Clinical Nutrition 38 (2019) 159–164

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Randomized Control Trials

A high whey protein, vitamin D and E supplement preserves muscle mass, strength, and quality of life in sarcopenic older adults: A double-blind randomized controlled trial

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ARTICLE INFO

Article history:
Received 25 July 2017
Accepted 30 December 2017

Keywords:
Older adults
Sarcopenia
Whey protein
Vitamin D
Vitamin E

SUMMARY

Objective: Sarcopenia, an age-related decline of muscle mass, strength, and physical function, was associated with falls, frailty, and poor quality of life. The aim of the current study is to examine the effect of nutritional supplement containing whey protein, vitamin D and E on measures of sarcopenia.

Methods: A total of 60 sarcopenic older adult subjects participated in the current randomized, double-blind, placebo-controlled (iso-caloric control product) trial for 6 months. Muscle mass [Relative skeletal mass index (RSMI) measured by bioimpedance analysis (BIA)], muscle strength (handgrip strength), physical function (6-m gait speed, chair stand test, and timed-up-and-go test, TUG), quality of life (measured by Short-Form 36-Item Health Survey, SF-36), and blood biochemical indexes were measured before and after the 6-month intervention.

Results: Compared to placebo group, nutritional supplementation improves RSMI (mean difference: 0.18 kg/m^2, 95%*CI*: 0.01–0.35, $P = 0.040$), handgrip strength (mean difference: 2.68 kg, 95%*CI*: 0.71–4.65, $P = 0.009$), SF-36 mental component summary (SF-36 MCS) (mean difference: 11.26, 95%*CI*: 3.86–18.65, $P = 0.004$), SF-36 physical component summary (SF-36 PCS) (mean difference: 20.21, 95%*CI*: 11.30–29.12, $P < 0.001$), serum IGF-1 (mean difference: 14.34 ng/mL, 95%*CI*: 2.06–26.73), IL-2 (mean difference: −575.32 pg/mL, 95%*CI*: −1116.94 ~ −33.70, $P = 0.038$), serum vitamin D_3 (mean difference: 11.01 ng/mL, 95%*CI*: 6.44–15,58, $P < 0.001$), and serum vitamin E (mean difference: 4.17 ng/L, 95%*CI*: 1.89–6.45, $P = 0.001$).

Conclusion: The current study demonstrated that the combined supplementation of whey protein, vitamin D and E can significantly improve RSMI, muscle strength, and anabolic markers such as IGF-I and IL-2 in older adults with sarcopenia. Further larger well-designed studies are warranted to evaluate whether long-term whey protein supplementation can blunt the declines of muscle function and mass in older adults with sarcopenia.

1. Introduction

Sarcopenia, a new geriatric syndrome, has been described as an age-related loss of muscle mass, strength, and function [1]. It may result in reduced mobility [2,3], poorer quality of life, falls and fractures [4], increased disability [5], impaired cardiopulmonary performance [6], increased hospital admission rates and long-term care placement [7], unfavorable metabolic effects [8], and reduced independence, as well as high health care expenditure [9]. The etiology and mechanisms of sarcopenia is multidimensional involving aging process, neuromuscular deterioration, altered metabolism, physical inactivity, chronic disease, marginal nutrient intakes and absorption [9]. There are growing researchers paying attention to the component of marginal nutrient intakes, which act as a modifiable risk factor of sarcopenia [10].

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https://doi.org/10.1016/j.clnu.2017.12.020
0261-5614/

Nutritional intervention for sarcopenia can have a positive effect on protein anabolism. Previous researches suggested that increased quantity and quality of dietary protein can stimulates muscle protein synthesis in the older adults [11,12]. Compared with other protein sources, whey protein, which is a high-quality protein characterized by high content of essential amino acids (eg. leucine, which is a powerful stimulator of muscle protein synthesis), faster digestion and absorption, has superior effects in promoting muscle protein synthesis for older adults [13–16]. Increased intake of vitamin D boosts muscle protein synthesis, stimulates gene expression, enhances strength and balance [17,18], and facilitates neuromuscular function [19,20]. Vitamin E intake is positively correlate with total physical performance score, knee extension strength [21], and muscle strength [22], Besides, vitamin E can promote adaptation against exercise induced-oxidative stress and reduce muscle damage [23].

Therefore, we inferred that combined supplementation of whey protein, vitamin D and E contributes to the improvements of muscle strength and function and the promoting of muscle protein synthesis. The efficacy of this concept was explored by comparing to an isocaloric placebo product for improvements of muscle mass (RSMI), muscle strength (handgrip strength), physical function (6-m gait speed, chair stand test, and timed-up-and-go test), blood biochemical indexes, and quality of life.

2. Methods

2.1. Participants

Sixty subjects aged 60–85 years old with sarcopenia participated in the study, which was approved by the ethical committee of Zhengzhou University, and written informed consent was obtained from all subject before the study. Sarcopenia was defined as: (1) the RSMI<5.7 kg/m^2 for women and <7.0 kg/m^2 for men using bioelectric impedance analysis (BIA, Inbody 720); (2) handgrip strength <18 kg for women and <26 kg for men, or 6-m usual walk speed<0.8 m/s for all included participants. Potential participants were excluded if they had mental disorders, had disabilities that significantly affect the data collected, such as severe deafness, blindness; had severe somatic diseases (diabetes with severe complications, patients with severe renal disease, or tumor); participating in other clinical trials; and other reasons that are not suitable for clinical trials. The current study was registered under the Chinese Clinical Trial Registry with the identifier: ChiCTR-IOR-16008155 (http://www.chictr.org.cn)

2.2. Design and randomization procedures

The 6-month double-blind, randomized, placebo-controlled trial was conducted to evaluate the effect of the combined supplementation containing whey protein, vitamin D and E (intervention group) or an isocaloric control product (placebo group) on muscle mass, muscle strength, physical function, nutritional status, inflammation, and quality of life in sarcopenic older adults.

Eligible subjects were randomly assigned in a 1:1 fashion to either intervention group or an iso-caloric placebo group by way of randomization envelopes with 2 different randomization codes stratified by age and sex. And the randomization sequence was computer-generated by a blinded statistician who did not involve in the conducting of the study.

Body composition, body weight, height, muscle strength, physical function, and serum markers was assessed at baseline and after 6 months of intervention.

2.3. Intervention

Both active and the iso-caloric control product were provided by the company of BY-HEALTH. They were similar in taste and appearance and were delivered as 40 g powder to be reconstituted with 100–150 mL water per serving. Subjects were asked to consume one serving before breakfast and another serving before dinner. The contents of both products are presented in Table 1. All subjects were instructed not to change their present diet and exercise habits in the study period. Besides, they were asked to record the intake of product in a diary and return empty flasks back to check compliance.

2.4. Outcome measures

The following outcomes were measured by blinded research staff during designated visits at month 0 and 6: Body composition, including appendicular skeletal muscle mass which was defined as the sum of lean mass of both arms and legs, was assessed by bioelectric impedance analysis (BIA, Inbody 720). And the relative muscle mass index (RSMI) was calculated by the following formula: RSMI (kg/m^2) = appendicular skeletal muscle mass (kg)/height^2 (m^2).

Hand grip strength was assessed with an electronic hand dynamometer (CAMRY; China) when the participant was in a sitting position. Two consecutive hand grip strength (kg) were measured to the nearest 0.1 kg for both hands, and all values were averaged.

6-m gait speed (6-m walk at the usual pace), timed-up-and-go test (participants standing up from a chair with armrests, walking 5 m, turning, walking back and sitting down) and chair stand test (time required to rise 5 consecutive times from a chair with armrests) were assessed twice, and all values were averaged [7].

Self-reported quality of life was measured by MOS item short form health survey (SF-36). Mini Nutritional Assessment Short-Form (MNA-sf) was used to measure the nutritional status. Product compliance was measured using self-recorded intake dairy. Dietary intake was assessed prospectively by 3-day diet records (1 weekend day and 2 week-days) at baseline and month 6. Besides, we also added additional protein and energy intake from supplementations (or placebo) to measure the total intakes.

Serum C- reactive protein, albumin, low density and high density lipoprotein cholesterol, total cholesterol, triglyceride, total protein, tumor necrosis factor alpha, insulin-like growth factor -1, interleukin-2, interleukin-6, 25-hydroxy-vitamin D_3, and vitamin E were measured at baseline and month 6.

2.5. Statistical analyses

Chi square test (for categorical variables) and independent-samples t test (for continuous variables) were used to compare the baseline characters. A paired samples t test was used to

Table 1
Nutrient composition of the dietary supplement.

Component	Intervention	Placebo
Energy, kcal	153	153
Protein, %	57.5	–
Carbohydrates, %	27.2	84.7
Fat, %	15.3	15.3
Protein, g	22	–
Carbohydrates, g	10.4	32.4
Fat, g	2.6	2.6
Vitamin D, IU	702	–
Vitamin E, mg	109	–

compare within-group differences. An ANCOVA with sex and age as covariates was used to compare between-group differences. All statistical analyses were carried out with SPSS version 21 for Windows software (IBM). A two-sided $P \leq 0.05$ was considered statistically significant.

3. Results

Eighty one subjects were enrolled and 60 were included in the current study; 21 were excluded because of: taking whey protein supplements (n = 5), refusal to participate (n = 9), diagnosed as tumors (n = 2), taking vitamin D or vitamin E supplements (n = 5) (Fig. 1). All subjects were recruited between April 1st 2016 and July 1st 2016. Their main characteristics are presented in Table 2. And there was no significant difference in their baseline characteristics between the two groups. Changes in energy, fat, and carbohydrates intake in intervention and placebo participants did not differ between groups either. However, because of the supplementation of whey protein, changes in protein intake in intervention group significantly increased than that in placebo group. The dietary intakes (including the supplementation and placebo) of both groups are presented in Table 3.

The 6-month nutritional intervention (between July 15,2016 and January 15, 2017) resulted in a significantly increased in body weight (Intervention change: 3.11 ± 1.67 kg, $P < 0.001$; placebo change: 4.91 ± 13.09 kg, $P = 0.049$), BMI (Intervention change: 1.23 ± 0.64 kg/m^2, $P < 0.001$; placebo change: 1.71 ± 4.02 kg/m^2, $P = 0.027$), and fat mass (Intervention change: 2.34 ± 2.23 kg, $P < 0.001$; placebo change: 2.74 ± 1.86 kg, $P < 0.001$) in the intervention and placebo groups, but without significant differences between the groups ($P > 0.05$) (Table 4).

No differences were observed in appendicular muscle mass, 6-m gait speed, time to complete 5 stands, time to stand up, serum C-reactive protein, albumin, high density lipoprotein cholesterol, total cholesterol, triglyceride, total protein, interleukin-6, tumor necrosis factor alpha, 25-hydroxy-vitamin D_3, and vitamin E between the intervention and placebo groups over time ($P > 0.05$) (Table 4).

When appendicular muscle mass was adjusted for height, the RSMI still showed no significant change in the intervention or placebo groups. However, compared with the placebo group, 6-month of nutritional intervention significantly increased RSMI (mean difference: 0.18 kg/m^2, 95%*CI*: 0.01–0.35, $P = 0.040$). Besides, compared with placebo group, 6-month of nutritional intervention also significantly improve handgrip strength (mean difference: 2.68 kg, 95%*CI*: 0.71–4.65, $P = 0.009$), SF-36 MCS(mean difference: 11.26, 95%*CI*: 3.86–18.65, $P = 0.004$), SF-36 PCS (mean difference: 20.21, 95%*CI*: 11.30–29.12, $P < 0.001$), serum IGF-1 (mean difference: 14.34 ng/mL, 95%*CI*: 2.06–26.73), IL-2 (mean difference: −575.32 pg/mL, 95%*CI*: −1116.94 ~ −33.70, $P = 0.038$), serum vitamin D_3 (mean difference: 11.01 ng/mL, 95%*CI*: 6.44–15,58, $P < 0.001$), and serum vitamin E (mean difference: 4.17 ng/L, 95%*CI*: 1.89–6.45, $P = 0.001$).

3.1. Adverse events

No serious side effects related to whey protein intake were reported. The most commonly reported adverse events was difficult defecation, which occurred in 3 subjects in the intervention group. Pain when urinating was reported by 1 subject in the placebo group and another one in the intervention group.

4. Discussion

This study found that the combined supplementation of whey protein, vitamin D and E can significantly improve muscle mass (RSMI) and strength (handgrip strength) in older adults with

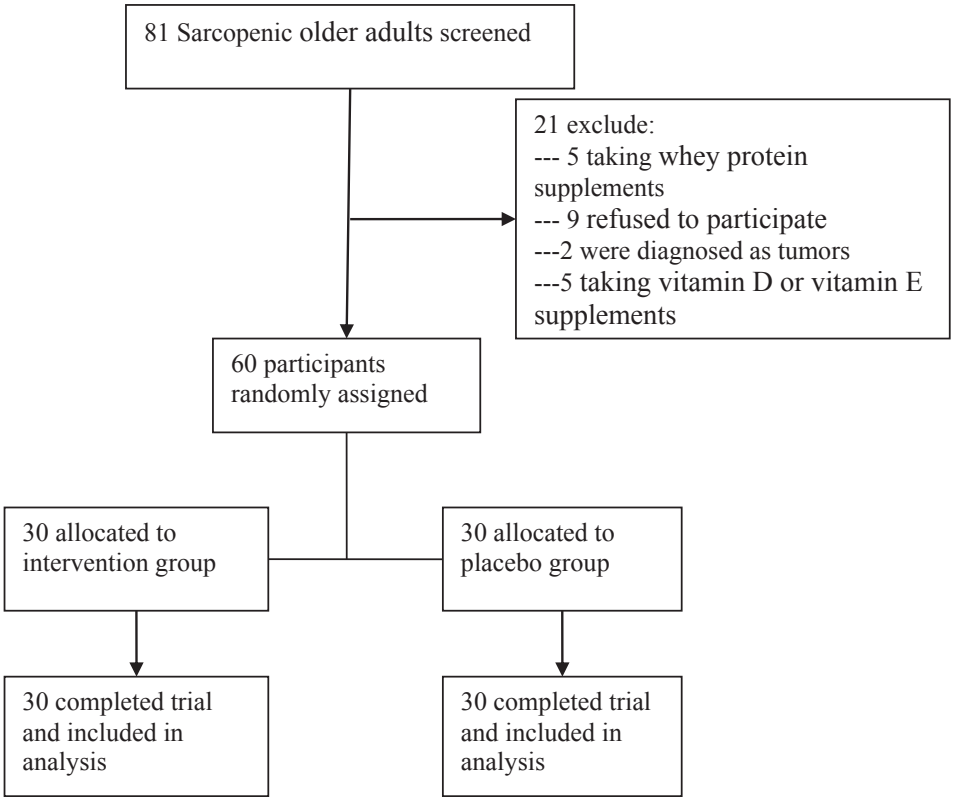

Fig. 1. Flow of participants participant screening, randomization and follow-up.

Table 2
Baseline demographic and clinical characteristics.

Characteristic	Intervention (n = 30)	Placebo (n = 30)	*P* value
Male sex, n (%)	13 (43.3)	14 (46.75)	0.795
Age, y	73.23 ± 6.52	74.83 ± 5.94	0.325
Height, cm	158.33 ± 7.29	160.33 ± 9.46	0.363
Body weight, kg	53.65 ± 8.33	50.45 ± 11.62	0.231
BMI, kg/m^2	21.34 ± 2.47	19.74 ± 4.05	0.071
Nutritional status			0.543
Well nourished, n (%)	21 (70.0)	17 (56.7)	
At risk of malnutrition, n (%)	8 (26.7)	12 (40.4)	
Malnutrition, n (%)	1 (3.3)	1 (3.3)	
Fat mass, kg	14.97 ± 5.40	13.45 ± 4.55	0.245
Appendicular muscle mass, kg	14.74 ± 2.79	15.21 ± 3.61	0.566
RSMI, kg/m^2	5.83 ± 0.69	5.83 ± 0.79	0.997
Handgrip strength, kg	19.50 ± 4.54	20.25 ± 5.89	0.586
6-m gait speed, m/s	7.48 ± 1.55	7.36 ± 1.58	0.747
Time to complete 5 stands, s	14.36 ± 3.99	14.53 ± 4.09	0.879
Time to stand up, s	13.03 ± 2.93	13.82 ± 2.58	0.648
SF-36 MCS score	66.40 ± 17.09	65.67 ± 20.04	0.881
SF-36 PCS score	80.65 ± 19.36	76.96 ± 17.85	0.446
C- reactive protein, mg/	3.01 ± 4.21	2.82 ± 6.98	0.903
Albumin, g/L	42.93 ± 6.89	42.33 ± 2.74	0.413
Total protein, g/L	70.37 ± 4.25	70.44 ± 3.76	0.946
LDL-C, mmol/L	2.92 ± 0.84	3.29 ± 0.96	0.114
HDL-C, mmol/L	1.28 ± 0.33	1.41 ± 0.39	0.204
Total cholesterol, mmol/L	4.41 ± 0.92	4.78 ± 1.10	0.156
Triglyceride, mmol/L	1.41 ± 0.79	1.16 ± 0.44	0.135
IGF-1, ng/mL	115.56 ± 43.87	96.18 ± 34.11	0.061
TNF-α, pg/mL	5.99 ± 1.52	6.36 ± 2.24	0.465
IL-2, pg/mL	833.16 ± 571.44	1277.03 ± 1576.51	0.153
IL-6, pg/mL	3.67 ± 1.69	6.40 ± 12.51	0.240
Vitamin D_3, ng/mL	21.29 ± 8.29	20.85 ± 7.72	0.830
Vitamin E, ng/L	11.35 ± 5.29	10.57 ± 3.42	0.499

LDL-C, Low density lipoprotein cholesterol; HDL-C, High density lipoprotein cholesterol; IGF-1, Insulin like growth factor-1; TNF-α, tumor necrosis factor-α; MCS, mental component summary; PCS, physical component summary; SF-36, Short-Form 36-Item Health Survey.

sarcopenia. Besides, the combined nutritional supplementation also improved some factors that contribute to sarcopenia. What's more, the nutritional supplementation can also enhance the anabolic growth hormone (GH) IGF-I hormone axis with significantly increased IGF-1 concentrations, attenuate the inflammatory (e.g. significant decrease of IL-2 concentration), and improve the quality of life assessed in sarcopenic older adult.

Previous study has demonstrated that high habitually protein intake was associated with the retention of appendicular muscle mass [11]. Before intervention, the habitually intakes of protein in both groups were below the recommended dietary allowance of 0.8 g kg BW^{-1} d^{-1} for adults [24]. During the 6-month nutritional supplementation, the whey protein group achieved a higher intake of total protein 1.02 ± 0.27 g kg BW^{-1} d^{-1}. Several recent studies have showed a muscle anabolic resistance in older adults, which implies that compared with young adults, the older adults have blunted postprandial response to protein (or amino acid) anabolic stimuli [13,25]. Whereas, providing older adults with enough amount of protein could stimulate the synthesis of muscle protein [26]. Breen et al. found that the intake of 20 g protein daily could leads to a significant muscle protein synthesis increase in older adults [25]. It has been demonstrated that the quality of protein had an important effect on muscle protein accretion. In addition, 20 g whey protein has been shown more effective in stimulating muscle protein synthesis than soy protein, casein, or hydrolysate casein in older men (14, 15). The muscle protein-stimulating effect of whey protein might be attributed to its delivering essential amino acids for protein synthesis, fast digestion [27] and the high content of leucine, which is a potent amino acid for muscle protein synthesis stimulating [28].

Additionally, it has been recognized that low vitamin D status could cause impaired muscle mass and function [19], moreover, vitamin D has also been found to facilitate muscle anabolic [29,30]. Vitamin D Supplementation might therefore be correlated with muscle mass preservation. The meta-analyses conducted by Muir et al. found that supplementing with vitamin D have beneficial effects on balance and strength [18]. The potential mechanism of vitamin D's positive on muscle protein is not yet fully elucidated. Recent studies have suggested that vitamin D signaling via vitamin D receptor (VDR) plays an important role in the regulation of myoblast proliferation and differentiation [30–33]. During the 6-month supplementation of 702 IU/d vitamin D in the intervention group, the concentration of serum 25-hydroxyvitamin D_3 in intervention group increased by 11.01 (95%*CI*: 6.44, 15.58) compared with placebo group. Blunting the vitamin D decrease, in combination with whey protein and vitamin E, could have contributed to the favorable effect we observed on patients with sarcopenia.

The intervention supplement in the current study also contained 109 mg vitamin E, which is a lipid soluble vitamin that exerts antioxidant properties, by scavenging reactive oxygen species (ROS) and boosting cellular antioxidative capacity to reduce oxidative damage [34]. The beneficial effects of vitamin E on muscle strength for the older adults has been demonstrated in human studies [22]. Several previous studies have suggested the beneficial effect of vitamin E in reversing muscle damage. A combination supplementation of 117.5 mg vitamin E and 500 mg vitamin C daily for 12 week blunted some muscular adaptations to strength training in older males [35]. In another study, 12-week 1000 IU/d vitamin E supplementation reduced lipid peroxidation in both quiescent condition and after exercise in the older adults, indicating that vitamin E reduces muscle damage and promotes adaptation against exercise induced-oxidative stress [23], supplementation with vitamin E might therefore facilitate muscle mass accretion, which is coordinate with current study.

Furthermore, the combined nutritional supplementation exerted a positive effect on IGF-I concentrations, which might make contribution to promoting muscle function by stimulating production of muscle contractile proteins and satellite cells [36]. The potential mechanism of the effect of IGF-1 on muscle retention

Table 3
Dietary intake in intervention and placebo group.

Dietary nutrition intake	Intervention group			Placebo group		
	Baseline	Change	*P*	Baseline	Change	*P*
Energy, kcal/d	1238.26 ± 395.89	−15.18 ± 590.63	0.893	1211.42 ± 540.67	18.82 ± 610.42	0.881
Proteins, g/d	41.34 ± 22.71	15.75 ± 27.67	0.006	38.78 ± 20.69	−7.78 ± 24.69	0.137
Protein, g · kg BW^{-1} · d^{-1}	0.76 ± 0.32	0.25 ± 0.08	0.003	0.71 ± 0.36	−0.14 ± 0.43	0.107
Fat, g/d	39.19 ± 17.89	−3.91 ± 24.85	0.404	41.84 ± 29.83	−0.65 ± 36.34	0.928
Carbohydrates, g/d	176.90 ± 62.46	8.78 ± 98.06	0.639	162.14 ± 67.41	29.64 ± 76.15	0.069

Values are means±SDs and significance level of estimate of change at month 6 by using a paired t test.

Table 4
Outcome measures for intervention and placebo groups with intervention effect.

	Intervention group[a]			Placebo group[a]			Intervention effect[b]	
	Baseline[c]	Change[c]	*P*	Baseline[c]	Change[c]	*P*	Mean difference (95% *CI*)[d]	*P*
Body weight, kg	53.65 ± 8.33	3.11 ± 1.67	<0.001	50.45 ± 11.62	4.91 ± 13.09	0.049	−1.64 (-6.50, 3.23)	0.503
BMI, kg/m[b]	21.34 ± 2.47	1.23 ± 0.64	<0.001	19.74 ± 4.05	1.71 ± 4.02	0.027	−0.43 (-1.94, 1.07)	0.566
Fat mass, kg	14.97 ± 5.40	2.34 ± 2.23	<0.001	13.45 ± 4.55	2.74 ± 1.86	<0.001	−0.43 (-1.50, 0.64)	0.428
Appendicular muscle mass, kg	14.74 ± 2.79	0.23 ± 1.07	0.246	15.21 ± 3.61	−0.25 ± 0.71	0.059	0.48 (0.00, 0.96)	0.050
RSMI, kg/m[b]	5.83 ± 0.69	0.08 ± 0.38	0.237	5.83 ± 0.79	−0.09 ± 0.26	0.051	0.18 (0.01, 0.35)	0.040
Handgrip strength, kg	19.50 ± 4.54	1.91 ± 4.24	0.020	20.25 ± 5.89	−0.88 ± 3.20	0.161	2.68 (0.71, 4.65)	0.009
6-m gait speed, m/s	7.48 ± 1.55	0.14 ± 0.15	<0.001	7.36 ± 1.58	0.08 ± 0.24	0.074	0.05 (-0.06, 0.15)	0.402
Time to complete 5 stands, s	14.36 ± 3.99	−2.79 ± 3.73	0.005	14.53 ± 4.09	−1.21 ± 6.28	0.507	−1.84 (-4.53, 0.85)	0.176
Time to stand up, s	13.03 ± 2.93	−1.36 ± 2.43	<0.001	13.82 ± 2.58	−0.68 ± 3.29	0.267	−0.67 (-2.20, −0.86)	0.383
SF-36 MCS score	80.65 ± 19.36	12.98 ± 13.70	<0.001	76.96 ± 17.85	2.20 ± 14.23	0.443	11.26 (3.86, 18.65)	0.004
SF-36 PCS score	66.40 ± 17.09	17.39 ± 16.92	<0.001	65.67 ± 20.04	−2.28 ± 17.01	0.468	20.21 (11.30, 29.12)	<0.001
C- reactive protein, mg/L	3.01 ± 4.21	−0.43 ± 3.93	0.555	2.82 ± 6.98	3.75 ± 24.18	0.402	−3.63 (-12.62, 5.36)	0.422
Albumin, g/L	42.93 ± 6.89	−1.42 ± 2.11	0.001	42.33 ± 2.74	−1.67 ± 3.05	0.006	0.28 (-1.11, 1.66)	0.693
Total protein, g/L	70.37 ± 4.25	0.19 ± 3.14	0.747	70.44 ± 3.76	−0.45 ± 4.18	0.563	0.77 (-1.13, 2.68)	0.418
LDL-C, mmol/L	2.92 ± 0.84	0.11 ± 0.53	0.269	3.29 ± 0.96	−0.21 ± 0.62	0.077	0.34 (0.06, 0.63)	0.019
HDL-C, mmol/L	1.28 ± 0.33	0.05 ± 0.17	0.129	1.41 ± 0.39	−0.02 ± 0.18	0.469	0.07 (-0.03, 0.16)	0.159
Total cholesterol, mmol/L	4.41 ± 0.92	0.28 ± 0.66	0.027	4.78 ± 1.10	0 ± 0.72	0.978	0.30 (-0.04, 0.64)	0.082
Triglyceride, mmol/L	1.41 ± 0.79	−0.23 ± 0.52	0.021	1.16 ± 0.44	0.01 ± 0.41	0.847	−0.23 (-0.46, 0.01)	0.059
IGF-1, ng/mL	115.56 ± 43.87	14.18 ± 28.33	0.010	96.18 ± 34.11	−1.13 ± 17.96	0.733	14.34 (2.06, 26.73)	0.023
TNF-α, pg/mL	5.99 ± 1.52	−0.14 ± 1.30	0.562	6.36 ± 2.24	−0.35 ± 1.37	0.175	0.22 (-0.47, 0.91)	0.522
IL-2, pg/mL	833.16 ± 571.44	40.17 ± 487.18	0.655	1277.03 ± 1576.51	633.3 ± 1362.27	0.016	−575.32 (-1116.94, −33.70)	0.038
IL-6, pg/mL	3.67 ± 1.69	−0.45 ± 1.33	0.076	6.40 ± 12.51	−1.23 ± 17.20	0.698	0.78 (-5.67, 7.27)	0.806
Vitamin D_3, ng/mL	21.29 ± 8.29	4.04 ± 9.77	0.031	20.85 ± 7.72	−6.83 ± 8.44	<0.001	11.01 (6.44, 15.58)	<0.001
Vitamin E, ng/L	11.35 ± 5.29	5.82 ± 5.28	<0.001	10.57 ± 3.42	1.67 ± 3.00	0.005	4.17 (1.89, 6.45)	0.001

LDL-C, Low density lipoprotein cholesterol; HDL-C, High density lipoprotein cholesterol; IGF-1, Insulin like growth factor-1; TNF-α, tumor necrosis factor-α; MCS, mental component summary; PCS, physical component summary; SF-36, Short-Form 36-Item Health Survey.
[a] Significance level of estimate of change at month 6 by using a paired t test.
[b] Effect of intervention treatment (compared with the placebo) was evaluated by using an ANCOVA with sex and age as covariates.
[c] Values are means±SDs.
[d] Values are means with upper and lower 95% CI bounds in parentheses.

possibly through the use of protein kinase B-mechanistic target of rapamycin-p70 ribosomal protein S6 kinase signaling [36,37].

This study is not without limitations. Firstly, we did not control our subjects' dietary intakes, although they were asked not to change their dietary habits during the entire study period. The second limitation is related to the effective and true control of the correct use of supplements, despite instructions on the route of administration, record product intake in a diary, and return of empty flasks indicating total consumption of the product.

In conclusion, this study demonstrated that the combined supplementation of whey protein, vitamin D and E supplementation can significantly improve RSMI, muscle strength, and anabolic markers such as IGF-I and IL-2 in older adults with sarcopenia. Further larger well designed studies are warranted to evaluate whether long-term whey protein supplementation can blunt the declines of muscle function and mass in older adults with sarcopenia.

Statement of authorship

Study design: Lu Quanjun.
Data collection: Bo Yacong, Liu Changfeng, Ji Zhe, Yang Ruihong, An Qianqian, Zhang Xueyuan, You Jie, Duan Dandan, Sun Yafei, Zhu Yiwei, Cui Han.
Manuscript writing: Bo Yacong, Liu Changfeng.
Statistical analysis: Bo Yacong.
Manuscript revision: Lu Quanjun

Conflict of interest

The authors declare no conflict of interest.

Funding

The present study was funded by Danone Institute Diet, Nutrition Research and Communication Grant (No. DIC2015-02).

Acknowledgement

We gratefully acknowledge the kindly provide of intervention and placebo product by company of BY-HEALTH.

Appendix A. Supplementary data

Supplementary data related to this article can be found at https://doi.org/10.1016/j.clnu.2017.12.020.

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