Original Article

Preliminary study on nutritional and exercise strategies to prevent and reverse sarcopenia in aging: an open-label single-arm trial

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Background and Objectives: Sarcopenia is the progressive loss of muscle mass and strength that can adversely affect an individual's health and quality of life. The objective of this study was to evaluate the effectiveness of a combined nutritional and exercise intervention among older adults who were at risk of sarcopenia. **Methods and Study Design:** 46 older adults were included in a 30±3 days intervention that combined oral nutritional supplements with resistance exercise. Parameters were measured at baseline (day 0) and after intervention (day 30±3), including routine parameters of sarcopenia, blood tests, and body measurements. The ITT analysis method was used, and the data were analysed using paired t-tests/paired Wilcoxon test, and ANOVA. **Results:** Among the 46 participants, there were no significant changes in hip circumference (HC), muscle mass of both lower limbs, appendicular skeletal muscle mass index (ASMI), and hemoglobin (Hb) after intervention. However, both hand grip strength (GS) significantly increased, as did muscle mass of both upper limbs and the total muscle strength. Blood tests showed a slight increase in albumin (ALB) levels and a significant increase in 25-OH-D levels, while the waist (WC) and calf circumferences (CC) also increased significantly after intervention. Somatic motor performance improved significantly in the 6-meter walk and 5 sit-ups tests. **Conclusions:** The combined nutritional and exercise intervention was feasible and effective in improving muscle mass and strength, especially in the upper limbs, as well as somatic motor performance among older adults at risk of sarcopenia. It could be beneficial among three stages of sarcopenia.

Key Words: sarcopenia, combined intervention, body composition analysis, resistance exercise, nutritional supplement

INTRODUCTION

According to China's Seventh Population Census, individuals aged 65 and over will account for 13.3% of Beijing's registered population by the end of 2020, nearly meeting the World Health Organization's advanced aging threshold of 14%.[1] Aging has been linked with an increased risk of falling, sarcopenia, physical dysfunction, and mortality.[2]

Sarcopenia, a geriatric disease characterized by a progressive loss of skeletal muscle mass (SMM), muscle function, and physical performance,[3] can begin as early as 40 years old,[4] indicating a need for early intervention. The prevalence of sarcopenia among the general older population in Asia has been illustrated by Asian Working Group of Sarcopenia (AWGS) 2014, ranging between 4.1% and 11.5%.[5] With the increasing prevalence, sarcopenia has been associated with poor quality of life,[6] a higher incidence of falls, higher hospitalization rates and increased healthcare cost.[7]

Sarcopenia's etiology and pathophysiology are multifactorial, including malnutrition, lack of exercise, immune imbalance, neuromuscular junction degeneration, and oxidative stress.[8] Nutrition and exercise are critical methods to prevent and treat sarcopenia as there is no specific pharmaceutical therapy approved for it, beneficial pharmaceutical treatment includes vitamin D, growth hormone, and oestrogen-progesterone/testosterone. Although pharmaceutical treatments including vitamin D, growth hormone, and oestrogen-progesterone/testosterone is beneficial, the effectiveness of such therapies is only visible in those who are older and insufficient in vitamin D/sexual hormone.[9]

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Sufficient energy intake, high-quality protein,[10] specific amino acids, vitamin D,[11] and omega-3 fatty acid[12] have been suggested suppressing the progressive reduction in SMM and muscle strength among older adults. Additionally, exercise involving aerobics exercise and resistance exercise significantly improves SMM and muscle strength in sarcopenia patients.[13] According to Chinese expert consensus on nutrition and exercise intervention for sarcopenia syndrome in 2021, For non-sarcopenia patients aged 60 years and older, a daily protein intake of 1.0-1.2 g/kg/d is recommended for prevention; for patients with diagnosis of sarcopenia, a daily protein intake of 1.2-1.5 g/kg/d is recommended; and for patients with sarcopenia combined with severe malnutrition, daily protein intake should be supplemented to exceed 1.5 g/kg/day. High-quality leucine-rich proteins can promote protein synthesis and reduce the occurrence of sarcopenia, and the recommended minimum intake of leucine for sarcopenic patients is 55 mg/kg/d, and vitamin D supplementation of 600-800 IU/d is recommended.[14] In addition, resistance training combined with nutritional supplementation including whey protein, branched-chain amino acids, vitamin D and HMB-fortified milk significantly improves activity function, muscle mass and strength.[15] Therefore, comprehensive intervention involving nutrition supplement and exercise is promising in prevention and treatment of sarcopenia.

The purpose of this study was to create a comprehensive exercise and nutritional intervention aimed at preventing and treating sarcopenia in a high-risk population. Additionally, we aimed to evaluate the feasibility and effectiveness of the intervention over a 30±3-day period using blood tests and body composition analysis.

METHODS

Participants

This study involved an open-label single-arm trial carried out in a single centre. The study population comprised participants from the Clinical Study of Comprehensive Intervention Techniques for Older Adults with a risk of sarcopenia at the People's Hospital of Peking University. Before and during intervention, participants underwent dietary surveys using food frequency questionnaire (FFQ) which was displayed in Supplementary Table 1 to calculate daily energy intake and make sure daily energy intake was no less than 70% of daily energy requirement (DER=weight*25 kcal/kg). For FFQ, it included frequency and intakes for each time among common food (rice, maize, other grain crops, yams, sorts of meats, eggs, aquatic products, animal viscera, light and coloured vegetables, fat and oil). The average intakes of each food category during the study period are presented in Supplementary Table 4. These averages were utilized to compute the daily energy intake, as detailed in Table 1. In addition, researchers ensured there are no changes in appetite, recipe, or dietary habits, as well as severe inadequate intake, resulted from any causes during intervention. The results of the dietary survey and the intakes of the three major nutrients are displayed in Table 1. Apart from FFQ, the study also involved body measurements, muscle strength, and somatic motor function measurements, such as time for 6m walking and time for 5 sit-ups, in addition to blood tests (n=40) and body composition analysis (n=43). These assessments were performed at baseline period and post-intervention period on 46 individuals who completed the intervention. The study aimed to evaluate variables related to sarcopenia.

The inclusion criteria of this research:

(1) Patients aged ≥60 years who have a self-reported decrease in exercise capacity; or patients who meet the criteria (GS <28 kg for male, <18 kg for female, or time for 5 sit-ups ≥12s) will be suggested suffering from possible sarcopenia (pSP); or who have a confirmed diagnosis of sarcopenia (SP) (according to the AWGS2019 criteria, i.e., ASMI <7.0 kg/m2 for male, <5.7kg/m^2 for female; GS <28 kg for male, <18 kg for female; 6m walking speed < 1.0m/s or > 6s; time for 5 sit-ups ≥12s).

(2) Patients who have no swallowing, digestion and absorption, and hepatic and renal dysfunction.

(3) Patients who have signed informed consent, adhering to a diet and exercise program and receiving regular checkups.

The exclusion criteria were as follows:

(1) Existence of impact factors for body composition analysis (such as metal or pacemaker implanted, disability to stand up).

(2) Patients who have severe cardiovascular disease.

Table 1. The study procedure

Items	Mean±SD/N (%)	*p*
Gender		0.984
Male	13 (28.3%)	
Female	33 (71.7%)	
Age/years (n=46)	79.8 ± 8.35	0.009*
Healthy	75.4 ± 9.07	
pSP	82.2 ± 6.88	
SP	85.8 ± 3.30	
Height/ m	1.61 ± 0.08	
Daily energy requirements (kcal)	1489 ± 254	
70% Daily energy requirements (kcal)	1042 ± 178	
		Percentage of energy (%)
Daily energy intake (kcal)	1278 ± 280	
Daily carbohydrate Intake (g)	160 ± 48.5	50.0±9.00
Daily protein intake (g)	57.8 ± 12.9	18.0±2.00
Daily fat intake (g)	45.3 ± 18.2	32.0±8.00

(3) Patients who are allergic to any composition of nutritional agents (mushroom protein, whey protein and soy protein).
(4) Presence of other circumstances that make intervention incomplete.

The exit criteria described as follows:
(1) Patients who have severe inadequate food intake resulted from any causes (less than 70% of need).
(2) Allergic reaction.
(3) Occurrence of gastrointestinal obstruction or an intestinal fistula.
(4) Deteriorated signs and symptoms impact on continuing research.
(5) Willingness to withdraw from the experiment.
(6) Poor adherence.
(7) Researchers-suggested participants who are not suitable for continued use of nutritional agents (JiaBeiSu Complex Protein & Fish Oil Nutritional Powder).
(8) Patients take supplements other than the experimental product.

This study was approved by the Ethics Committee of Peking University People's Hospital, and all patients signed informed consent forms. This study was performed in accordance with the modified Helsinki Declaration.

Combined exercise and nutritional intervention

Participants visited our institute weekly for 30±3 days. In each exercise program, the forms of exercise and continuous time were as follows:

(1) Aerobic training (brisk walking or bicycle for 20 min);

(2) Resistance training (tension band, weightlifting, or sit-up with lifting leg for 5 min);

(3) Balance training (Taiji, folk dancing, walk for 20 min, or one-legged stand for 5 min);

(4) Flexibility and mobility training (stretching before and after exercise for 10 min).

Only participants without swallowing, digestion and absorption, and hepatic or renal dysfunction were provided daily with a total nutritional powder, JiaBeiSu Complex Protein & Fish Oil Nutritional Powder, which contains a total energy content of 215 kcal/ 50g/ serving. This powder includes protein (15g/ serving at 29 EN%), fat (particularly omega-3 fatty acid (260mg): 6.8g/ serving/ 50g at 28 EN%), carbohydrate (22g/ serving/ 50g at 41 EN%), and dietary fibre (2.4g/ serving/ 50g at 2 EN%), calcium beta-hydroxy-beta-methylbutyrate (HMB-Ca) (1.5g), as well as a protein powder component, whey protein peptide complex powder, which provides 16.38 g of high-quality protein and abundant essential amino acids, particularly leucine (2.08g/ 20g). Additionally, participants were independently supplemented with 600 IU of vitamin D for 30 ± 3 days, and those with impairments mentioned above changed their nutritional supplements, as appropriate, according to Figure 1. Nutritional agents were scheduled to be taken twice daily and researchers provided participants with monitoring forms to record their daily consumption. The adherence to exercise and nutrition interventions was continuously monitored by researchers, and participants with less than 80% adherence were considered lost to follow-up. A single group of researchers conducted the exercise and nutrition interventions, whereas other researchers conducted data collection, measurement, and analysis.

Data measurement and collection

Each outcome variable and its measurement method were described in detail in the study protocol paper. The primary outcome measure was the body composition analysis, which involved measuring total skeletal muscle mass (TSMM), skeletal muscle mass (SMM) of the limbs, and appendicular skeletal muscle mass index (ASMI) using the InBody 770 body composition analyzer produced by Shanghai Sanwei Medical Equipment Co. Ltd. These measurements were taken at baseline (day 0) and post-intervention (day 30±3).

Measurement methods of other outcome were described as follow:

(1) Physical measurements included height, weight, waist circumference (WC), hip circumference (HC), calf circumference (CC), grip strength (GS), 6-meter walking speed, and 5-time sit-to-stand test. GS was measured (1) using a CAMRY EH101 electronic GS meter, with units

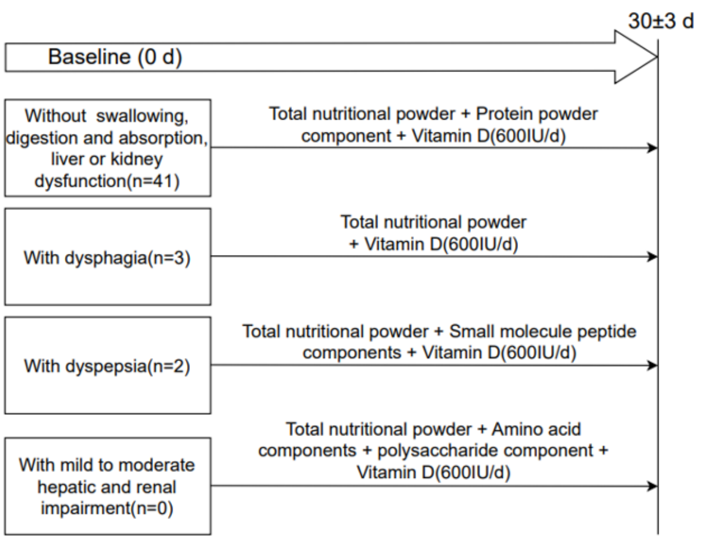

Figure 1. Description of different interventions for participants with different impairments

of kg/lb and an allowable error of ±0.5 kg/lb.

(2) Supplementary Table 2 shows each blood test factor and its normal range. The researchers collected whole blood samples and performed analysis in the laboratory.

In case of the occurrence of a serious adverse event (SAE), including but not limited to death, hospitalization or prolonged hospitalization, permanent or significant disability/functional impairment, and life-threatening situations, the State Food and Drug Administration, the sponsor, and the unit responsible for the study must be notified within 24 hours, and the study must be promptly terminated.

Data analysis and statistics

The data analysis followed the ITT principle. Data characteristics were described using means ± standard deviations/median (P25, P75) for continuous data and frequency (percentage) for categorical variables of the entire sample. Paired t-tests/paired Wilcoxon tests were used to compare normal/non-normal data between two time points, after confirming normality. Independent t-tests were performed for continuous variables between two groups, and Pearson's chi-square or Fisher's exact tests were performed for categorical variables between two groups. ANOVA was used to compare continuous variables between more than two groups. A two-sided p value < 0.05 was considered statistically significant. All statistical analyses were performed using SPSS statistical software (Version 24.0; IBM Corp., New York).

Ethical approval

All procedures performed in studies involving human participants were in consistent with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki declaration and its later amendments or comparable ethical standards. This article does not contain any studies with animals performed by any of the authors. Written informed consent was obtained from all participants in this study. The survey was registered on the Ethical Review Committee of Peking University People`s Hospital (2021PHB119-001) and this clinical experiment was registered on https://www.chictr.org.cn with a clinical trial registration number for ChiCTR2100046367.

RESULTS

Participants

Figure 2 depicts the recruitment and intervention flowchart. A total of 71 individuals were enrolled in the intervention; however, after exclusions based on the inclusion criteria and incomplete interventions, 46 individuals were included, comprising of 13 males and 33 females. Table 1 showed the mean age of the participants as 79.85 years; the maximum being 92 years and the minimum being 62 years. There was a significant trend of ages displayed as SP> pSP >Healthy. The baseline period measurement (day 0) and post-intervention measurement (day 30±3) were completed on all 46 participants. Out of these, 40 participants completed pre- and post-intervention blood sampling (6 did not have post-

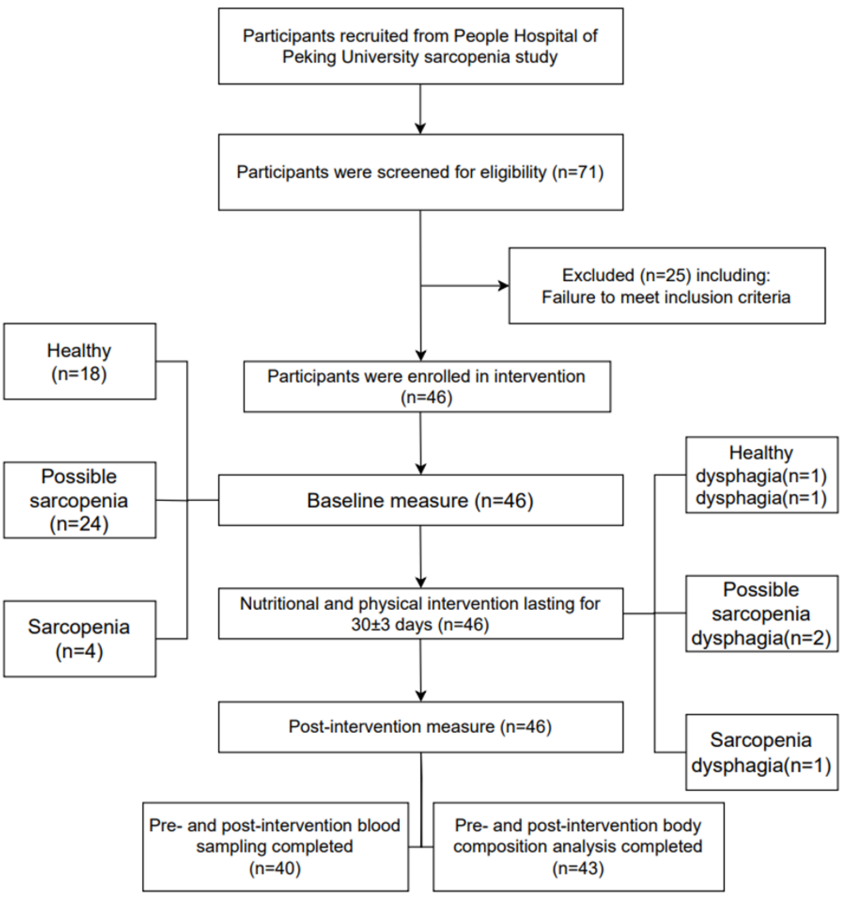

Figure 2. Flowchart of inclusion and intervention in the study

intervention blood sampling), and 43 completed pre- and post-intervention body composition analysis (3 did not have post-intervention body composition analysis). The sample comprised of 18 healthy individuals, 24 with pSP, and 4 with SP. During the study period, there were no dropouts due to poor compliance, and no adverse events related to the sports and nutritional intervention were reported, and also no significant change in appetite, recipe, or dietary habits, as well as severe inadequate intake were observed.

Evaluation for effectiveness of intervention

Table 2 illustrated variables of all participants, including body measurements, blood tests, measurement of GS, and SMM. A comparison was made between pre-intervention and post-intervention results. Improvement and degradation expressed as a percentage (calculated by mean or median). Results of body measurements suggest significant improvement in WC, CC, 6-meter walk, and 5 sit-ups. Blood test results showed that Hb, ALB, and Alanine aminotransferase (ALT) did not display any significant changes during the study period. However, Prealbumin (PA) increased significantly from 250±29.3 to 259±27.6, and 25-OH-D increased significantly from 20.1±9.97 to 21.6±9.18. Significant increases in blood Urea and Aspartate aminotransferase (AST) levels were also observed, while the decreases in blood creatinine (Cr) levels showed significance from 82.5±22.7 to 78.0±25.1. The mean/median values of the above-mentioned variables did not exhibit any abnormal increase or decrease. Muscle strength also showed an increase in GS of both hands. Additionally, there were no significant changes in left lower limb (LLL) and right lower limb (RLL) SMM, nor were there significant changes in ASMI. However, SMM of the left upper limb (LUL), right upper limb (RUL), and TSMM were significantly increased from 1.90 (1.50, 2.33) to 1.94 (1.54, 2.41), from 1.91 (1.56, 2.31) to 1.94 (1.59, 2.41), and from 20.60 (18.50, 23.80) to 21.10 (19.10, 24.10), respectively. It can be observed that the intervention favoured an increase in SMM, especially in both upper limbs.

Table 3 compares the data on healthy, pSP and SP participants between pre- and post-intervention. Significant differences in WC, 5 sit-ups, 25-OH-D, AST, Cr, Urea, GS of the left hand, and SMM of the LUL, RUL, and TSMM were observed between pre- and post-intervention in healthy participants. Some results overlapped between Table 2 and Table 3, suggesting that the intervention may also be effective for healthy individuals. Furthermore, a significant effect of improvement can be observed in WC, CC, time for 6-meter walk, time for 5 sit-ups, ALB, PA, Hb, 25-OH-D, and GS of both left and right hands in pSP participants. Although the factors of SMM were not significantly altered by the intervention, a tendency towards higher levels was observed in most SMM factors, indicating that the intervention may enhance the nutritional status of pSP and potentially improve SMM. Then significantly increased levels can be observed in ALB, PA, Hb,

Table 2. Pre- and post-intervention comparisons for all participants

Items	Pre-intervention	Post-intervention	Percentage (%)	*p*
Body measurements (n=46)				
Weight (kg)	58.3 (50.5,70.7)	59.3 (50.9, 67.5)	+1.72	0.469
BMI (kg/m^2)	23.4 (20.5, 26.1)	23.4 (20.8, 25.9)	+0.00	0.834
WC (cm)	91.9 (85.3, 99.2)	92.2 (85.3, 99.4)	+0.32	<0.001*
HC (cm)	97.0 (89.6, 104)	97.2 (89.7, 104)	+0.15	0.198
CC (cm)	33.6 (31.6, 35.5)	33.9 (89.7, 35.5)	+1.04	0.008*
Time for 6 meter walk (s)	9.01 (7.02, 12.4)	9.00 (7.18, 11.7)	-0.11	0.001*
Time for 5 sit-ups (s)	13.4 (10.1, 16.9)	11.9 (9.55, 15.5)	-11.1	<0.001*
Blood test (n=40)				
ALB (g/L)	43.8 (42.3,45.8)	44.2 (42.8,46.0)	+0.91	0.110
PA (mg/L)	250±29.3	259±27.6	+3.53	0.002*
Hb (g/L)	131±12.5	132±10.9	+1.03	0.157
25-OH-D (ng/mL)	20.1±9.98	21.6±9.18	+7.45	0.001*
ALT (U/L)	19.0 (15.0, 23.5)	18.0 (13.0, 22.0)	-5.26	0.736
AST (U/L)	21.0 (17.2, 30.0)	23.0 (21.0, 29.0)	+9.52	0.001*
Cr (umol/L)	82.5±22.7	78.0±25.1	-5.39	<0.001*
Urea (mmol/L)	7.31±2.51	8.19±3.19	+12.0	0.005*
Muscle strength (n=46)				
GS of left hand (kg)	17.5 (13.4, 23.0)	18.4 (15.0, 23.9)	+4.79	<0.001*
GS of right hand (kg)	18.4 (16.2, 24.2)	18.8 (16.5, 24.1)	+2.45	0.001*
SMM (n=43)				
LUL (kg)	1.90 (1.50, 2.33)	1.94 (1.54, 2.41)	+2.11	0.028*
RUL (kg)	1.91 (1.56, 2.31)	1.94 (1.59, 2.41)	+1.57	0.011*
LLL (kg)	6.10 (5.19, 6.94)	6.10 (5.20, 7.33)	0.00	0.722
RLL (kg)	6.06 (5.29, 7.03)	5.97 (5.32, 7.04)	-1.49	0.763
ASMI (kg/m^2)	7.25 (6.62, 8.16)	7.33 (6.63, 8.22)	+1.10	0.094
TSMM (kg)	20.6 (18.5, 23.8)	21.1 (19.1, 24.1)	+2.43	0.007*

WC: Albumin, HC: Hip circumference, CC: Calf circumference, ALB: Albumin, PA: Prealbumin, Hb: Hemoglobin, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, Cr: Creatine, GS: Grip strength, SMM: Skeletal muscle mass, LUL: Left upper limb, RUL: Right upper limb, LLL: Left lower limb, RLL: Right lower limb, ASMI: Appendicular skeletal muscle mass index, TSMM: Total skeletal muscle mass.

Table 3. Pre-/post-intervention comparisons and ANOVA analysis among three groups

Items	Healthy		pSP		SP		ANOVA
Body measurements		*p* (n=18)		*p* (n=24)		*p* (n=4)	*p* (n=46)
Weight (kg)							
	60.3±9.50	0.202	62.0±11.4	0.837	48.0±2.18	1.000	<0.001*
	60.7±9.27		61.7±11.0		48.0±2.32		0.047*
BMI (kg/m^2)							
	23.0±3.62	0.272	25.0 (21.6,27.3)	0.794	20.0±2.22	0.955	0.137
	23.1±3.49		24.4 (22.0,26.7)		20.0±2.19		0.144
WC (cm)							
	90.5 (84.8,97.6)	0.004*	94.6 (86.0,102)	0.027*	88.6±7.42	0.514	0.378
	90.6 (84.8,98.1)		94.6 (86.2,101)		88.7±7.27		0.386
HC (cm)							
	96.2±9.72	0.116	98.6±10.1	0.575	91.0±3.33	0.547	0.317
	96.3±9.61		98.7±9.91		91.0±3.53		0.302
CC (cm)							
	34.2 (33.0,35.7)	0.653	33.4 (31.5,35.5)	0.012*	32.4±0.98	0.135	0.604
	34.1 (32.9,35.6)		33.9 (32.0,36.6)		32.7±0.93		0.610
Time for 6 meter walk (s)							
	6.94 (6.10,8.54)	0.286	11.0 (8.26,15.2)	0.003*	10.8±3.33	0.062	0.013*
	7.50 (6.18,8.83)		10.6 (8.11,14.4)		9.93±2.81		0.021*
Time for 5 sit-ups (s)							
	9.74 (8.55,10.4)	0.001*	14.1 (13.8,18.1)	0.001*	19.6±4.41	0.072	0.001*
	9.10 (8.30,9.88)		13.9 (12.2,16.3)		18.8±4.43		0.001*
Blood test		*p* (n=16)		*p* (n=20)		*p* (n=4)	*p* (n=40)
ALB (g/L)							
	46.2 (44.4,47.3)	0.49	43.0±2.02	0.041*	42.3±2.44	0.045*	<0.001*
	45.6 (43.8,46.4)		43.8±2.12		43.7±3.26		0.137
PA (mg/L)							
	257±31.4	0.299	246±26.2	0.037*	249±40.4	0.023*	0.185
	261±28.4		255±26.0		276±33.6		0.389
Hb (g/L)							
	138±11.0	0.152	126 (117,133)	0.047*	132±7.62	0.009*	0.015*
	136±11.2		129 (120,135)		138±6.18		0.036*
25-OH-D (ng/mL)							
	18.6±11.4	0.030*	19.7±8.96	0.026*	28.1±7.38	0.027*	0.290
	20.2±10.1		21.0±7.92		30.4±9.22		0.125

WC: Waist circumference, HC: Hip circumference, CC: Calf circumference, ALB: Albumin, PA: Prealbumin, Hb: Hemoglobin, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, Cr: Creatine, GS: Grip strength, SMM: Skeletal muscle mass, LUL: Left upper limb, RUL: Right upper limb, LLL: Left lower limb, RLL: Right lower limb, ASMI: Appendicular skeletal muscle mass index, TSMM: Total skeletal muscle mass.

Table 3. Pre-/post-intervention comparisons and ANOVA analysis among three groups (cont.)

Items	Healthy		pSP		SP		ANOVA
Blood test		*p* (n=16)		*p* (n=20)		*p* (n=4)	*p* (n=40)
AST (U/L)							
	24.8±8.17	0.009*	21.0 (18.2,30.0)	0.064	30.0±8.91	0.351	0.655
	26.5±10.1		23.0 (21.5,26.0)		32.8±10.5		0.276
Cr/umol/L							
	80.1±14.1	<0.001*	85.0±25.8	0.022*	94.0±36.2	0.023*	0.534
	70.7±11.6		81.2±29.5		89.2±35.4		0.305
Urea (mmol/L)							
	6.61±1.72	0.023*	7.71±2.62	0.073	8.38±3.81	0.620	0.364
	7.39±2.38		8.61±3.58		9.05±3.89		0.461
Muscle strength		*p* (n=18)		*p* (n=24)		*p* (n=4)	p(n=46)
GS of left hand (kg)							
	21.2 (17.7,25.8)	0.025*	15.2 (12.5,20.5)	0.002*	14.1±2.52	0.051	0.001*
	22.9 (18.4,26.4)		16.8 (12.9,21.9)		14.7±2.83		0.002*
GS of right hand (kg)							
	22.5 (18.4,27.9)	0.349	17.1 (14.9,19.5)	0.001*	15.3±4.61	0.266	0.001*
	21.1 (19.6,26.6)		17.2 (15.6,22.4)		15.8±3.91		0.004*
SMM		*p* (n=16)		*p* (n=23)		*p* (n=4)	*p* (n=43)
LUL (kg)							
	2.01±0.39	0.037*	1.87 (1.52,2.42)	0.211	1.48 (1.29,1.72)	1.000	0.107
	2.05±0.38		1.72 (1.54,2.47)		1.44 (1.30,1.45)		<0.001*
RUL (kg)							
	2.01±0.39	0.019*	2.01±0.48	0.243	1.40±0.14	0.858	0.096
	2.07±0.37		2.07±0.61		1.41±0.06		<0.001*
LLL (kg)							
	6.43 (5.65,7.23)	0.776	6.31 (5.19,7.44)	0.527	4.55±0.52	0.253	0.050*
	6.20 (5.68,7.51)		6.18 (5.20,7.33)		4.64±0.48		0.055
RLL (kg)							
	6.40 (5.71,7.20)	0.570	6.30 (5.28,7.50)	0.267	4.65±0.69	0.302	0.067
	6.23 (5.66,7.33)		6.26 (5.30,7.22)		4.74±0.63		0.073
ASMI (kg/m^2)							
	7.45±0.81	0.238	7.47 (6.97,8.20)	0.230	5.11±0.29	0.467	0.001*
	7.57±0.91		7.40 (6.85,8.35)		5.20±0.17		0.009*
TSMM (kg)							
	22.0±2.93	0.041*	20.6 (18.8,25.8)	0.065	17.5 (15.3,20.4)	1.000	0.059
	22.4±2.76		20.9 (19.2,25.3)		16.9 (15.3,18.0)		0.001*

WC: Waist circumference, HC: Hip circumference, CC: Calf circumference, ALB: Albumin, PA: Prealbumin, Hb: Hemoglobin, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, Cr: Creatine, GS: Grip strength, SMM: Skeletal muscle mass, LUL: Left upper limb, RUL: Right upper limb, LLL: Left lower limb, RLL: Right lower limb, ASMI: Appendicular skeletal muscle mass index, TSMM: Total skeletal muscle mass.

and 25-OH-D in SP participants, suggesting that the intervention may enhance the nutritional status of sarcopenia patients. However, there was no significant effect on muscle strength or SMM.

Differences of parameters

Details on the differences in all factors among the three groups in pre-/post-intervention assessments were also shown in Table 3. Significant differences in weight, time for 6-meter walk, time for 5 sit-ups, Hb, GS of both left and right hands, and ASMI were observed in both pre- and post-intervention assessments. Additionally, differences in ALB and SMM of the LLL were only observed in pre-intervention assessments, whereas differences in SMM of the LUL, RUL, and TSMM were witnessed in post-intervention assessments, particularly.

Regarding muscle strength and SMM, GS was significantly different in both pre-intervention values and post-intervention values, and the trends were consistent with the diagnostic criteria. Although some SMM parameters did not show significant differences, they mostly followed the trend of healthy> pSP> SP. Moreover, LSD comparisons also revealed that the SMM parameters were significantly higher in both the healthy and pSP groups than in the SP group.

Effect of intervention

Table 4 compared the efficacy of intervention among the three groups. Differences are calculated as pre-intervention values minus post-intervention values, so negative values suggest improvement (except for the 6 m walk and 5 sit-ups which are positive values suggesting improvement). Significant differences in the degree of change for ALB and Hb were observed among the three groups. Differences were also observed for PA, although these were not statistically significant. (LSD comparisons suggest that the degree of improvement in PA was significantly better in SP patients than in the healthy population, *p*=0.017). The lack of improvement in ALB of the healthy population may be attributed to the absence of inadequate protein intake/synthesis in this group, whereas the degree of improvement in SP was better than that in pSP, which could suggest that SP may be suffering from inadequate protein intake/synthesis. Furthermore, although none of the three groups were anemic, the best degree of improvement in Hb was observed in the SP group, followed by pSP, with no improvement seen in the healthy population. There was no significant difference seen in the degree of improvement in muscle strength and SMM.

DISCUSSION

Sarcopenia is characterized as an age-related progressive loss of muscle mass, along with reduced muscle strength and/or physical performance. Studies have confirmed that sarcopenia is associated with adverse events such as a higher rate of falls, disability, and mortality.[16] Table 1 displayed a significant trend of age, with the SP group showing greater age than the pSP and Healthy groups. Therefore, prevention and treatment of sarcopenia is in-

Table 4. Comparison of intervention effect among three groups

Items	Healthy	pSP	SP	*p*
Body measurements (n=46)				
Weight (kg)	-0.42±1.33	0.28±3.34	0.00±0.29	0.691
BMI (kg/m²)	-0.13±0.50	0.18±1.31	0.00±0.13	0.620
WC (cm)	-0.23±0.30	-0.16±0.33	-0.10±0.27	0.649
HC (cm)	-0.13±0.34	-0.05±0.43	0.08±0.22	0.576
CC (cm)	0.05±0.42	-0.26±0.77	-0.23±0.22	0.262
Time for 6 meter walk (s)	-0.15±1.52	0.45±0.91	0.85±0.59	0.159
Time for 5 sit-ups (s)	0.54±0.82	0.58±2.37	0.74±0.54	0.980
Blood test (n=40)				
ALB (g/L)	0.81±2.20	-0.82±1.73	-1.43±0.86	0.023*
PA (mg/L)	-3.73±13.4	-9.05±18.5	-26.8±12.5	0.054
Hb (g/L)	2.07±5.28	-2.95±5.73	-5.75±1.89	0.009*
25-OH-D (ng/mL)	-1.59±2.55	-1.27±2.43	-2.30±3.41	0.753
ALT (U/L)	-2.20±8.87	2.71±11.0	0.50±6.81	0.354
AST (U/L)	-2.80±3.59	-1.00±6.20	-2.75±4.99	0.567
Cr (umol/L)	5.33±3.92	3.76±6.97	4.75±2.22	0.715
Urea (mmol/L)	-0.93±1.41	-0.90±2.17	-0.67±2.43	0.972
Muscle strength (n=46)				
GS of left hand (kg)	-0.49±1.25	-1.01±1.62	-0.61±0.39	0.492
GS of right hand (kg)	0.06±1.53	-1.01±1.75	-0.51±0.75	0.114
SMM (n=43)				
LUL (kg)	-0.05±0.08	-0.05±0.22	0.00±0.09	0.896
RUL (kg)	-0.06±0.1	-0.06±0.23	-0.01±0.09	0.903
LLL (kg)	-0.03±0.4	-0.09±0.39	-0.09±0.09	0.879
RLL (kg)	0.00±0.41	-0.09±0.37	-0.09±0.12	0.757
ASMI (kg/m²)	-0.09±0.32	-0.18±0.66	-0.08±0.16	0.865
TSMM (kg)	-0.42±0.78	-0.67±1.92	-0.10±0.52	0.771

WC: Waist circumference, HC: Hip circumference, CC: Calf circumference, ALB: Albumin, PA: Prealbumin, Hb: Hemoglobin, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, Cr: Creatine, GS: Grip strength, SMM: Skeletal muscle mass, LUL: Left upper limb, RUL: Right upper limb, LLL: Left lower limb, RLL: Right lower limb, ASMI: Appendicular skeletal muscle mass index, TSMM: Total skeletal muscle mass.

creasingly important. Our study has shown that comprehensive intervention (including exercise and nutritional supplements) can delay the progression of sarcopenia and even improve variables associated with sarcopenia. To our knowledge, our research is one of the few studies to include a healthy population in the intervention of sarcopenia and to compare its efficacy with that of the SP group. This emphasized the effectiveness of prevention in the subclinical stage of sarcopenia

In this open-label, single-arm prospective study with 46 older adults, despite the short period, the combined exercise and nutrition intervention could be practicable and helpful in improving parameters of body measurements, including WC, CC, time for 6-meter walk and time for 5 sit-ups. These results indicated improvements in somatic and motor function of participants as CC has been shown to predict muscle performance in older people (cut-off point <31 cm).[17,18] This finding contradicts a previous randomized controlled trial conducted by J. Zhao and Y. Huang, which suggested that significant improvements were not observed in parameters such as time for 5-time chair stand test, 6-meter walk speed, WC, and HC. However, the improvement in CC was consistent with the results of our study.[19] Y.L. Lin and C.H. Wang's work has suggested that incorporating CC (with a cut-off point of ≤34 cm) into the SARC-F tool improves its overall accuracy and specificity in screening for sarcopenia among patients undergoing peritoneal dialysis. This new tool, known as SARC-CalF, has been built upon the original SARC-F and shown promising results in their study.[20] The research conducted by K. Borges and R. Artacho showed that the predictive performance of CC (with a cut-off point of <31 cm) was considerable in screening for sarcopenia among older adults with hip fracture, compared to the SARC-F or SARC-CalF tools. These findings suggest that measuring CC could potentially serve as a simple and effective screening tool for sarcopenia in this population.[21] As a result, our intervention was able to prevent and alleviate sarcopenia by improving CC.

Similar improvements can be observed in the nutritional status of individuals through blood tests such as ALB, Hb, and 25-OH-D, which have been shown to provide protection against risk factors for sarcopenia.[22] The observed increases in ALB and Hb levels are consistent with the findings of S. Tan and Q. Meng's research, which demonstrated that oral nutritional supplements were beneficial for patients at nutritional risk following colorectal cancer surgery.[23] In sarcopenia, insufficient total protein resulting from an imbalance of protein synthesis and degradation, as well as inadequate protein intake, can lead to significant decreases in muscle strength.[24] Our intervention demonstrates the ability to limit this contribution. Furthermore, given the high prevalence of vitamin D deficiency among older adults, supplementing with vitamin D would be beneficial for overall muscle quality in advanced age.[25]

Furthermore, noteworthy enhancements in GS and SMM, particularly for the upper extremities, were observed in the entire cohort (Table 2). These findings are consistent with those of M. Rondanelli., who identified the efficacy of a nutritional supplement containing omega-3 fatty acids and leucine in mitigating the decline of SMM and hand GS in older adults.[26] This aligns with the conclusion of a meta-analysis, which demonstrated that omega-3 fatty acid supplementation may confer benefits on SMM and overall physical performance in older adults.[27] Additionally, these also accord with analysis of E. Cereda, which showed that a muscle-targeted oral nutritional supplementation based on whey-protein, leucine- and vitamin D-enriched formula was an effective therapy for older adults with sarcopenia.[28] Nonetheless, these results contradict previous studies that have reported no significant effects on GS or SMM following a 12-24 week intervention involving combined exercise and nutrition.[29] One possible explanation for this could be that individuals in the healthy and pre-sarcopenia population may have a greater potential to make progress in GS and SMM due to being in a subclinical stage of sarcopenia. In such cases, preventive measures focused on essential amino acids, omega-3 fatty acids, and vitamin D for sarcopenia may be more effective.

Additionally, our study also revealed differences in all parameters among the three groups, with some exhibiting trends that align with established diagnostic criteria and risk factors of sarcopenia, such as weight, time for 5 sit-ups, ALB, and GS. As illustrated in Table 3, there is a trend towards lower weight, with a pattern of healthy/ pSP> SP, which suggests that becoming thin is a risk factor of sarcopenia. Long-term weight loss and involuntary weight loss have been shown to be associated with malnutrition, which is a critical cause of sarcopenia.[30] A similar pattern was also evident in the time for 5 sit-ups and ALB levels, with healthier and pre-sarcopenic individuals exhibiting better results than those with sarcopenia. This trend aligns with the diagnostic criteria for pre-sarcopenia and sarcopenia and suggests that low protein levels may be a risk factor for sarcopenia. However, our findings are inconsistent with those of a cross-sectional study conducted by Ruby Yu,[31] their results showed that protein and vitamin D intake were not significantly associated with sarcopenia incidence or its reversibility. This discrepancy could be attributed to lower protein intake before intervention in our participants. However, another study showed a strong recommendation for consuming adequate dietary protein intake, specifically with high-quality proteins, as being essential for preserving muscle mass.[32] Finally, when comparing the efficacy of the intervention among the three groups, no significant difference was found in all patient groups (healthy and sarcopenic).

This study is subject to several limitations. Firstly, since our cohort consisted mostly of healthy older adults, it is possible that they may have displayed higher levels of compliance, both subjectively and objectively, compared to older adults with pre-sarcopenia or sarcopenia, which may have amplified the effect of the intervention. Secondly, the small sample size of the SP group (n=4) may have limited the effectiveness of the experiment in this subgroup. Thirdly, our research design only included a single-arm study, which limited our ability to make comparisons between pre- and post-intervention values in different populations. Additionally, all participants were recruited from Beijing, the northern capital of China, and therefore, the results of this study may not be generalizable to other populations in different regions with varying dietary patterns and living habits. Therefore, future studies with larger-scale, multi-stage randomized controlled trials are needed to confirm the findings of this study.

Conclusion

In summary, our study demonstrated that comprehensive exercise and nutritional intervention for older adults at risk of sarcopenia is not only feasible, but also beneficial in terms of improving somatic and motor function, protein and vitamin D levels, as well as muscle strength and SMM, especially in upper limbs. Furthermore, our intervention showed the potential to prevent sarcopenia in its subclinical stage. Nevertheless, further long-term controlled trials are necessary to validate the efficacy of this comprehensive intervention.

DATA AVAILABILITY STATEMENT

The data that support the findings of this research are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

CONFLICT OF INTEREST AND FUNDING DISCLOSURE

The authors declare no conflict of interest.

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Supplementary Tables

Supplementary Table 1. Food frequency questionnaire (FFQ) used in dietary surveys

Food type	Dietary frequency				Average serving size
	Times/day	Times/week	Times/month	Times/year	
Rice	( )	( )	( )	( )	( )*50g/time
Flours	( )	( )	( )	( )	( )*50g/time
Grains (millet, corn)	( )	( )	( )	( )	( )*50g/time
Potatoes	( )	( )	( )	( )	( )*50g/time
Red meat (pork, beef and mutton)	( )	( )	( )	( )	( )*50g/time
White meat (chick-en, duck)	( )	( )	( )	( )	( )*50g/time
Visceral	( )	( )	( )	( )	( )*50g/time
Fish and shrimp	( )	( )	( )	( )	( )*50g/time
Soybean products	( )	( )	( )	( )	( )*50g/time
Dairy products	( )	( )	( )	( )	( )*50g/time
Eggs	( )	( )	( )	( )	( )*50g/time
Dark Vegetables	( )	( )	( )	( )	( )*50g/time
Light-colored vege-tables	( )	( )	( )	( )	( )*50g/time
Pickles	( )	( )	( )	( )	( )*50g/time
Fruits	( )	( )	( )	( )	( )*50g/time
Tea/water	( )	( )	( )	( )	( )*250mL/time
Oil					Serving size for the whole family ( )kg/month ( )persons in family

Supplementary Table 2. Normal range of items in blood tests

Items	Normal range
Albumin (ALB) (serum)	40.0–55.0 g/L
Prealbumin (PA) (serum)	
Male	160–450mg/L
Female	150–380mg/L
Hemoglobin (Hb) (whole blood)	
Male	120–160 g/L
Female	110–150 g/L
25-OH-D (serum)	20.00–30.00 ng/mL
ALT (serum)	
Male	9–50 U/L
Female	7–40 U/L
AST (serum)	
Male	15–40U/L
Female	13–35 U/L
Creatine (Cr) (serum)	
Male	57–111umol/L
Female	41–81umol/L
Urea (serum)	
Male	3.60–9.50mmol/L
Female	3.10–8.80mmol/L

Supplementary Table 3. Pre- and post-intervention comparisons for female participants

Items	Pre-intervention	Post-intervention	Percentage (%)	*p*
Body measurements (n=33)				
Weight (kg)	58.8±10.2	58.6±9.76	-0.37	0.672
BMI (kg/m^2)	23.8±3.88	23.6±3.67	-0.55	0.519
WC (cm)	91.7±10.0	91.9±10.1	+0.21	0.003*
HC (cm)	97.2±10.6	97.3±10.5	+0.10	0.139
CC (cm)	34.0±3.02	34.2±3.10	+0.53	0.182
Time for 6 meter walk (s)	10.3±4.80	10.1±4.37	-2.14	0.356
Time for 5 sit-ups (s)	14.4±5.75	13.5±5.79	-6.32	<0.001*
Blood test (n=30)				
ALB (g/L)	44.4±2.16	44.7±1.93	+0.63	0.473
PA (mg/L)	250±26.2	258±26.4	+2.95	0.018*
Hb (g/L)	129±10.6	130±9.98	+0.72	0.363
25-OH-D (ng/mL)	20.4±10.8	21.8±9.91	+6.61	0.006*
ALT (U/L)	23.7±15.7	22.6±15.7	-4.64	0.615
AST (U/L)	26.0±8.50	27.3±8.55	+5.16	0.233
Cr (umol/L)	76.4±16.6	71.4±16.7	-6.63	<0.001
Urea (mmol/L)	6.96±2.10	7.53±2.44	+8.19	0.096
Muscle strength (n=33)				
GS of left hand (kg)	16.4±4.25	17.3±4.15	+5.17	<0.001*
GS of right hand (kg)	17.7±4.34	18.1±3.97	+2.21	0.079*
SMM (n=31)				
LUL (kg)	1.81±0.40	1.83±0.41	+1.10	0.388
RUL (kg)	1.82±0.34	1.86±0.40	+2.20	0.180
LLL (kg)	5.79±0.85	5.84±0.93	+0.86	0.315
RLL (kg)	5.83±0.85	5.86±0.90	+0.51	0.609
ASMI (kg/m^2)	7.11±1.08	7.21±1.30	+1.41	0.193
TSMM (kg)	20.2±2.49	20.6±3.12	+1.98	0.078*

WC: Waist circumference, HC: Hip circumference, CC: Calf circumference, ALB: Albumin, PA: Prealbumin, Hb: Hemoglobin, ALT: Alanine aminotransferase, AST: Aspartate aminotransferase, Cr: Creatine, GS: Grip strength, SMM: Skeletal muscle mass, LUL: Left upper limb, RUL: Right upper limb, LLL: Left lower limb, RLL: Right lower limb, ASMI: Appendicular skeletal muscle mass index, TSMM: Total skeletal muscle mass.

Supplementary Table 4. Average daily intakes of each item in food frequency questionnaire (FFQ)

Food type	Average intakes
Rice (g)	85.8±47.0
Flours (g)	108±55.2
Grains (millet, corn) (g)	42.9±28.5
Potatoes (g)	40.3±29.0
Red meat (pork, beef and mutton) (g)	63.0±34.4
White meat (chicken, duck) (g)	32.2±28.1
Visceral (g)	0.20±0.60
Fish and shrimp (g)	24.7±19.1
Soybean products (g)	31.6±23.2
Dairy products (g)	167±137
Eggs (g)	64.5±28.9
Dark Vegetables (g)	112±178
Light-colored vegetables (g)	150±114
Pickles (g)	20.4±23.0
Fruits (g)	108±80.3
Tea/water (mL)	1166±384
Oil (g)	9.80±5.00