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Per meal dose and frequency of protein consumption is associated with lean mass and muscle performance☆

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SUMMARY

Background: It has been hypothesized that for older adults evenly distributing consumption of protein at 30–40 g per meal throughout the day may result in more favorable retention of lean mass and muscular strength. Such a thesis has not, to our knowledge, been tested outside of short-term studies or acute measures of muscle protein synthesis.
Aims: To examine whether the number of times an individual consumed a minimum of 30 g of protein at a meal is associated with leg lean mass and knee extensor strength.
Methods: Data from the 1999–2002 NHANES were used, with 1081 adults (50–85 y) constituting the analytic sample. A "multiple pass" 24-h dietary interview format was used to collect detailed information about the participants' dietary intake. Knee extensor strength was assessed objectively using the Kin Com MP dynamometer. Leg lean mass was estimated from whole-body dual-energy X-ray absorptiometry (DXA) scans.
Results: Participants with 1 vs. 0 ($\beta_{adjusted} = 23.6$, $p = 0.002$) and 2 vs. 0 ($\beta_{adjusted} = 51.1$, $p = 0.001$) meals of $\geq$30 g protein/meal had greater strength and leg lean mass (1 vs. 0, $\beta_{adjusted} = 1160$, $p < 0.05$ and 2 vs. 0, $\beta_{adjusted} = 2389$, $p < 0.05$). The association of protein frequency with leg lean mass and strength plateaued at ~45 g protein/meal for those consuming 2 vs. 0 meals above the evaluated protein/meal threshold. However, for those with only 1 meal at or above the evaluated threshold, the response plateaued at 30 g/meal. Leg lean mass mediated the relationship between protein frequency and strength, with the proportion of the total effect mediated being 64%.
Conclusions: We found that more frequent consumption of meals containing between 30 and 45 g protein/meal produced the greatest association with leg lean mass and strength. Thus, the consumption of 1–2 daily meals with protein content from 30 to 45 g may be an important strategy for increasing and/or maintaining lean body mass and muscle strength with aging.

1. Introduction

Dietary protein intake, and the per meal distribution of that protein, throughout the day have received increasing interest in the literature due to the potential influence on health-related outcomes such as body composition, muscle mass and functional capacity [1–5]. For adults, the Recommended Dietary Allowance (RDA) for protein is 0.8 g/kg body mass/d, however, a number of researchers have proposed that the RDA is not adequate for older adults [1,2,6] and recent studies in older women support this conclusion [7]. Additionally, the RDA for protein does not provide specific guidance on a per-meal recommendation for protein intake. Such a recommendation may be important as there is no capacity for storage of diet-derived amino acids beyond their almost immediate use in protein synthetic or amino acid-requiring processes. As such, an even distribution of protein throughout the day to, for example, maximally stimulate muscle protein synthesis

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(MPS) at each meal, may enhance the preservation of muscle mass over time [6,8]. This would be a particularly important strategy in older individuals experiencing sarcopenia and obese individuals losing lean mass during energy-restricted diets.

The hypothesis for an even distribution of dietary protein on a per meal basis is based on the existence of a saturable dose-response relationship between the protein ingested, and subsequent aminoacidemia, and the muscle protein synthetic response [9–12]. Several studies have demonstrated that as the amount of protein consumed in a single bolus increases, there is a graded rise in the rate of MPS up to a maximally effective protein dose [9–12]. Beyond this optimal protein dose MPS cannot be stimulated further despite consumption of larger protein servings [11,13]. For example, Symons et al. [14] observed that a serving of beef providing 30 g of protein was sufficient to maximally stimulate protein synthesis and giving a dose higher than this did not further augment the response.

Providing support for the importance of the distribution of protein intake, rather than simply the total amount of protein consumed over the day, Mamerow et al. [15] recently reported that the consumption of ~30 g of protein at breakfast, lunch and dinner stimulated 24-h mixed MPS to a greater extent than a 'skewed' isonitrogenous protein intake weighted, as is commonly consumed, towards the evening meal (i.e. 10 g at breakfast, 15 g at lunch and 65 g dinner) in younger adults. Furthermore, recent work in energy restricted overweight/obese older men showed that evenly distributing 75 g of whey protein (3 $\times$ 25 g doses) throughout the day stimulated myofibrillar protein synthesis more effectively than the traditional skewed distribution of protein (i.e. 10 g at breakfast, 15 g at lunch, 50 g at dinner). This, however, was not observed during conditions of energy balance [16].

The majority of Americans consume a high percentage of their daily protein at the last meal of the day [17]; thus, we aimed to investigate whether the frequency of a dose of dietary protein of 30–45 g per meal was related to lean mass and muscular strength; two outcomes important for metabolism and functional ability. Our working hypothesis, was that the highest association between leg lean mass and strength would be between 30 and 45 g of protein. Moreover, that a greater number of meal occasions at this dose would also be related to lean mass and strength.

2. Methods

2.1. Design and participants

Data were extracted from the 1999–2002 NHANES (only cycles with lower extremity muscle strength data). NHANES evaluates a representative sample of non-institutionalized U.S. civilians, selected by a complex, multistage probability design. NHANES is conducted by the National Center for Health Statistics (NCHS), and all procedures for data collection were approved by the NCHS ethics review board [18]. Analyses were based on data from 1081 consented adults (50–85 y) who provided data for the study variables and who did not have a physician-diagnosis of diabetes, coronary artery disease, musculoskeletal conditions (e.g., arthritis), on statin or anti-hypertensive medication, or consumed <600 or >4000 kcal/day. Notably, only those 50 and older were eligible for the muscle strength assessment.

2.2. Frequency of protein consumption

A "multiple pass" 24-h dietary interview format was used to collect detailed information about the participant's dietary behavior [18]. This multiple pass format included asking participants to recall all foods and beverages consumed in a 24-h period the day before the interview; report the time in which each food was eaten and what they would call the eating occasion for the food (e.g., breakfast); food probes were used to collect detailed information for each food reported; and the final reported foods were reviewed with the respondent in chronological order. Herein, we report the total daily consumption of protein (g), carbohydrate (g), total fat (g) and energy (kcal). Given the study aim of examining the association of the consumption of protein frequency on leg strength and leg lean mass, we created a 'protein frequency' variable by summing the number of meals individuals consumed $\geq$30 g of protein per meal. This protein frequency variable could range from 0 to 6 (breakfast, brunch, lunch, snack, dinner, evening snack), but because of small cell size issues at greater protein frequency, we recoded this protein frequency variable as 0, 1, and 2 or more occasions. Information about protein quality was not available.

2.3. Peak knee extensor muscle strength

A Kin Com MP dynamometer (Chattanooga Group, Inc.) was used to assess voluntary peak isokinetic knee extensor strength in Newtons (at a speed of 60°/second). A total of 6 measurements of muscle strength of the right quadriceps were taken: three warm-up trial measurements followed by 3 outcome measurements. If a participant completed 4–6 measures, the highest peak force was selected from trials 4 to 6; however, if a participant completed fewer than 4 measures, the highest peak force from the warm-up trials was selected. All values were gravity corrected for limb and lever arm weight [19].

2.4. Leg lean mass

Leg lean mass was estimated using whole-body dual-energy X-ray absorptiometry (DXA) scans using the Hologic QDR 4500A fan beam X-ray bone densitometer (Hologic, Inc, Bedford, Massachusetts). Multiple imputation was used for missing data and as a result, exact-p-values are not provided, but rather, whether the association was significant ($P < 0.05$) or not ($p \geq 0.05$) [20]. The "IMPUTE" module, as implemented in SAS, was used for the sequential regression multivariate imputation and details on generating estimates from the NHANES multiple imputed DXA data are provided elsewhere [20]. Lower extremity lean mass was calculated by summing the lower extremity lean mass (excluding bone mineral content) of the left and right legs.

2.5. Statistical analyses

All analyses were performed in Stata (v. 12) and accounted for the complex survey design employed in NHANES, with population-based estimates generated using the dietary-specific NCHS sample weights. Two separate multivariable linear regression analyses were computed that examined the association of frequency of protein consumption $\geq$30 g of protein per meal (range: 0–2+, with "0" serving as the referent group) with peak leg strength and lower extremity lean mass; for each model the protein frequency variable was the independent categorical variable (0, 1, or 2+). In addition to linear regression models, a Barron and Kenny mediational analysis examined whether lower extremity lean mass mediated the relationship between frequency of protein consumption (independent variable) and peak leg strength (outcome variable). Barron and Kenny mediation analyses were computed which includes a 3-step regression process (1. IV $\rightarrow$ DV; 2. IV $\rightarrow$ M; 3. M $\rightarrow$ DV while controlling for IV); indirect effects were calculated using the product of coefficients approach, with bootstrapping used to calculate confidence intervals [21].