Prospective associations of protein intake parameters with muscle strength and physical performance in community-dwelling older men and women from the Quebec NuAge cohort

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ABSTRACT

Background: Dietary protein has been related to muscle function in aging. Beyond total intake, parameters such as protein distribution across meals might also be important.

Objectives: We aimed to examine prospective associations of different protein intake parameters with muscle strength and physical performance in community-dwelling older men and women.

Methods: In total, 524 men and 574 women aged 67–84 y at baseline (T1) were followed annually for 3 y (T2, T3, T4). Outcomes included handgrip strength (kPa), knee extensor strength (kg), and physical performance (Timed Up and Go, s) at T4, and their 3-y changes (T4 minus T1). Protein intake parameters were assessed using nine 24-h recalls collected over 3 y (T1, T2, T3) and included daily total intake (g/d), number of protein-providing meals and snacks, and protein distribution across meals (expressed as CV). Associations were examined by multivariable linear regression models including all protein intake parameters simultaneously. Also, the optimal protein dose (g) per meal for the maximum effect size of total daily intake was determined.

Results: Higher daily protein intake was associated with better knee extensor strength and physical performance at T4 in both sexes and less physical performance decline in women. Optimal protein doses per meal were 30–35 g in men and 35–50 g in women for these outcomes. In men, more uneven protein distribution was associated with better physical performance at T4 and less handgrip strength decline. In women, a higher number of protein-providing snacks was associated with better handgrip strength and knee extensor strength at T4 and less handgrip strength decline. In neither sex was number of protein-providing meals associated with outcomes.

Conclusions: Higher daily protein intake, up to 30–50 g protein/meal, may contribute to better knee extensor strength and physical performance in generally well-functioning older men and women. More aspects of protein intake may contribute to muscle strength and physical performance than solely the daily quantity, notably the protein dose per meal. Am J Clin Nutr 2021:00:1–12.

Keywords: diet, eating occasion, food patterns, muscle health, old age

Introduction

Skeletal muscle strength diminishes with age (1) and contributes to decreased physical performance (2) and higher risks of frailty, disability, and mortality in older adults (3, 4). Dietary protein intake is a key modifiable factor affecting muscle metabolism (3–5). Nonetheless, there is an ongoing debate on protein recommendations for older people, which currently vary from 0.8 to 1.2 g · kg body weight (BW)−1 · d−1 (6–8). Protein recommendations may be difficult to establish because, beyond daily quantity, other protein intake parameters have been evoked as being potentially involved in the anabolic response to protein (6, 9). These include notably the evenness of protein intake distribution across meals (6, 10, 11) and an optimal protein dose per meal of 25–35 g beyond which muscle protein synthesis (MPS) is no further increased in older adults (6, 10, 11). Studies have also indicated that more frequent consumption of protein-providing meals may be related to better muscle strength (11, 12). However, such protein intake parameters may not be independent from one another. Reaching 30 g protein per meal 3 times/d probably leads to an even protein intake distribution across meals and a relatively high daily protein intake (13–16). Furthermore, protein requirements may differ between men and women owing to sex-differences in body composition (17), hormonal milieu (17, 18), MPS rate (19, 20), and sensitivity to anabolic stimuli (18, 19). Insight into the independent role of

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each protein intake parameter in the muscle strength and physical performance of older men and women is required to optimize protein recommendations.

Observational studies are most appropriate to investigate how protein intake parameters relate to relevant clinical outcomes such as muscle strength and physical performance. However, most studies have focused only on daily protein intake—many of them being limited by the use of FFQs, which cannot estimate protein intake per eating occasion. Moreover, only 4 studies have presented prospective data. One prospective study showed that higher protein intake was associated with less decline in handgrip strength in both sexes (21). Others observed no association with change in handgrip strength (22, 23), knee extensor strength (24), or physical performance (23). Only 2 prospective studies have examined other parameters of protein intake, specifically its distribution across meals. One showed that a more even protein intake distribution across meals was associated with higher handgrip strength throughout a 3-y follow-up in the NuAge (Quebec Longitudinal Study on Nutrition and Successful Aging) cohort, but not with knee extensor strength or physical performance (25). The other observed no associations with change in handgrip strength or physical performance (23). Among the factors that may explain this discrepancy is the fact that not all protein intake parameters were examined simultaneously in 1 model.

A better understanding of how protein intake parameters can contribute to maintenance of muscle strength and physical performance requires studies having access to detailed dietary data and considering multiple protein intake parameters simultaneously while avoiding collinearity. Hence, the aim of the present study was to examine prospective associations of different protein intake parameters with muscle strength and physical performance after 3 y of follow-up, and with their 3-y change, in community-dwelling older men and women.

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Supplemental Tables 1–7 and Supplemental Methods are available from the "Supplementary data" link in the online posting of the article and from the same link in the online table of contents at https://academic.oup.com/ajcn/.

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Abbreviations used: aBW, adjusted body weight; BW, body weight; MPS, muscle protein synthesis; NuAge, Quebec Longitudinal Study on Nutrition and Successful Aging; PASE, Physical Activity Scale for the Elderly; TUG, Timed Up and Go; T1, baseline; T2, 1-y follow-up; T3, 2-y follow-up; T4, 3-y follow-up; VIF, variance inflation factor; 3MS, Modified Mini-Mental State Examination.

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Methods

Study population

Data used in the present study were from the NuAge Database and Biobank, containing detailed information on 1754 participants of the NuAge Study. A complete description of the NuAge Study can be found elsewhere (26). In short, the NuAge cohort consists of generally healthy Quebec community-dwelling older men and women aged 67–84 y at baseline (2003–2005; T1). Inclusion criteria were, among others, able to walk without help, able to walk 100 m or climb 10 stairs without rest, free of disabilities in activities of daily living, and not cognitively impaired [Modified Mini-Mental State Examination (3MS) score > 79]. People suffering from severe health conditions were excluded (26). Baseline measurements (T1) took place at the Research Centers of either of the Montreal and Sherbrooke Geriatric University Institutes. Follow-up data were retrieved annually for 3 subsequent years (T2, first annual follow-up; T3, second annual follow-up; T4, third annual follow-up). At each clinic visit, nutritional, functional, medical, and social variables were assessed by trained research dietitians and nurses using computer-assisted personal interview software. Dietary intake was assessed annually with 3 consecutive 24-h dietary recalls. The NuAge Database and Biobank as well as the present study have been approved by the Research Ethics Board of the Centre intégré universitaire de santé et de services sociaux de l’Estrie—Centre hospitalier universitaire de Sherbrooke.

Analytic sample

We aimed to examine associations of different protein intake parameters with J) muscle strength and physical performance after 3 y of follow-up (i.e., at T4) and 2) the 3-y change in muscle strength and physical performance (i.e., T4 minus T1). To this end, we excluded participants with missing data on muscle strength or physical performance at T1 (n = 23) or T4 (n = 473). Furthermore, we excluded participants with ≥1 missing 24-h dietary recalls at T1, T2, or T3 to achieve the most precise estimate of each protein intake parameter (n = 116) and then excluded those with missing data on covariates that were considered important confounders (n = 44), leaving an analytic sample of 524 male and 574 female participants (Figure 1).

Assessment of muscle strength and physical performance

Measures of muscle strength included handgrip strength and knee extensor strength, assessed according to a predefined standardized protocol in NuAge. Handgrip strength was assessed using a pneumatic dynamometer (Martin vigorimeter), which measures the force of compression (kPa). Participants were seated with the shoulder adducted and the elbow flexed at 90 degrees and were encouraged to squeeze the bulb at maximal force for a maximum of 6 s. Three maximum contractions were recorded at each hand, starting with the dominant side. Knee extensor strength was assessed using the dynamometer Microfet2 (Hogган Industries, Inc.) and the belt-resisted method (27), which measures the strength of the quadriceps (kg). Participants were seated with the knee flexed at 120 degrees and the foot on
the ground, and encouraged to push at maximum force against the dynamometer placed in the center of the distal third of their leg (27). Three maximum contractions were recorded for each leg. For both muscle strength measures, the highest value of the 6 attempts at T4, regardless of the side, was used in our study. The 3-y changes in muscle strength were calculated as T4 minus T1, by using for T1 the highest value of the same side as T4 to ensure consistency. Physical performance was assessed using the Timed Up and Go (TUG) test, which measures the time (s) it takes to rise from a chair without using arms, walk 3 m at usual pace, turn, return to the chair, and sit down (28). Participants were allowed to use walking aids but not any assistance from the examiner. The test was completed once per participant after a practice trial. A higher value indicates more time to complete the TUG test, so lower physical performance. The 3-y change in physical performance was calculated as T4 minus T1.

Dietary assessment

Three nonconsecutive 24-h dietary recalls (1 face-to-face during the annual visit and 2 by telephone, within 2 mo after the annual visit) were collected on 2 randomly chosen weekdays and 1 weekend day. The 24-h dietary recalls were administered by extensively trained registered dietitians. They followed the USDA 5-step multiple-pass method (26, 29) and used portion-size models and pictures of standardized food portions to increase the precision of the food estimates. Dietary intake data were obtained for 7 eating occasions, including 3 meals (breakfast, lunch, and dinner) and 4 snack moments (morning, afternoon, evening, and night snack) (Supplemental Table 1). Intakes of energy and nutrients were calculated using the CANDAT nutrient analysis program (version 10; Godin London), which was based on the 2007 Canadian Nutrient File. For the present study, we used all 24-h dietary recalls collected before measurement of outcomes at T4, so 9 in total (from T1, T2, and T3). Recalls from T4 were not used because they were collected over the weeks after the measurement of outcomes at T4.

Composition of protein intake parameters

Five protein intake parameters were investigated: daily protein intake, number of protein-providing meals, number of protein-providing snacks, evenness of protein intake distribution across meals, and the optimal protein dose per meal (i.e., the amount of protein to be reached in each meal to maximize the effect size on muscle strength and physical performance, assuming that an optimal dose actually exists). Except for the latter, all protein intake parameters were determined for each recall day separately and the mean of the 9 d was used for analyses, as a reflection of the usual intake pattern.

Daily protein intake (g/d) comprised all protein consumed in 1 d (Supplemental Table 2). Absolute values of protein intake per day were used in analyses, which were adjusted for factors related to energy expenditure and energy balance over time as described in the Statistical analyses section below. Protein-providing meals or snacks included those with >0 g protein (Supplemental Table 2). Because there is no literature that specifies what minimal amount of protein intake is needed to stimulate MPS, we assumed that any amount of protein would contribute to the muscle synthetic response and we therefore chose >0 g of protein per eating occasion as the cutoff for defining a protein-providing meal or snack. The mean number of protein-providing meals
consumed per day was categorized into 3.00 meals/d (i.e., 3 meals/d on all recall days), 2.86–2.99 meals/d (i.e., equivalent to 3 meals/d on 6 d/week and 2 meals/d on 1 d/week), and <2.86 meals/d. Number of protein-providing snacks was categorized into sex-specific tertiles. Evenness of protein intake distribution across meals was determined using the CV. For this, the SD of the mean protein intake from meals (g/d) was divided by the mean protein intake from meals (g/d) (see Supplemental Methods and Supplemental Table 3). A higher CV indicates less evenness of protein intake across the meals. Because previous studies have indicated that the number of protein-providing meals may be related to muscle strength (12, 30), we calculated the CV only indicated that the number of protein-providing meals (which may differ per individual and per recall day) in order to obtain a CV that was independent of the number of meals with protein.

To investigate the optimal protein dose per meal, we recalculated the daily protein intake and the CV for each recall day of each participant, after having truncated the protein intake per eating occasion to a given maximal value (i.e., the potential optimal dose), presuming that any protein intake beyond this dose provides no additional benefit to muscle strength or physical performance. The Supplemental Methods and Supplemental Tables 4–6 detail an example of the truncation approach. Because of the uncertainty about the existence of an optimal dose per meal (31, 32), let alone the exact amount of this dose, we applied potential values varying from 15 to 70 g with 5-g increments. Each of these threshold values was applied in a separate model. The highest value of 70 g corresponded to the 95th percentile of the distribution of protein intake per meal in our analytic sample. By this approach, we postulated that if an optimal protein dose per meal actually exists, daily protein intake and CV will be calculated with increasing error when truncating the protein dose per meal with values under or above the so-called optimal dose, translating into attenuated estimates of regression coefficients in statistical analyses (33).

Assessment of covariates

Sex, years of education, smoking status, and number of prescribed medications were self-reported. Any alcohol consumption in the past 3 y (yes compared with no) was based on the 24-h dietary recall data. Habitual physical activity was estimated using the Physical Activity Scale for the Elderly (PASE) questionnaire (34, 35). The PASE asks for the daily time spent on leisure activities, household activities, and occupational activities in the previous week. A higher score (range 0–793) indicates more physical activity. Body height (m) was measured using a stadiometer and BW was measured with a beam balance with participants dressed in light indoor clothing without shoes (26). BMI was calculated as BW divided by body height squared (kg/m²). To minimize the influence of shrinking due to aging on BMI, body height measured at T1 was used for the calculation of BMI at T1 and T4. Weight change over the 3-y follow-up was calculated as T4 minus T1 (absolute difference). Number of chronic diseases was assessed by the participant’s self-report of the presence of each of 20 chronic conditions (36). Level of pain was assessed using the SF-36 bodily pain index [from the 36-item Short form health survey (37)]. A higher score (range 0–100) indicates less pain. Depressive symptoms were assessed using the Geriatric Depression Scale (38) and cognitive status using the 3MS (39). All covariates were determined at each annual visit except for sex and education level, which were only assessed at baseline. For descriptive purposes, we expressed daily total protein intake relative to actual BW (g/kg BW) and adjusted body weight (aBW) (g/kg aBW). aBW is the nearest (ideal) BW that would place a participant with an undesirable BMI into the healthy range of 18.5–25.0 for adults aged <71 y or 22.0–27.0 for adults aged ≥71 y (40). This controlled for the deficit or excess in BW of underweight and overweight people, respectively.

Statistical analyses

Changes in dietary intake characteristics and other variables between T1 and T4 were tested using Student’s paired-samples t test for continuous variables, the Sign test for categorical variables, and the McNemar test for dichotomous variables. Differences in dietary intake characteristics and other variables between sexes were tested using Student’s independent-samples t test for normally distributed continuous variables, the Mann–Whitney U test for nonnormally distributed continuous variables, and Pearson’s chi-square test for categorical variables.

Linear regression analyses were performed to examine the associations of the 5 protein intake parameters with handgrip strength, knee extensor strength, and physical performance (TUG) at T4, and with their 3-y change. All analyses were a priori stratified by sex because of presumed sex-differences in body composition (17), hormonal milieu (17, 18), and MPS rates (19, 20). Also, higher muscle strength values indicate better muscle strength, whereas higher TUG values indicate lower physical performance. Thus, in the regression models all TUG values were multiplied by −1 such that positive coefficients (β) always represent better outcomes, which eases the interpretation and graphical display of the results.

The regression models included all 4 protein intake parameters simultaneously, i.e., daily protein intake (g/d), number of protein-providing meals, number of protein-providing snacks, and evenness of protein intake distribution across meals. In addition, all regression models were adjusted for the T4 values (or stated otherwise) of the following potential confounding variables: age, body height (T1), BW, habitual physical activity (mean PASE scores of T1–T4), education level (T1), smoking, alcohol use (mean of T1–T3), 3-y weight change, cognition, number of medications, and pain. Because all models were adjusted for factors accounting for energy expenditure (i.e., age, body height, BW, habitual physical activity) as well as energy balance over time (i.e., 3-y weight change), we did not further adjust for energy intake (41, 42). When assessing the associations with 3-y change in outcomes, the models were also adjusted for the outcome value at T1.

The statistical procedure was as follows (and described in more detail in Supplemental Methods). We first examined the associations of 4 protein intake parameters (i.e., daily protein intake, number of protein-providing meals, number of protein-providing snacks, and evenness of protein intake distribution across meals; independent variables) with 1 of the outcomes (handgrip strength, knee extensor strength, and physical performance at T4, or their 3-y change; dependent variables). Subsequently, we reran the aforementioned model after replacing the actual values of daily protein intake and evenness of protein intake distribution across
meals (CV) by the truncated values. This means that for each potential optimal protein dose a separate regression model was run. For each model, we noted the \( t \) statistic value—which reflects the statistical significance of an association—for the association between daily protein intake and the outcome. We compared these \( t \) values to determine if there was an optimal protein dose per meal for a maximum effect size on muscle strength and physical performance, and what this dose would be. Under the hypothesis that higher daily protein intake would be associated with better muscle strength and physical performance—which would be reflected by a positive \( t \) value—the threshold applied in the model with the highest statistically significant \( t \) value (i.e., the most statistically significant result) was considered the optimal protein dose per meal. If we observed the highest \( t \) value in the model with the actual (nontruncated) values or if \( t \) values did not reach statistical significance in any model, we assumed that no optimal dose actually existed. In fact, if there were no association between daily protein intake and muscle strength or physical performance, then the protein dose per meal would not matter. Final results for all 4 protein intake parameters were presented from the model with the optimal protein dose or if we did not determine an optimal protein dose, from the model with the actual values. We performed this procedure for each of the 3 outcomes at T4 and for the 3-year change in each outcome.

Spearman rank correlation coefficients were calculated to examine correlations between the 4 protein intake parameters (i.e., daily protein intake, number of protein-providing meals, number of protein-providing snacks, and evenness of protein intake distribution across meals). Multicollinearity was also checked by the variance inflation factor (VIF). In all models, the VIF for the protein intake parameters was <4 and thus multicollinearity was considered weak (43), allowing the inclusion of all protein parameters in a single model. Normality and linearity were checked by visual inspection of histograms and scatterplots, respectively. Statistical analyses were performed using SPSS Statistics version 26.0 (IBM Corp.). Results were considered statistically significant at \( P < 0.05 \) (2-sided).

### Results

#### Participant characteristics

Men (\( n = 524 \)) and women (\( n = 574 \)) had a mean ± SD age of 74.8 ± 4.0 y and 75.2 ± 4.2 y at T1, respectively (Table 1). Mean BMI ± SD at T1 was 28.1 ± 4.0 in men and 27.4 ± 4.7 in women, and was slightly lower at T4. Number of chronic diseases and medication use increased over the 3-year follow-up, with more than half of the men and women having ≥3 chronic diseases and using ≥5 medications at T4.

During the 3-year follow-up, handgrip strength, knee extensor strength, and physical performance (TUG) declined (all \( P < 0.001 \) based on Student’s paired-samples \( t \) test), with mean ± SD 3-year changes of −6.9 ± 9.6 kPa, −4.9 ± 15.8 kg, and +0.6 ± 1.8 s in men and −4.9 ± 10.6 kPa, −2.4 ± 10.4 kg, and +0.6 ± 2.1 s in women, respectively. Expressed as percentages, the mean ± SD 3-year changes in the 3 outcomes were

### Table 1: General characteristics at T1 and T4 of the community-dwelling older men and women from the NuAge (Quebec Longitudinal Study on Nutrition and Successful Aging) cohort

|                  | Men (\( n = 524 \)) | Women (\( n = 574 \)) |
|------------------|----------------------|-----------------------|
|                  | \( n \) | T1 | \( n \) | T4 | \( n \) | T1 | \( n \) | T4 |
| **Ages, y**      | 524 | 74.8 ± 4.0 | 524 | 77.9 ± 4.0* | 574 | 75.2 ± 4.2 | 574 | 78.3 ± 4.2* |
| **Years of education** | 524 | 12.0 ± 5.1 | — | — | 574 | 11.6 ± 3.9 | — | — |
| **Current smoker** | 516 | 33 (6.4) | 524 | 33 (6.3) | 562 | 16 (2.8)† | 574 | 18 (3.1)† |
| **Consumed alcohol in past 3 y** | — | — | 524 | 416 (79.4) | — | — | 574 | 365 (63.6)† |
| **Physical activity (PASE score; 0–793)** | 524 | 118 ± 55 | 491 | 112 ± 53* | 574 | 94 ± 45† | 544 | 84 ± 42† |
| **BMI, kg/m²**   | 524 | 28.1 ± 4.0 | 524 | 27.9 ± 4.1* | 574 | 27.4 ± 4.7† | 574 | 27.2 ± 4.8† |
| **Weight change over past 3 y, kg** | — | — | 524 | −0.7 ± 3.4* | — | — | 574 | −0.5 ± 3.4* |
| **Chronic diseases, \( n \)** | 524 | 516 | 574 | 562 |
| **Medications, \( n \)** | 524 | 524 | 574 | 574 |
| **Pain (SF-36 pain index; 0–100)** | 523 | 75.9 ± 24.4 | 524 | 72.5 ± 25.3* | 574 | 67.5 ± 25.3† | 574 | 63.9 ± 25.6† |
| **Depressive symptoms (GDS score; 0–30)** | 524 | 4.3 ± 3.7 | 507 | 4.2 ± 4.0 | 574 | 5.0 ± 4.3† | 563 | 5.0 ± 4.2† |
| **Cognitive status (3MS score; 0–100)** | 523 | 93.7 ± 44 | 524 | 91.6 ± 6.1* | 574 | 95.0 ± 3.8† | 574 | 93.5 ± 5.6† |
| **Handgrip strength, kPa** | 524 | 78.4 ± 17.6 | 524 | 71.5 ± 17.6* | 574 | 59.3 ± 16.3† | 574 | 54.3 ± 16.0† |
| **Knee extensor strength, kg** | 524 | 72.8 ± 20.7 | 524 | 67.9 ± 19.8* | 574 | 43.6 ± 14.0† | 574 | 41.2 ± 12.7† |
| **Physical performance, Timed Up and Go, s** | 524 | 10.0 ± 1.8 | 524 | 10.6 ± 2.2* | 574 | 10.6 ± 2.1† | 574 | 11.2 ± 2.8† |

*Statistically significant difference between T1 and T4 within sex groups (\( P < 0.05 \)) estimated using Student’s paired-samples \( t \) test for continuous variables, the Sign test for categorical variables, and the McNemar test for dichotomous variables.

†Statistically significant difference between men and women at the given time point (\( P < 0.01 \)) estimated using Student’s independent-samples \( t \) test or Pearson’s chi-square test for categorical variables.

GDS, Geriatric Depression Scale; PASE, Physical Activity Scale for the Elderly; SF-36, 36-item Short Form Health Survey Questionnaire; T1, baseline; T4, 3-year follow-up; 3MS, Modified Mini-Mental State Examination.
Dietary characteristics

Mean ± SD daily protein intake was higher in men (82.7 ± 19.4 g/d) than in women (68.3 ± 15.0 g/d), but was similar relative to aBW (1.13 ± 0.27 and 1.12 ± 0.26 g · kg aBW$^{-1}$ · d$^{-1}$, respectively) and only slightly differed when expressed as percentage of energy (16.1% ± 2.5 and 16.6% ± 2.5%, respectively) (Table 2). Also, mean daily protein intake did not differ significantly between T1, T2, and T3 (data not shown), suggesting stable intake over time. Most participants consumed 3 protein-providing meals every day. Snacks contributed ~5% to daily protein intake. The vast majority of consumed meals (96%) and snacks (76%) provided ≥5 g of protein. The evenness of protein intake distribution across meals was comparable in men and women. Correlation between each of the protein intake parameters was low: $r ≤ 0.136$ in men and $r ≤ 0.192$ in women (Supplemental Table 7).

Protein intake parameters, muscle strength, and physical performance in men

Regression models were run in order to identify whether truncating the protein intake per meal to a maximal value would lead to stronger associations between daily protein intake and the outcomes, and thus suggest an optimal protein dose per meal to exist. As expected, with an increase in the maximal value applied, the $t$ values for daily protein intake increased, reached a maximum (i.e., the optimal dose), and continued on a plateau or slightly decreased (Figure 2). For men, optimal protein doses per meal of 30–35 g were observed for knee extensor strength and physical performance at T4 (Figure 2A). Applying these thresholds led to statistically significant associations of higher daily protein intake with better knee extensor strength ($β$: 0.218; 95% CI: 0.015, 0.422) and physical performance ($β$: 0.029; 95% CI: 0.002, 0.057) (Table 3) at T4, whereas the models based on the actual (nontruncated) intakes failed to show significant associations (Figure 2A, B). We observed no associations—and thus no optimal protein dose per meal—of daily protein intake with handgrip strength at T4 or with the 3-y change in these outcomes.

The number of protein-providing meals and the number of protein-providing snacks were not associated with any outcome (e.g., association between number of protein-providing snacks and handgrip strength at T4, tertile 2 compared with tertile 1: $β$: −0.586; 95% CI: −3.842, 2.670; tertile 3 compared with tertile 1: $β$: −1.140; 95% CI: −4.464, 2.184) (Table 3). Evenness of protein intake distribution across meals was not associated with handgrip or knee extensor strength at T4, but a more uneven protein intake distribution across meals was associated with better physical performance at T4 ($β$: 1.768; 95% CI: 0.051, 3.484). Also, a more uneven protein intake distribution across meals was associated with less 3-y decline in handgrip strength ($β$: 6.670; 95% CI: 1.406, 11.935), but not with the 3-y change in knee extensor strength ($β$: 4.545; 95% CI: −3.699, 12.790) or physical performance ($β$: 0.372; 95% CI: −0.621, 1.365).

Protein intake parameters, muscle strength, and physical performance in women

In women, optimal protein doses per meal varied from 35 to 50 g, depending on the outcome. Specifically, an optimal protein dose per meal was observed for knee extensor strength (40 g) and
The present study is the first that we know of to examine prospective associations of different protein intake parameters simultaneously with muscle strength and physical performance in community-dwelling older men and women. Higher daily protein intake was associated with higher knee extensor strength and better physical performance (TUG) in both sexes and with less 3-y decline in physical performance in women. We observed optimal protein doses per meal of ~30–35 g in men and ~35–50 g in women for these outcomes. Daily protein intake was not associated with handgrip strength. In men, more uneven protein intake distribution across meals was associated with better physical performance and with less decline in handgrip strength. In women, higher number of protein-providing snacks was associated with higher handgrip strength and knee extensor strength and with less decline in handgrip strength. In neither sex was the number of protein-providing meals associated with outcomes.

Higher daily protein intake was associated with higher knee extensor strength and physical performance (TUG) at T4 in both sexes. Although these findings confirm our hypothesis, very few studies have examined protein intake in relation to these outcomes specifically. Our results are nonetheless similar to the findings of Farsijani et al. (25), also from the NuAge data, but contrast with the (cross-sectional) null findings of Granic et al. (23). This discrepancy may be explained by differences in age, follow-up time, dietary assessment, and adjustment for different confounders, but also by the fact that Granic et al. did not account for the potential ceiling effect of protein intake per meal. Regression coefficients were indeed severely attenuated in the present study when no maximal value of protein per meal was applied (Figure 2).

**Discussion**

The present study is the first that we know of to examine prospective associations of different protein intake parameters simultaneously with muscle strength and physical performance in community-dwelling older men and women. Higher daily protein intake was associated with better knee extensor strength and better physical performance (TUG) in both sexes and with less 3-y decline in physical performance in women. We observed optimal protein doses per meal of ~30–35 g in men and ~35–50 g in women for these outcomes. Daily protein intake was not associated with handgrip strength. In men, more uneven protein intake distribution across meals was associated with better physical performance and with less decline in handgrip strength. In women, higher number of protein-providing snacks was associated with higher handgrip strength and knee extensor strength and with less decline in handgrip strength. In neither sex was the number of protein-providing meals associated with outcomes.

Higher daily protein intake was associated with higher knee extensor strength and physical performance (TUG) at T4 in both sexes. Although these findings confirm our hypothesis, very few studies have examined protein intake in relation to these outcomes specifically. Our results are nonetheless similar to the findings of Farsijani et al. (25), also from the NuAge data, but contrast with the (cross-sectional) null findings of Granic et al. (23). This discrepancy may be explained by differences in age, follow-up time, dietary assessment, and adjustment for different confounders, but also by the fact that Granic et al. did not account for the potential ceiling effect of protein intake per meal. Regression coefficients were indeed severely attenuated in the present study when no maximal value of protein per meal was applied (Figure 2).

Surely, more prospective research is needed...
## TABLE 3  Associations of protein intake parameters with handgrip strength, knee extensor strength, and physical performance at T4, and with their 3-y change, in community-dwelling older men from the NuAge (Quebec Longitudinal Study on Nutrition and Successful Aging) study1

| Outcome at T4 3 | Handgrip strength, kPa | Knee extensor strength, kg | Physical performance, TUG, 2 s |
|----------------|------------------------|---------------------------|-----------------------------|
|                | β (95% CI)             | P value                   | β (95% CI)                  | P value                   | β (95% CI)                  | P value                   |
| Daily protein intake, g | 0.000 (−0.074, 0.073) | 0.993                     | 0.218 (0.015, 0.422)        | 0.035                     | 0.029 (0.002, 0.057)        | 0.038                     |
| Mean number of protein-providing meals: 2.86–2.99 vs. <2.86 meals/d 5 | 1.143 (−0.198, 3.331)  | 0.555                     | −2.310 (−7.985, 3.365)      | 0.424                     | −0.599 (−1.231, 0.032)      | 0.063                     |
| Mean number of protein-providing meals: 3.00 vs. <2.86 meals/d 5 | −0.378 (−4.147, 3.390) | 0.844                     | −4.169 (−8.764, 0.425)      | 0.075                     | −0.357 (−0.872, 0.159)      | 0.175                     |
| Mean number of protein-providing snacks: tertile 2 vs. tertile 1 6 | −0.586 (−3.842, 2.670) | 0.724                     | 0.384 (−3.450, 4.217)       | 0.844                     | −0.109 (−0.531, 0.313)      | 0.612                     |
| Mean number of protein-providing snacks: tertile 3 vs. tertile 1 6 | −1.140 (−4.464, 2.184) | 0.501                     | −0.393 (−4.393, 3.608)      | 0.847                     | −0.173 (−0.622, 0.275)      | 0.448                     |
| Evenness of protein intake distribution across meals, CV 7 | 7.009 (−2.035, 16.053) | 0.128                     | 11.427 (−3.156, 26.011)     | 0.124                     | 1.768 (0.051, 3.484)        | 0.044                     |
| 3-y change in outcome 3,7 | Optimal dose per meal 4 no | Optimal dose per meal 4 no | Optimal dose per meal 4 no | Optimal dose per meal 4 no | Optimal dose per meal 4 no | Optimal dose per meal 4 no |
| Daily protein intake, g 4 | 0.006 (−0.037, 0.049)  | 0.781                     | −0.013 (−0.080, 0.055)      | 0.712                     | 0.005 (−0.003, 0.013)       | 0.212                     |
| Mean number of protein-providing meals: 2.86–2.99 vs. <2.86 meals/d 5 | 0.897 (−3.670, 1.877)  | 0.526                     | −1.976 (−6.321, 2.368)      | 0.372                     | −0.174 (−0.697, 0.349)      | 0.513                     |
| Mean number of protein-providing meals: 3.00 vs. <2.86 meals/d 5 | −1.106 (−3.300, 1.088) | 0.322                     | −1.641 (−5.079, 1.797)      | 0.349                     | −0.107 (−0.520, 0.306)      | 0.611                     |
| Mean number of protein-providing snacks: tertile 2 vs. tertile 1 6 | 1.574 (−0.326, 3.474)  | 0.104                     | 0.575 (−2.393, 3.543)       | 0.704                     | −0.044 (−0.401, 0.312)      | 0.807                     |
| Mean number of protein-providing snacks: tertile 3 vs. tertile 1 6 | 1.694 (−0.249, 3.637)  | 0.087                     | −0.067 (−3.097, 2.964)      | 0.965                     | −0.050 (−0.414, 0.315)      | 0.790                     |
| Evenness of protein intake distribution across meals, CV 7 | 6.670 (1.406, 11.935)  | 0.013                     | 4.545 (−3.699, 12.790)      | 0.279                     | 0.372 (−0.621, 1.365)       | 0.462                     |

1n = 524. TUG, Timed Up and Go; T4, 3-y follow-up.

2To facilitate comparison of the results, all TUG values were multiplied by −1 so that positive coefficients (β) always represent better outcomes.

3Results are from the fully adjusted regression models, including age, body height, body weight, physical activity, education level, smoking, alcohol use, weight change, cognition, number of medications, and pain.

4Results are from the regression analyses with the optimal dose of protein per meal as specified, i.e., the optimal protein dose per meal was defined as the dose applied in the model with the highest statistically significant t value for daily protein intake.

5Number of protein-providing meals was categorized based on the mean number of meals per day over the 9 recall days.

6Number of protein-providing snacks was categorized based on the mean number of snacks per day over the 9 recall days into tertiles: tertile 1 (≥0.67 snacks/d), tertile 2 (≥0.78 and ≤1.22 snacks/d), and tertile 3 (≥1.33 snacks/d).

7In addition adjusted for outcome value at baseline.
**TABLE 4** Associations of protein intake parameters with handgrip strength, knee extensor strength, and physical performance at T4, and with their 3-y change, in community-dwelling older women from the NuAge (Quebec Longitudinal Study on Nutrition and Successful Aging) study

| Outcome at T4 | Handgrip strength, kPa | Knee extensor strength, kg | Physical performance, TUG, ² s |
|---------------|-------------------------|----------------------------|-------------------------------|
|               | β (95% CI)               | P value                    | β (95% CI)                    | P value | β (95% CI)                    | P value |
| Daily protein intake, g | 0.008 (−0.081, 0.097) | 0.863                      | 0.103 (0.003, 0.203) | 0.043 | 0.017 (0.000, 0.034) | 0.050 |
| Mean number of protein-providing meals: 2.86–2.99 vs. <2.86 meals/d | 3.404 (−1.523, 8.331) | 0.175                      | 0.946 (−2.896, 4.787) | 0.629 | 0.515 (−0.268, 1.299) | 0.197 |
| Mean number of protein-providing meals: 3.00 vs. <2.86 meals/d | 0.251 (−3.887, 4.390) | 0.905                      | 0.190 (−3.055, 3.435) | 0.908 | −0.031 (−0.691, 0.629) | 0.927 |
| Mean number of protein-providing snacks: tertile 2 vs. tertile ³ | 4.817 (1.732, 7.902) | 0.002                      | 3.045 (0.639, 5.451) | 0.013 | −0.027 (−0.517, 0.464) | 0.915 |
| Mean number of protein-providing snacks: tertile 3 vs. tertile ³ | 4.103 (0.965, 7.240) | 0.010                      | 2.120 (−0.323, 4.563) | 0.089 | 0.098 (−0.401, 0.596) | 0.701 |
| Evenness of protein intake distribution across meals, CV | −1.792 (−10.876, 7.292) | 0.698                      | −2.049 (−10.405, 6.307) | 0.630 | 0.698 (−0.877, 2.273) | 0.384 |
| ³-y change in outcome | Optimal dose per meal: ⁴ no | Optimal dose per meal: ⁴ 40 g | Optimal dose per meal: ⁴ 50 g |
| Daily protein intake, g | −0.021 (−0.079, 0.037) | 0.476                      | 0.027 (−0.026, 0.079) | 0.317 | 0.019 (0.000, 0.039) | 0.049 |
| Mean number of protein-providing meals: 2.86–2.99 vs. <2.86 meals/d | 1.069 (−2.134, 4.271) | 0.512                      | 1.333 (−1.566, 4.232) | 0.367 | 0.100 (−0.541, 0.740) | 0.760 |
| Mean number of protein-providing meals: 3.00 vs. <2.86 meals/d | −1.349 (−4.037, 1.340) | 0.325                      | 1.034 (−1.401, 3.470) | 0.404 | −0.292 (−0.834, 0.249) | 0.289 |
| Mean number of protein-providing snacks: tertile 2 vs. tertile ³ | 2.091 (0.079, 4.103) | 0.042                      | 1.033 (−0.792, 2.859) | 0.267 | 0.205 (−0.605, 0.196) | 0.316 |
| Mean number of protein-providing snacks: tertile 3 vs. tertile ³ | 1.113 (−0.934, 3.161) | 0.286                      | 1.403 (−0.444, 3.251) | 0.136 | −0.018 (−0.425, 0.388) | 0.929 |
| Evenness of protein intake distribution across meals, CV | −0.950 (−6.847, 4.946) | 0.752                      | −4.651 (−9.995, 0.693) | 0.088 | 0.779 (−0.698, 2.257) | 0.301 |

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1 n = 574. TUG, Timed Up and Go; T4, 3-y follow-up.

2 To facilitate comparison of the results, all TUG values were multiplied by −1 so that positive coefficients (β) always represent better outcomes.

3 Results are from the fully adjusted regression models, including age, body height, body weight, physical activity, education level, smoking, alcohol use, weight change, cognition, number of medications, and pain.

4 Results are from the regression analyses with the optimal dose of protein per meal as specified, i.e., the optimal protein dose per meal was defined as the dose applied in the model with the highest statistically significant r value for daily protein intake.

5 Number of protein-providing meals was categorized based on the mean number of meals per day over the 9 recall days.

6 Number of protein-providing snacks was categorized based on the mean number of snacks per day over the 9 recall days into tertiles: tertile 1 (≤0.67 snacks/d), tertile 2 (≥0.78 and ≤1.22 snacks/d), and tertile 3 (≥1.33 snacks/d).

7 In addition adjusted for outcome value at baseline.
to replicate our findings. Our observed associations for daily protein intake were not statistically significant when analyzed in relation to the 3-y change in outcomes, except for physical performance in women. The decline in muscle strength and physical performance in our analytic sample (1%–9%) may have been too small over the limited follow-up to observe associations, and this change might be at the lower limit of what is considered clinically meaningful (44). Moreover, participants included in the present study had relatively high habitual daily protein intake compared to the current RDA. The potential contribution of protein to muscle strength and physical performance may then have already been integrated within the outcome measurements at baseline. Also, we did not measure change in protein intake over time, but it would unlikely have explained the 3-y changes in outcomes because our data showed that daily protein intake remained stable over years. The decrease in muscle strength and physical performance in the NuAge cohort may likely be related to the aging process per se and other factors (e.g., sedentary behavior) than changes in dietary protein.

The observed optimal protein doses per meal of 30–35 g in men and 35–50 g in women indicate that older men and women should aim for ≥30 g and ≥35 g protein/meal, respectively—and no more than 35 g and 50 g—to optimize their muscle strength and physical performance. These results were comparable with findings from metabolic dose-response studies (45–47). For example, Cuthbertson et al. (45) observed a significant increase in MPS up to intakes of 10 g essential amino acids (~20 g whey protein or ~30 g protein in a regular meal) in older men (mean ± SD age: 70 ± 6 y) at rest, but no significant additional effect for higher intakes. Similarly, Yang et al. (47) found the optimal dose of whey protein for maximal MPS in older men (mean ± SD age: 71 ± 4 y) to be 20 g at rest and 40 g in combination with leg-based resistance exercise. Only 1 (cross-sectional) observational study addressed this protein intake parameter and showed that more frequent consumption of meals was most strongly associated with higher muscle strength when protein doses were 30–45 g/meal (30), which is also consistent with our results. The higher optimal protein dose per meal observed in women than in men may be due to the reduced sensitivity (i.e., lower MPS response to anabolic stimuli) reported in older women (18, 19). Hence, women might need higher protein doses to reach optimal MPS than men. Moreover, women in the NuAge cohort appear on average less physically active than men, whereas physical activity was reported to potentially overcome anabolic resistance in older adults (48). Therefore, although physical activity might further increase MPS rates reached by protein provision only (47, 48), it may also decrease the amount of protein needed to preserve optimal muscle and physical function in aging.

Number of protein-providing meals was not associated with any outcome in both sexes. This might be the result of the observation that most NuAge participants had 3 meals/d most (if not all) of the time, making it difficult to reveal any association. However, number of protein-providing snacks was associated with (change in) muscle strength in women, independently of daily protein intake. Snacking may provide more anabolic opportunities during the postprandial state when MPS reduces and muscle protein breakdown elevates. Interestingly, in our analytic sample, women consumed more protein-providing snacks than men, which may explain our results. Women also had a smaller protein dose per meal, which may make the role of snacking more important than in men. No previous studies to our knowledge examined the effect of snacking in older adults, so future research is needed to explore this hypothesis.

In the present study, we did not observe that a more even distribution of protein across meals was associated with better muscle strength or physical performance, as some authors have postulated (6, 10, 11). On the contrary, we observed either no association or that a more uneven protein intake distribution was better in terms of muscle strength and physical performance (only in men). Our results are in contrast with those reported by Farsi jani et al. (25) and Granic et al. (23), who did not observe an association between evenness of protein intake distribution and physical performance. As for daily protein intake, these discrepancies might be partly explained by the optimal protein dose per meal we took into account in calculations of the CV. Also, daily protein intake is slightly negatively correlated with CV. As such, the CV may have captured the effect of daily protein intake in these studies, leading to results suggesting beneficial effects of even distribution. The sex-difference observed in our study may be explained by women being probably less likely to reach the optimal protein dose per meal as often as men given their lower daily protein intake and higher optimal protein dose per meal. In fact, especially when daily protein intake is low, it is likely that reaching the optimal dose in a meal and a consequent uneven distribution is related to better physical performance. More research focused on protein intake distribution—dependent of total protein intake and number of meals—in relation to functional outcomes is required.

The main strength of the present study is the in-depth and unique approach to studying protein intake, i.e., considering its multiple facets. Moreover, dietary intake was assessed using nine 24-h recalls collected over 3 y, which provided representative estimates of habitual dietary habits. Indeed, all significant coefficients were severely attenuated when calculations of the protein intake parameters were estimated based on only 3 recalls (1 from each of T1, T2, and T3; data not shown). Other strengths included the prospective study design and large number of potent confounders that we adjusted for. Some limitations must be discussed as well. First, the truncation approach is a new method that has not been validated per se. Nevertheless, we observed that for most of the outcomes, the t values increased up to a certain point and started to reach a plateau or slightly decrease once this point was passed, which supports our method (face validity). More studies using this approach are needed to replicate our findings. Second, this cohort comprised predominantly Caucasians, so caution is required when applying these results to other ethnicities. Third, other protein intake parameters may be related to muscle strength and physical performance, such as the protein quality (49, 50). Last, owing to the observational nature of this study, the causal relation remains uncertain and any residual confounding (e.g., by fat mass) cannot be dismissed.

To conclude, in this cohort of community-dwelling older adults being generally well-functioning at baseline, higher daily protein intake over 3 y, up to a maximum per-meal dose of 30–35 g in men and 35–50 g in women, was associated with better knee extensor strength and physical performance, but not handgrip strength. The role of protein intake distribution across meals or the number of protein-providing meals or snacks remains uncertain and could depend on the protein dose per meal. Nevertheless, our results imply that more aspects of protein intake may contribute
to muscle strength and physical performance than solely the daily protein quantity, notably the protein dose per meal. Our results also support the idea that large quantities of protein in 1 meal (>50 g) might not provide further benefits for muscle and physical function. Future prospective research should examine the role and interplay of different protein intake parameters in maintenance of function outcomes in older adults to further refine protein recommendations.

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The authors’ responsibilities were as follows—LMH and NP: conceived and designed the study, analyzed the data, and drafted the manuscript; SC, MV, and PG: critically revised the manuscript for important intellectual content; PG: was the principal investigator of the NuAge Study; NP: was the administrator of the NuAge Database and takes full responsibility for the integrity of the data and the conduct of the study; and all authors: interpreted the data and read and approved the final manuscript. The authors report no conflicts of interest.

Data Availability

Data described in the article can be made available upon request pending application and approval by the NuAge team via nuage-cdrv@usherbrooke.ca. The code book and analytic code can be obtained by contacting the corresponding author.

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