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Research Article

Protein Intake and Functional Integrity in Aging: The Framingham Heart Study Offspring

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Abstract

Background: Higher protein intake is linked to maintenance of muscle mass and strength, but few studies have related protein to physical function and disability in aging.

Methods: In participants of the Framingham Heart Study Offspring, we examined associations between protein intake (g/d), estimated from food frequency questionnaires, and maintenance of functional integrity, as a functional integrity score based on responses to 17 questions from Katz Activities of Daily Living, Nagi, and Rosow-Breslau questionnaires, repeated up to five times (1991/1995–2011/2014) over 23 years of follow-up. Cox proportional hazard models were used to estimate risk of incident loss of functional integrity (functional integrity score ≤ 15th percentile).

Results: In 2,917 participants (age 54.5 [9.8] years), baseline protein intake was 77.2 (15.6) g/d. The functional integrity score (baseline, mean 98.9, range 82.4–100.0) was associated with objective performance (gait speed, grip strength) and lower odds of falls, fractures, and frailty. Across follow-up, there were 731 incident cases of loss of functional integrity. In fully adjusted models, participants in the highest category of protein intake (median 92.2 g/d) had 30% lower risk of loss of functional integrity (hazard ratio [95% confidence interval] 0.70 [0.52, 0.95], p trend = .03), versus those with the lowest intake (median 64.4 g/d). However, sex-stratified analyses indicated the association was driven by the association in women alone (hazard ratio [95% confidence interval] 0.49 [0.32, 0.74], p trend = .002) and was nonsignificant in men (hazard ratio [95% confidence interval] 1.14 [0.70, 1.86], p trend = .59).

Conclusions: Higher protein intake was beneficially associated with maintenance of physical function in middle-aged, high-functioning U.S. adults over the span of two decades. This association was particularly evident in women.

Keywords: Diet, Dietary protein, Physical function, Activities of daily living, Frailty

Although healthy aging is easily recognized, it is not readily characterized. Aging is associated with many physiologic and metabolic changes that are in part nutritionally driven. To date, the role of dietary protein in aging has focused largely on musculoskeletal aging. Evidence suggests benefits of protein intake on preservation of lean mass and strength (ie, attenuating risk of sarcopenia) (1–6) and bone health (7–10). Minimizing these age-related musculoskeletal changes is a central component of maintaining physical function and independence, and preventing frailty, in older individuals.

However, evidence of a role for protein and/or protein above recommended intake in preventing frailty, disability, or physical dysfunction is limited. Several cross-sectional studies have reported inverse relationships between protein intake and frailty (11–14), physical function (15), and quality of life (15). A recent secondary analysis of 4-month combined protein and resistance training trial in older women observed better health-related quality of life, specifically in the domain of physical quality of life (ie, function), with higher protein intake (16). But there are relatively few longer-term

prospective studies of protein intake and frailty (17–19) or function and/or mobility (20–26). All but one (21) of these studies observed favorable associations with higher protein intake; three (17,20,22) were conducted in women only, another (19) only in men; several focused on narrow functional domains such as strength and/or mobility (21–24); and all but three (20,25,26) involved less than 10 years of follow-up.

Within subsets of the present study population, the Framingham Heart Study Offspring cohort, we have previously observed beneficial associations of protein intake and leg lean mass and isometric quadriceps strength (2), as well as 6-year change in grip strength (5). In the present study, we use data from a very long prospective cohort (>20 years) to examine associations between protein intake and maintenance of physical function and prevention of physical disability with the hypothesis that higher intake would be associated with maintenance of function.

Methods

Study Participants

The Framingham Heart Study Offspring cohort of the National Heart, Lung, and Blood Institute is a community-based, longitudinal study of cardiovascular disease that began in 1971 (27). In the fifth examination cycle ("baseline," 1991–1995) of the Offspring cohort, 3,799 participants underwent a standard medical examination, consisting of laboratory and anthropometric assessments, as well as dietary intake assessment. Participants were followed through the ninth exam (2011–2014). Individuals were excluded from the present analysis if they had missing or invalid dietary data (baseline excluded n = 381); were missing covariates (baseline excluded n = 140); or had the primary outcome at baseline (n = 361). The final sample size in the primary analysis was 2,917 participants.

The original Offspring protocols were approved by the Institutional Review Board at Boston University Medical Center, and written informed consent was obtained from all participants. The present study protocol was reviewed and approved by the Tufts University Health Sciences Institutional Review Board.

Dietary Measures

The Harvard semiquantitative, 126-item food frequency questionnaire (FFQ) was used to assess dietary intake at each exam, beginning at exam 5 (28). The FFQs included lists of foods for which participants were asked to report the frequency of consumption of standard serving sizes of each item over the previous year. The range of possible responses was never or less than one time per month to greater than or equal to six times daily. Invalid FFQs were those that estimated daily caloric intake as less than 600 kcal/d, or greater than or equal to 4,000 kcal/d for women, greater than or equal to 4,200 kcal/d for men, or those that had greater than or equal to 12 blank items (29). Total protein intake was calculated as the sum of protein intake from contributions to protein from individual line items. The validity and reliability of the FFQs have been described (28,30–32). The relative validity of the FFQ for protein intake shows reasonable correlation with estimates from dietary records and urinary nitrogen (28,30–32).

All foods and nutrients, including protein intake, were energy adjusted using the residual method (29,33). We created quartile categories of the cumulative average of the reported intake from each exam. Other dietary factors derived from the FFQ included estimated intake of energy, carbohydrates, fats, and the dietary Glycemic Index (34,35).

Measures of Functional Integrity and the Functional Integrity Score

There were 17 measures of function consistently inquired about and therefore available, in principle, for analysis at every exam: five measures of Activities of Daily Living (dressing, bathing, eating, transferring, toileting); three Rosow-Breslau measures (heavy work, walk up and down flight of stairs, walk a half mile without help); and nine Nagi measures (pulling/pushing large objects, stooping, reaching below shoulder, reaching above shoulder, writing or fingering small objects, standing for long periods, sitting for long periods, lifting or carrying less than 10 and greater than 10 lbs; Supplementary Table 1).

The range of ease or difficulty associated with the functional measures was typically assigned values from 0 to 3 for Activities of Daily Living and Nagi measures and 0 or 1 for Rosow-Breslau measures (Supplementary Table 1). Each of these measures was standardized from lowest to highest function with a minimum score of 0 and a maximum score of 10. The standardized measures were then summed (possible range 0–170 when all 17 questions were answered). This sum was then expressed as a percentage of the maximum sum of answered questions, to generate the functional integrity score (FIS). For example, if a participant answered 15 out of 17 questions and expressed independent abilities (ie, 10 points) for each question, with no obvious systematic basis for nonresponse to the two unanswered questions, that participant's total possible score was (15 questions × 10 points per question)/150 possible points, with FIS equal to 100%. We explored three possible cut points of the score: the 10th, 15th, and 20th percentiles. Because all cut points displayed a similar degree of validity, we selected the intermediate cut point of less than or equal to 15th percentile as our primary outcome to balance the degree (and prevalence) of baseline and incident loss of function and to maintain a sufficient sample size of participants with and without baseline and incident loss of function. Sensitivity analyses included cut points of less than or equal to 10th percentile and less than or equal to 20th percentile. The influence of each individual functional component of the FIS on the total FIS is presented in Supplementary Table 2.

Measures Related to Validity of the FIS

We assessed the validity of the FIS in the total population and sex specifically in several ways: (a) using logistic regression, examining the odds of reporting a fall or a fracture in the prior year, per FIS category (eg, ≤15th vs >15th percentile); (b) using logistic regression, examining the odds of being frail as defined by Fried and colleagues (36), at exam 8 or 9; and (c) assessing adjusted means of objective physical performance—gait speed and grip strength—in FIS categories at exams 8 and 9. We would expect that if the FIS is a valid measure of functional integrity, a higher score would be associated with lower odds of falls, fractures, and frailty and with faster gait and stronger grip.

Timed walk tests and hand-grip tests were conducted at exams 8 and 9. Gait speed (meter per second) was derived from the average of two timed walk tests, measured to the nearest hundredth of a second, in which participants walked 4 m at a usual pace. Quick gait speed (meter per second) was derived from a single timed walk of 4 m at a rapid pace ("as fast as you can"). To assess grip strength (kilogram), participants were asked to squeeze a Jamar dynamometer (Lafayette Instrument Co, Lafayette, IN) as hard as possible, three times in each hand. The average of the three values for each hand was calculated.

We used an assessment of frailty previously used in the Framingham Heart Study (37), which included meeting greater than or equal to three of five primary characteristics characterized by Fried and colleagues (36): (a) unintentional weight loss; (b) exhaustion/fatigue; (c) low physical activity; (d) slow gait speed; and (e) weak hand-grip strength.

Falls (exams 5–7) and fractures (exams 5–9) were defined as self-reports of "yes," "not sure," or "maybe" responses to "In the past year have you accidentally fallen and hit the floor or ground?" and "Since your last clinic visit have you broken any bones?", respectively.

Covariates

Potential confounders of the relationship between protein intake and functional integrity, as well as other risk factors for loss of functional integrity, were considered as covariates, including age (year); sex (male/female); body mass index (BMI; calculated as measured weight [kilogram] divided by height [meter] squared [kilogram per square meter]); waist circumference (centimeter); smoking status (in the prior year, yes/no); systolic blood pressure (millimeters of mercury); total:high-density lipoprotein (HDL) cholesterol; pharmacological treatment for hypertension, dyslipidemia, or diabetes (all yes/no); physical activity (score based on sum of moderate and vigorous metabolic equivalent task-hours per week), highest educational level completed; self-rated health status (four categories ranging from "poor" to "excellent," derived from the self-administered Short Form [SF]-12 Health Survey question); and the date of the first adjudicated cardiovascular event (including myocardial infarction, angina pectoris, intermittent claudication, cerebrovascular accident, stroke, intracerebral or subarachnoid hemorrhage, transient ischemic attack, cerebral embolism, or congestive heart failure). Baseline values, as well as updated values of each potential covariate except for sex, were used in analyses. Missing values for covariates at a given exam were carried forward from the prior exam.

Statistical Analysis

The validity of the FIS was assessed as described earlier, using multivariate logistic regression for self-reported measures. Baseline (exam 5) participant characteristics adjusted for age, sex, and energy intake are presented across quartile categories of average protein intake. Tests for linear trend across increasing categories of intake were performed by assigning the median value of intake within each category and treating these as a continuous variable.

To assess the relationship between protein intake in quartile categories and risk of loss of functional integrity, we used Cox proportional hazards regression models, with the primary outcome of falling into FIS less than or equal to 15th percentile for 10-year age group. The lowest quartile category of protein intake was considered as reference group. Follow-up time was calculated from the date of exam 5 attendance to the date of the event (ie, the exam date at which the FIS ≤ 15th percentile), date of the last follow-up exam (up to exam 9 date), or date of death.

Statistical models were adjusted as follows: Model 1 was adjusted for baseline age, sex, cumulative average energy intake, and the baseline FIS. Model 2 (risk factor model) was adjusted as for model 1, plus baseline and updated variables of BMI, waist circumference, systolic blood pressure, treatment for hypertension, total:HDL cholesterol, treatment for hyperlipidemia, smoking status, and physical activity. Model 3 (diet model) was further adjusted for cumulative average intake of saturated fat, monounsaturated fat, polyunsaturated fat, and the Glycemic Index of the overall diet. Model 4 (socioeconomic risk model) was adjusted as for model 3, plus highest education completed and the most recent self-rated health status. Model 5 further adjusted for a cardiovascular event prior to the outcome. All models were stratified by 5-year age groups. *p* Values for trend across quartile categories of intake were estimated using the median value in each quartile category, modeled as a continuous variable.

Although we did not have a priori hypotheses regarding differential associations by age or by sex, we reran primary analyses within strata of age (median cut point) and sex and tested for statistical interaction by age and by sex. In addition, we ran sensitivity analyses using outcomes of alternative FIS cut points of less than or equal to 10th percentile and less than or equal to 20th percentile. In secondary analyses, we analyzed protein intake expressed in grams per kilogram body weight per day, which is the unit used in U.S. dietary recommendations (38). The same models were used, except change in weight was substituted for updated BMI values to avoid confounding by the shared kilogram units in the exposure.

All analyses were conducted in SAS (version 9.4, SAS Institute, Cary, North Carolina). Two-tailed statistical significance was set at *p* value of less than .05.

Results

Baseline Age and Sex-Adjusted Characteristics of Participants

At baseline, participants were, on average, age 54.5 [9.8] years (range 26.0–81.0 years), 51% were female, and the average BMI was 27.2 [4.7] kg/m^2. Average protein intake at baseline was 77.2 [15.6] g/d or 1.04 (0.31) g/kg body weight/d, and 78% of participants met the recommended dietary allowance. The baseline FIS was 98.9 [2.1] (range 82.4–100.0). Across increasing quartile categories of protein intake, participants tended to be younger, have a higher BMI, waist circumference, and total:HDL cholesterol ratio, and were less likely to be current smokers; they were more likely to be female, have type 2 diabetes, and report consuming fewer carbohydrates and more fat (Table 1).

Validity of the FIS

Higher FIS was associated with significantly lower odds of having reported a fall (exams 5, 6, or 7) or a fracture (every exam) occurring in the prior year, adjusting for age, sex, and BMI. This was consistent at across FIS cut points (ie, 10th, 15th, and 20th percentiles; Supplementary Table 3). In addition, higher FIS was strongly associated with lower odds of frailty as defined by Fried and colleagues (36) (Supplementary Table 4). For example, those with FIS greater than 15th percentile had 14% of the odds (odds ratio [95% confidence interval] 0.14 [0.09, 0.22]) of being frail compared with those with FIS less than or equal to 15th percentile.

With respect to the objective functional measures of gait speed and grip strength at exams 8 and 9, those with less than or equal to 15th percentile of FIS at each of exams 8 and 9 had slower gait speeds and weaker grip strengths than those with greater than 15th percentile (Supplementary Table 5). Results of validity analyses were broadly consistent in both sexes when assessed separately (data not shown).

Protein Intake and Functional Integrity

Across up to 23 years of follow-up, there were 731 incident losses of functional integrity, defined as FIS less than or equal to 15th percentile.

Table 1. Adjusted Means of Baseline Characteristics per Quartile Category of Cumulative Averaged Protein Intake in 2,917 Participants of the Framingham Heart Study Offspring Cohort[a]

Characteristic	Quartile Category of Protein Intake (Median Energy Adjusted, g/d)				*p* Trend
	64.4	74.4	82.0	92.2	
n	717	725	752	723	
Age, y	56.2 (0.4)	54.4 (0.4)	54.4 (0.4)	53.1 (0.4)	<.001
Sex, % female	39 (2)	47 (2)	56 (2)	62 (2)	<.001
BMI, kg/m^2	26.6 (0.2)	26.6 (0.2)	27.6 (0.2)	27.9 (0.2)	<.001
Weight, kg	75.7 (0.5)	75.6 (0.5)	78.7 (0.5)	79.3 (0.5)	<.001
Waist circumference, in	36.1 (0.2)	35.9 (0.2)	36.7 (0.2)	36.8 (0.2)	<.001
Smoking, current, %	27 (1)	17 (1)	16 (1)	15 (1)	<.001
Physical activity, MET-h/wk	35.2 (0.2)	35.1 (0.2)	34.8 (0.2)	34.8 (0.2)	.18
Clinical characteristics					
Cholesterol, mg/dL	205.1 (1.3)	203.6 (1.3)	205.5 (1.3)	203.6 (1.3)	.63
HDL cholesterol, mg/dL	50.9 (0.5)	50.0 (0.5)	50.1 (0.5)	49.4 (0.5)	.04
Total:HDL cholesterol	4.4 (0.1)	4.4 (0.1)	4.5 (0.1)	4.5 (0.1)	.04
LDL cholesterol, mg/dL	126.3 (1.2)	126.2 (1.2)	127.0 (1.2)	126.5 (1.2)	.80
Triglycerides, mg/dL	145.2 (3.8)	140.5 (3.8)	145.6 (3.7)	145.5 (3.8)	.76
Dyslipidemia treatment, %	7 (1)	7 (1)	7 (1)	8 (1)	.70
SBP, mmHg	126.5 (0.7)	126.2 (0.6)	125.2 (0.6)	126.5 (0.7)	.73
DBP, mmHg	74.4 (0.4)	74.5 (0.4)	74.5 (0.4)	75.1 (0.4)	.19
Hypertension treatment, %	18 (1)	19 (1)	19 (1)	19 (1)	.74
Diabetes, %	1 (1)	3 (1)	3 (1)	5 (1)	<.001
Reported falling in prior year, %	17 (1)	15 (1)	20 (1)	19 (1)	.05
Reported fracture in prior year, %	5 (1)	7 (1)	5 (1)	5 (1)	.53
Functional integrity score	98.9 (0.1)	99.1 (0.1)	98.8 (0.1)	99.0 (0.1)	.80
Dietary characteristics					
Energy, kcal/d	1,930 (23)	1,795 (22)	1,809 (22)	1,973 (23)	.16
Protein, g/d	61.9 (0.4)	73.5 (0.4)	80.8 (0.4)	92.6 (0.4)	<.001
Protein, g/kg body weight/d	0.86 (0.01)	1.01 (0.01)	1.08 (0.01)	1.22 (0.01)	<.001
Protein, % energy	13.5 (0.1)	16.0 (0.1)	17.6 (0.1)	19.9 (0.1)	<.001
Meets RDA for protein, %	53 (1)	79 (1)	86 (1)	93 (1)	<.001
Carbohydrates, g/d	248.0 (1.5)	242.8 (1.5)	235.8 (1.5)	225.4 (1.5)	<.001
Carbohydrates, % energy	52.9 (0.3)	51.9 (0.3)	50.4 (0.3)	48.3 (0.3)	<.001
Glycemic Index	55.3 (0.1)	55.2 (0.1)	54.4 (0.1)	53.9 (0.1)	<.001
Fat, g/d	60.2 (0.5)	62.4 (0.5)	63.6 (0.5)	63.8 (0.5)	<.001
Fat, % energy	29.0 (0.2)	30.0 (0.2)	30.4 (0.2)	30.5 (0.2)	<.001
PUFA, g/d	11.6 (0.1)	12.0 (0.1)	12.3 (0.1)	12.3 (0.1)	<.001
MUFA, g/d	22.4 (0.2)	23.1 (0.2)	23.5 (0.2)	23.6 (0.2)	<.001
SFA, g/d	21.2 (0.2)	21.8 (0.2)	22.1 (0.2)	22.0 (0.2)	.01

Notes: BMI = body mass index; DBP = diastolic blood pressure; HDL = high-density lipoprotein; LDL = low-density lipoprotein; MET = metabolic equivalent task; MUFA = monounsaturated fatty acid; PUFA = polyunsaturated fatty acid; RDA = recommended dietary allowance; SBP = systolic blood pressure; SFA = saturated fatty acid. Values presented as the adjusted mean (*SE*), unless otherwise noted.

[a]Characteristics were age and sex adjusted, except for age and sex, which were mutually adjusted. Dietary characteristics were further adjusted for energy intake.

In a model adjusted for baseline age, sex, cumulative average energy intake, and the baseline FIS, higher protein intake was associated with lower risk of loss of function (model 1, Q4 vs Q1 [ref], hazard ratio [95% confidence interval] 0.67 [0.55, 0.83], *p* trend < .001; Table 2). This trend remained after further adjusting for baseline and updated health-related factors, including BMI, as well as other dietary variables. Trends in the total population were largely consistent when loss of functional integrity was defined at alternate cut points of FIS less than or equal to 10th percentile or less than or equal to 20th percentile (Supplementary Table 6). Tests of interaction were not statistically significant between protein intake and age (*p* interaction > .10).

However, tests of interaction were statistically significant between protein intake and sex (*p* interaction < .05; Table 3). In stratified analyses by sex, associations were statistically significant in women only across all models (model 5, Q4 vs Q1 [ref], hazard ratio [95% confidence interval] 0.49 [0.32, 0.74], *p* trend = .002) and not significant in men.

When protein intake was expressed in grams per kilogram of body weight per day, a significant linear trend was evident in the basic and risk factor models in the total population (Supplementary Table 7). However, after adjusting for other dietary characteristics, linear trends were attenuated. However, again, there was a significant statistical interaction with sex. Stratified analyses indicated results consistent with protein expressed in grams per day, with significant inverse associations in women across all models (model 5, Q4 vs Q1 [ref], hazard ratio [95% confidence interval] 0.60 [0.33, 1.09], *p* trend = .01) and no associations in men (Supplementary Table 8).

Discussion

In this analysis of participants in the Framingham Heart Study Offspring, we observed that higher protein intake across adulthood was associated with significantly lower risk of losing functional integrity in aging, an association that was evident only among women

Table 2. Hazard Ratios (95% Confidence Intervals) of Incident Loss of Functional Integrity by Quartile Category of Average Protein Intake in Participants of the Framingham Heart Study Offspring Cohort[a]

	Quartile Category of Average Protein Intake (Median, g/d)				
	64.4	74.4	82.0	92.2	*p* Trend
Events, *n*	199	171	188	173	
Person-years	9,653	10,666	11,072	10,580	
Crude rate per 100 person-years	2.06	1.60	1.70	1.64	
Model 1	1 (ref)	0.75 (0.61, 0.93)	0.73 (0.59, 0.89)	0.67 (0.55, 0.83)	<.001
Model 2	1 (ref)	0.79 (0.60, 1.04)	0.73 (0.56, 0.95)	0.56 (0.42, 0.74)	<.001
Model 3	1 (ref)	0.84 (0.64, 1.10)	0.83 (0.63, 1.09)	0.67 (0.50, 0.91)	.01
Model 4	1 (ref)	0.87 (0.66, 1.15)	0.91 (0.69, 1.21)	0.70 (0.51, 0.94)	.03
Model 5	1 (ref)	0.90 (0.68, 1.19)	0.92 (0.70, 1.22)	0.70 (0.52, 0.95)	.03

Note: [a]Models were adjusted as follows: model 1 was adjusted for baseline age, sex, cumulative average energy intake, and the baseline functional integrity score. Model 2 (risk factor model) was adjusted as for model 1, plus baseline and updated variables of body mass index, waist circumference, systolic blood pressure, treatment for hypertension, total:high-density lipoprotein cholesterol, treatment for hyperlipidemia, smoking status, and physical activity. Model 3 (diet model) was further adjusted for cumulative average intake of saturated fat, monounsaturated fat, polyunsaturated fat, and the Glycemic Index of the overall diet. Model 4 (socioeconomic risk model) was adjusted as for model 3, plus highest education completed and the most recent self-rated health status. Model 5 further adjusted for a cardiovascular event prior to the outcome. All models were stratified by 5-y age groups. *p* Values for trend across quartile categories of intake were estimated using the median value in each quartile category, modeled as a continuous variable.

Table 3. Hazard Ratios (95% Confidence Intervals) of Incident Loss of Functional Integrity by Quartile Category of Average Protein Intake in Participants of the Framingham Heart Study Offspring Cohort, Stratified by Sex[a]

	Quartile Category of Average Protein Intake (Median, g/d)				
Sex	64.4	74.4	82.0	92.2	*p* Trend
Female					
Events, *n*	120	91	125	116	
Person-years	3,542	4,836	6,133	6,597	
Crude rate per 100 person-years	3.39	1.88	2.04	1.76	
Model 1	1 (ref)	0.57 (0.43, 0.76)	0.61 (0.47, 0.79)	0.51 (0.39, 0.66)	<.001
Model 2	1 (ref)	0.57 (0.38, 0.83)	0.59 (0.42, 0.84)	0.39 (0.27, 0.57)	<.001
Model 3	1 (ref)	0.62 (0.42, 0.91)	0.70 (0.48, 1.01)	0.49 (0.32, 0.74)	.002
Model 4	1 (ref)	0.63 (0.43, 0.94)	0.74 (0.51, 1.08)	0.48 (0.32, 0.73)	.002
Model 5	1 (ref)	0.64 (0.43, 0.95)	0.75 (0.52, 1.10)	0.49 (0.32, 0.74)	.002
Male					
Events, *n*	79	80	63	57	
Person-years	6,111	5,830	4,939	3,983	
Crude rate per 100 person-years	1.29	1.37	1.28	1.43	
Model 1	1 (ref)	1.01 (0.73, 1.38)	0.93 (0.66, 1.31)	1.03 (0.73, 1.46)	.99
Model 2	1 (ref)	1.17 (0.78, 1.74)	1.02 (0.66, 1.56)	0.92 (0.59, 1.45)	.64
Model 3	1 (ref)	1.21 (0.81, 1.81)	1.09 (0.71, 1.70)	1.03 (0.65, 1.64)	.96
Model 4	1 (ref)	1.27 (0.84, 1.91)	1.24 (0.79, 1.96)	1.16 (0.71, 1.88)	.54
Model 5	1 (ref)	1.31 (0.87, 1.98)	1.23 (0.78, 1.94)	1.14 (0.70, 1.86)	.59

Note: [a]*p* Interaction between protein and sex < .05. Models were adjusted as follows: model 1 was adjusted for baseline age, cumulative average energy intake, and the baseline functional integrity score. Model 2 (risk factor model) was adjusted as for model 1, plus baseline and updated variables of body mass index, waist circumference, systolic blood pressure, treatment for hypertension, total:high-density lipoprotein cholesterol, treatment for hyperlipidemia, smoking status, and physical activity. Model 3 (diet model) was further adjusted for cumulative average intake of saturated fat, monounsaturated fat, polyunsaturated fat, and the Glycemic Index of the overall diet. Model 4 (socioeconomic risk model) was adjusted as for model 3, plus highest education completed and the most recent self-rated health status. Model 5 further adjusted for a cardiovascular event prior to the outcome. All models were stratified by 5-y age groups. *p* Values for trend across quartile categories of intake were estimated using the median value in each quartile category, modeled as a continuous variable.

in sex-stratified analyses. This association was apparent even after adjusting for other major age-related disease characteristics, such as diabetes and heart disease, as well as other dietary components, and when considering protein expressed in terms of grams per kilogram of body weight, the protein unit used by United States and other dietary guidelines (38). Our results are consistent with a large body of literature that indicates that higher protein intake is related to lower risk of frailty, and adds to the nascent body of evidence relating protein intake to the maintenance of functional aspects of daily living and independence.

To our knowledge, our results present the longest duration of follow-up in a longitudinal study to examine associations between protein intake and measures of self-reported function including activities of daily living in community-based populations. Three other studies have looked at protein intake and function over follow-up periods of more than 10 years (20,25,26). Beasley and

colleagues examined protein intake in more than 110,000 women (age 50–79 years at baseline) participating in the Women's Health Initiative (WHI) study in relation to self-reported physical function, finding that across approximately 11 years of follow-up, women in the highest category of protein intake (15.4%–22.3% energy [81.7 (9.9) g/d, 1.19 (0.20) g/kg body weight/d]) experienced about half the rate of annual decline in their self-reported function compared with those with the lowest intake (6.6%–13.1% energy [71.5 (12.1) g/d, 0.97 (0.17) g/kg body weight/d]; 20). In addition, in a subset of women with available measures, those with the highest protein intake experienced slower declines in grip strength.

Two recent analyses in the Framingham Heart Study Offspring Cohort have examined either protein food sources (25) or total protein intake (26) earlier in the life course in relation to functional declines over later adulthood. Using dietary data from 3-day food records collected up to 12 years preceding assessment of incident functional decline, the authors reported that those with the highest intakes of animal food sources of protein (≥7 vs <7 ser/d in men, ≥6 vs <6 ser/d in women) had 29%–35% lower risk of functional decline (25), and those with the highest total protein intake (≥1.2 vs <0.8 g/kg body weight/d) had 41% lower risk of becoming dependent in at least one functional task (26) over a median 13 years of follow-up. Together with our results, these data appear to indicate that higher protein intake, especially in women, during periods both earlier in the life course and across adulthood, may be related to subsequent lower risks of functional decline.

Other studies have examined these relationships over shorter follow-up periods, with results broadly supporting a role of higher protein in mitigating incident disability. Houston and colleagues examined protein intake and self-reported mobility across 6 years of follow-up in older men and women (age 70–79 years) in the Health, Aging, and Body Composition (Health ABC) study, observing that those with the lowest protein intake (<0.7 g/kg body weight/d) had 86% higher risk of incident limitation than those in the highest protein intake category (≥1.0 g/kg body weight/d) (23). Importantly, mean protein intake was 0.91 g/kg body weight/d, and 43% reported intake less than the recommended 0.8 g/kg body weight/d.

In contrast, a study conducted in 2,726 older Chinese adults (age >65 years) examining protein intake in relation to 4-year changes in mobility reported no association between total, animal, or vegetable protein intake and change in any mobility measure (21). Notably, protein intake was quite high in this cohort: Median intake was 1.3 and 1.1 g/kg body weight/d in men and women, respectively, which may underlie the lack of observed associations.

Other longitudinal studies in community-based populations have used objective measures (eg, strength, speed) to assess function and/or mobility and protein intake. As mentioned, within subsets of the present study population, we previously observed beneficial associations of protein intake and quadriceps strength (2), as well as 6-year change in grip strength (5). A recent study in 1,741 community-dwelling older Quebecois adults (age 67–84 years) observed beneficial associations between total protein intake and 3-year changes in physical performance as assessed by a composite score of muscle strength in both men and women (24). Mean protein intake was approximately 1.05 and 1.04 g/kg body weight/d in men and women, respectively.

Another study of 554 women (age 65–72 years) assessed 3-year changes in strength and mobility observed that those with higher protein intake (≥1.2 g/kg body weight/d) experienced less decline in grip strength and 6-m tandem walking speed, compared with those with moderate (0.81–1.19 g/kg body weight/d) or low intakes (≤0.8 g/kg body weight/d) (22). However, these associations were not significant after controlling for fat mass and were generally more evident in women without sarcopenia, suggesting fat mass may be playing a mediating role between protein intake and some aspects of function.

Interestingly, although our results in the total population were statistically significant, stratified analyses indicated that these results were driven by associations in women, rather than in men, despite few differences in baseline FIS or protein intake. However, a differential association by sex was observed in the Health ABC study (23) and in a prior Framingham Offspring report (26) discussed earlier. In both cases, the authors have suggested that such differences may be explained in part by differences in body composition (either fat-free mass or skeletal muscle mass) between men and women. Health ABC investigators have observed that lean mass adjusted for adiposity better predicted physical functioning deficits and incident disability than lean mass alone (39), whereas Framingham investigators have observed that skeletal muscle mass may be a mediator of protein-function relationships (26). These and other phenomena (25,40) potentially underlying observed differences are also supported by prior studies indicating that protein intake in men may have weaker associations with frailty (19), disability (25,26), and health-related quality of life, fatigue, or well-being (41). Furthermore, there is mixed evidence on the effects of protein or high-protein food intake on strength and lean mass in men alone or when compared with women (6,25,41).

With regard to frailty, in the present study, we validated our score of functional integrity against a commonly used measure of frailty, the Fried frailty phenotype (36). Although the concept of function captures related but distinct aspects of well-being, including mobility and independence, that are not part of the typical frailty phenotype, the relationship between protein intake and frailty merits at least brief discussion in the present context. A 2017 systematic review reported that three out of five studies examining protein intake and frailty in older adults found a beneficial association with higher intake (42). Notably, only one of these five studies was longitudinal (19). The three cross-sectional studies showing an association between protein intake and frailty generally observed benefits accruing at levels of at least 1 g/kg body weight/d (11), or 70 g/d (women) (13), or risk increasing at <66 g/d (men) or <55 g/d (women) (12). The remaining cross-sectional study conducted in 194 community-dwelling older participants reported that although total protein intake was not significantly associated with frailty, its mealtime distribution was a factor (14).

In the context of these previous findings, the present results have several implications, among them, that self-reports of physical impairment/difficulty are useful outcomes in observational cohorts, even in the absence of muscular/bone health measures or mass, or objective functional measures such as grip strength and walking speed. Indeed, gait speed and the physical function domain of the SF-36 were very recently shown to have similar predictive ability of future preclinical mobility disability in the Women's Health Initiative (43). From a clinical perspective, our results suggest that patient responses to these relatively straightforward questions could indicate a need for clinicians to pay greater attention to the protein content of their patients' diets to help preserve function for as long as possible, especially in women, although the sex-based differences we observed require further investigation in both observational and experimental settings. Furthermore, these data highlight the need to identify and target nutrition and exercise therapies within (and potentially before) the fifth decade of life to optimize healthy aging.

Our study has several strengths. First, we used repeated measures of both dietary intake and self-reported assessments of function across several decades of follow-up, thereby informing a life-course picture of dietary protein in relation to function into mid- and late-old age. To our knowledge, our results represent one of the most comprehensive, repeated self-reported assessments of dietary protein intake and function over time. We were able to validate our FIS at two time points against objective functional measures, as well as with self-reported falls and/or fractures at each exam.

The present study also has several limitations. Dietary data were derived from self-report by FFQs, thus come with inherent self-report biases and other limitations. However, no cost-effective or feasible alternative to measuring diet over this time period in this size of a population has been identified. We also could not assess the potential impact of mealtime distribution of protein, given our dietary assessment instrument, and mealtime distribution of protein may be relevant to frailty and related functional measures (44). The primary outcome of functional integrity was based on self-reported measures, which are subject to misreporting. However, previous studies support that such self-reports have a physiologic basis and can accurately predict preclinical disability and related impairment (45). In addition, it would have been interesting to assess the mediating impact of both body mass compartments (eg, lean vs fat mass) and muscle strength on the association between protein intake and functional measures because relationships between protein intake and strength and function may be partially, but not entirely mediated through muscle mass (44) and may help explain the differential associations by sex. However, such measures were available either at limited time points or in limited subsamples of the cohort, precluding a meaningful analysis across the entire population in the timeframe of interest. Women remain an understudied population within aging literature, and these results further underscore the need to include women as part of intervention studies related to diet, physical function, and aging. Finally, as with all observational studies, we cannot infer a causal relationship between protein intake and functional integrity and residual confounding is possible.

In conclusion, we observed favorable associations between protein intake and functional integrity over a greater than 20-year timeframe, notably in women. Our approach was one that relied on function-related questions, such as the ability to pull, lift, and do housework, asked repeatedly, and validated against objective physical performance as well as a well-characterized frailty phenotype. Optimizing protein intake may therefore play a role not just in preserving muscle mass and/or strength in aging, but also in maintaining functional integrity, protecting against frailty and falls, and ensuring independence.

Supplementary Material

Supplementary data is available at *The Journal of Gerontology, Series A: Biological Sciences* online.

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Conflict of Interest

P.F.J. is a member of the Danone North America Essential Dairy and Plant-Based Advisory Board. S.S. has received institutional grants from the Dairy Management and is also a member of the Nutrition Research Scientific Advisory Committee, National Dairy Council. D.B. is an employee of Danone North America. The authors declare no conflicts of interest.

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