protein intake (g/kg/day)	1.10 ± 0.51	1.60 ± 0.35	<0.001 ^a	63.52 ± 58.20
Carbohydrate (g)	160.31 ± 55.54	210.65 ± 38.37	<0.001 ^a	38.27 ± 39.80
Fat (g)	39.22 ± 14.41	62.10 ± 15.21	<0.001 ^a	72.84 ± 64.30
Albumin (g/dL)	4.39 ± 0.29	4.35 ± 0.27	0.267 ^b	-0.74 ± 5.52
Total Lymphocytes Counts (cell/μL)	1,773.48 ± 688.49	1,794.52 ± 680.07	0.713 ^a	4.36 ± 23.92
Vitamin D (ng/ml)	32.03 ± 13.01	35.58 ± 11.26	0.011 ^a	15.67 ± 29.25
Parathyroid Hormone (pg/mL)	52.11 ± 24.66	42.96 ± 20.63	<0.001 ^a	-11.90 ± 20.04
Vitamin B12 (pg/ml)	498.38 ± 246.25	604.34 ± 307.49	<0.001 ^a	25.38 ± 32.24
Homocysteine (μmol/L)	12.43 ± 5.12	11.15 ± 4.63	<0.001 ^a	-8.30 ± 16.94
Total cholesterol (mg/dL)	193.41 ± 47.47	195.47 ± 44.00	0.845 ^b	0.63 ± 10.15
HDL (mg/dL)	63.36 ± 14.25	64.77 ± 15.97	0.787 ^b	0.13 ± 9.64
LDL (mg/dL)	116.37 ± 42.31	116.50 ± 40.16	0.312 ^b	-0.45 ± 17.40
Triglyceride (mg/dL)	105.54 ± 49.03	119.66 ± 80.99	0.092 ^a	12.23 ± 35.61
Fasting Blood Glucose (mg/dL)	99.51 ± 29.34	101.49 ± 31.36	0.018 ^a	2.36 ± 7.61
Creatinine (mg/dL)	0.83 ± 0.27	0.81 ± 0.26	0.103 ^a	-1.76 ± 8.68

Plus-minus values are means ± SD.

^a Compared with baseline values determined by Wilcoxon Signed Ranks test.

^b Compared with baseline values determined by Paired-Samples t-test.

Table 5. Comparison of quality of life and frailty values between week 0 (baseline) and week 12

Quality of life and frailty assessment	week0	week12	p-value	% change
SarQoL	71.87 ± 12.16	83.07 ± 9.74	<0.001 ^b	16.94 ± 19.79
- Physical and mental health	67.82 ± 15.84	83.46 ± 11.58	<0.001 ^b	28.22 ± 31.45
- Locomotion	74.53 ± 17.36	84.34 ± 15.25	0.001 ^b	19.66 ± 33.07
- Body composition	62.41 ± 15.87	81.82 ± 12.65	<0.001 ^b	39.23 ± 45.29
- Functionality	75.83 ± 13.67	85.50 ± 10.59	<0.001 ^b	14.84 ± 19.81
- Activities of daily living	72.24 ± 17.54	83.34 ± 14.47	0.001 ^b	16.71 ± 30.25
- Leisure activities	47.75 ± 20.98	63.10 ± 22.84	0.001 ^b	43.69 ± 71.44
- Fears	83.51 ± 10.46	80.11 ± 14.09	0.030 ^b	-4.71 ± 15.68
Frail scale	0.57 ± 0.85	0.11 ± 0.39	0.001 ^b	-89.47 ± 26.77
Thai Barthel Activities of Daily Living	98.40 ± 3.77	99.66 ± 1.67	0.005 ^b	1.52 ± 3.63

Plus-minus values are means ± SD.

^b Compared with baseline values determined by Paired-Samples t-test.

6. Discussion

In Thailand, sarcopenic-malnutrition has been commonly found in older people [19]. This problem has led to poor health outcomes and increased mortality rates [20,21]. An exercise program combined with the administration of nutritional supplements in older people diagnosed with sarcopenia has helped patients gain more muscle mass, improve muscle strength, and increase walking speed [22]. Our study is the first nutritional-exercise intervention involving Thai older adults. The results indicate that a high protein, leucine-enriched oral nutritional supplement, taken together with resistance exercise, could improve physical performance, muscle strength, muscle mass, and the quality of life in malnourished older adults diagnosed with sarcopenia. The outcomes of previous studies have indicated the same trend [22-27].

The consumption of adequate amounts of nutrients is essential for improving nutritional status. A recent systematic review reported that older adults with sarcopenia had significantly lower caloric, macronutrient, and micronutrient consumption than those without sarcopenia [25]. In our study, nutritional intervention that involved two servings per day of specific ONS improved caloric and protein intake to the recommended daily energy and protein requirements of 30 kcal/kg BW and 1.2 g protein/kg BW for those presenting with sarcopenia and/or frailty, respectively [26]. As a result, all subjects recovered from malnutrition or the risk of suffering from malnutrition. That indicated not only protein intake but also total calorie intake contributed to improve muscle function and nutritional status. Tieland et al. [28] compared resistance exercise with and without dietary protein supplementation in frail older adults. Lean body mass rose significantly in the protein-supplemented group but not in the placebo group. However, Kim et al. [24] found that amino acid supplementation and exercise improved only leg muscle mass and walking speed but not knee extension strength. This may have resulted from the ingestion of suboptimal protein supplements (6 g/day) and engagement in less frequent exercise routines (2 days/week). In our study, we provided two servings of a supplement that contained a total of 34 grams of protein and 6 grams of leucine. This would indicate that adequate protein intake is needed for muscle protein synthesis (MPS) in older people. Whey protein contains high amounts of branched-chain amino acids and leucine, which activates the mammalian target of the Rapamycin (mTOR) pathway and then mediates mRNA translation for MPS. To overcome the anabolic resistance of aged muscles and enhance MPS throughout the day, 25–30 g of high-quality protein and up to 2.8–3 g of leucine should be provided at each meal and at least twice daily [17]. This has proven to be sufficient to promote a positive impact on protein anabolism.

Besides the macronutrient benefits that improve sarcopenia and nutritional status, vitamins contribute to the support of a variety of physical functions. Vitamin D is known to play a role in MPS. Rondanelli et al. [29] demonstrated that the consumption of a vitamin D-enriched protein supplement, combined with exercise intervention, improved muscle mass and hand grip strength in sarcopenic older adults. Our nutritional intervention

comprised of two servings of the supplement provided 800 IU of vitamin D, which is the daily recommended requirement according to the Institute of Medicine (IOM) [30]. Serum vitamin D levels increased significantly, whereas serum parathyroid hormone decreased significantly at the end of this study. Older adults are typically depleted in vitamin B12 due to either malabsorption or low consumption of B12. This can cause methionine synthase enzyme inactivity, which inhibits important methylation processes and elevates blood homocysteine [31]. High homocysteine levels have been associated with dementia in older people [32]. Serum vitamin B12 levels in our subjects increased, while serum homocysteine levels declined significantly after 12 weeks of ONS.

The following points are considered the strengths of this study. First, this is the first multicenter study involving the intake of high protein supplements and exercise intervention in Thai older adults diagnosed with malnutrition-sarcopenia syndrome. Second, two servings of ONS per day provided subjects with adequate amounts of energy and protein while meeting the recommendations of the ESPEN guidelines (>400 kcal/day and ≥ 30 g protein/day) [26]. Third, the exercise protocol employed in this study was a progressive resistance training program that was adjusted to each participant over a 12-week period. This provided optimal intensity of exercise leading to good compliance and improvement in all muscle outcomes. Finally, this study measured the relevant outcomes pertaining to physical activities, body composition, biochemistry, and quality of life.

Limitations of this study were that the study was only conducted among community-dwelling older individuals who were willing and able to adhere to the outlined exercise protocol, especially during the coronavirus pandemic. Therefore, some visits were followed up by video calls instead of home visits. Regardless of these obstacles, the subjects still performed their resistance exercises correctly with good compliance until the end of the study.

7. Conclusions

In conclusion, this research study concluded that the high-protein, leucine-enriched oral supplement, administered in conjunction with resistance training, enhanced physical performance in malnourished, sarcopenic older people. Individuals who took the oral supplement for 12 weeks showed improvement in physical functions, muscle mass, and muscle strength, as well as recovering from malnutrition while achieving improved quality of life. No severe adverse effects were reported. Accordingly, oral dietary supplements and optimal resistant exercise regimens are helpful in the management of malnourished-sarcopenic older people.

Statement of Competing Interests

The authors declared no potential conflicts of interest with respect to the research, authorship and/or publication of this article.

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References

- [1] L. K. Chen, J. Woo, P. Assantachai, T. W. Auyeung, M. Y. Chou, K. Iijima, *et al.*, "Asian Working Group for Sarcopenia: 2019 Consensus Update on Sarcopenia Diagnosis and Treatment", *Journal of the American Medical Directors Association*, 21(3), 300-307, March 2020.
- [2] G. Shafiee, A. Keshtkar, A. Soltani, Z. Ahadi, B. Larijani, and R. Heshmat, "Prevalence of sarcopenia in the world: a systematic review and meta-analysis of general population studies", *Journal of Diabetes & Metabolic Disorders*, 16(1), May 2017.
- [3] G. C. Ligthart-Melis, Y. C. Luiking, A. Kakourou, T. Cederholm, A. B. Maier, and M. A. E. de van der Schueren, "Frailty, Sarcopenia, and Malnutrition Frequently (Co-) occur in Hospitalized Older Adults: A Systematic Review and Meta-analysis", *Journal of the American Medical Directors Association*, 21(9), 1216-1228, September 2020.
- [4] A. S. Sousa, R. S. Guerra, I. Fonseca, F. Pichel, and T. F. Amaral, "Sarcopenia among hospitalized patients - A cross-sectional study", *Clinical Nutrition*, 34(6), 1239-1244, December 2015.
- [5] N. Khongsri, S. Tongsuntud, P. Limampai, and V. Kuptniratsaikul, "The prevalence of sarcopenia and related factors in a community-dwelling elders Thai population", *Osteoporosis and Sarcopenia*, 2(2), 110-115, June 2016.
- [6] M. Locquet, C. Beaudart, M. Hajaoui, J. Petermans, J. Y. Reginster, and O. Bruyère, "Three-Year Adverse Health Consequences of Sarcopenia in Community-Dwelling Older Adults According to 5 Diagnosis Definitions", *Journal of the American Medical Directors Association*, 20(1), 43-46, January 2019.
- [7] D. Sanchez-Rodriguez, M. Locquet, J. Y. Reginster, E. Cavalier, O. Bruyère, and C. Beaudart, "Mortality in malnourished older adults diagnosed by Espen and glim criteria in the sarcophage study", *Journal of Cachexia, Sarcopenia and Muscle*, 11(5), 1200-1211, July 2020.
- [8] A. J. Cruz-Jentoft, G. Bahat, J. Bauer, Y. Boirie, O. Bruyère, T. Cederholm, *et al.*, "Sarcopenia: revised European consensus on definition and diagnosis", *Age Ageing*, 48(1), 16-31, January 2019.
- [9] H. Arai, H. Wakabayashi, Y. Yoshimura, M. Yamada, H. Kim, and A. Harada, "Chapter 4 Treatment of sarcopenia", *Geriatrics & Gerontology International*, 18(Suppl. 1), 28-44, May 2018.
- [10] C. K. Liu, and R. A. Fielding, "Exercise as an intervention for frailty", *Clinics in Geriatric Medicine*, 27(1), 101-110, February 2011.
- [11] E. Rosendahl, N. Lindelöf, H. Littbrand, E. Yifter-Lindgren, L. Lundin-Olsson, L. Häglin, *et al.*, "High-intensity functional exercise program and protein-enriched energy supplement for older persons dependent in activities of daily living: a randomised controlled trial", *Australian Journal of Physiotherapy*, 52(2), 105-113, 2006.
- [12] K. L. Timmerman, S. Dhanani, E. L. Glynn, C. S. Fry, M. J. Drummond, K. Jennings, *et al.*, "A moderate acute increase in physical activity enhances nutritive flow and the muscle protein anabolic response to mixed nutrient intake in older adults", *The American Journal of Clinical Nutrition*, 95(6), 1403-1412, June 2012.
- [13] N. E. P. Deutz, J. M. Bauer, R. Barazzoni, G. Biolo, Y. Boirie, A. Bosy-Westphal, *et al.*, "Protein intake and exercise for optimal muscle function with aging: recommendations from the ESPEN Expert Group", *Clinical Nutrition*, 33(6), 929-936, December 2014.
- [14] N. M. Cermak, P. T. Res, L. C. P. G. M. de Groot, W. H. M. Saris, and L. J. C. van Loon, "Protein supplementation augments the adaptive response of skeletal muscle to resistance-type exercise training: a meta-analysis", *The American Journal of Clinical Nutrition*, 96(6), 1454-1464, December 2012.
- [15] S. Morris, J. D. Cater, M. A. Green, A. M. Johnstone, J. M. Brunstrom, E. J. Stevenson, *et al.*, "Inadequacy of Protein Intake in Older UK Adults", *Geriatrics (Basel, Switzerland)*, 5(1), March 2020.
- [16] L. M. Hengeveld, J. M. A. Boer, P. Gaudreau, M. W. Heymans, C. Jagger, N. Mendonça, *et al.*, "Prevalence of protein intake below recommended in community-dwelling older adults: a meta-analysis across cohorts from the PROMISS consortium", *Journal of Cachexia, Sarcopenia and Muscle*, 11(5), 1212-1222, October 2020.
- [17] M. S. Borack, and E. Volpi, "Efficacy and Safety of Leucine Supplementation in the Elderly", *Journal of Nutrition*, 146(12), 2625-2629, December 2016.
- [18] Y. Park, J. E. Choi, and H. S. Hwang, "Protein supplementation improves muscle mass and physical performance in undernourished prefrail and frail elderly subjects: a randomized, double-blind, placebo-controlled trial", *American Journal of Clinical Nutrition*, 108(5), 1026-1033, November 2018.
- [19] J. Sri-On, Y. Fusakul, T. Kredarunsooksree, T. Paksopis, and R. Ruangsiri, "The prevalence and risk factors of sarcopenia among Thai community-dwelling older adults as defined by the Asian Working Group for Sarcopenia (AWGS-2019) criteria: A cross-sectional study", *BMC geriatrics*, 22(1), October 2022.
- [20] S. Sobestiansky, A. C. Åberg, and T. Cederholm, "Sarcopenia and malnutrition in relation to mortality in hospitalised patients in geriatric care - predictive validity of updated diagnoses", *Clinical nutrition ESPEN*, 45, 442-448, October 2021.
- [21] X. Hu, L. Zhang, H. Wang, Q. Hao, B. Dong, and M. Yang, "Malnutrition-sarcopenia syndrome predicts mortality in hospitalized older patients", *Scientific reports*, 7(1), June 2017.
- [22] D. Luo, Z. Lin, S. Li, and S. J. Liu, "Effect of nutritional supplement combined with exercise intervention on sarcopenia in the elderly: A meta-analysis", *International Journal of Nursing Sciences*, 4(4), 389-401, October 2017.
- [23] S. Shahar, N. S. Kamaruddin, M. Badrasawi, N. I. Sakian, Z. Abd Manaf, Z. Yassin, *et al.*, "Effectiveness of exercise and protein supplementation intervention on body composition, functional fitness, and oxidative stress among elderly Malays with sarcopenia", *Clinical Interventions in Aging*, 8, 1365-1375, 2013.
- [24] H. K. Kim, T. Suzuki, K. Saito, H. Yoshida, H. Kobayashi, H. Kato, *et al.*, "Effects of exercise and amino acid supplementation on body composition and physical function in community-dwelling elderly Japanese sarcopenic women: A randomized controlled trial", *Journal of the American Geriatrics Society*, 60(1), 16-23, January 2012.
- [25] E. C. S. Santiago, A. K. C. Roriz, L. B. Ramos, A. J. F. Ferreira, C. C. Oliveira, and M. Gomes-Neto, "Comparison of calorie and nutrient intake among elderly with and without sarcopenia: A systematic review and meta-analysis", *Nutrition Reviews*, 79(12), 1338-1352, December 2021.
- [26] D. Volkert, A. M. Beck, T. Cederholm, A. Cruz-Jentoft, S. Goisser, L. Hooper, *et al.*, "ESPEN guideline on clinical nutrition and hydration in geriatrics", *Clinical Nutrition*, 38(1), 10-47, February 2019.
- [27] C. D. Liao, H. C. Chen, S. W. Huang, and T. H. Liou, "The role of muscle mass gain following protein supplementation plus exercise therapy in older adults with sarcopenia and frailty risks: A systematic review and meta-regression analysis of randomized trials", *Nutrients*, 11(8), July 2019.

- [28] M. Tieland, M. L. Dirks, N. van der Zwaluw, L. B. Verdijk, O. van de Rest, L. C. P. G. M. de Groot, *et al.*, "Protein supplementation increases muscle mass gain during prolonged resistance-type exercise training in frail elderly people: a randomized, double-blind, placebo-controlled trial", *Journal of the American Medical Directors Association*, 13(8), 713-719, 2012, [Internet]. October 2012.
- [29] M. Rondanelli, C. Klersy, G. Terracol, J. Talluri, R. Maugeri, D. Guido, *et al.*, "Whey protein, amino acids, and vitamin D supplementation with physical activity increases fat-free mass and strength, functionality, and quality of life and decreases inflammation in sarcopenic elderly", *American Journal of Clinical Nutrition*, 103(3), 830-840, March 2016.
- [30] E. Azzam, N. Elsabbagh, N. Elgayar, and D. Younan, "Relation between vitamin D and geriatric syndrome", *Clinical Nutrition ESPEN*, 35, 123-127. February 2020.
- [31] C. F. Hughes, M. Ward M, L. Hoey, and H. McNulty, "Vitamin B12 and ageing: current issues and interaction with folate", *Annals of Clinical Biochemistry*, 50(4), 315-329, July 2013.
- [32] A. D. Smith, H. Refsum. "Homocysteine, B Vitamins, and Cognitive Impairment", *Annual Review of Nutrition*, 36, 211-239, July 2016.

Supplementary Figure 1. Resistance exercise protocol

Resistance exercise training sessions

1. Chair squat



7. Triceps Extension



2. Chest press



8. Calf Raise



3. Lateral Shoulder Raise



9. Hip extension



4. Sitting Abduct



10. Bicep Curl



5. Sitting Adduct



11. Wall Plank



6. Band pull apart



Monday	Chair Squat	Triceps Extension	Calf Raise
Wednesday	Sitting Abduct	Sitting Adduct	Band pull apart
Friday	Chair Squat	Triceps Extension	Calf Raise
Saturday	Hip extension	Bicep Curl	Wall Plank

Exercise protocol

Ten training sessions (1-10) beginning with 1 set of 8-10 repetitions in the first week and stepping up to 1-2 sets of 10-12 repetitions and then 2-3 sets of 10-12 repetitions using elastic exercise bands (Theraband®) under the supervision of a physical therapist. The elastic bands had several resistance levels indicated by the different colors listed below.

Theraband® color	Resistance weight (kg)
Yellow	1.3
Red	1.7
Green	2.1
Blue	2.6

A subsequent session (11) was initiated with 1 time/set of the ‘Wall Plank’ exercise. The frequency was then increased to three times/set. All subjects were assigned to participate in resistance exercises four days per week.



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