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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[1,2]

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ABSTRACT

Background: Interventions to attenuate the adverse effects of age-related loss of skeletal muscle and function include increased physical activity and nutritional supplementation.

Objective: This study tested the hypothesis that nutritional supplementation with whey protein (22 g), essential amino acids (10.9 g, including 4 g leucine), and vitamin D [2.5 μg (100 IU)] concurrent with regular, controlled physical activity would increase fat-free mass, strength, physical function, and quality of life, and reduce the risk of malnutrition in sarcopenic elderly persons.

Design: A total of 130 sarcopenic elderly people (53 men and 77 women; mean age: 80.3 y) participated in a 12-wk randomized, double-blind, placebo-controlled supplementation trial. All participants concurrently took part in a controlled physical activity program. We examined body composition with dual-energy X-ray absorptiometry, muscle strength with a handgrip dynamometer, and blood biochemical indexes of nutritional and health status, and evaluated global nutritional status, physical function, and quality of life before and after the 12 wk of intervention.

Results: Compared with physical activity and placebo, supplementation plus physical activity increased fat-free mass (1.7-kg gain, $P < 0.001$), relative skeletal muscle mass ($P = 0.009$), android distribution of fat ($P = 0.021$), handgrip strength ($P = 0.001$), standardized summary scores for physical components ($P = 0.030$), activities of daily living ($P = 0.001$), mini nutritional assessment ($P = 0.003$), and insulin-like growth factor I ($P = 0.002$), and lowered C-reactive protein ($P = 0.038$).

Conclusion: Supplementation with whey protein, essential amino acids, and vitamin D, in conjunction with age-appropriate exercise, not only boosts fat-free mass and strength but also enhances other aspects that contribute to well-being in sarcopenic elderly. This trial was registered at clinicaltrials.gov as NCT02402608. *Am J Clin Nutr* doi: 10.3945/ajcn.115.113357.

Keywords: amino acids, dietary supplement, elderly, insulin-like growth factor I, fat-free mass, relative skeletal muscle mass, sarcopenia, vitamin D, whey protein

INTRODUCTION

Human aging involves changes in body structure and function. Older adults experience a progressive, generalized loss of skeletal muscle and a decrease in physical function, with an inherent risk of disability, poor quality of life, and death (1). Rosenberg (2) proposed the term "sarcopenia" to describe this age-related depletion of skeletal muscle mass and loss of strength.

The etiology and mechanisms of sarcopenia are complex and multifactorial (3). Primary sarcopenia is a consequence of the aging process (e.g., reduced neurological function, altered muscle fiber type distribution, and increased protein turnover). Secondary sarcopenia, however, is linked with inactivity (e.g., bed rest or a sedentary lifestyle) or chronic disease (e.g., organ failure, malignancy, inflammation, or endocrine disease). There is growing evidence that nutritional factors (e.g., an inadequate intake of protein, energy, and certain micronutrients; malabsorption; and drug-induced anorexia) contribute to secondary sarcopenia (4).

Interventions for sarcopenia include nutrition, because nutrition can have a positive impact on protein anabolism. Increasing the quantity (e.g., in excess of the recommended dietary intake) (5) and quality (e.g., essential amino acids, specifically leucine) of dietary protein stimulates muscle protein synthesis in the elderly (6). Increased intake of vitamin D stimulates gene expression and boosts muscle protein synthesis, facilitates neuromuscular function (7, 8), and enhances strength and balance (9, 10). It also reduces the inflammation that is associated with decreased muscle strength in the elderly (11). Because older adults risk having a low intake of high-quality protein, as well as

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vitamin D insufficiency, strategies to boost intake are recommended to attenuate the loss of muscle and its adverse effects in these people (12, 13).

Physical activity can also slow the loss of muscle mass and improve function. Strength training with resistance exercise universally strengthens muscles (14). The addition of dynamic exercise to resistance training also contributes substantial benefits to physical function (15).

The complex etiology of sarcopenia calls for integrated interventions in a practical approach (16). We therefore designed a randomized, double-blind, placebo-controlled supplementation trial that combined whey protein, essential amino acids, and vitamin D with regular physical activity for all participants. We set out to test whether, compared with placebo, supplementation would increase fat-free mass (FFM)[10] (primary outcome) while improving strength, nutritional status, inflammation, and measures of quality of life and physical function (secondary outcomes).

METHODS

Participants

The study was approved by the institutional review board at the University of Pavia and was conducted after approval from the ethics committee of the Department of Internal Medicine and Medical Therapy at the University of Pavia. Participants gave their written consent to this study (NCT02402608).

We evaluated elderly men and women admitted to the geriatric physical medicine and rehabilitation division at the Santa Margherita Hospital, Azienda Human Service of Pavia in Pavia, Italy. Before participation, each person had complete medical screening, including vital signs, blood tests, urine tests, and a 12-lead electrocardiogram. Anyone with evidence of heart disease, kidney or liver disease, or any other disease that might influence the results of the study was excluded. Data were gathered from the end of January 2013 to the end of June 2014. Eligible persons were aged ≥65 y and had an appendicular skeletal FFM divided by height squared that was 2 SD below the mean for young adults (17), hence, relative muscle mass <7.26 kg/m^2 for men and <5.5 kg/m^2 for women. They had to have no acute illness or severe liver, heart, or kidney dysfunction, and body weight had to have been stable for 6 mo. Anyone with altered glycometabolic control, thyroid disorders, other endocrinopathies, or cancers, and any patients treated with steroids and heparin or who had total walking incapacity were excluded. The participants selected had to have similar physical ability, assessed with the activities of daily living (ADL) score, and normal cognitive function or only mild cognitive disturbance as defined by a Mini-Mental State Examination >20 (18).

Body composition, nutritional status, and food intake

Body composition (FFM, fat mass, and gynoid and android fat distribution) was measured by dual-energy X-ray absorptiometry (DXA) with the use of a Lunar Prodigy DXA (GE Medical Systems). The in vivo CVs were 0.89% and 0.48% for whole-body fat (fat mass) and FFM, respectively. The relative skeletal muscle mass (RSMM) was taken as the sum of the fat-free soft tissue mass of arms and legs (19).

Body weight was measured to the nearest 0.1 kg on a precision scale with the participants wearing light clothing, without shoes, with the use of a standardized technique (20). Waist measurements were taken at the midpoint between the lowest rib and the top of the hip bone (iliac crest), with the use of a standardized technique (20).

We assessed the hydration of these elderly adults with bioelectrical impedance, because changes in fluid status affect the soft tissue composition estimated by DXA (21, 22). Whole-body resistance and reactance were measured with the patient lying supine on a nonconductive surface with the use of a phase-sensitive, single-frequency impedance plethysmograph [400-μA, 50-kHz alternating current (BIA-101; RJL/Akern Systems)]. Adhesive surface electrodes were placed on the right hand and foot, and measurements were taken according to the guidelines of the NIH Technology Assessment Conference Statement (23).

Resistance and reactance were standardized by the standing height of each individual (i.e., resistance divided by height and reactance divided by height), expressed in ohms/m and plotted on the resistance-reactance graph (24). Bioelectrical impedance vector analysis (BIVA) expresses tissue hydration status and body cell mass solely while considering the impedance vector relative to a population of healthy individuals (24); this was a valid method for detecting changes in hydration (classified as under-, normal or overhydration) and body fluid volume changes (25). Sex-specific bivariate reference intervals were available for the Italian healthy population as 50%, 75%, and 95% tolerance ellipses on the resistance-reactance graph.

A mini nutritional assessment (MNA) was done for all participants (26). The MNA uses simple measurements and a brief questionnaire involving an anthropometric assessment (weight, height, and weight loss), a general assessment (lifestyle, medication, and mobility), and a dietary assessment (number of meals, food and fluid intake, self-assessment of autonomy of eating, and self-perception of health and nutrition). Patients ate 3 meals daily.

Dietary schedule

Food intake was based on a balanced diet (with standard caloric and macro- and micronutrient content) provided by the hospital kitchen, which consisted of a repeating 4-wk rotating menu, so the diet remained similar throughout the study.

A trained dietitian used a calibrated dietetic spring scale to weigh all foods served and returned for 3 consecutive days at the beginning and end of the study. Nurses who served any foods to the participants between meals recorded the amount eaten, in household measurements. A computer program (DR3 v3.1.0; Sintesi Informatica) was used to calculate the energy and the macronutrient content of food consumed.

Handgrip

The JAMAR Hand Dynamometer (Jamar 5030J1; Sammons Preston Rolyan; accuracy 0.6 N) was used to assess muscle function with the use of a standardized procedure (27).

[10] Abbreviations used: ADL, activities of daily living; CRP, C-reactive protein; DXA, dual-energy X-ray absorptiometry; FFM, fat-free mass; GH, growth hormone; IGF-I, insulin-like growth factor I; MNA, mini nutritional assessment; PCS, physical component summary; PRT, progressive resistance training; RSMM, relative skeletal muscle mass; SF-36, Short-Form 36-Item Health Survey.

Biochemical analyses

Fasting venous blood samples were drawn with the participants seated. Blood was collected and handled under strictly standardized conditions. Blood samples were collected into vacuum tubes without anticoagulant, left for 1 h at room temperature, and then centrifuged for 15 min at 1500 × *g* at 20°C. The serum was then transferred into plastic tubes, rapidly frozen, and stored at –80°C until analysis (<1 mo later). Whole blood (with the use of EDTA as an anticoagulant) was used for hematologic variables. Clinical chemistry markers were detected on the Roche Cobas Integra 400 plus analyzer (Roche Diagnostics), with specially designed commercial kits provided by the manufacturer. Cobas Integra 400 is a random, continuous-access, sample-selective analyzer that provides absorbance photometry for measuring enzymes and substrates, turbidimetry for specific proteins, and ion-selective electrode potentiometry for serum electrolytes. Serum total and LDL cholesterol, triglycerides, HDL cholesterol, total proteins, total bilirubin, iron, glucose, uric acid, creatinine, and liver enzymes such as alanine transaminase, aspartate transaminase, and γ-glutamyltransferase were measured by enzymatic-colorimetric methods. C-reactive protein (CRP) was determined by a nephelometric high-sensitivity CRP (Dade Behring).

Erythrocyte, white blood cell, and platelet counts; hemoglobin concentrations; mean cell volumes; and mean cell hemoglobin concentrations were measured with the use of a Coulter automated cell counter (MAX-M; Beckman Coulter). Serum albumin was analyzed with the use of a nephelometric method (Behring Nephelometric Analyzer II, Behring Diagnostics), with a 2% CV.

Serum samples for insulin-like growth factor I (IGF-I) assay were collected at admission and after the 12 wk of treatment; samples were pretreated to release IGF-I from binding proteins and then assayed with quality controls. Serum IGF-I concentrations were measured with the use of a solid-phase quantitative ELISA kit (R&D Systems); the minimum detectable dose of IGF-I was 0.026 ng/mL. Intra- and interassay CVs were 4% and 7.9%, respectively.

Health-related quality of life

The participants were tested with the Short-Form 36-Item Health Survey (SF-36) (28) to assess their quality of life. This questionnaire is a valid generic measure that is used for rating health-related quality of life in several research fields because of its validity, high internal consistency, and high test–retest reliability. The SF-36 scales were summarized in 2 dimensions. The first 5 make up the "physical health" dimension, and the last 5 the "mental health" dimension. The vitality and general health scales are parts of both dimensions. Thus, each dimension includes 3 specific and 2 overlapping scales. The standardized summary scores for physical and mental components were calculated and used separately as outcome measures. The quality-of-life SF-36 was administered before and after the treatment period.

Function

The participants' ability to care for themselves was assessed with the Katz Index of Independence in Activities of Daily Living (29).

Intervention

Physical activity

A comprehensive physical fitness and muscle mass enhancement training program of moderate intensity was provided for all participants (30). The exercise intervention was supervised by trained personnel and consisted of 20-min exercise sessions daily, 5 times/wk for 12 wk. Each session consisted of a 5-min warm-up, 5 min of strengthening exercises, 5 min of balance and gait training, and 5 min of cool-down. The strengthening exercises were done in a progressive sequence from seated to standing positions (31). For each type of exercise, participants were instructed to repeat the movements ≤8 times. Intensity was maintained at ~12–14 on the Borg Rate of Perceived Exertion scale (32). The principal investigator, with the exercise instructor and assistant trainers, assessed each individual's ability to increase intensity.

For the chair exercise, repetitions of toe raises, heel raises, knee lifts, knee extensions, and others were done while seated on a chair. Hip flexions, lateral leg raises, and repetitions of other exercises were done while standing upright behind the chair, holding the back of the chair for stability.

For the ankle-weight exercise, to strengthen the legs, a fixed weight was placed on the ankle while participants did strengthening exercises. Weights of 0.50, 0.75, 1.00, and 1.50 kg were prepared and used in accordance with each participant's strength as the resistance progressively increased. The exercises with the use of these ankle weights included seated knee flexion and extension and standing knee flexion and extension.

In the exercises with the use of a resistance band, resistance bands were used to strengthen the upper and lower body. Lower-body exercises included leg extension and hip flexion. Upper-body exercises included double-arm pull-downs and biceps curls.

For balance and gait training, exercises included standing on one leg, multidirectional weight shifts, a tandem stand, and a tandem walk. Participants practiced proper gait mechanics that focused on maintaining stability during walking and increasing stride length, toe elevation of the forward limb, heel elevation of the rear limb, frequency of stepping, and heel–floor angle. Exercises included raising the toes (dorsiflexion) during the forward swing of the leg, kicking off the floor with the ball of the foot, walking with directional changes, and gait pattern variations. In spring and summer, these exercises were done outdoors.

Dietary supplement

The intervention treatment included an oral essential amino acid, whey protein, and vitamin D mixture (**Tables 1** and **2**). The control group was given a placebo that consisted of an isocaloric amount of maltodextrin with the same flavor and appearance as the intervention product. Subjects were randomly assigned to receive one portion containing the dietary supplement or placebo (32 g) orally 1 time/d at 1200 with meals for 12 wk.

Participants were assigned to a treatment according to a coded (AB) block randomization table prepared by an independent statistician. Investigators were blinded to the randomization table, the code assignments, and the procedure. As people were enrolled they were assigned a progressive number. A research dietitian, blinded to the randomization schedule provided by the statistician, distributed the supplements to participants each day. Supplements were in powder form and packed in numerically

TABLE 1
Nutritional content of the dietary supplement[1]

	Energy value		% RDA[2] per 32-g dose
	Per 100 g	Per 32 g	
Kilojoules	1466	469	
Kilocalories	351	112	
Nutrients, g			
Whey protein	68.9	22	
Lipids	1.1	0.4	
SFAs	0.2	0.0	
Total carbohydrates	14.8	4.7	
Simple carbohydrates	2.6	0.8	
Complex carbohydrates	3.9	1.2	
Polyols	8.3	2.7	
Fiber	6.9	2.2	
Fructo-oligosaccharides	3.2	1.0	
Minerals, mg			
Calcium	25.8	8.3	1
Phosphorus	76.3	24.4	3
Sodium	917.4	293.6	
Magnesium	140.7	45.0	12
Iron	0.8	0.3	2
Vitamins, μg (IU)			
D_3 (cholecalciferol)	7.8 (312)	2.5 (100)	50

[1]SAI Nutrition.
[2]RDA, Recommended Dietary Allowance.

coded packages. Instructions on each bottle included the amount of water to be added; the water and contents of the bottle were then mixed and stirred for 60 s until the product was ready for consumption. Participants were instructed to eat their normal amounts of food in addition to the dietary supplement. All supplements were provided by SDM, Savigliano, Italy.

Safety was judged based on the absence of serious side effects with the supplement, i.e., gastrointestinal symptoms such as nausea and diarrhea. Every day, after administering the supplement, the dietitian asked about any unwanted side effects. No participant refused to take the supplement, and no side effects were reported.

Statistical analysis

Study design

This was a randomized, controlled, double-blind, parallel-group superiority clinical trial to compare the efficacy of whey protein, essential amino acid, and vitamin D supplementation or placebo in improving FFM or strength in sarcopenic elderly people in a hospital and rehabilitation division. The primary endpoint of the study was comparison of the increase in FFM after supplementation in the 2 groups. Secondary endpoints included the comparison of anthropometric characteristics (RSMM, fat mass, gynoid and android fat, and waist circumference), muscle strength (handgrip), quality of life [SF-36 mental component summary and physical component summary (PCS)], hormonal status (IGF-I), inflammation (CRP), and ADL. Finally, we assessed the correlations between several biomarkers, independently of treatment assignment, as exploratory endpoints.

To understand the links between variables better, we investigated the correlations between measures of primary endpoint muscle mass (FFM and RSMM), strength (handgrip), inflammation (CRP), and quality of life (SF-36 mental component summary and PCS), then between muscle mass (FFM and RSMM), strength (handgrip), function (ADL), and hormonal status (IGF-I). Finally, we analyzed the correlations between primary endpoint muscular mass (FFM and RSMM) and strength (handgrip).

Sample size

We based our sample size calculation on the findings of Borsheim et al. (6), and considered an expected mean ± SD increase of 1.1 kg ± 1.2 kg in the supplement group, and 0.5 kg ± 1.2 kg in the placebo group, with a power of 80% and an α level (2-tailed) of 5%, as well as 10% attrition. This gave a sample size of 140 patients (70/group).

Random assignment and masking

A random-blocks 1:1 random assignment list was prepared by a statistician. The treatment assignment sequence was masked to the investigator with the use of opaque envelopes. Blindness was maintained by providing the patients with undistinguishable products.

Statistical analysis

We used Stata 13. A 2-sided P value < 0.05 was considered to be significant. Continuous variables were summarized by treatment groups as means ± SDs or medians (25th, 75th percentiles), and categorical variables were summarized as counts and percentages. To compare changes in FFM between groups, a general linear regression model was fitted with FFM as the dependent variable, and treatment, time, and the interaction of treatment with time were used as independent variables. Huber–White robust SEs were computed with subject as the cluster variable to account for within-patient correlations of measurements and clustering for patients. Similarly, changes within groups also were analyzed by fitting to each group the same model with time as the sole independent variable. Mean

TABLE 2
Aminogram of the essential amino acids (g) in the dietary supplement

Amino acid	Value
Essential amino acids/g of product ready for use (32 g)	
L-Ile	1.0
L-Leu	4.0
L-Lys	1.5
L-Thr	1.1
L-Trp	0.3
L-Val	1.0
Nonessential amino acids/g of product ready for use (32 g)	
DL-Met	0.6
L-Cys	0.4
L-Phe	0.5
L-Tyr	0.5
Asp	1.8
Ser	0.8
Glu	5.2
Pro	1.0
Gly	0.3
Ala	0.8
Arg	0.8

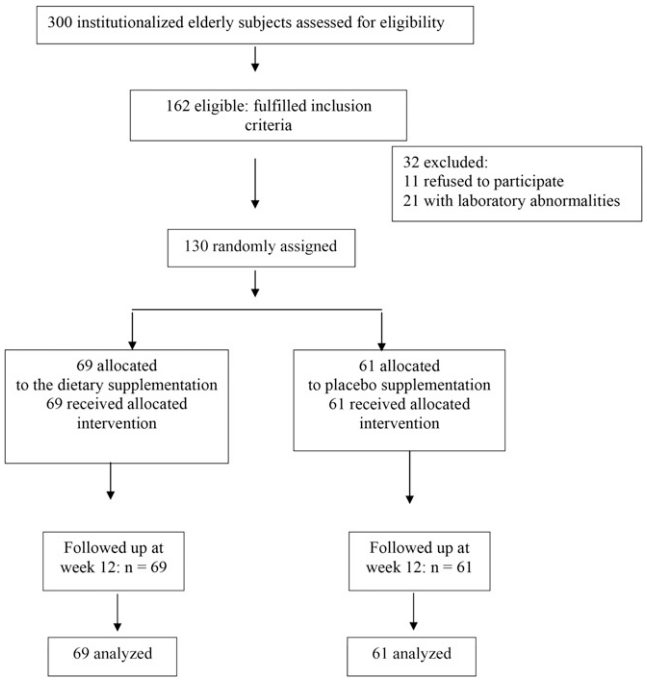

FIGURE 1 Flow diagram of trial supplementation with dietary supplement compared with placebo in sarcopenic elderly people. The diagram indicates the number of individuals analyzed for the main outcome (effect on fat-free mass).

differences (changes) and 95% CIs were computed. Secondary endpoints were analyzed similarly.

For tertiary endpoints, partial correlations (and 95% CIs) were computed by fitting linear regression models while adjusting for treatment and time. Robust SEs were computed because of heteroscedasticity. All model assumptions were verified graphically (residuals compared with fitted plot) and were satisfied. Finally, a Fisher's exact test was used for comparison of the proportion of patients who became nonsarcopenic.

We used a Hotelling's T^2 (33) test on the calculated BIVA distribution with the use of pretreatment and compared the vectors to the population vector of healthy Italian adults to detect possible imbalances in the hydration levels of the study participants. Impedance individual vectors were plotted with BIVA software (25).

RESULTS

In all, 162 people were enrolled and 130 were randomly assigned; 32 were excluded because of refusal to participate (n = 11) or laboratory abnormalities (n = 21) (**Figure 1**). Participants were recruited from January 2013 through June 2014. Their main details are shown in **Table 3**. The placebo and supplement groups were similar on all counts.

The FFM response was significantly different between the 2 groups, with a mean difference of 1.7 kg (95% CI: 0.9, 2.5; P-interaction for treatment × time < 0.001). Fat-free mass increased significantly in the supplemented group (1.4 kg, $P <$ 0.001), with no noteworthy change in the placebo group (−0.3 kg) (**Table 4**). Among secondary endpoints, responses were also significantly different in the 2 groups for treatment × time, as follows: RSMM, P-interaction = 0.009; android distribution of fat, P-interaction = 0.021; handgrip, P-interaction = 0.001; PCS, P-interaction = 0.030; ADL, P-interaction = 0.001; MNA, P-interaction = 0.003; IGF-I, P-interaction = 0.002; and CRP, P-interaction = 0.038. Specifically, RSMM, handgrip, PCS, ADL, MNA, and IGF-I increased significantly in the treatment group, whereas this was not the case in the placebo group. PCS slightly increased in the treatment group (P = 0.06), but not in the placebo group. Conversely, percentage android distribution of fat significantly decreased in the treatment group, but not in the placebo group, whereas CRP slightly decreased in the treatment group and slightly increased in the placebo group, although not significantly in either case (Table 4). The substantial improvements in RSMM and muscle strength in the supplementation group improved the classification of 68% of the elderly people in that group from sarcopenic to nonsarcopenic, but none of the participants in the placebo group showed improvement (Fisher's exact test $P <$ 0.001).

No treatment effects were seen for waist circumference, fat mass, or gynoid percentage distribution of fat (Table 4), although gynoid fat percentage decreased significantly in both the supplemented and the placebo groups. Routine blood test results for clinical chemistry did not differ with respect to changes over time (data not shown). Changes in nutritional intake in treated and control patients did not differ between groups either. The dietary intake of both groups (not including the supplementation or placebo) is shown in **Table 5**.

TABLE 3
Baseline characteristics of the study participants[1]

Characteristic	Dietary supplement group (n = 69)	Placebo group (n = 61)
Age, y	80.77 ± 6.29	80.21 ± 8.54
Male	29 (42)	24 (39)
Smoker	3 (4)	5 (8)
Level of schooling, y	7 (3–11)	5 (2–9)
Fat-free mass, g	39,895 ± 8132	38,714 ± 8371
Fat mass, g	17,813 ± 6780	19,210 ± 9182
Gynoid, %	35.79 ± 9.67	37.67 ± 10.60
Android, %	34.21 ± 10.76	34.26 ± 12.85
RSMM, kg/m^2	6.60 ± 1.19	6.36 ± 1.32
MNA score	17.84 ± 3.07	17.84 ± 3.57
Weight, kg	59.47 ± 11.16	59.39 ± 13.51
BMI, kg/m^2	23.85 ± 3.63	23.93 ± 4.60
Wrist circumference, cm	16.29 ± 1.75	16.08 ± 1.42
Arm circumference, cm	25.22 ± 3.36	25.02 ± 3.80
Calf circumference, cm	30.43 ± 3.13	29.95 ± 4.55
Waist circumference, cm	88.95 ± 9.74	89.01 ± 10.15
MMSE score	21.78 ± 3.70	20.5 ± 4.93
ADL score	3.97 ± 1.19	4.03 ± 1.08
SF-36 MCS score	46.65 ± 10.7	44.0 ± 9.7
SF-36 PCS score	34.1 ± 10.2	37.1 ± 11.0
Proteins, g/dL	6.67 ± 0.55	6.56 ± 0.61
Albumin, g/dL	3.76 ± 0.54	3.6 ± 0.55
Creatinine, mg/dL	0.95 ± 0.7	0.91 ± 0.38
CRP, mg/L	0.30 (0.14–1.23)	0.33 (0.16–1.03)
IGF-I, ng/mL	80.6 ± 33.8	82.7 ± 38.8
Handgrip, kg	16.63 ± 4.99	19.62 ± 6.01

[1]Data are means ± SDs, medians (25–75th percentiles), or n (%). ADL, activities of daily living; CRP, C-reactive protein; IGF-I, insulin-like growth factor I; MCS, mental component summary; MMSE, Mini-Mental State Examination; MNA, mini nutritional assessment; PCS, physical component summary; RSMM, relative skeletal muscle mass; SF-36, Short-Form 36-Item Health Survey.

TABLE 4
Effects of supplementation compared with placebo in exercise-trained elderly people[1]

	Dietary supplement group (n = 69)		Placebo group (n = 61)		Treatment effect	
Variable	Mean change (95% CI)	Intragroup P^2	Mean change (95% CI)	Intragroup P^2	Mean difference (95% CI)	P^3
Fat-free mass,[4] g	1382 (847, 1918)	<0.001	−312 (−930, 307)	0.316	1695 (892, 2498)	<0.001
Fat mass, g	−345 (−747, 57.18)	0.092	−484 (−1049, 81.74)	0.092	−114 (−786, 559)	0.689
Gynoid, %	−1.39 (−2.22, −0.56)	0.001	−0.92 (−1.83, −0.02)	0.046	0.54 (−0.67, 1.75)	0.451
Android, %	−2.03 (−2.99, −1.06)	0.001	−0.26 (−1.43, 0.92)	0.66	1.80 (0.30, 3.29)	0.021
RSMM, kg/m^2	0.21 (0.07, 0.35)	0.004	−0.06 (−0.21, 0.90)	0.42	0.27 (0.07, 0.47)	0.009
MNA score	1.76 (1.23, 2.28)	<0.001	0.24 (−0.63, 1.11)	0.585	1.52 (0.51, 2.52)	0.003
Weight, kg	1.12 (0.37, 1.87)	0.004	−0.89 (−1.62, −0.15)	0.019	2.00 (0.97, 3.04)	<0.001
BMI, kg/m^2	0.42 (0.11, 0.72)	0.008	−0.42 (−0.70, −0.14)	0.004	0.84 (0.43, −1.25)	<0.001
Waist circumference, cm	4.93 (−0.86, 10.72)	0.094	2.27 (−1.72, 6.25)	0.259	2.67 (−4.29, 9.62)	0.449
ADL score	0.54 (0.39, 0.68)	<0.001	−0.61 (−0.79, −0.42)	<0.001	1.14 (0.91, 1.38)	<0.001
SF-36 MCS score	4.50 (2.68, 6.32)	<0.001	2.48 (0.21, 4.75)	0.033	2.02 (−0.85, 4.89)	0.166
SF-36 PCS score	1.32 (−0.05, 2.68)	0.059	−0.77 (−2.10, 0.58)	0.249	2.09 (0.21, 3.97)	0.030
CRP, mg/dL	−0.19 (−0.57, 0.19)	0.329	0.44 (−0.02, 0.90)	0.061	0.63 (0.04, 1.22)	0.038
IGF-I, ng/mL	20.7 (11.0, 30.4)	<0.001	1.8 (−4.2, 7.8)	0.541	19.7 (7.1, 32.3)	0.002
Handgrip, kg	3.20 (2.23, 4.18)	<0.001	−0.47 (−1.07, 0.12)	0.117	3.68 (2.55, 4.81)	<0.001

[1]ADL, activities of daily living; CRP, C-reactive protein; IGF-I, insulin-like growth factor I; MCS, mental component summary; MNA, mini nutritional assessment; PCS, physical component summary; RSMM, relative skeletal muscle mass; SF-36, Short-Form 36-Item Health Survey.
[2]From within-treatment regression model—test for main effect of time within each treatment arm.
[3]From between-treatment regression model—test for treatment × time interaction.
[4]Primary endpoint. Regression model for repeated measures.

The reference bivariate tolerance ellipses (50%, 75%, and 95% of the distribution of the values for the general Italian population) for elderly men were used for the qualitative and semiquantitative assessment of body composition and hydration in each individual. The 95% CI ellipses for the mean vectors of the treated group before and after supplementation were drawn to compare these groups. SDs between the mean vectors were found with Hotelling's T^2 test for vector analysis, which is a multivariate extension of Student's test for unpaired data in comparisons of mean vectors from 2 groups. Two mean vectors have a significantly different ($P < 0.05$) position in the resistance-reactance graph if their 95% CI ellipses are separated according to Hotelling's T^2 test. Overlapping ellipses are not significantly different ($P > 0.05$). Ellipses were plotted with BIVA software (25). Mean group vectors before and after treatments were within the reference sex–specific 50% tolerance ellipse. Thus, hydration was classified as normal. Hotelling's T^2 test indicated a nonsignificant (T^2= 1.7756, $P > 0.05$) difference between the study group vector and the reference population 50% CI ellipse, confirming that the hydration of FFM was normal, so the DXA results were not influenced by altered soft-tissue hydration (21, 22).

Among the exploratory endpoints, a weak but statistically significant correlation was found between handgrip strength and RSMM (R = 23%; 95% CI: 11%, 34%; P = 0.0014) (**Figure 2**), between handgrip strength and FFM (R = 27%; 95% CI: 16%, 38%; P = 0.003) (**Figure 3**), and between IGF-I and FFM (R = 15%; 95% CI: 2%, 29%; P = 0.041) (**Figure 4**), while adjusting for treatment and time.

The dietary supplement was well tolerated, and there were no serious adverse events. Compliance was 100%.

DISCUSSION

This study found a significant beneficial effect of supplementation with whey protein, essential amino acids, and vitamin D compared with placebo in elderly sarcopenic adults

TABLE 5
Nutritional intake of supplemented and control participants at beginning of study and after 12 wk[1]

	Placebo group (n = 69)		Dietary supplement group (n = 61)			
Daily nutritional intake	Baseline	12 wk	Baseline	12 wk	P^2	P^3
Energy, kcal/d	1622 ± 350	1615 ± 273	1600 ± 215	1573 ± 339	NS	NS
Proteins, g/d	59 ± 8	60 ± 9	54 ± 12	55 ± 11	NS	NS
Fat, g/d	54 ± 12	55 ± 11	52 ± 9	53 ± 14	NS	NS
Carbohydrates, g/d	225 ± 4	220 ± 5	214 ± 3	212 ± 4	NS	NS
Vitamin D, IU/d	299 ± 79	298 ± 87	301 ± 92	296 ± 89	NS	NS

[1]Data are means ± SDs with the use of the Carnovale E Marletta L food composition tables, Italian National Institute of Nutrition, Rome, 1997.
[2]From within-treatment regression model—test for main effect of time within each treatment arm.
[3]From between-treatment regression model—test for treatment × time interaction.

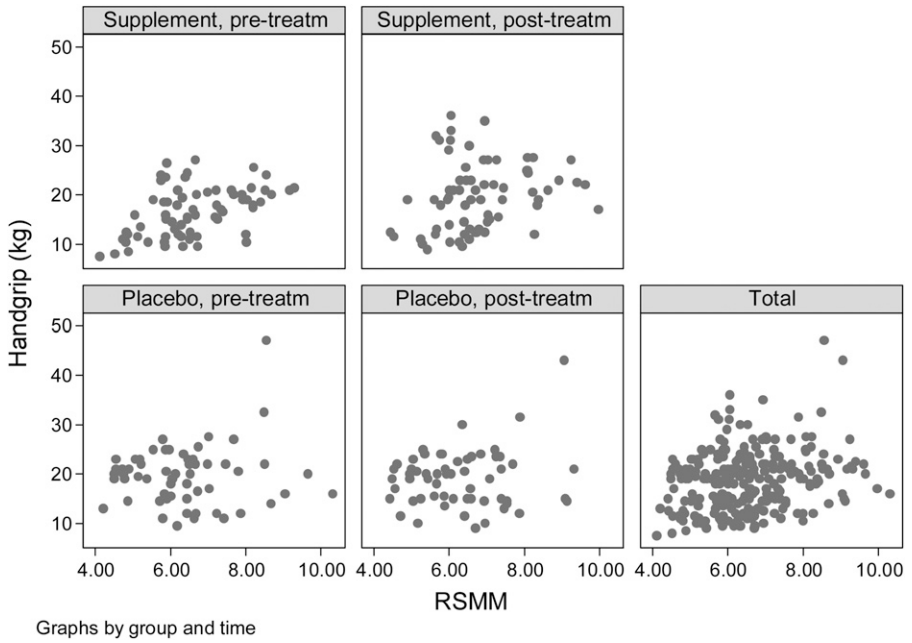

FIGURE 2 Correlation between handgrip strength and RSMM (R_{total} = 0.23; 95% CI: 0.11, 0.34; P = 0.0014) for each treatment arm, before and after treatment, and overall. After dropping 2 outliers for handgrip (>40), the results were the same. R is computed as the partial correlation, adjusted for treatment and time, from the repeated-measures model; thus, each individual is represented twice in the "total" graph. RSMM, relative skeletal muscle mass; treatm, treatment.

participating in controlled resistance training, with a gain of 1.7 kg in FFM. Supplementation significantly improved RSMM and muscle strength and, in fact, 68% of sarcopenic people became nonsarcopenic (19).

Key strengths of the study included the comprehensive assessment of the main causal factors of sarcopenia in a well-defined elderly population. Nutritional supplementation, independently of increased physical activity, also improved some factors that contribute to sarcopenia. Supplementation attenuated the inflammatory state, as seen by the significant drops in CRP concentrations, and enhanced the anabolic growth hormone (GH) IGF-I hormone axis, with significant increases in IGF-I concentrations and a reduction in the indexes of malnutrition assessed with the MNA. Dietary supplementation also boosted various

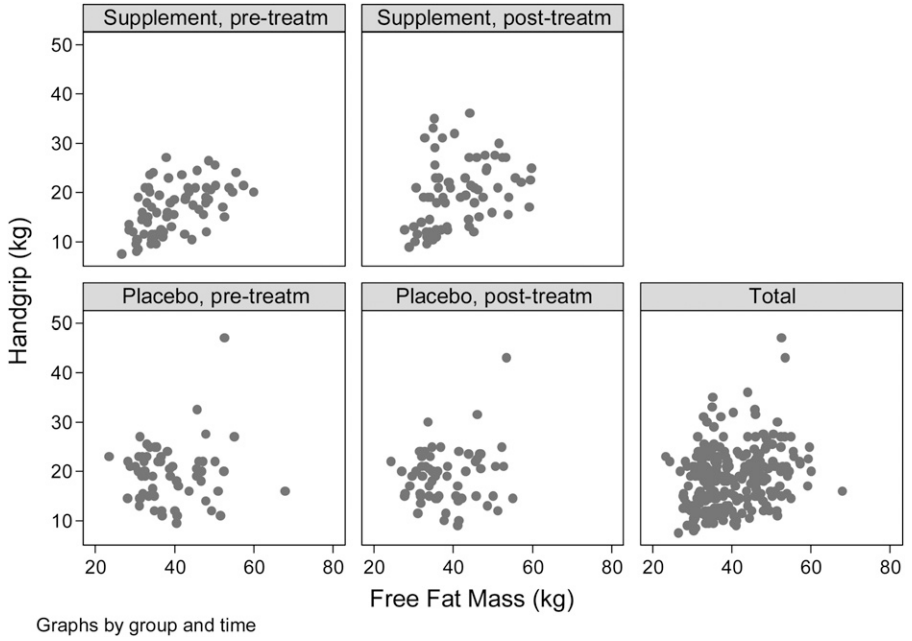

FIGURE 3 Correlation between handgrip strength and fat-free mass (R_{total} = 0.27; 95% CI: 0.16, 0.38; P = 0.003) for each treatment arm, before and after treatment, and overall. After dropping 2 outliers for handgrip (>40), the results were the same. R is computed as the partial correlation, adjusted for treatment and time, from the repeated-measures model; thus, each individual is represented twice in the "total" graph. treatm, treatment.

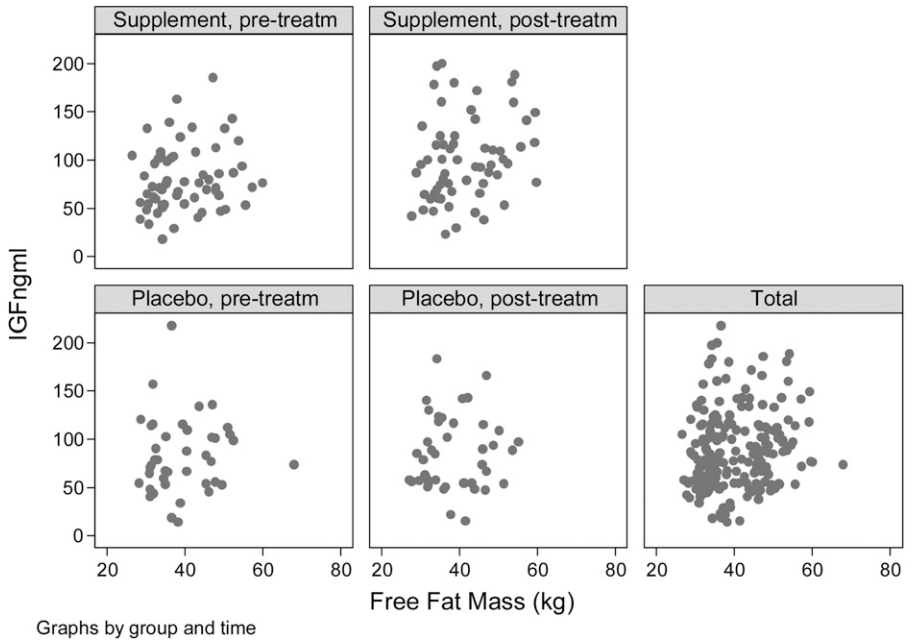

FIGURE 4 Correlation between IGF-I and fat-free mass (R_{total} =0.15; 95% CI: 0.2, 0.29; P = 0.041) for each treatment arm, before and after treatment, and overall. After dropping 2 outliers for handgrip (>40), the results were the same. R is computed as the partial correlation, adjusted for treatment and time, from the repeated-measures model; thus, each individual is represented twice in the "total" graph. IGF-I, insulin-like growth factor I; treatm, treatment.

measures of function in the participants, assessed by the ADL, and their quality of life, particularly its physical component, assessed by the physical component of the SF-36.

Both groups followed a physical activity plan, but beneficial results were seen only in the supplemented group, indicating that physical activity is important, but not sufficient to achieve a significant result. However, the physical activity was gentle and nonintensive and this might have explained the lack of increase in FFM in the placebo group. These results are in agreement with the study by Raguso et al. (34), which showed that leisure-time physical activity does not seem to prevent loss of muscle mass.

We decided on 12 wk of resistance training on the basis of previous research showing that substantial muscle hypertrophy can occur within this period (34, 35) and that substantial diet-related differences in muscle hypertrophy responses may also arise within this time frame (34, 36).

Both aerobic and resistance-type exercise training have been shown to improve the rate of decline in muscle mass and strength with age (37). Progressive resistance training (PRT) is the most commonly used resistance therapy in older people. A Cochrane review of 121 randomized controlled trials of PRT in older people showed that doing PRT 2–3 times/wk improved physical function, gait speed, timed get-up-and-go, climbing stairs, and balance, and, more importantly, had a significant effect on muscle strength, especially in the high-intensity training groups (38). Even in very old nursing home residents, PRT achieved substantial improvements in muscle fiber cross-sectional area (3–9%), muscle strength (100%), and physical performance such as gait speed and stair climbing (38, 39).

The majority of studies show that resistance exercise training must be carried out at high intensity to achieve substantial improvements in muscle strength, but, for sarcopenic people, high-intensity resistance training may not be realistic or practical. The elderly people recruited for this study would not have been able to maintain high-intensity resistance exercise training. We therefore selected an age-appropriate, tolerable, and sustainable exercise program that consisted of resistance and aerobic exercises. Compliance was 100%. These age-related resistance and aerobic exercises in older people increased their strength by 38% and resulted in significant reductions in CRP (40).

Reducing inflammation is one mechanism that can improve age-related muscle loss through either direct catabolic effects or indirect mechanisms (through higher GH and IGF-I concentrations, less anorexia, etc.) (41)

Protein supplementation combined with physical exercise, particularly resistance training, has yielded mixed results on body composition, muscle hypertrophy, strength, and physical function in the elderly (6, 39, 42–47), even though most studies have focused on healthy older adults, with limited data from trials on sarcopenic individuals, in which nutritional but not specifically protein and amino acid supplementation was a focus (39). Moreover, in these studies, the doses of protein supplemented varied between 7.4 and 15 g/serving. These differences make it difficult to compare studies.

Another key finding is the positive effect of nutritional supplementation on IGF-I concentrations. IGF-1 contributes to improving muscle function by increasing production of muscle satellite cells and stimulating production of muscle contractile proteins. The age- related decline in GH concentrations, combined with lower IGF-I concentrations contributes to the development of sarcopenia (48). IGF-I is perhaps the most important mediator of muscle growth and repair (49), possibly through the use of protein kinase B–mechanistic target of rapamycin–p70 ribosomal protein S6 kinase signaling.

The composition and timing of the supplement are novel aspects of this study. To counteract protein catabolism, the elderly must increase the anabolic stimulus, consuming 30 g protein/meal (50–52). The combination of whey protein and essential

amino acids providing Leu is important (53, 54). Whey protein increases postprandial plasma amino acid availability, further stimulating muscle protein synthesis (53, 55–58), more than casein (59, 60). Whey contains a high concentration of Leu, which stimulates skeletal muscle protein synthesis (61). Thus, whey protein and essential amino acids that contain Leu are recommended interventions for sarcopenia (62, 63), and the effect of nutritional therapies for sarcopenia can be enhanced by a comprehensive approach (64).

A low-caloric dietary supplement can be taken with a meal without problems of gastric emptying; the supplement can even be taken by overweight or obese sarcopenic individuals (sarcopenic obesity), because being sarcopenic does not necessarily mean being underweight (65).

The present findings are in agreement with previous reports of improvements in muscle strength with exercise and whey protein supplementation (20 g/d) in frail elderly people after ~12 wk of supplementation, as in this study (6) and in an acute situation (66, 67). Verreijen et al. (68) reported that a high whey protein, Leu, and vitamin D–enriched supplement similar to the supplement used in this study—except for the vitamin D content—compared with isocaloric control preserved appendicular muscle mass in obese older adults during a hypocaloric diet and resistance exercise program (3 times/wk) for 13 wk and might therefore reduce the risk of sarcopenia. However, Verreijen et al. (68) found no beneficial effect of supplementation on muscle strength or function—which contrasts with the findings of the present study. Whether differences in the physical activity interventions explain the lack of functional improvement remains to be established.

The effect of nutritional therapies for sarcopenia can be enhanced by a comprehensive approach (64). This is why the supplement we used also contained vitamin D. A recent meta-analysis (69) of the results of 30 randomized, double-blind, placebo-controlled clinical trials indicated that daily supplementation with ≥400 IU of vitamin D_3 increased skeletal muscle strength on average by 17%. The intervention supplement we used contained 2.5 μg (100 IU) vitamin D. We selected this dose of vitamin D for the supplement, whereas the participants receiving the control diet consumed 120 g halibut 2 times/wk, canned tuna 2 times/wk, cod 2 times/wk, and 2 eggs/wk, which provided them with a mean of 300 IU of vitamin D daily; they also participated in balance and gait training outdoors. Our result is in line with the meta-analysis by Beaudart et al. (69), because the increase in muscle strength averaged 21%. This gain in muscle strength has been suggested as the mechanism behind a reduction in falls of 23–53%, in addition to a reduction in fractures in older nursing or residential home residents given vitamin D (70–72).

A potential implication of our findings is that patients with sarcopenia should consider the use of specific supplements combined with appropriate physical activity to attenuate loss or increase skeletal muscle mass.

A limitation of this study was that we did not assay blood vitamin D concentrations. Vitamin D status and its relation to physical training with and without supplementation are important questions that await investigation. Another important limitation was that we were not able to assess the effects of vitamin D supplementation separately from essential amino acid supplementation, although this type of experimental design would require many more participants than were available and a new sample size calculation.

In conclusion, aging causes the loss of many of the anabolic signals and an increase in catabolic signals to muscle that are present in young adulthood, but this study suggests that whey protein, essential amino acid, and vitamin D supplementation, together with gentle physical activity, can produce changes in catabolic mediators, lowering inflammatory markers such as CRP, and improving anabolic markers such as IGF-I. This shift results in a significant increase in FFM (+1.7 kg) and muscle strength, proving effective in the treatment of sarcopenia, with improvements in physical function and quality of life.

The authors' responsibilities were as follows—MR, CK, GT, JT, and RM: designed the study; MAF and SP: conducted the study; CK and DG: analyzed the data; BSS and MF: conducted the blood tests; MR and CK: wrote the manuscript; MR: had primary responsibility for the design and final content of the article; HL: revised the manuscript; and all authors: read and approved the final manuscript. None of the authors reported a conflict of interest related to the study.

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