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Protein Intake and Muscle Strength in Older Persons: Does Inflammation Matter?

Benedetta Bartali, PhD¹, Edward A. Frongillo, PhD^{2,3}, Martha H. Stipanuk, PhD³, Stefania Bandinelli, MD⁴, Simonetta Salvini, RD⁵, Domenico Palli, MD, PhD⁵, Jose A. Morais, MD⁶, Stefano Volpato, MD, MPH⁷, Jack M. Guralnik, MD, PhD⁸, and Luigi Ferrucci, MD, PhD⁹

¹Channing Laboratory, Brigham and Women's Hospital and Harvard Medical School, Boston, MA

²Department of Health Promotion, Education, and Behavior, Arnold School of Public Health, University of South Carolina, SC

³Division of Nutritional Sciences, Cornell University, NY, CT

⁴Tuscany Regional Health Agency, Florence, Italy

⁵Molecular & Nutritional Epidemiology Unit, Cancer Research and Prevention Institute - ISPO, Florence, Italy

⁶Division of Geriatric Medicine, McGill University, MUHC-Royal Victoria Hospital, Quebec

⁷University of Ferrara, Italy

⁸Epidemiology and Demography Section, National Institute on Aging, NIH, MD

⁹Longitudinal Studies Section, National Institute on Aging, NIH, MD

Abstract

BACKGROUND/OBJECTIVES—The effect of dietary protein intake on muscle strength in older persons is unknown. The objective of this study was to examine whether protein intake is associated with change in muscle strength in older persons. Because systemic inflammation has been associated with protein catabolism, we also evaluated whether a synergistic effect exists between protein intake and inflammatory markers on change in muscle strength using a longitudinal study of community-dwelling persons aged 65 years or older.

DESIGN—Longitudinal.

Address correspondence: Benedetta Bartali, Brigham and Women's Hospital and Harvard Medical School, 181 Longwood Ave, Boston, MA 02115, Phone: 617-525-2021, Fax: 617-525-2008, benedetta.bartali@channing.harvard.edu.

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Author Contributions:

Bartali: study concept and design, analysis and interpretation of data, and preparation of manuscript.

Frongillo: study concept and design, analysis and interpretation of data, and preparation of manuscript.

Stipanuk: study concept and design, analysis and interpretation of data, and preparation of manuscript.

Bandinelli: acquisition of subjects and/or data, analysis and interpretation of data.

Salvini: analysis and interpretation of data.

Palli: analysis and interpretation of data.

Morais: analysis and interpretation of data.

Volpato: analysis and interpretation of data.

Guralnik: acquisition of subjects and/or data, analysis and interpretation of data.

Ferrucci: acquisition of subjects and/or data, analysis and interpretation of data.

SETTING—The InCHIANTI Study.

PARTICIPANTS—Five hundred and ninety-eight persons.

MEASUREMENTS—Knee extension strength was measured at baseline (1998–2000) and during 3-year follow-up (2001–2003) using a hand-held dynamometer. Protein intake was assessed using a very detailed food frequency questionnaire. The inflammatory markers included in this study were C-reactive protein (CRP), Interleukin-6 (IL-6), and Tumor Necrosis Factor- α (TNF- α).

RESULTS—The main effect of protein intake on change in muscle strength was not significant, but we found a significant interaction between protein intake and CRP, IL-6 and TNF- α ($p=0.003$, $p=0.049$ and $p=0.019$, respectively), indicating that a lower protein intake was associated with a greater decline in muscle strength in persons with high levels of inflammatory markers.

CONCLUSION—Selectively in older persons with a pro-inflammatory state, low protein intake was associated with accelerated decline in muscle strength. These results may help to understand the factors contributing to decline in muscle strength and to identify the target population of older persons who may benefit from nutritional interventions aimed at preventing or reducing age-associated muscle impairments and its detrimental consequences.

Keywords

Muscle Strength; Protein Intake; Inflammatory markers

INTRODUCTION

The decline in muscle strength that occurs with aging(1) is a risk factor for the development of frailty and disability, and predicts hospitalization in older persons (2–4). Although the etiology of the decline in muscle strength is not fully understood, poor nutrition and responsiveness to nutrients are considered potential factors contributing to its development and progression (1).

In previous studies, we found that a low protein intake was associated with frailty (5), and that a low concentration of specific micronutrients was a predictor of decline in physical function (6) and disability (7) in older persons living in the community. Although dietary protein intake is essential for protein muscle anabolism (8) and has been positively associated with muscle mass (9), its effect on muscle strength in the general older population is unknown.

This study was aimed at addressing this gap in knowledge by providing empirical evidence on the effect of protein intake on longitudinal changes in muscle strength in a representative sample of older persons living in the community. Because aging is often associated with the development of a mild pro-inflammatory state that may lead to reduced muscle strength, muscle wasting and muscle protein catabolism (10–11), we also examined whether the effect of protein intake on muscle strength depends on the levels of inflammatory markers.

METHODS

InCHIANTI (Invecchi are in Chianti, aging in the Chianti area) is a study of risk factors contributing to the decline in mobility in older persons, conducted in two municipalities adjacent to the city of Florence (Italy), more details have been described elsewhere (12). In brief, 1299 participants aged 65 years or older were randomly selected from the population registry. Of the 1260 persons who were eligible (39 had died or moved away from the area), 1155 (91.6%) participated in the study. After excluding those with disability at baseline ($n=116$), those with missing information on knee muscle strength at baseline ($n=158$) or at

follow-up (n=156), and those who refused (n=71), emigrated (n=10), or died (n=46) during the three-year follow-up, the final analytical sample included 598 persons.

Trained interviewers administered two structured questionnaires at the participant's home: 1) a questionnaire to collect information on education, socio-economical status, household composition, physical activity, functional and health status; 2) a detailed food frequency questionnaire to collect data on dietary intake. Medical and physical assessments were performed in the study clinic by trained geriatricians and therapists, respectively.

Dietary intake

Data on dietary intake were collected using the food-frequency questionnaire developed for the European Prospective Investigation into Cancer and nutrition (EPIC) study (13). Although the EPIC questionnaire was originally developed for and validated in middle-aged persons, our previous study suggested that this tool provides valid estimates of dietary intake when administered to older persons (14). Specific software created for EPIC was used to transform data on food consumption into daily intake of energy, macro- and micro-nutrients. A detailed description of this food-frequency questionnaire has been published elsewhere (14).

Muscle strength

Knee extension strength was measured with a hand-held dynamometer (Nicholas Muscle Tester; Sammon Preston Inc, Chicago, IL) according to a standard assessment protocol (15) that has been shown to be highly reliable (test-retest reliability 0.85, inter-rater reliability 0.74). The participants lay down in lateral decubitus (opposite to the examined limb) with the hip and knee in 45° and 60° flexed positions, respectively. The subjects were instructed to exert maximal effort against the dynamometer. Strength was measured as the peak force that the examiner had to apply to break the isometric contraction, indicated by a slight movement of the subject's leg in the direction opposite to the voluntary movement. The subject was also instructed to maintain a fixed posture during the entire testing procedure. The test was repeated thrice and the mean value of knee strength of the dominant leg was used for the present analyses. In previous studies, the intraclass correlation coefficients for test-retest measures of knee extension isometric strength ranged from 0.89 to 0.99 in older persons, and the coefficient of variation was 4.6 (15–16).

Markers of Inflammation

Blood samples were obtained from participants after a 12-hour fast and 15-minute rest. Aliquots of serum were stored at -80°C and not thawed until analyzed.

C-reactive protein (CRP) was measured in duplicate by enzyme linked immunosorbent assay (ELISA) high sensitivity test using purified protein and polyclonal anti-CRP antibodies (Calbiochem, San Diego, CA) with standardization according to the World Health Organization 1st International Reference Standard. The minimum detectable concentration was 0.03 mg/L, and the inter assay coefficient of variation was 5%. Interleukin 6 (IL-6) and Tumor Necrosis Factor- α (TNF- α) were assessed in duplicate by ELISA using high sensitive commercial kits (Human Ultrasensitive, BIOSOURCE International Inc., Camarillo California USA). The minimum detectable concentrations were 0.10 pg/ml for IL-6 and 0.09 pg/ml for TNF-alpha, and the coefficient of variation (CV) was 7%.

Other variables

Presence of major chronic conditions was ascertained by trained geriatricians according to standard algorithms based on information on medical history, drug treatments, signs and symptoms, medical documents, and hospital discharge records (12). Diagnostic algorithms

were modified versions of those created for the Women's Health and Aging Study (17). Chronic conditions considered for the present analysis were: hypertension, diabetes, peripheral artery disease (PAD), stroke, angina pectoris, congestive heart failure (CHF), myocardial infarction, chronic obstructive pulmonary disease (COPD), Parkinson, cancer, and arthritis. The number of chronic conditions was used in the present analyses as a continuous variable.

Weight and height were measured according to standard protocols (18) and body mass index (BMI) was calculated as kg/m^2 . Smoking habits were classified as never smoked, former smoker, and current smoker. Physical activity was defined as a) sedentary: completely inactive or light physical activity (i.e., walking) for less than 1 hour/week; b) light:light physical activity for 2–4 hours/week; c) moderate: light physical activity for more than 4 hours/week or moderate physical activity (i.e., gymnastics, swimming etc.) 1–2 hours/week; d) Intense: moderate physical activity for 3 or more hours/week or walk 5 or more km/day (19).

Statistical Analysis

General linear models were used to evaluate the effect of protein intake at baseline on change in muscle strength over three years of follow-up. We used muscle strength at follow-up as dependent variable, and protein intake at baseline as main independent variable. Muscle strength at baseline was entered in the model as independent variable to account for differential subsequent change in muscle strength depending on the initial status. The model was adjusted for age, sex, BMI, physical activity, energy intake, the presence of chronic conditions and smoking. In addition, adjusted general linear models were used to examine the interaction between protein intake and CRP, IL-6, and TNF- α at baseline on muscle strength at follow-up. We included a 3-way interaction (protein intake*markers of inflammation*muscle strength at baseline) in the general linear model to verify whether the effect of the interaction between protein intake and inflammatory markers on change in muscle strength depended on the initial level of muscle strength. Furthermore, a 3-way interaction (protein intake*markers of inflammation*sex) was used to verify whether the analyses needed to be stratified by sex. In addition, we examined whether the effect of the interaction between protein intake and markers of inflammation on muscle strength at follow-up depended on the presence of chronic conditions (protein intake*markers of inflammation*chronic conditions). Since CRP, IL-6, and TNF- α were not normally distributed, these variables were log-transformed. All analyses were performed using the SAS statistical software, version 8.1 (20).

RESULTS

The comparison of characteristics between participants included and not included in the study is reported in Table 1. Participants excluded from the study were older, mostly female, with lower economical status, more sedentary, with more chronic conditions, higher levels of CRP, and TNF- α and with lower intake of energy and protein. The mean age of participants included in the study was 73 years and 53% were women. As shown in Table 2, after adjustment for age, sex, BMI, physical activity, energy intake, chronic conditions, smoking, and muscle strength at baseline, protein intake was not associated overall with change in muscle strength over three years of follow-up ($p=0.78$). We found, however, a significant interaction between protein intake and CRP, IL-6 and TNF- α on change in muscle strength ($p=0.003$; $p=0.050$; and $p=0.019$; respectively). In persons with high levels of inflammatory markers, lower protein intake was associated with a greater decline in muscle strength. These results were not attributable to the presence of chronic conditions (Table 2). We also repeated the analyses selectively in a subsample of participants without chronic conditions ($n=188$) and the result on the interaction between protein intake and

inflammatory markers on muscle strength did not substantially change (i.e. for CRP: Beta for Protein = -0.009, Beta for CRP = -1.48, Beta for Protein*CRP = 0.023, $p=0.03$; for TNF- α : Beta for Protein = -0.014, Beta for TNF- α = -1.840, Beta for Protein*TNF- α = 0.022; $p=0.03$) with the only exception for IL-6 (Beta for Protein = 0.003, Beta for IL-6 = -0.45, Beta for Protein*IL-6 = 0.011; $p=0.35$) likely because of the lack of statistical power. In addition, the 3-way interaction term with muscle strength at baseline (protein intake*markers of inflammation*muscle strength at baseline) was not significant. Thus, the effect of the interaction between protein intake and inflammatory markers on change in muscle strength did not depend on the initial level of muscle strength. Similarly, the 3-way interaction with sex (protein intake*markers of inflammation*sex) was not significant and, consequently, the analyses were not stratified by sex.

DISCUSSION

This study examined the effect of protein intake on decline in muscle strength in older persons, and whether this effect was dependent on inflammation. The overall effect of protein on subsequent decline in muscle strength was not significant. We found, however, a significant interaction between protein intake and markers of inflammation on muscle strength during three years of follow-up. In persons with high levels of inflammatory markers, lower protein intake was associated with a greater decline in muscle strength.

Previous studies found a significant association between oral aminoacids supplementation and muscle strength in older persons (21). To our knowledge, however, this is the first longitudinal study on the relationship between dietary protein intake and muscle strength using a representative sample of community-living older persons. The mechanism for the interplay between protein intake and inflammatory markers on age-associated changes in muscle strength could be attributable, at least in part, to the complexity of cellular and metabolic activities involved in the pro-inflammatory state that may alter the pattern of regulation for protein metabolism and may impose priorities. Indeed, the systemic inflammatory response is associated with oxidative stress (22) and stimulates muscle protein turnover (8) to allow for the adaptation to this stressful condition. Although this process is a protein- and energy-demanding response (8, 23) the amino acids released by tissue protein breakdown represent a substrate for the synthesis of numerous proteins and peptides involved in the immune system (24) with consequent reduction in plasma amino acid concentration. Previous studies have shown that amino acid availability is critical in the regulation of muscle protein metabolism (25). Of note, lower rates of muscle protein synthesis with aging have been associated with up-regulation of the NF- κ B pathway, which is the critical intra-cellular gate for the inflammatory response (26). Thus, a pro-inflammatory state as indicated by high levels of inflammatory markers in older persons may result in an increased requirement for dietary intake of proteins to create a more anabolic environment and to counteract muscle protein breakdown.

A limitation of this study is that participants who were not included in the analyses were older, more sedentary, with more chronic conditions and higher levels of inflammatory markers (CRP and TNF- α) compared with those who were included and this may have biased our results. In longitudinal studies of older persons, however, age-related problems - such as morbidity, morbidity-related factors and mortality - are inevitable causes of attrition leading to loss of power and underestimation of decline in muscle strength over time (27). Another limitation is that protein intake was assessed using a food frequency questionnaire. Thus, the accuracy of the observed associations might be affected by reporting bias. The distribution of errors, however, is unlikely to be related to the outcome. Consequently, the observed results may be underestimated.

An important strength of this study is that we used an objective measure of muscle strength that is highly reliable (15–16). Furthermore, the analyses were adjusted for total energy intake and, consequently, our results 1) are unlikely explained by a possible loss of appetite, and in turn a low dietary intake of protein, associated with high levels of inflammatory markers; and 2) show the separate effect of protein intake on muscle strength, independent of the intake of energy provided by other sources.

In conclusion, we found a significant interaction between protein intake and markers of inflammation on muscle strength at follow-up, after adjustment for muscle strength at baseline. In persons with high levels of inflammatory markers, lower protein intake was associated with a greater decline in muscle strength, independent of the presence of chronic conditions. These findings suggest that high levels of markers of inflammation may alter protein metabolism and the efficiency of protein utilization. Since this is the first longitudinal study on the effect of protein intake and inflammatory markers on muscle strength in older persons, further studies are needed to confirm these results, and caution should be used in recommending higher protein intakes to older persons with specific chronic conditions (e.g., kidney diseases). These results may help to understand the etiology of the decline in muscle strength with aging and to develop intervention strategies aimed at preventing or delaying its debilitating consequences.

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Table 1Main characteristics of the study participants (mean \pm SD or %)

	Included in the Study (n=598)	Excluded from the Study (n=557)	p^I
Age (years; range: 65.1–92.8)	72.9 \pm 5.6	79.3 \pm 8.2	<.001
Gender (Female, %)	52.8	60.9	0.006
Education (years)	5.7 \pm 3.3	5.3 \pm 3.3	0.542
Living Alone (%)	16.9	20.7	0.102
Economical Status (sufficient, %)	68	49	<.001
Smoking (pack-year)	12.4 \pm 20.3	12.4 \pm 22.5	0.119
Physical Activity (sedentary, %)	10.25	40.9	<.001
Body Mass Index (kg/m ²)	27.5 \pm 3.8	27.4 \pm 8.2	0.175
Number of Chronic Conditions	1.2 \pm 1.1	4.6 \pm 6.2	<.001
CRP (mg/L)	3.98 \pm 5.06	7.4 \pm 13.2	<.001
IL-6 ultra-sensitive (pg/ml)	1.86 \pm 3.6	2.9 \pm 5.07	0.171
TNF- α (pg/ml)	3.38 \pm 4.82	4.47 \pm 6.43	0.008
Energy Intake (kcal/day)	1999 \pm 568	1776 \pm 533	0.003
Total Protein Intake (g/day)	77 \pm 20	70.5 \pm 20.5	0.024
Vegetable Protein (g/day)	29.0 \pm 9.5	25.5 \pm 9.6	0.006
Animal Protein (g/day)	48.5 \pm 15.0	45.0 \pm 14.7	0.182
Muscle Strength at Baseline (kg)	15.9 \pm 5.6	---	---
Decline in Muscle Strength (%)	43	---	---

^I Adjusted for age (except age comparison)

Table 2

Effect of protein intake on subsequent change in muscle strength

	Beta (SE)	p¹
Protein Intake (g/day) ²	-0.004 (0.013)	0.777
Protein * Log(CRP) ³	0.020 (0.007)	0.003
Protein * Log(IL-6) ⁴	0.016 (0.008)	0.050
Protein * Log(TNF-a) ⁵	0.016 (0.007)	0.019
Protein * Log(CRP) * sex	-0.002 (0.004)	0.530
Protein * Log(IL-6) * sex	-0.003 (0.005)	0.507
Protein * Log(TNF-a) * sex	-0.001 (0.003)	0.895
Protein * Log(CRP) * chronic conditions	-0.002 (0.002)	0.199
Protein * Log(IL-6) * chronic conditions	-0.002 (0.002)	0.234
Protein * Log(TNF-a) * chronic conditions	-0.001 (0.002)	0.372

¹ Adjusted for age, sex, BMI, energy intake, chronic conditions, physical activity, smoking, and muscle strength at baseline

² Intercept (SE)= 24.9 (2.8)

³ Intercept (SE)= 26.3 (2.7); Beta Protein=-0.021; Beta CRP=-1.41

⁴ Intercept (SE)=25.3 (2.7); Beta Protein=-0.009; Beta IL-6 =- 1.28

⁵ Intercept (SE)=27.1 (4.0); Beta Protein=-0.016; Beta TNF-a =- 1.44