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Biomarker-Calibrated Protein Intake and Physical Function in the Women’s Health Initiative

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Abstract

OBJECTIVES—Preserving physical function with aging may be partially met through modification in dietary protein intake.

DESIGN—Women’s Health Initiative Clinical Trials (CT) and Observational Study (OS).

SETTING & PARTICIPANTS—Women age 50–79 y ($n=134,961$) with dietary data and 1 physical function measure.

MEASUREMENTS—Physical function was assessed by short form RAND-36 at baseline and annually beginning in 2005 for all WHI participants, and at closeout for CT participants (average ~7 y after baseline). In a subset of 5346 participants, physical performance measures (grip strength, number of chair stands in 15 seconds, and timed 6-meter walk) were assessed at baseline and years 1, 3, and 6. Calibrated energy and protein intake were derived from regression equations using baseline food frequency questionnaire data collected on the entire cohort and doubly labeled water and 24-hour urinary nitrogen collected from a representative sample as reference measures. Associations between calibrated protein intake and each of the physical function measures were assessed using generalized estimating equations.

RESULTS—Calibrated protein intake ranged from 6.6 to 22.3% energy. Higher calibrated protein intake at baseline was associated with higher self-reported physical function [quintile (Q) 5 vs. Q1: 85.6 (95% CI, 81.9 to 87.5) vs. 75.4 (73.2 to 78.5), $P_{\text{trend}}=0.002$] and a slower rate of functional decline [Q5 vs. Q1 annualized change: -0.47 (-0.63 to -0.39) vs. -0.98 (-1.18 to -0.75), $P_{\text{trend}}=0.022$]. Women with higher calibrated protein intake also had higher grip strength at baseline [Q5 vs. Q1: 24.7 (24.3 to 25.2) vs. 24.1 (23.6 to 24.5), $P_{\text{trend}}=0.036$] and showed slower declines in grip strength [Q5 vs. Q1 annualized change: -0.45 kg (-0.39 to -0.63) vs. -0.59 kg (-0.50 to -0.66), $P_{\text{trend}}=0.028$]. Additionally, women with higher calibrated protein intake completed more chair stands at baseline [Q5 vs. Q1: 7.11 (6.91 to 7.26) vs. 6.61 (6.46 to 6.76), $P_{\text{trend}}=0.002$].

CONCLUSION—Higher calibrated protein intake is associated with greater physical function and performance and slower rates of decline in postmenopausal women.

Keywords

dietary protein intake; physical performance; physical function; grip strength

INTRODUCTION

Women’s Health Initiative (WHI) investigators previously reported that a 20% higher uncalibrated protein intake (% energy) was associated with a 12% (95% CI, 8 to 16%) decrease in frailty, while 20% higher calibrated protein intake (i.e. corrected for measurement error using biomarkers of energy and protein intake) was associated with a 32% (23 to 44%) decrease in frailty¹. Understanding the pathways through which higher dietary protein intake may reduce frailty risk would inform future intervention study designs and could contribute greatly to public health, since frailty is associated with increased risk of falls, fractures, disability, institutionalization, and death^{2–4}. Data from several highly controlled clinical studies support a beneficial role of increased protein intake in reducing loss of lean body mass^{5–7}. Whether the beneficial effect of higher protein intake also translates into clinically important differences in preservation of physical function over time on a population level is less clear.

The WHI is the largest study of postmenopausal women’s health ever undertaken in the U.S. Further, the study provides a particularly robust description of diet and health indicators

over a prolonged follow-up period, including data on protein intake and physical function. Here we examined total biomarker-calibrated protein intake in relation to baseline and changes in physical function, as measured by both self-report (short form RAND-36 Physical Function Score) and three objective measures (grip strength, number of chair stands in 15 seconds, and timed 6-meter walk) over a mean follow-up of 11.5 ± 3.1 y in a large sample of postmenopausal women.

METHODS

Study population

The WHI Observational Study (OS) of 93,676 women, along with 68,132 women enrolled in the Clinical Trials (CT), comprised women age 50–79 y when recruited between 1993 and 1998 from 40 clinical centers across the U.S. Over 80% of WHI CT and OS women also enrolled in an Extension Study (WHI Extension) to ascertain additional health outcomes beginning in 2005. Women were eligible for study inclusion if they were postmenopausal, of stable residence, and unlikely to die within 3 y. Additional eligibility criteria were assigned to each CT for reasons of safety, competing risk, and adherence/retention. Further details regarding the design, recruitment strategy, and data collection methods have been published^{8,9}. The study was reviewed and approved by institutional review boards at each participating institution, and all participants signed written, informed consent.

The study population for this analysis includes all WHI OS and CT women with at least one physical function measure (either self-reported or objective). Additionally, women must have completed a food frequency questionnaire (FFQ) at baseline with reported energy intake between 600 and 5000 kcal/d, and they must have had available information necessary to compute calibrated protein intake: age, race/ethnicity, body mass index (BMI), recreational physical activity, and smoking status ($n=134,961$). Self-reported physical function scores were available for at least two visits for 75.0% and three visits for 70.2% of participants.

Objective physical function measures were available for a subset of the study population. A 25% random sample of CT participants age ≥ 65 y completed measures of grip strength ($n=5,331$), chair stands ($n=5,294$), and timed walk ($n=5,335$), for a total of 5,346 women completing at least one of the three measures at one or more time points. Among the subset of CT women who completed physical performance measures, data were available for at least two visits for 94.8%, 93.6%, and 95.1% and three visits for 84.0%, 81.4%, and 84.6% of participants for grip strength, chair stands, and timed walk, respectively. The subset of women completing physical performance measures were generally older, were more likely to be obese, had a lower income and education, were more likely to live alone, and reported more co-morbidities (i.e. arthritis, diabetes) than the overall study population (data not shown).

Physical function measures

Self-reported physical function was assessed using a summary measure, a subscale from the RAND Short Form-36 (SF-36)^{10,11}, that included 10 items related to the ability to engage in activities (i.e. vigorous and moderate activities, lifting/carrying groceries, climbing stairs, bending, walking, and bathing/dressing oneself) and is scored from 0 to 100, with a higher score indicating greater physical function capacity. The SF-36 was assessed at baseline and annually beginning in 2005 for all WHI participants, and at closeout for CT participants (average ~ 7 y after baseline). For this analysis, women were followed for a mean total follow-up of 11.5 ± 3.1 y.

Physical performance measures (grip strength, chair stands, and timed walk) were assessed at baseline, at follow-up years 1, 3, and 6 in the CT, and at follow-up year 3 in the OS by trained, certified study staff using standard protocols. Hand grip strength was measured using a handheld dynamometer (Jamar hand dynamometer; Lafayette Instruments, Lafayette, IN). Two measurements were taken using the dominant hand with staff coaching for maximal performance, and the higher score was used in the analysis.

Repeated chair stands and the timed walk, which represent two of three items of the Short Physical Performance Battery (SPPB)¹² commonly employed to assess function in aging populations, correlate with disability and/or mortality in older adults^{13,14}. The chair-stand test was conducted if the participant was able to stand at least once without using hands or arms from a straight-backed, non-padded, flat-seated, armless chair. Two 15-second trials of repeated chair stands were performed with arms folded across the chest, with a 1–2 minute rest between trials, and the score with the greater number of chair stands was included in the analysis. The 6-meter timed walk was performed with women walking at usual speed, using ambulatory aids as needed. The test was repeated, and the faster of the two measured times was included in this analysis.

Dietary protein

The 122-item WHI FFQ was self-administered at baseline for all participants¹⁵. Daily energy (kcal) and protein (g) intake were estimated for individual foods/food groups, 19 adjustment items, and summary questions (i.e. uncalibrated energy and protein). The WHI Nutritional Biomarkers Study ($n=544$) was conducted to evaluate accuracy of self-reported energy and protein consumption from the FFQ using biomarkers (doubly labeled water for energy and urinary nitrogen for protein) of “true” intake¹⁶.

“Uncalibrated” protein intake refers to the amount calculated directly on the FFQ, whereas “biomarker-calibrated” protein intake refers to the adjusted levels obtained after using regression equations that were developed based upon the NBS. These regression equations incorporate participant characteristics like BMI, age, race/ethnicity, and smoking status¹⁷. The biomarker-calibrated values are more accurate than the uncalibrated values, as they acknowledge differential reporting errors in dietary data based upon participant characteristics. Protein intake measures were categorized into quintiles separately for the self-reported (median calibrated protein intake of 14.3% energy) and objective physical function (median calibrated protein intake of 13.7% energy) measures based on distributions among the women included in each analysis.

Potential confounders

Demographic characteristics (age, family income, education, race/ethnicity), medical history [hip fracture, emphysema, treated diabetes, hypertension (on medication and/or blood pressure >140/90 mmHg), arthritis, cancer], and other health-related factors (having a current health care provider, number of falls, living alone, ability to perform activities of daily living, depression) were self-reported at baseline. BMI was computed using measured height (m) and weight (kg) at baseline (kg/m^2). Smoking status was self-reported and classified as current, past, or never. Depressive symptoms were assessed by 6-item short form of the Center for Epidemiologic Studies Depression (CESD) Scale²⁴. Postmenopausal hormone therapy was self-reported and categorized as current, past, or never use, with separate indicator variables for unopposed estrogen and estrogen + progestin.

Analytic approach

Baseline characteristics were examined by quintile of calibrated protein intake (% energy). Multivariate adjusted linear generalized estimating equations (GEE) with an exchangeable

covariance matrix were used to examine the longitudinal association between baseline protein intake and each outcome (self-reported physical function and three physical performance measures), including a protein-by-time interaction to allow for varying slopes, with time modeled as a continuous variable. The models test whether the mean scores on these outcome measures differ at baseline (intercept) or with respect to annual change over time (slope) according to quintile of protein intake. The reasonableness of linear fit was confirmed by comparing these estimates to results obtained by treating time as a categorical variable. Models were adjusted for previously identified independent predictors of frailty: age, income, education, race/ethnicity, BMI, smoking status, alcohol consumption, physical activity, hormone therapy use, whether the participant lived alone, having a healthcare provider, number of falls, disability, depression, and self-reported history of medical conditions (emphysema, diabetes, hypertension, arthritis, and cancer)³. Models were also adjusted for calibrated total energy intake and clinical trial arm. To evaluate potential effect modification, the relationship between calibrated protein intake and each of the four outcome measures was stratified by median age, recreational physical activity, BMI, or protein source (animal: total protein ratio).

Bootstrapping procedures were conducted to approximate variance around the estimated physical function outcomes across levels of calibrated protein intake. First, variance in the coefficients for the calibration regression equations in the NBS was determined by constructing 1000 bootstrap replicates using their original dataset. Next, using the dataset for the current study¹⁶, we constructed 1000 bootstrap replicates, and each new randomly selected sample of participants was paired with a new set of regression coefficients from the first bootstrapping procedure to re-calculate calibrated protein and energy intake. Then, the previously described GEE models were repeated for each sample, and 95% confidence intervals were determined from the distribution of 1000 new estimates. Tolerance (i.e. relative change in the coefficient vector from one iteration to the next) was set to 10^{-4} during bootstrapping to ensure consistent model convergence. A trend in the relationship between protein quintile (ordinal) and each estimated physical function score at baseline (or annualized change) was assessed by calculating the slope between these variables for each bootstrap replicate. These slopes were used to construct a distribution to determine if there was a significant trend in physical function across levels of protein intake. Statistical analyses were conducted using Stata 12.1 (StataCorp, College Station, TX) and SAS 9.3 (SAS Institute, Cary, NC); all reported *P*-values are two-sided.

RESULTS

At baseline, mean calibrated protein intake was $14.3 \pm 1.4\%$ total energy. Factors associated with higher protein intake included younger age, lower BMI, non-Hispanic white race/ethnicity, higher socioeconomic status, and higher recreational physical activity (Table 1). Similar trends were observed when characterizing protein intake as g/kg body weight and when restricting the study population to the subset of women with physical performance measures (data not shown).

Women in the highest quintile of calibrated protein intake reported a 14% higher level of physical function at baseline than women in the lowest quintile (Table 2). Further, the rate of self-reported annual decline in physical function was 52% lower in women in the highest compared with the lowest quintile of calibrated protein intake. The positive association between self-reported physical function and protein intake was consistent across the continuum of calibrated protein intake. Results were similar when characterizing protein intake as g/kg body weight (consistent with dietary reference intake estimates) instead of % energy; however, there was no association for grams of absolute protein intake and self-reported physical function (Figure 1). Furthermore, the positive associations between protein

intake and physical function were evident using either uncalibrated or calibrated protein intake as g/kg body weight; however, the strength of the associations was substantially weaker for uncalibrated protein intake.

With respect to objective measures of physical function, mean grip strength at baseline was slightly higher among women with higher calibrated protein intake ($P=0.036$), and these women experienced smaller declines in grip strength over time than those with low calibrated protein intake ($P=0.028$) (Table 2). Additionally, women in the highest quintile of calibrated protein intake completed on average 0.5 more chair stands at baseline than women in the lowest quintile ($P=0.002$). In contrast, there was no significant association between calibrated protein intake and the timed 6-meter walk in either cross-sectional or longitudinal analyses ($P=0.748$ and 0.080 , respectively). Consistent with the self-reported analyses, estimates for the association between protein intake and objective measures of physical function did not change substantively when characterizing protein intake as g/kg body weight instead of % energy, but grams of absolute protein intake was not significantly associated with any of the objective measures (data not shown). Furthermore, associations between protein intake and grip strength or chair stands were substantially stronger using calibrated versus uncalibrated protein intake (Figure 2). For self-reported and objective measures, no substantive interactions were detected between calibrated protein intake and age, BMI, or physical activity (data not shown).

DISCUSSION

In this large, prospective study of postmenopausal women with a mean 11.5 y of follow-up, women with higher calibrated protein intake demonstrated smaller declines in self-reported physical function as well as two physical performance measures (grip strength and repeated chair stands). There was no association between calibrated protein intake and gait speed, suggesting that factors related to preserving mobility may be less closely related to protein intake. Therefore, improvements in physical function, particularly with respect to factors related to strength, may partially explain the protein-frailty association. Over a 4-y follow-up period, a one-point lower SPPB¹² score, which is frequently used as a summary measure of physical function, was associated with 15% (95% CI, 6 to 24%) higher risk of frailty and 25% (95% CI, 6 to 47%) higher risk of mortality in the Einstein Aging Study¹⁷. Two randomized, controlled trials of protein supplementation (30 g/day) and protein supplementation (30 g/day) plus exercise observed ~1 point increases in SPPB after 24 intervention weeks compared to no changes in the placebo group^{18,19}. These data support the hypothesis that greater protein intake as % energy reduces the risk of frailty by maintaining functional status, muscle strength, and balance in postmenopausal women.

Given the large sample size of the current study, it is important to consider whether our statistically significant findings are also clinically relevant. Over a 5-y period, average self-reported physical function declined by 2 versus 5 points among women in the highest versus lowest quintile of calibrated protein intake, respectively. Likewise, over a 5-y period, average grip strength declined by 2.3 kg versus 3.0 kg in the highest versus lowest quintile of calibrated protein intake. These differences are larger than what has been defined as substantially clinically important changes in a cohort using somewhat different (i.e. SPPB, gait speed, timed walk) but analogous measures of physical function^{20,21}.

One issue to be resolved is determining the optimal percentage of energy as protein associated with greater physical function in aging women^{22,23}. In our study higher self-reported physical function was demonstrated with higher protein intake through the upper bound of intake (top quintile median of 16.0% energy or 1.18 g/kg body weight). While there remains insufficient evidence overall in regard to specific levels of protein intake

required to optimize health status in aging women, our findings are supported by those from available epidemiological studies and clinical trials^{24–27}. A recent meta-analysis of randomized, controlled trials for protein supplementation reported that participants consuming high-protein diets (mean intake 1.25 ± 0.17 g/kg body weight) increased grip strength by 1.76 kg (95% CI, 0.36 to 3.17) compared with control groups (four studies, $n=219$, mean age 65 y, age range 53–85 y, 60% female)²⁸. Similar to our findings, however, prior studies of protein intake and mobility reported no significant effects^{29–31}. Mean intake in the high-protein diets in the meta-analysis was slightly higher than the mean protein intake in the top quintile for this study (1.19 ± 0.20 g/kg body weight), and the mean intake for the comparison group was 0.72 ± 0.09 g/kg body weight, which was lower than the bottom quintile for this study (0.97 ± 0.17 g/kg body weight).

Low grip strength is a predictor of disability, mortality, and other poor outcomes in older adults³². Evidence suggests that reductions in frailty risk can be achieved through physical activity interventions by directly targeting inactivity and improving strength and motor performance³³. In the current study, each MET-hr./week of recreational physical activity was associated with a 0.2-unit increase in self-reported physical function ($P<0.001$), no significant difference in grip strength, 0.02-unit increase in the number of chair stands completed ($P<0.001$), and 0.01-second decrease in the timed walk ($P=0.001$). However, a 3-y prospective study of 92 women (mean age 71 ± 4 y) enrolled in a twice-weekly fitness program documented declines in grip strength (-3.2 ± 5.0 kg), increases in walking time (0.71 ± 0.9 seconds), and declines in energy (-345 ± 533 kcal/d) and protein (-9.5 ± 14.7 g/d) intake³⁴. A stratified analysis of protein intake (% energy) by median recreational physical activity level in our sample showed that the protein associations with physical function were independent of activity (data not shown), suggesting that women may benefit from consuming higher amounts of protein irrespective of physical activity level. An intervention combining protein and physical activity would exclude older adults who are on bed rest, yet evidence from clinical trials suggests that essential amino acid supplementation helps to preserve muscle mass and improve function among individuals during bed rest^{25,35}.

Strengths of this study include the prospective design, wide age range in this well characterized sample of postmenopausal women, ability to correct for measurement error in protein and energy intake using biomarker-derived calibration equations, availability of multiple standardized physical performance measures over a 6-y period as well as annual repeat measures of self-reported physical function spanning more than a decade, and ability to adjust for a large number of covariates that may be confounders. However, similar to the general U.S. population³⁶, the range of protein intake was not highly variable and cannot inform on aging women with chronic intake outside this range (e.g. high-protein diets or animal product-restricted vegetarian diets). Furthermore, given the differences in participant characteristics among women consuming low versus high protein intake, it is possible factors other than protein intake are partially explaining observed associations, though we did our best to account for potential confounders. Additionally, there is substantial measurement error in self-reported physical activity, physical activity measurement was restricted to recreational/leisure time, and protein intake is positively correlated with physical activity. Thus, we cannot rule out the possibility of residual confounding by physical activity. Analyses incorporating objective measures of physical activity could better elucidate the relationship between protein intake and physical function. The possibility of selection bias also must be considered, as participants enrolled in WHI for up to almost 20 y may differ from the general population of postmenopausal women. However, women in this study had considerable variation in physical function measures at baseline, and the change over time was sufficient in magnitude, to detect significant differences with respect to protein intake.

In summary, this prospective study of well functioning postmenopausal women showed that higher biomarker-calibrated protein intake was associated with smaller declines in self-reported physical function and objective measures of physical performance over an average 11.5-y time period. Given the multi-factorial nature of age and disease-related functional decline, modification of one potential factor may not be sufficient to delay decline. However, our data suggest that efforts to intervene in relation to protein intake in this subgroup of the population are warranted and should be rigorously evaluated. Randomized, controlled trials could also provide important information regarding optimal dose, composition, and effects of protein intake on aging.

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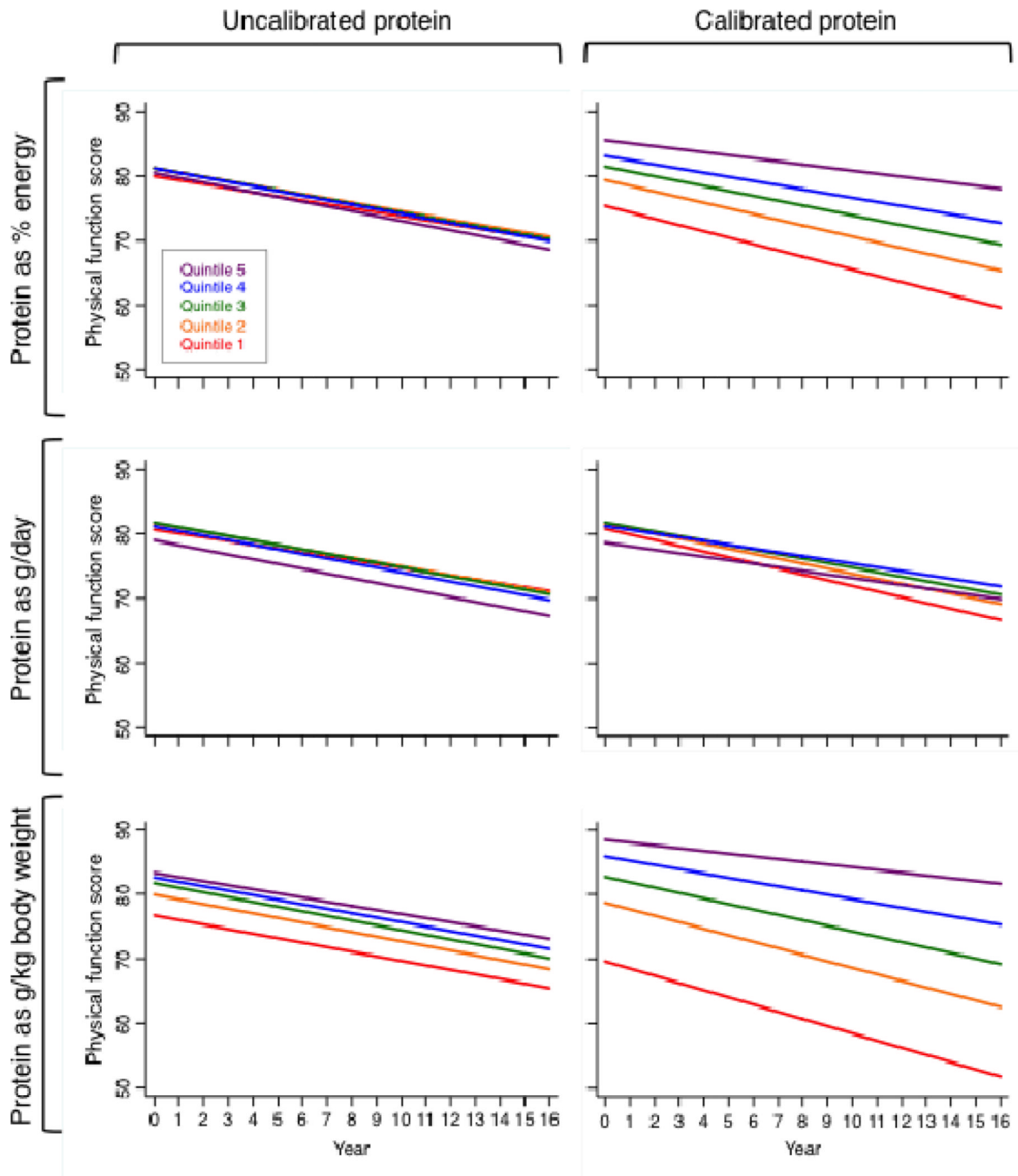


Figure 1. Self-reported physical function score over time, by quintiles of protein intake, calculated using GEE. Models were adjusted for age, income, education, race/ethnicity, BMI (height only for g/kg models), smoking status, alcohol consumption, physical activity, hormone therapy use, whether the participant lived alone, having a healthcare provider, number of falls, disability, depression, and self-reported history of medical conditions (emphysema, diabetes, hypertension, arthritis, and cancer), calibrated total energy intake, and clinical trial arm.

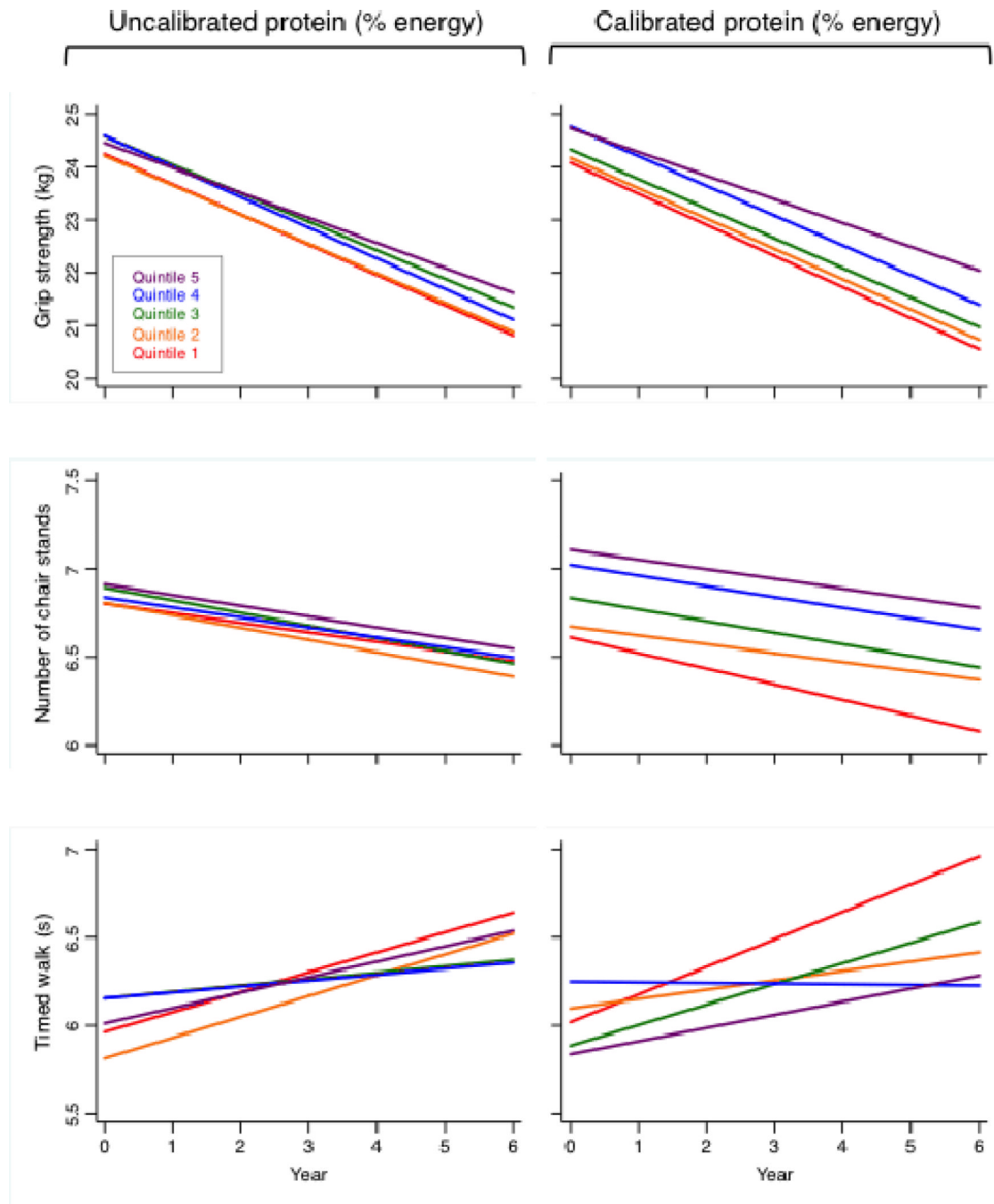


Figure 2. Physical performance measures over time, by quintiles of protein intake, calculated using GEE. Models were adjusted for age, income, education, race/ethnicity, BMI, smoking status, alcohol consumption, physical activity, hormone therapy use, whether the participant lived alone, having a healthcare provider, number of falls, disability, depression, and self-reported history of medical conditions (emphysema, diabetes, hypertension, arthritis, and cancer), calibrated total energy intake, and clinical trial arm.

Table 1
 Baseline Characteristics of Women’s Health Initiative Participants, by Quintile of Calibrated Protein Intake (% Energy)

Characteristic	Quintile 1 6.6–13.1% n = 26,994	Quintile 2 13.1–13.9% n = 26,991	Quintile 3 13.9–14.6% n = 26,992	Quintile 4 14.6–15.4% n = 26,992	Quintile 5 15.4–22.3% n = 26,992
Age (y), mean ± SD	66.0 ± 7.2	64.9 ± 7.0	63.6 ± 6.9	61.9 ± 6.7	59.5 ± 6.3
Body mass index (kg/m ²), mean ± SD	29.2 ± 7.0	28.6 ± 6.1	28.0 ± 5.7	27.5 ± 5.4	26.6 ± 4.9
Underweight (< 18.5), %	0.93	0.78	0.68	0.79	1.11
Normal (18.5 to < 25), %	28.7	30.8	33.7	36.6	42.4
Overweight (25.0 to < 30), %	33.0	34.4	35.2	35.6	35.2
Obese (≥ 30), %	37.4	34.0	30.5	27.1	21.3
Ethnicity, %					
White	78.1	83.4	85.4	86.5	85.6
Black	14.1	9.04	7.28	6.09	5.90
Hispanic	3.70	3.29	3.18	3.63	3.86
American Indian	0.54	0.46	0.34	0.31	0.41
Asian/Pacific Islander	2.32	2.66	2.71	2.54	3.15
Other	1.27	1.15	1.11	0.96	1.08
Income, %					
< \$20,000	25.5	18.2	15.0	12.0	9.40
\$20,000 to \$34,999	29.0	27.4	24.9	21.9	18.0
\$35,000 to \$49,999	19.7	21.1	21.4	21.2	19.7
\$50,000 to \$74,999	15.1	18.4	20.4	22.6	24.1
≥ \$75,000	10.8	14.9	18.2	22.3	28.9
Education, %					
High school diploma or GED	28.4	23.7	21.2	18.6	15.6
Some college	40.5	38.8	37.7	36.7	36.0
College degree	31.1	37.5	41.2	44.8	48.5
Physical activity (MET-hr/wk.), mean ± SD	9.9 ± 12.5	11.5 ± 13.1	12.5 ± 13.6	13.4 ± 13.7	14.9 ± 14.8
Live alone, %	38.2	32.7	29.5	26.3	23.3
Have current healthcare provider, %	92.6	94.3	94.7	94.9	94.5
Number of falls in past year, %					

Characteristic	Quintile 1 6.6–13.1% n = 26,994	Quintile 2 13.1–13.9% n = 26,991	Quintile 3 13.9–14.6% n = 26,992	Quintile 4 14.6–15.4% n = 26,992	Quintile 5 15.4–22.3% n = 26,992
0	67.5	67.3	67.8	67.7	69.2
1	19.7	20.3	19.9	20.1	19.3
2	8.44	8.37	8.35	8.18	7.64
3+	4.35	4.04	3.94	4.02	3.85
1 activity of daily living disability, %	2.21	1.65	1.63	1.49	1.28
Depression score, %					
0	26.7	27.1	28.3	28.3	29.0
1–2	38.4	41.0	40.9	41.5	41.1
3–4	17.9	17.3	17.5	17.0	16.8
5–18	17.1	14.5	13.3	13.3	13.2
Smoking, %					
Never	41.6	51.6	53.2	53.4	53.3
Past	35.1	41.6	44.0	45.3	46.2
Current	23.3	6.79	2.79	1.30	0.50
Unopposed estrogen use, %					
Never	64.6	63.6	63.8	63.4	65.3
Past	15.2	13.8	12.5	12.1	10.3
Current	20.2	22.6	23.7	24.5	24.4
Estrogen + progesterone use, %					
Never	81.6	76.5	72.7	69.6	65.6
Past	6.96	8.18	9.09	9.38	9.89
Current	11.4	15.3	18.2	21.0	24.5
Medical history, %					
Arthritis	53.0	49.9	48.5	46.0	40.6
Diabetes (treated with pills/shots)	4.05	4.30	4.45	4.42	4.46
Cancer	10.4	9.81	9.34	8.84	8.95
Hypertension	39.4	36.7	34.3	31.2	27.1
Emphysema	5.40	3.83	3.47	2.96	2.76
Hip fracture	1.05	1.02	0.91	0.86	0.57
Dietary intake, mean ± SD					

Characteristic	Quintile 1 6.6–13.1% n = 26,994	Quintile 2 13.1–13.9% n = 26,991	Quintile 3 13.9–14.6% n = 26,992	Quintile 4 14.6–15.4% n = 26,992	Quintile 5 15.4–22.3% n = 26,992
Calibrated energy (kcal/d)	2069 ± 245	2069 ± 218	2071 ± 203	2075 ± 188	2071 ± 171
Calibrated Protein (g/d), Calibrated Protein (g/kg body weight/d)	71.5 ± 12.1	74.7 ± 11.0	76.7 ± 10.5	79.0 ± 10.1	81.7 ± 9.9
Animal protein (g/d)	0.97 ± 0.17	1.03 ± 0.17	1.07 ± 0.17	1.12 ± 0.18	1.19 ± 0.20
Vegetable protein (g/d)	35.5 ± 19.0	43.2 ± 20.7	48.1 ± 21.4	52.9 ± 22.2	60.5 ± 25.2
Fruits/vegetables (servings/d)	19.3 ± 8.8	20.8 ± 8.9	21.0 ± 8.8	20.9 ± 8.6	19.7 ± 8.4
Calcium (mg/d)	3.8 ± 2.2	4.1 ± 2.1	4.2 ± 2.1	4.2 ± 2.1	4.2 ± 2.1
Vitamin D (mcg/d)	666 ± 359	777 ± 400	839 ± 424	896 ± 458	960 ± 535
Alcohol (g/d)	3.3 ± 2.3	3.9 ± 2.6	4.4 ± 2.8	4.8 ± 3.1	5.5 ± 3.8
	7.0 ± 15.2	5.5 ± 10.7	5.2 ± 9.4	4.8 ± 8.6	4.0 ± 7.2

Table 2

Baseline Physical Function Measures and Annualized Change (and 95% Confidence Intervals) by Quintile of Calibrated Protein Intake^a

Physical function measure	Quintile of calibrated protein intake (% energy)					<i>P</i> _{trend}
	1	2	3	4	5	
Self-reported score (<i>n</i> = 112,213)						
Baseline	75.4 (73.2 to 78.5)	79.4 (78.4 to 80.7)	81.5 (81.2 to 81.9)	83.1 (81.8 to 84.4)	85.6 (81.9 to 87.5)	0.002
Annualized change	-0.98 (-1.18 to -0.75)	-0.88 (-1.03 to -0.70)	-0.75 (-0.82 to -0.70)	-0.64 (-0.69 to -0.59)	-0.47 (-0.63 to -0.39)	0.022
Grip strength, kg (<i>n</i> = 4,527)						
Baseline	24.1 (23.6 to 24.5)	24.2 (23.7 to 24.5)	24.3 (24.0 to 24.9)	24.8 (24.3 to 25.2)	24.7 (24.3 to 25.2)	0.036
Annualized change	-0.59 (-0.66 to -0.50)	-0.57 (-0.66 to -0.50)	-0.56 (-0.65 to -0.47)	-0.56 (-0.63 to -0.49)	-0.45 (-0.53 to -0.38)	0.028
Chair stands, <i>n</i> (<i>n</i> = 4,497)						
Baseline	6.61 (6.46 to 6.76)	6.67 (6.55 to 6.86)	6.83 (6.71 to 6.99)	7.02 (6.79 to 7.13)	7.11 (6.91 to 7.26)	0.002
Annualized change	-0.09 (-0.11 to -0.05)	-0.05 (-0.08 to -0.03)	-0.07 (-0.10 to -0.04)	-0.06 (-0.09 to -0.03)	-0.05 (-0.08 to -0.03)	0.306
Timed walk, seconds (<i>n</i> = 4,533)						
Baseline	6.02 (5.81 to 6.37)	6.10 (5.70 to 6.27)	5.89 (5.67 to 6.31)	6.25 (5.87 to 6.50)	5.84 (5.62 to 6.22)	0.748
Annualized change	0.16 (0.05 to 0.22)	0.05 (0.01 to 0.17)	0.12 (0.00 to 0.18)	0.00 (-0.06 to 0.09)	0.07 (0.00 to 0.14)	0.080

^a Estimates derived from GEE adjusted for age, income, education, race/ethnicity, BMI, smoking status, alcohol consumption, physical activity, hormone therapy use, whether the participant lived alone, having a healthcare provider, number of falls, disability, depression, self-reported history of medical conditions (emphysema, diabetes, hypertension, arthritis, and cancer), calibrated total energy intake, and clinical trial arm. Confidence intervals were calculated using 1000 bootstrap replicates to account for uncertainty in the calibrated estimates of protein intake.