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Authors

Langsetmo, Lisa
Harrison, S
Jonnalagadda, S
et al.

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LOW PROTEIN INTAKE IRRESPECTIVE OF SOURCE IS ASSOCIATED WITH HIGHER MORTALITY AMONG OLDER COMMUNITY-DWELLING MEN

L. LANGSETMO[1], S. HARRISON[2], S. JONNALAGADDA[3], S.L. PEREIRA[3], J.M. SHIKANY[4], S. FARSIJANI[5], N.E. LANE[6], J.A. CAULEY[5], K. STONE[2], P.M. CAWTHON[2] FOR THE OSTEOPOROTIC FRACTURES IN MEN (MROS) RESEARCH GROUP

1. Division of Epidemiology and Community Health, University of Minnesota, Minneapolis, MN, USA; 2. California Pacific Medical Center Research Institute, San Francisco Coordinating Center, San Francisco, CA, USA; 3. Abbott Nutrition Inc., Columbus, OH, 43054, USA; 4. Division of Preventive Medicine, School of Medicine, University of Alabama at Birmingham, Birmingham, AL, USA; 5. Department of Epidemiology, University of Pittsburgh, Graduate School of Public Health, Pittsburgh, PA, USA; 6. Department of Internal Medicine, University of California Davis Health, Davis, CA, USA. Corresponding author: Lisa Langsetmo, Ph.D., Division of Epidemiology and Community Health, University of Minnesota, 1300 S. 2nd St., Suite 300, Minneapolis, MN, USA, 55454. E-mail: langs005@umn.edu

Abstract: *Objectives:* Our aim was to determine the association between protein intake (overall and by source) and all-cause and cause-specific mortality among older men. *Design:* Prospective cohort study. *Setting:* 5790 ambulatory community-dwelling older men from multicenter Osteoporotic Fractures in Men (MrOS) study. *Measurements:* Total energy and protein intake, and protein intake by source (dairy, non-dairy animal, plant) were assessed using a 69-item food frequency questionnaire. We included up to 10-year follow-up with adjudicated cardiovascular, cancer and other mortality outcomes. We used time-to-event analysis with protein exposures, mortality outcome, and adjusted for possible confounders including age, center, education, race, smoking, alcohol use, physical activity, weight, total energy intake (TEI), and comorbidities. Hazard ratios were expressed per each unit=2.9% TEI decrement for all protein intake variables. *Results:* The mean (SD) baseline age of 5790 men was 73.6 (5.8) y. There were 1611 deaths and 211 drop-outs prior to 10 years, and 3868 men who were alive at the 10-year follow-up. The mean (SD) total protein intake was 64.7 (25.8) g/d, while the mean (SD) intake expressed as percent of total energy intake (%TEI) was 16.1 (2.9) %TEI. Lower protein intake was associated with an increased risk of death, with unadjusted HR=1.11 (95% CI: 1.06, 1.17) and adjusted HR=1.09 (95% CI: 1.04, 1.14) and the associations for protein intake by source were similar. The adjusted HR for cancer mortality was HR=1.13 (95% CI: 1.03, 1.25) while the association for CVD mortality was HR=1.08 (95% CI: 0.99, 1.18). *Conclusions:* Low protein intake, irrespective of source, was associated with a modest increase in risk of all-cause and cause-specific mortality among older men. Special consideration should be given to level of protein intake among older adults.

Key words: Protein intake, older men, all-cause mortality, cause-specific mortality.

Introduction

Studies have shown that higher protein intake is associated with longitudinal preservation of muscle mass (1), strength (2), physical performance (3, 4) and functional integrity (5). Low protein intake, and the associated loss of muscle strength, may also be associated with an increase in falls (6), loss of mobility and functional limitations (7), and disability. We have previously shown associations between lower dietary protein intake and lower bone strength (8) as well as greater frailty (9) and higher fracture risk (10). It is hypothesized that frailty involves a vicious cycle where there is a dynamic feedback between components of frailty (11). Thus lower dietary intake could lead to low energy and low protein intake and decrease in amino acid supply needed for muscle anabolism, eventually leading to declining muscle mass and strength. Frailty is linked to important clinical outcomes including falls, disability, and fracture (12).

Based on these studies, one might hypothesize that higher protein intake would be associated with lower all-cause mortality. However, many studies in non-geriatric adults have shown that higher protein intake is associated with higher mortality (13, 14). Differences in associations might be attributable to changes in nutritional intake or requirements with advancing age. An interaction between age and protein intake, where the associations are reverse in older vs younger adults, has been observed in a population-based US study (15)

The objective of this study was to determine the association of total energy intake, total protein intake and protein intake by source (dairy, non-dairy animal, plant) with 10-year all-cause and cause specific mortality among community-dwelling older men. In a secondary analysis, we determined the association between protein intake and mortality using categories of protein intake determined by the Recommended Dietary Allowance (RDA) threshold and a plausible higher threshold.

Material and Methods

Between 2000 and 2002 the Osteoporotic Fractures in Men (MrOS) study enrolled 5994 ambulatory men aged 65 years and older who lived in one of six U.S. metropolitan areas. The study sample for this analysis included men who completed a food frequency questionnaire (FFQ) at baseline with fewer than 10 percent missing responses, who had a

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reported energy intake between 500 and 5000 kcal/d and who were not using an androgen or androgen agonist. Further details concerning study cohort recruitment and methods have been published (16, 17). Study information is also available on line at http://mrosdata.sfcc-cpmc.net. All participants gave written informed consent; the study was conducted in accord with the Helsinki Declaration, and ethics approval was granted through institutional review boards at the respective study centers.

Protein intake

Participants completed a brief version of the original Block FFQ at study baseline (18). The brief FFQ queried the intake of 69 individual food items over the previous year. There were nine frequency responses and four portion size responses for each item. A graphic representation of standard portion sizes was included with the questionnaire. An additional 13 questions about food preparation and low-fat foods were used to refine the nutrient calculations. Total energy intake, total protein intake and protein intake by source were derived from the responses to the questionnaire by Block Dietary Data Systems (Berkeley, CA, USA), with dietary reference data from the United States Department of Agriculture Database for Standard Reference for Version 12 and the 1994–1996 Continuing Survey of Food Intakes by Individuals database. We considered the following subcategories of intake: protein from dairy products (milk, yogurt, cheese, ice cream), non-dairy animal protein (meat, fish, poultry, eggs), and protein from plant sources (legumes, grains, nuts).

Mortality outcomes

Study participants were contacted by mail every 4 months. Deaths were reported by proxy respondent and were confirmed with hospital discharge records and/or death certificate. Underlying cause of death was centrally adjudicated by physician review of discharge records or death certificates which were then grouped into three categories (cardiovascular disease, cancer, or other deaths). We include all deaths up to and including 10th anniversary of study entry.

Other covariates

Information on demographics, lifestyle, medical and family history was obtained by questionnaire and interview by trained clinical staff. Race/ethnicity and physician diagnosed medical conditions were self-reported. Participants were classified into ever (>100 lifetime cigarettes) vs. never smokers. Self-reported alcohol intake was divided into two categories: < 2 drinks per day vs ≥ 2 drinks per day. The baseline clinical exam included assessment of height (Harpenden stadiometer) and weight (balance beam or electronic scale). Body mass index (BMI) was calculated from measured height and weight using the formula BMI = weight (kg)/height (m)^2. Physical activity was assessed by computing the Physical Activity Scale for the Elderly (PASE) (19). Participants were asked to bring all current (any use within the past 30 days) prescription medications to the clinic. All prescription medications were recorded in an electronic medication inventory database and matched to their ingredients(s) based on the Iowa Drug Information Service drug vocabulary (College Pharmacy, University of Iowa, Iowa City, IA).

Statistical methods

We used ANOVA for comparisons of continuous variables by protein intake category and chi-square tests for comparisons of categorical variables by protein intake category. We used a time-to-event (Cox) model to determine the association between protein intake and risk of mortality. Participants were followed until death, loss to follow-up, or 10 year follow-up, whichever occurred first. We considered crude and full confounder-adjusted models. Total protein was the primary exposure variable, while protein intake by source (dairy, non-dairy animal, plant) were secondary exposure variables. Our analysis considered protein intake (and protein intake by source) as a percent of total energy intake (%TEI), while TEI was included as an adjustment variable in all multivariate models to account for the correlations between energy intake and the exposure and outcome. Models were run with total protein intake %TEI as a standardized variable with SD=2.9%TEI. For comparison purposes, models considering protein by source were parameterized to have the same units as for total protein, i.e., 1 unit=2.9%TEI, and all three variables were included in a single model. We assessed all continuous variables for possible non-linearity using higher order terms and fractional polynomials. The RDA for dietary protein is 0.8 g/kg/d since requirements vary by body size. It has been suggested that this threshold may be too low (20, 21), and therefore we considered 3 groups base on intake: low (<0.8g/kg/d), intermediate (≥0.8 g/kg/d and <1.0 g/kg/d), and high (≥1.0 g/kg/d).

Results

The baseline cohort had mean (SD) age of 73.6 (5.8) years, while the mean (SD) energy intake was 1621 (610) kcal/d, and the mean (SD) total protein intake was 64.7 (25.8) g/d and 0.79 (0.33) g/kg/d. Protein intake was low compared to the RDA of 0.8 g/kg/d, with 3329 (57.5%) men having intakes below the RDA. Converting to %TEI, the mean (SD) total protein intake was 16.1 (2.9) %TEI, while the mean (SD) dairy protein intake was 3.6 (2.1) %TEI, the mean non-dairy animal protein intake was 6.2 (2.9) %TEI, and the mean plant protein intake was 6.3 (1.7) %TEI. Baseline characteristics of the study sample stratified by level of protein intake are shown in Table 1. Participants with higher protein intake by category compared to those below the RDA were more active, and had lower mean height, weight, and BMI and were less likely to have 2 or more alcoholic drinks per day. The number of comorbidities, race, education, marital status, and smoking history did not vary by protein intake category.

Table 1
Baseline Demographics Overall and by Groups Determined by Protein Intake (N=5790)

Mean ± SD N (%)	Baseline Cohort N=5790	Protein Intake <0.8 g/kg N=3329	 0.8 g/kg to 0.99 g/kg N=1175	 ≥ 1.0 g/kg N=1286	p-value
Age (y)	73.6 ± 5.8	73.5+/- 5.7	73.8+/- 5.9	74.0 +/- 6.1	0.02
Non-Hispanic White	5194 (89.7)	2965 (89.1)	1071 (91.2)	1158 (90.1)	0.12
College educated	4412 (76.2)	2535 (76.2)	891 (75.8)	986 (76.7)	0.88
Married	4767 (82.3)	2754 (82.7)	969 (82.5)	1044 (81.2)	0.46
Ever smoker	3622 (62.6)	2091 (62.8)	735 (62.5)	796 (61.9)	0.84
Alcohol use ≥ 2 drinks/day	672 (11.6)	416 (12.5)	139 (11.8)	117 (9.1)	0.005
Height (cm)	174.2 +/- 6.8	174.6 +/- 6.8	174.1 +/- 6.6	173.1 +/- 6.7	<0.0001
Weight (kg)	83.2 +/- 13.3	85.8+/- 13.4	81.3 +/- 12.2	78.3 +/- 12.2	<0.0001
Body mass index (kg/m^2)	27.4 +/- 3.8	28.1 +/- 3.9	26.8 +/- 3.5	26.1 +/- 3.5	<0.0001
PASE score	146.5 +/- 68.3	143.5 +/- 67.2	148.2 +/- 66.6	152.8 +/- 72.1	0.0001
Comorbidities					
0	1602 (27.7)	926 (27.8)	330 (28.1)	346 (26.9)	0.21
1	1997 (34.5)	1119 (33.6)	399 (34.0)	479 (37.3)	
2+	2191 (37.8)	1284 (38.6)	446 (38.0)	461 (35.8)	
Total energy intake (kcal/d)	1621 +/- 610	1289.31 +/- 373.3	1806.23 +/- 394.58	2311.6 +/- 613.8	<0.0001
Protein intake (g/d)	64.7 +/- 25.8	49.0 +/- 13.5	72.4+/- 11.8	98.5 +/- 23.6	<0.0001
Dairy protein intake (g/d)	14.0 +/- 8.6	11.0 +/- 6.4	16.1 +/- 8.3	19.8 +/- 10.2	<0.0001
Non-dairy animal protein intake (g/d)	25.2+/- 15.7	18.1 +/- 9.0	28.1 +/- 11.6	40.8 +/- 19.8	<0.0001
Plant protein intake (g/d)	25.6 +/- 12.0	19.9 +/- 7.2	28.3 +/- 8.6	37.9 +/- 14.3	<0.0001
Protein intake (% TEI)	16.1 +/- 2.9	15.5 +/- 2.8	16.4 +/- 2.7	17.4 +/- 2.9	<0.0001
Dairy protein intake (% TEI)	3.6 +/- 2.1	3.5 +/- 2.1	3.7 +/- 2.1	3.6 +/- 2.0	0.02
Non-dairy animal protein intake (% TEI)	6.2 +/- 2.9	5.7 +/- 2.6	6.4 +/- 2.9	7.3 +/- 3.4	<0.0001
Plant protein intake (% TEI)	6.3 +/- 1.7	6.2 +/- 1.64	6.3 +/- 1.6	6.6 +/- 1.9	<0.0001

* Expressed as a percentage of total energy intake (TEI); **ANOVA for continuous variables, chi-square for categorical variables

Protein and Mortality

During a maximum follow-up of 10 years there were 1611 deaths (473 cancer, 589 cardiovascular disease [CVD], and 549 other deaths). Table 2 shows the association between total protein intake and by source and all-cause and cause-specific mortality. Lower protein intake was associated with an increased risk of death, with unadjusted HR=1.11 (95% CI: 1.06, 1.17) and adjusted HR=1.09 (95% CI: 1.04, 1.14) per each unit=2.9% TEI decrement in protein intake. The association between protein intake and mortality did not vary by protein source; e.g. the adjusted HR for dairy protein was HR=1.08 (95% CI: 1.02, 1.14) per each unit=2.9% TEI decrement, and the estimates for non-dairy animal protein and plant protein were similar. The association between protein intake and cause-specific mortality varied slightly according to outcome; e.g., adjusted HR=1.13 (95% CI: 1.03, 1.25), HR=1.08 (95% CI: 0.99, 1.18), and HR=1.09 (95% CI: 1.00, 1.18) per each unit=2.9% TEI for cancer, CVD, and other mortality, respectively.

Table 3 shows the association between protein intake groups (below RDA, intermediate, high ≥1.0 g/kg/d). There were no statistically significant associations between protein intake groups and all-cause mortality, but those with protein intake below the RDA had nominally higher risk of mortality as compared with those with protein intake ≥1.0 g/kg/d, while those with intermediate protein intake had similar mortality risk. There was a statistically significant association between protein intake and cancer mortality; those with protein intake below the RDA had a higher risk of mortality HR=1.58 (95% CI: 1.12, 2.23) compared with those with protein intake above 1.0 g/kg/d, while those with moderate protein intake (e.g., at least the RDA but below 1.0 g/kg/d had similar mortality risk.

Table 2

The Association between Dietary Protein Intake (Total and by Source) and All-Cause and Cause-specific Mortality (N=5790)

Hazard Ratio (95% Confidence Interval)		All-cause Mortality N=1611	Cancer Mortality N=473	CVD Mortality N=589	Other Mortality N=549
Total protein*	Crude	1.11 (1.06, 1.17)	1.16 (1.06, 1.27)	1.10 (1.01, 1.19)	1.13 (1.03, 1.22)
	MV	1.09 (1.04, 1.15)	1.13 (1.03, 1.25)	1.08 (0.99, 1.18)	1.09 (1.00, 1.18)
*Protein intake by source***					
Dairy protein	Crude	1.04 (0.99, 1.10)	1.14 (1.03, 1.27)	1.00 (0.92, 1.10)	1.03 (0.94, 1.12)
Non-dairy animal protein		1.13 (1.06, 1.19)	1.14 (1.03, 1.27)	1.12 (1.02, 1.24)	1.16 (1.06, 1.28)
Plant Protein		1.09 (1.03, 1.15)	1.10 (0.99, 1.21)	1.13 (1.02, 1.24)	1.07 (0.98, 1.17)
Dairy protein	MV	1.08 (1.02, 1.14)	1.14 (1.02, 1.27)	1.04 (0.95, 1.15)	1.07 (0.97, 1.17)
Non-dairy animal protein		1.08 (1.02, 1.15)	1.12 (1.00, 1.24)	1.08 (0.98, 1.19)	1.08 (0.98, 1.20)
Plant Protein		1.07 (1.01, 1.13)	1.07 (0.96, 1.19)	1.09 (0.99, 1.21)	1.04 (0.95, 1.15)

* Calculated as a percentage of total energy intake (%TEI) and hazard ratio shown per 1 SD decrease =2.9% TEI in total protein intake; ** Protein intake by source variables all used same scale as for total protein, i.e. hazard ratio per 1 unit (2.9% TEI) decrease, and were all included in the same model; MV: Multivariate models adjusted for age, center, education, race, smoking, alcohol use, physical activity, weight, total energy intake, and comorbidities (diabetes, stroke, Parkinson's disease, hypertension, angina, myocardial infarction, heart failure, cancer, COPD).

Table 3

The Association between Protein Intake Categories and All-cause and Cause Specific Mortality Among Community Dwelling Older Men (N=5790)

		All-cause Mortality N=1611	Cancer Mortality N=473	CVD Mortality N=589	Other Mortality N=549
Low protein vs reference*	Crude	0.93 (0.82, 1.05)	1.02 (0.82, 1.28)	0.99 (0.80, 1.21)	0.81 (0.67, 0.99)
Moderate protein vs reference**		0.88 (0.76, 1.03)	0.90 (0.68, 1.20)	0.87 (0.67, 1.14)	0.87 (0.69, 1.11)
Low protein vs reference*	MV	1.18 (0.98, 1.42)	1.58 (1.12, 2.23)	1.04 (0.76, 1.43)	1.11 (0.82, 1.50)
Moderate protein vs reference**		1.00 (0.85, 1.18)	1.11 (0.81, 1.52)	0.94 (0.71, 1.25)	1.04 (0.79, 1.35)

* Low protein (<0.8 g/kg) vs reference (≥ 1.0 g/kg); ** Moderate protein intake (0.8 g/kg to 1.0 g/kg) vs reference (≥ 1.0 g/kg); MV: Multivariate models adjusted for age, center, education, race, smoking, alcohol use, physical activity, weight, total energy intake, and comorbidities (diabetes, stroke, Parkinson's disease, hypertension, angina, myocardial infarction, heart failure, cancer, COPD).

Discussion

We found that low protein intake as %TEI was associated with modestly increased mortality among a group of community-dwelling men age 65 y and older and did not vary by source of protein intake (dairy vs non-dairy animal vs plant). These results support the findings by Levine et al who investigated protein intake and mortality in the Third National Health and Nutrition Examination Survey (NHANES III) cohort and found that there was a statistically significant interaction between age and protein for cancer mortality and all-cause mortality (15). In particular, among adults age 50-65, higher animal protein intake was associated with an increased risk of all-cause mortality, whereas there was no association among adults age 66 and over. Our findings are contrary to many of the findings in non-geriatric populations (13, 14, 22), in which high protein intake was associated with increased, not decreased risk of mortality.

The non-geriatric studies referred to above are based on studies of adults who were younger than those in the present cohort; hence, even if an age interaction was tested they would be limited by the age range in the source population. In addition, many studies combined men and women, and while many assessed for a sex interaction, few considered more complicated interactions involving sex and other variables. Other studies have noted possible non-linear relationships. Hernandez-Alonso et al examined the association between protein intake and mortality among 7447 men and women with mean age 67 y and found that there was a non-linear association between protein intake and mortality so that in the unadjusted model low intakes were associated with higher mortality, while in fully adjusted models higher intake were associated with higher mortality (23).

At present the RDA for protein intake for adults is 0.8 g/kg/d (Dietary Reference Intakes). Some professional societies have endorsed increasing the recommended protein intake among older adults to 1.0 g/kg/d (20, 21) based on further assessment of protein requirements. Protein requirements vary by age and

health status, including chronic condition, immobility, and critical illness. Many conditions are associated with higher requirements with the notable exception of kidney disease, in which protein intake should be limited. In this study men who had an intake below the RDA (expressed as g/kg body weight) had a nominally but not significant higher risk of mortality compared with those with intakes above 1.0 g/kg/d, thus showing a possible gradient of mortality risk present between low intake (below the RDA) and high intake (above 1.0 g/kg/d) groups. Furthermore, when considering cancer mortality, men who had an intake below the RDA had a substantially higher risk of mortality compared to those with intakes above 1.0 g/kg/d. There were no conclusive findings with respect to mortality for those in the intermediate group, whose mortality more nearly matched those with high protein intake.

Several studies have shown that the association between protein intake and mortality may vary according to protein source. Etemadi et al examined the association of meat intake specifically with mortality in a cohort (316 505 men and 220 464 women) aged 50-71 years and found that a higher intake of red meat (processed or unprocessed), heme iron, and nitrates/nitrite were all associated with increased risk of mortality whereas higher intake of other meats (e.g. chicken, turkey, fish) was associated with lower mortality (24). A study by Farvid et al examined the association between various high-protein foods and mortality and found differential effects, notably an increased mortality risk associated with higher intake of eggs and a lower mortality risk association with higher intake of fish (25). Schwingshackl et al performed a meta-analysis of prospective studies and findings were consistent with both of the studies (26). Such findings may be attributable to the specific food source per se or may be attributable to other foods with correlated intake and are consistent with associations found using dietary patterns (i.e., egg intake may be a marker for a Western dietary pattern while fish intake may be a marker for a Prudent dietary pattern). Several meta-analysis have looked at the associations between red-and processed meat, noting overall adverse effects together with substantial uncertainty (27, 28). As noted above, there are plausible sources of heterogeneity related to age and sex. Experimental studies have shown that specific amino acid profile or protein type impacts subsequent protein metabolism (29, 30). These studies have limited generalizability to diet, since it is not possible to separate out protein profile in particular from the other nutrients found in the same food source, i.e., high protein of meats might be offset by high saturated fat.

The strengths of this study are the inclusion of major potential confounders and a large sample of community-dwelling older men with a long follow-up and more than adequate number of events. We assessed protein intake by source, accounting for different nutrient profiles (i.e., micronutrients and distribution of specific proteins) of the three major sources (dairy, non-dairy animal, plant). Confounding by age and sex is limited to due study sample. Study limitations include the use of a FFQ at a single point in time to assess long-term dietary intake which may had led to misclassification of protein intake. We also did not assess the timing of protein consumption throughout the data and therefore are unable to address questions regarding quantity vs. timing. The study design was an observational cohort study with all the usual limitations, including the possibility of selection bias and residual confounding. The generalizability of the present study is limited to healthy community-dwelling older men. The cohort was also mostly non-Hispanic white, and therefore we were unable to assess potential racial/ethnic differences.

In summary, we found that lower protein intakes regardless of source was associated with a modestly increased risk of mortality among community-dwelling older men. This increased risk was especially notable for cancer mortality.

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