Applied nutritional investigation

Higher protein intake is associated with improved muscle strength in elite senior athletes

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ABSTRACT

Objective: The optimal protein intake for elderly individuals who exercise regularly has not yet been clearly defined. The aim of this study was to test the hypothesis that protein intake level is associated with muscle strength in elderly elite athletes.
Methods: We evaluated 50 elite senior athletes (38 men and 12 women) participating in the European Master Games 2011 in an observational cross-sectional study. Participants were divided into two groups—lower (LPI) or higher (HPI) protein intake—according to the median value of their ratio of urinary urea nitrogen to urinary creatinine (i.e., 8.8 g/L), as a marker of protein intake. A dietary interview confirmed differences in protein consumption between the LPI and HPI groups. We also evaluated body composition (bioimpedance), muscle strength, and hematochemical indices.
Results: LPI and HPI groups were homogeneous for age (72 [68–74] and 71 [68–74] y, respectively), fat-free mass index (18.4 [17–19.4] and 18.2 [17–19.1] kg/m^2), body fat (18.3% [12.3–20.7%] and 16.6% [13.6–21.2%]), and glomerular filtration rate (57.7 [53.8–64.9] and 62.7 [56.1–69.3] mL/min/1.73 m^2). The HPI group showed greater leg and trunk muscle strength (N) compared with the LPI group (left leg extension, 339 [238–369] versus 454 [273–561], respectively, $P < 0.05$; right leg extension, 319 [249–417] versus 432 [334–635], $P \leq 0.05$; trunk extension, 435 [370–467] versus 464 [390–568], $P \leq 0.05$).
Conclusions: Higher protein intake in elite senior athletes is associated with a greater muscle strength.

Introduction

Aging is associated with progressive changes in body composition, with sarcopenia being the most relevant. This syndrome, induced by an interplay of multiple factors, is defined as significant loss of muscle mass and function [1]. Moreover, sarcopenia leads to increased risk for adverse outcomes, such as physical disability, metabolic complications, poor quality of life, and death [1,2]. Longitudinal studies in free-living populations have

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confirmed the association between body composition changes and functional losses in the elderly [3]. In both young and elderly healthy adults, resistance and endurance exercise acutely stimulates muscle protein synthesis [4,5], whereas long-term training allows muscle hypertrophy and strength enhancement [6,7]. However, in the elderly, training is associated with a lower increase in muscle mass, possibly related to the so-called "anabolic resistance," both to physical activity and protein intake [8–14].

Increasing protein intake above the recommended dietary allowances allows enhancement of muscle protein anabolism and reduction of anabolic resistance. Therefore, many authors now consider protein requirement in the elderly to be higher than in younger adults (age <65 y) [15]. Elderly individuals may show a neutral/positive nitrogen balance but only because they have already lost lean body muscle mass. As a consequences, their protein intake is apparently adequate Elderly individuals may show a neutral/positive nitrogen balance but only because they have already lost lean body muscle mass. As a consequences, their protein intake is apparently adequate [14,16]. Dynapenic postmenopausal women had a habitual lower protein intake compared with a nondynapenic group of the same lean body mass [17]. The hypothesis that the association of resistance training and higher protein intake (from diet or dietary supplements) have a synergic anabolic effect, has been confirmed, both in young and elderly, in acute exercise conditions [18–20]. Such a relation, however, remains controversial in long-term physical activity protocols involving elderly participants [20–23]. In healthy, metabolically stable individuals, the ratio of urinary urea nitrogen to urinary creatinine (UUN/UCr), in a postabsorptive spot urine specimen, is a reliable index of recent dietary protein intake [24–27]. In the present study, we tested the hypothesis that higher protein intake is associated with greater muscle strength in elderly elite athletes. We divided 50 elite senior athletes into two groups: higher (HPI) or lower (LPI) protein intake, according to the median value of the UUN/UCr ratio (as quantitative marker of protein intake). Differences in protein consumption were also confirmed by the results of a dietary interview. Thereafter, we evaluated the effects of different levels of protein intake on body composition, skeletal muscle strength, and blood biochemistry, including plasma free amino acid concentration, lipid pattern, insulin sensitivity, and oxidative stress.

Methods

The study followed an observational cross-sectional design. Fifty elite senior athletes (38 men and 12 women, between 65 and 81 y of age) were recruited from among participants in the European Master Games 2011 (held in Lignano, Italy), which was open to individuals ≥65 y of age. The study was approved by the Italian National Olympic Committee and the Ethical Committee of the University of Trieste, in agreement with the Declaration of Helsinki standard and its amendments. Each volunteer signed an informed consent. A physician completed a medical history and a physical examination of all volunteers to exclude those with chronic and acute illnesses, pharmacologic treatment, and current smoking. All individuals were eligible to participate in the study.

Participants were involved in different sports (e.g., running, cycling, pentathlon, fencing, and weightlifting), which were classified, according to Mitchell et al. [28], into categories based on the level of intensity (low, medium, high) of dynamic or static exercises required to perform that sport during competition (Table 1). We also recorded the number of years in training.

Using a dietary interview, a dietitian assessed the participants' habitual food intake over a 1-wk period [24–27]. A dedicated software (Dietosystem, DSmedica, Milan, Italy) was used to analyze the diet nutrient content.

All analyses were performed 36 h after competition, during the recovery phase, in the morning after an overnight fast.

Anthropometric and body composition indices

Standard methods were used to measure anthropometric data (body weight, height, waist and hip circumference). Athletes were weighed on an electronic scale, wearing only their underwear, after voiding their bladder. Height was measured without shoes with a stadiometer. We assessed waist and hip circumferences with the individuals in a standing position. Body composition indices (i.e., fat-free mass [FFM] and fat mass [FM]), were obtained using a multifrequency bioimpedance (BIA-Human Implus–DSmedica, Milan, Italy). An experienced dietitian obtained bioelectrical impedance measurements in the morning, after an overnight fast. We asked volunteers to refrain from strenuous exercise beginning the night before and to void the bladder before examination. Measurements were conducted according to manufacturer's instructions. Software supplied by the manufacturer was used to calculate FM and FFM. The FFM index was determined as FFM (kg) divided by the squared height in meters (m^2). None of the participants reported recent use of medications that might interfere with body water compartments.

Table 1
Number of elite senior athletes with a lower (LPI) or higher (HPI) protein intake practicing different sport specialties categorized according to Mitchell classification

Sport*	LPI (n = 25; female/male: 7/19)	HPI (n = 25; female/male: 6/18)
MD-MS (sprint running; jumping; fencing)	13	12
HD-LS (middle-long distance running; race-walking; cycling)	9	11
LD-HS (weightlifting; powerlifting; archery; throwing)	3	2
Years of training†	20.5 (1547)	20 (14–35)

HD-LS, high dynamic–low static; LD-HS, low dynamic–high static; MD-MS, moderate dynamic–moderate static; UUN/UCr, urinary urea nitrogen/urinary creatinine
Participants stratified in two groups (LPI and HPI) according to the UUN/UCr ratio median (i.e., 8.8 g/L) as cutoff value
* Sport specialties categorized following Mitchell classification [28].
† Years of training expressed as median value (interquartile range).

Skeletal muscle strength and quality

Muscle strength of trunk (flexion and extension) and both legs (extensions) was measured by a force gauge dynamometer (Imada co. LTD, Aichi, Japan), whereas hand flexion was measured by a handgrip dynamometer (Lafayette, Lafayette, IN, USA). Whole-body skeletal muscle quality was determined as the ratio between the sum of muscle strength (N) of the different muscle groups (hand + trunk + legs) and FFM (kg) [29–31].

Biochemical indices

Blood samples were taken from the forearm vein of all participants and centrifuged at 3000g at 4°C for 10 min. After separation, plasma and erythrocytes were treated according to the analytical protocols and immediately stored at –80°C until laboratory measurements were taken. Blood biochemistry included routine indices, insulin, and free amino acid plasma levels and red blood cell glutathione concentration as index of oxidative stress. Total cholesterol, high- and low-density lipoprotein cholesterol, triacylglycerol, insulin, and fasting glucose plasma levels were analyzed by standard procedures by a certified external laboratory (Synlab Italia Srl, Italy). Insulinemia and glycemia were used to calculate insulin resistance; and the homeostatic model assessment was used to calculate sensitivity. Amino acid concentrations were measured by gas chromatography mass-spectrometry (HP5890, Agilent Technologies, Santa Clara, CA, USA), using the internal standard technique, as previously described [32]. For each compound, a known amount of stable isotope (Cambridge Isotope Laboratories) was added as an internal standard to a known volume of plasma. Silylated derivatives were measured under electron-impact ionization by selective ion monitoring. Total glutathione concentrations in erythrocytes were evaluated by gas chromatography mass-spectrometry using the internal standard technique, as previously described [33].

Urine samples were collected to measure the UUN/UCr ratio as a marker of recent protein intake.

The Modification of Diet in Renal Disease formula was used to calculate the glomerular filtration rate [34].

Data presentation and statistics

We excluded seven subjects from data analysis because they did not perform the dietary interview.

Table 2
Characteristics of elite senior athletes with a lower (LPI) or higher (HPI) protein intake

	LPI (n = 25; female/male: 7/19)	HPI (n = 25; female/male: 6/18)	*P*-value*
Age (y)	72 (68–74)	71 (68–74)	0.97
Weight (kg)	72.9 (65.3–78.7)	73.8 (62.4–78.3)	0.71
Height (m)	1.74 (1.70–1.78)	1.74 (1.67–1.78)	0.67
BMI (kg/m^2)	24 (23–25)	24 (23–25)	0.79
FFMI (kg/m^2)	18.4 (17–19.4)	18.2 (17.1–19.1)	0.52
FM (kg)	18.3 (12.3–20.7)	16.6 (13.6–21.2)	0.88
WC (cm)	83 (81–92)	87 (79–95)	0.67
W/H ratio	0.86 (0.85–0.90)	0.88 (0.85–0.97)	0.16
GFR (mL/min/1.73 m^2)†	57.7 (53.8–64.9)	62.7 (56.1–69.3)	0.21
UUN/UCr ratio	7.2 (6.6–7.9)	10.5 (9.8–11.2)	–

BMI, body mass index; FFMI, fat-free mass index; FM, fat mass; GFR, glomerular filtration rate; UUN/UCr, urinary urea nitrogen/urinary creatinine; W/H ratio, waist-to-hip ratio; WC, waist circumference.
Data expressed as median value (interquartile range)
* Mann–Whitney test. Data were log-transformed when appropriate. Participants stratified in two groups (LPI and HPI) according to the UUN/UCr ratio median (i.e., 8.8 g/L) as cutoff value.
† GFR assessed trough the modification of diet in renal disease (Modification of Diet in Renal Disease) formula.

We divided the remaining volunteers into LPI and HPI groups according to the median values of the UUN/UCr ratio (i.e., 8.8 g/L) as a marker of recent protein intake.

Data are expressed as median values with interquartile range in parentheses. Data were log-transformed where appropriate. The differences between the two groups were evaluated through the Mann–Whitney non-parametric test for independent samples. The Spearman test was used to investigate the non-parametric correlations between protein intake calculated by the UUN/UCr ratio and that evaluated from the dietary interview, protein intake as assessed by the UUN/UCr ratio, and whole-body skeletal muscle quality. Statistical analysis was performed using SPSS software version 12 (SPSS, Inc., Chicago, IL, USA). $P \leq 0.05$ was considered statistically significant.

Results

Both groups were homogeneous for sex distribution (LPI group, female/male: 7/19; HPI group, female/male: 6/18), age, anthropometric and body composition indices, kidney function (Table 2), sporting specialties, and years of training (Table 1). Table 3 presents nutrient intake. Dietary protein content is significantly higher in the HPI group, both as percentage of total energy and as g/kg of body weight. We did not observe any significant differences between groups for other nutrients or total calories. We found a significant direct correlation between the protein intake assessed by either UUN/UCr ratio or by dietary interview ($r = 0.55$; $P < 0.01$).

Table 3
Dietary nutrient intake in elite senior athletes with a lower (LPI) or higher (HPI) protein intake

	LPI (n = 25)	HPI (n = 25)	*P*-value*
Protein	1.21 (1.05–1.29) g/kg BW	1.34 (1.19–1.58) g/kg BW	0.01
	16 (15–19)%†	23 (20–27)%†	0.001
Lipid (%)†	26 (22–30)	22 (20–24)	0.13
Carbohydrate (%)†	51 (48–55)	49 (44–53)	0.80
Total energy (kcal/d)	2137 (1796–2395)	2028 (1737–2347)	0.25

BW, body weight; UUN/UCr, urinary urea nitrogen/urinary creatinine
Data expressed as median value (interquartile range)
* Mann–Whitney test. Data were log-transformed when appropriate. Participants stratified in two groups (i.e., LPI and HPI) according to the UUN/UCr ratio median (i.e., 8.8 g/L) as cutoff value.
† Percent of total energy intake.

Table 4
Skeletal muscle strength in elite senior athletes with a lower (LPI) or higher (HPI) protein intake

	LPI (n = 25)	HPI (n = 25)	*P*-value*
Left leg extension (N)	339 (238–369)	454 (273–561)	0.05
Right leg extension (N)	319 (249–417)	432 (334–635)	0.01
Trunk extension (N)	435 (370–467)	464 (390–568)	0.05
Trunk flexion (N)	280 (230–313)	271 (250–363)	0.43
Hand grip (N)	446 (392–500)	441 (343–461)	0.55

Data expressed as median value (interquartile range)
* Mann–Whitney test. Data were log-transformed when appropriate. Participants stratified in two groups (i.e., LPI and HPI) according to the urinary urea nitrogen/urinary creatinine ratio median (i.e., 8.8 g/L) as cutoff value.

The HPI group showed greater muscle strength in the right and left leg and trunk extension, although there were no significant differences between the two groups for the trunk and hand flexion (Table 4). A significant difference was observed between the two groups in terms of whole-body skeletal muscle quality (LPI, 31.7 [29.4–35] and HPI, 37.8 [33.8–41.7]; $P = 0.002$; Fig. 1). We found a significant direct correlation between whole-body skeletal muscle quality and UUN/UCr ratio as a marker of protein intake ($r = 0.33$; $P = 0.03$; Fig. 2).

Data on amino acid concentration and oxidative stress (Table 5), as well as lipid pattern and glucose/insulin levels (Table 6), showed no significant differences between the two groups.

Discussion

Sarcopenia causes loss of strength and function, disability, dependency, reduced health, and worsening of quality of life [2]. As such, ways to improve muscle mass and function are of great relevance in the aging population. Exercise and protein intake

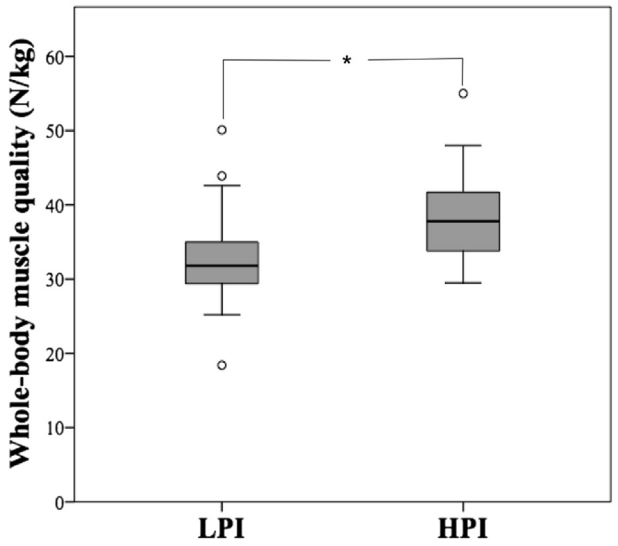

Fig. 1. Whole-body skeletal muscle quality in elite senior athletes with a lower (LPI) or a higher (HPI) protein intake. LPI group, n = 25; HPI group, n = 25. Data expressed as median value (interquartile range). A higher protein intake is associated with enhanced whole-body muscle quality. Whole-body muscle quality is defined as the ratio between the sum of muscle strength (N) of the different muscle groups (hand grip + trunk extension and flexion + right and left leg extension) and fat-free mass (kg). *$P < 0.05$.

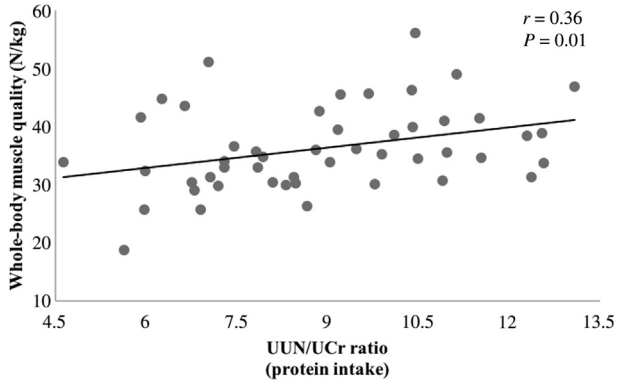

Fig. 2. Correlation between whole-body muscle quality and urinary urea nitrogen/urinary creatinine (UUN/UCr) as a quantitative marker of protein intake in pooled elite senior athletes (N = 50).

may have a synergic effect on muscle mass and function [14,18,19]. In the present study, unlike other protocols that have applied exercise, protein or amino acid supplementation, or both to sedentary or moderately active untrained elderly individuals [14,18,19], we evaluated the relationships among different levels of protein intake and physical function, body composition, and metabolic markers in well-trained elite senior athletes.

We divided the enrolled volunteers into two groups (HPI and LPI) according to the median values of the UUN/UCr ratio, which is a quantitative marker of recent protein intake [24–27]. The groups were homogeneous for age, sex, years of training, and sport specialties (Tables 1 and 2).

Recent studies that have considered anabolic resistance to dietary protein and the lower effects of training in aging skeletal muscle have reported an increase in recommended protein intake for healthy elderly from 0.83 to 1 to 1.2 g/kg daily and to ≥1.2 g/kg daily for healthy and regularly active aging individuals [14,15,35,36]. The American College of Sport Medicine's position on dietary practices for adult athletes [37] recommends a daily protein intake of 1.2 to 1.7 g/kg; however, no indications are available for elite senior athletes. In the present study, data from dietary interviews show that individuals in the LPI group had an average daily protein intake of 1.2 ± 0.2 g/kg, whereas the HPI group's protein intake was 1.4 ± 0.3 g/kg. These values are highly correlated with those obtained from the UUN/UCr ratio ($r = 0.66$; $P < 0.01$).

Table 5
Plasma amino acid and erythrocyte GSH concentrations in elite senior athletes with a lower (LPI) or higher (HPI) protein intake

	LPI (n = 25)	HPI (n = 25)	*P*-value*
Alanine (μmol/L)	348 (276–388)	345 (299–407)	0.41
Glycine (μmol/L)	217 (195–249)	204 (187–265)	0.65
Leucine (μmol/L)	124 (110–139)	129 (124–149)	0.22
Phenylalanine (μmol/L)	62 (56–67)	66 (58–73)	0.09
Glutamic acid (μmol/L)	52 (47–61)	48 (44–59)	0.30
Glutamine (μmol/L)	581 (549–615)	595 (562–622)	0.48
Tyrosine (μmol/L)	63 (53–70)	67 (60–79)	0.12
Threonine (μmol/L)	126 (107–139)	132 (109–145)	0.61
Serine (μmol/L)	99 (86–105)	95 (84–103)	0.87
Proline (μmol/L)	200 (160–227)	209 (190–238)	0.40
Cysteine (μmol/L)	302 (296–320)	304 (291–332)	0.68
Methionine (μmol/L)	27 (24–29)	31 (27–33)	0.01
Homocysteine (μmol/L)	17 (14–19)	17 (14–19)	0.61
GSH (μmol/L)	2139 (2010–2222)	2159 (2079–2211)	0.59

GSH, glutathione
Data expressed as median value (interquartile range)
* Mann–Whitney test. Data were log-transformed when appropriate. Participants stratified in two groups (i.e., LPI and HPI) according to the urinary urea nitrogen/urinary creatinine ratio median (i.e., 8.8 g/L) as cutoff value.

Table 6
Fasting data on glucose metabolism and plasma lipid pattern in elite senior athletes with a lower (LPI) or higher (HPI) protein intake

	LPI (n = 25)	HPI (n = 25)	*P*-value*
Insulin (mUI/L)	5.7 (4.0–8.2)	4.6 (3.0–6.2)	0.56
Glucose (mg/dL)	96 (91–107)	93 (87–105)	0.81
HOMA	1.2 (0.8–1.9)	1.1 (0.8–1.6)	0.66
Total cholesterol (mg/dL)	179 (166–216)	192 (185–218)	0.30
HDL cholesterol (mg/dL)	54 (44–60)	57 (50–64)	0.41
LDL cholesterol (mg/dL)	114 (97–143)	121 (104–142)	0.65
Triacylglycerols (mg/dL)	70 (54–91)	81 (63–105)	0.29

HDL, high-density lipoprotein; HOMA, homeostatic model assessment; LDL, low-density lipoprotein
Data expressed as median value (interquartile range)
* Mann–Whitney test. Data were log-transformed when appropriate. Participants stratified in two groups (i.e., LPI and HPI) according to the urinary urea nitrogen/urinary creatinine ratio median (i.e., 8.8 g/L) as cutoff value.

In the present study, higher protein intake was associated with greater muscle strength, which could not be attributed to a higher skeletal muscle mass because no differences were observed in body composition indices between the two groups. This is in agreement with studies on aging that report that the loss of muscle strength is three times higher than the decline in muscle mass [3]. Furthermore, other studies showed that in nonsarcopenic elderly individuals, a protein intake higher than the recommended levels improved function but caused modest or no gain in skeletal muscle mass [6,27,38,39]. This higher muscle strength may be related to multiple factors, such as modification in muscle fiber morphology or contractile kinetics, nutrition quality and quantity, type and intensity of physical activity, variability in the individual response to training, and enhancement of skeletal muscle quality [6,27,38,39].

We found a significant difference in whole-body skeletal muscle quality between the two groups, with the HPI group (greater UUN/UCr ratio) having higher values. This finding was confirmed by a significant direct correlation between UUN/UCr ratio as an index of protein intake and muscle quality in pooled data (Fig. 2).

High-protein diets could have negative effects on kidney function, which is known to decline with aging [40]; however, we did not observe any glomerular filtration rate differences between the two groups, as assessed by Modification of Diet in Renal Disease formula [14], indicating the safety of the higher protein intake. Indeed, impairment of renal function from dietary protein has not been shown with an intake within 2 g/kg daily in healthy elderly individuals [40].

The present study had some limitations: the relative small number of cases, the cross-sectional nature of the protocol, and the use of simple methodologies, compared with higher level evaluation methods such as muscle biopsies. Still, compared with other studies, this is one of the larger ones on elite senior athletes. Furthermore, the short stay of the participants, who came from different European countries, did not allow a prospective approach.

Conclusion

Higher protein intake in elite senior athletes is associated with enhanced strength and muscle quality, without changes in muscle mass or renal function.

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