Clinical Nutrition 35 (2016) 1506–1511

Contents lists available at ScienceDirect

Clinical Nutrition

journal homepage: http://www.elsevier.com/locate/clnu

Original article

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

Jeremy P. Loenneke [a,*], Paul D. Loprinzi [b], Caoileann H. Murphy [c], Stuart M. Phillips [c]

[a] Kevser Ermin Applied Physiology Laboratory, Department of Health, Exercise Science, and Recreation Management, The University of Mississippi, University, MS, USA
[b] Center for Health Behavior Research, Department of Health, Exercise Science, and Recreation Management, The University of Mississippi, University, MS, USA
[c] Exercise Metabolism Research Group, Department of Kinesiology, McMaster University, Hamilton, ON, Canada

ARTICLE INFO

Article history:
Received 7 December 2015
Accepted 1 April 2016

Keywords:
Protein distribution
Muscle mass
Sarcopenia
Epidemiology
Muscle strength

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 ≥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

☆ This manuscript was not supported by funding.
* Corresponding author. Kevser Ermin Applied Physiology Laboratory, The University of Mississippi, 231 Turner Center, University, MS, 38677, USA. Tel.: +1 (662) 915 5567; fax: +1 (662) 915 5525.
E-mail address: jploenne@olemiss.edu (J.P. Loenneke).

http://dx.doi.org/10.1016/j.clnu.2016.04.002
0261-5614/

(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 × 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 ≥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 ≥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 → DV; 2. IV → M; 3. M → DV while controlling for IV); indirect effects were calculated using the product of coefficients approach, with bootstrapping used to calculate confidence intervals [21].

For all models (both linear regression and mediational models), covariates included relative protein intake (g/kg), total daily carbohydrate (g), total daily fat (g), age (continuous; y), gender (male/female), race-ethnicity (Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black, and other), mean arterial pressure (continuous; mmHg), self-reported smoking status (current, former, never), and participation in moderate-to-vigorous physical activity in the past 30 days (yes/no). Relative protein, daily carbohydrate, and daily fat intake were co-varied to allow for the question of protein dose per meal to be better answered. The remaining covariates were included to control for any condition that may directly or indirectly influence skeletal muscle form and/or function [16].

3. Results

3.1. Descriptive characteristics

Table 1 displays the weighted characteristics of the study variables. The mean age of the sample was 60.7 y with the majority (82.3%) of the sample being non-Hispanic white.

3.2. Association between protein frequency and muscular-related parameters

Table 2 displays the weighted multivariable linear regression results examining the association between protein frequency (number of meals/day with at least 30 g of protein/meal) with peak knee strength and leg lean mass. For the entire sample, and after complete adjustment (including daily relative protein intake [g/kg]), participants with 1 ($\beta_{adjusted}$ = 23.6, 95% CI: 9.5, 37.7, $p = 0.002$) and 2 ($\beta_{adjusted}$ = 51.1, 95% CI: 19.3, 83.0, $p = 0.001$) meals of ≥30 g protein/meal were associated with higher peak knee extensor strength than those who did not consume any meals of ≥30 g protein/meal; notably, a greater magnitude of association occurred for those consuming 2 (vs. 0) meals of ≥30 g protein/meal compared to 1 (vs. 0) meal of ≥30 g protein. Similar to peak knee extensor strength, greater frequency of protein consumption was associated with larger leg lean mass. Additional analyses were computed that changed the referent group to those consuming 1 meal of ≥30 g protein/meal. Participants consuming ≥2 meals ≥30 g protein/meal, compared to those with 1 meal of ≥30 g protein/meal, were associated with higher peak knee extensor strength and a larger leg lean mass (Table 2). In line with the results for the entire sample, similar associations were observed when employing effect modification analyses among overweight/obese participants and those above and below 65 y of age; multiplicative interaction models (cross-product term plus their main variables and covariates in the model) confirmed these effect modification results (*P* interaction terms > 0.05).

Table 2
Multivariable linear regression association (β, 95% CI) between frequency of protein consumption ≥ 30 g/meal with peak isokinetic knee extensor strength and leg lean tissue mass (g; left + right leg lean mass excluding bone mineral content; N = 1081). *denotes significant differences at $p < 0.05$.

	# Of meals/day with protein consumption ≥30 g protein/meal[a]		
Entire sample	0 (n = 349)	1 (n = 560) vs. 0	2 (n = 172) vs. 0
Strength (N)	Referent	23.6 (9.5, 37.7)*	51.1 (19.3, 83.0)*
Leg lean mass (g)	Referent	1160 (678, 1643)*	2389 (1702, 3076)*
Age-group			
50–64 y			
Strength (N)	Referent	32.7 (13.1, 52.3)*	49.6 (9.4, 89.8)*
Leg lean mass (g)	Referent	1141 (536, 1746)*	2370 (1525, 3215)*
65–85 y			
Strength (N)	Referent	10.5 (−12.6, 33.7)	64.2 (30.6, 97.8)*
Leg lean mass (g)	Referent	1133 (534, 1732)*	2564 (1628, 3501)*
Overweight/obese sample (BMI >25 kg/m²)			
Strength (N)	Referent	14.2 (−4.2, 32.7)	54.4 (23.5, 85.3)*
Leg lean mass (g)	Referent	1003 (511, 1495)*	2211 (1304, 3118)*

[a] All associations (β) were adjusted for relative protein intake (g/kg), total daily carbohydrate (g), total daily fat (g), age (continuous; y), gender (male/female), race-ethnicity (Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black, and other), mean arterial pressure (continuous; mmHg), self-reported smoking status (current, former, never), and participation in moderate-to-vigorous physical activity in the past 30 days (yes/no).

3.3. Mediation effects of lean muscle mass on the relationship between protein frequency and muscle strength

Given that frequency of protein consumption was positively associated with peak knee strength and leg lean mass, this suggests that leg lean mass may mediate the relationship between

Table 1
Weighted characteristics of the study variables for the total sample (N = 1081) and separated by those who met the 30 g protein per meal threshold at 0 (n = 349), 1 (n = 560), and 2+ (n = 172) times per day. Data are presented as means and stand errors.

Variables	Total	0 times	1 time	2+ times
Age (y)	60.7 (0.3)	62.0 (0.7)	60.6 (0.4)	58.3 (0.7)
% Female	52.7 (1.9)	72.8 (2.8)	47.6 (2.5)	31.2 (5.8)
% Non-hispanic white	82.3 (1.7)	79.3 (2.9)	83.2 (2.1)	78.7 (3.4)
% Current smoker	18.6 (1.7)	16.7 (2.0)	19.1 (2.0)	20.0 (4.0)
Mean arterial pressure (mmHg)	93.5 (0.5)	94.5 (1.2)	93.0 (0.6)	93.3 (1.1)
% Engaged in MVPA in past 30 days	62.6 (2.2)	60.9 (4.1)	62.2 (2.4)	66.6 (4.5)
Body mass index (kg/m²)	27.2 (0.2)	27.0 (0.3)	27.0 (0.2)	27.9 (0.5)
Body mass (kg)	77.0 (0.6)	73.7 (1.0)	77.3 (0.8)	82.2 (1.7)
Peak knee extensor strength (N)	377 (5)	333 (7)	383 (7)	434 (15)
Leg lean mass (g)	15,017 (140)	13,740 (257)	15,246 (203)	16,656 (368)
Relative protein intake (g/kg)	1.02 (0.01)	0.64 (0.01)	1.06 (0.01)	1.4 (0.03)
Total daily protein (g)	74.5 (0.8)	45.3 (0.9)	78.8 (1.0)	114.7 (2.4)
Breakfast (g)	11.1 (0.3)	8.8 (0.5)	11.4 (0.4)	14.2 (1.7)
Brunch (g)	1.5 (0.2)	0.8 (0.2)	1.1 (0.3)	3.9 (1.0)
Lunch (g)	18.6 (0.7)	11.3 (0.7)	18.1 (0.8)	33.5 (2.3)
Snack (g)	9.5 (0.4)	7.5 (0.6)	9.1 (0.6)	14.1 (1.4)
Dinner (g)	32.9 (0.6)	16.1 (0.5)	38.2 (1.0)	47.7 (2.0)
Evening snack (g)	0.6 (0.1)	0.4 (0.1)	0.5 (0.1)	1.1 (0.3)
Total daily carbohydrate intake (g)	241.7 (4.4)	199.5 (5.4)	252.7 (7.1)	285.4 (9.1)
Total daily fat intake (g)	72.9 (1.2)	52.1 (1.2)	75.5 (1.3)	102.7 (3.6)

MVPA, Moderate-to-vigorous physical activity.

frequency of protein consumption and knee extensor strength. Results indicated that the indirect effect of leg lean mass on the relationship between protein frequency and peak knee strength was significant ($\beta = 0.08$; bootstrapped 95% CI: 0.05, 0.10; $p < 0.05$), with a relatively large effect. The proportion of the total effect mediated was 64%, the ratio of the indirect to direct effect was 1.80, and the ratio of the total to direct effect was 2.80.

3.4. Dose-response association between protein frequency and leg lean mass

Figure 1 and Table 3 display the dose-response association between protein frequency number meals/day above various thresholds (e.g., 15 g/meal, 20 g/meal, 25 g/meal, etc.) with leg lean body mass and knee extensor strength. When compared to those consuming 0 meals above the evaluated protein/meal threshold, a clear dose-response association, up to 45 g/meal, was visually observed for those consuming 2 or more meals above the evaluated threshold. The association between protein frequency and leg lean mass and strength appeared to visually plateau at ~45 g/meal. However, when comparing consuming only 1 meal at or above the evaluated threshold, the response plateaued at 30 g/meal. With regard to model stability, and particularly, whether the mean strength/lean mass estimates were deemed reliable across the different protein thresholds, we applied the Healthy People 2010 criteria for data suppression for NHANES data (i.e., relative standard error [RSE] <30% is considered a reliable estimate); notably, for all estimates, the RSE was <30% [22].

4. Discussion

The aim of the present study was to determine whether the frequency of consumption of a meal containing the quantity of protein previously shown to maximize MPS (≥30 g of protein) was associated with leg lean mass and knee extensor strength using a nationally representative sample of U.S. adults. We found that more frequent consumption of meals containing at least 30 g of protein was associated with greater leg lean mass and knee extensor muscle strength. This finding was apparent across different age groups and BMI classifications. Further, our analysis suggests that the relationship between protein frequency and knee extension strength may be mediated by the change in leg lean mass. To explore this phenomenon further, we examined different 'per meal' protein intakes (from 15 to 60 g protein/meal) to see how it affected the association with the outcome variables of leg lean mass and knee extension strength. We found a clear dose response association in those consuming 2 or more meals at or above the evaluated threshold up to approximately 45 g of protein per meal. For those consuming only 1 meal at or above the threshold, we observed that the dose-response relationship plateaued at approximately 30 g of protein per meal, with no further increase in leg lean mass and knee extension strength with higher per meal protein intakes.

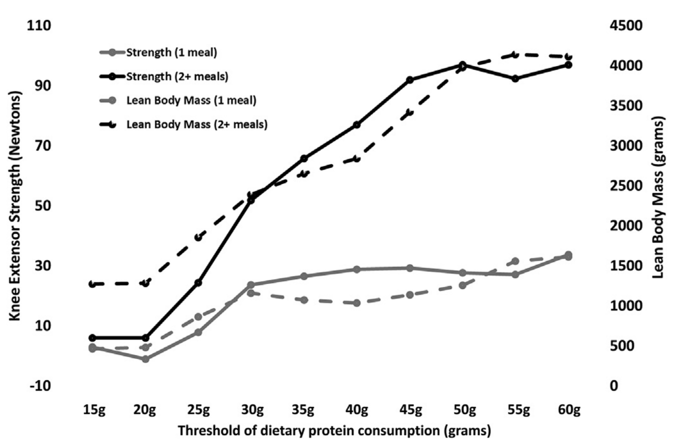

Fig. 1. Dose-response association between protein frequency (# meals/day above various thresholds [e.g., 15 g/meal, 20 g/meal, 25 g/meal, etc.) and knee extensor strength and leg lean mass. Strength and leg lean mass results are reported for 1 (vs. 0) and 2 (vs. 0) meals above various protein thresholds (per meal). With regard to 1 (vs. 0) meal, strength (grey line) and lean mass (grey dash) appear to level off at approximately 30 g/meal. With regard to 2 (vs. 0) meals, strength (black line) and lean mass (black dash) appear to level off at approximately 45 g/meal. All associations (β) were adjusted for relative protein intake (g/kg), total daily carbohydrate (g), total daily fat (g), age (continuous; y), gender (male/female), race-ethnicity (Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black, and other), mean arterial pressure (continuous; mmHg), self-reported smoking status (current, former, never), and participation in moderate-to-vigorous physical activity in the past 30 days (yes/no).

To date, several studies have been conducted in an attempt to determine the quantity of protein required to maximally stimulate MPS per meal. In younger adults, MPS shows a dose-dependent increase from 0 to 20 g, and then plateaus despite doubling the protein serving to 40 g [11]. In middle-aged and older adults higher protein intakes in the range of ~30–40 g appear to be necessary to maximize the response [12,23,24]. In agreement with the results from these experimental studies, we observed that higher frequency of consuming at least 30 g of protein per meal was associated with greater leg lean mass and strength in this cohort of 50–85 year olds. Moreover, this association assumed a classical curvilinear dose response that was saturable when the protein per meal protein intake was increased from 15 to 60 g/meal. Among those consuming ≥2 meals at or above the evaluated per meal protein threshold, the positive association between protein quantity per meal and leg lean mass/strength plateaued after 45 g protein/meal. While 45 g protein/meal is slightly greater than the dose of protein shown to maximize MPS in older adults in some studies [12,14], it is important to note that the available studies determining the dose of protein required to maximally stimulate MPS have been conducted using either isolated proteins or protein-rich foods (i.e. beef) [9–12]. In free-living individuals, such as those represented in the current study, protein-containing foods would usually have been consumed in the context of a mixed meal. The co-ingestion of substantial amounts of other nutrients such as fat, carbohydrate, and/or fiber with the protein may affect rates of digestion and subsequent aminoacidemia [25]. Given the apparent role of the pattern of aminoacidemia in influencing the MPS response [26] it remains possible that higher protein doses of ~45 g are needed to optimize the MPS response, and possibly modify the per meal anti-catabolic response [27], to food-based meals in middle-aged and older adults.

We are unable to explain why, among those consuming only 1 meal at or above the evaluated threshold, the dose-response relationship between protein intake per meal and leg lean mass and strength plateaued at 30 g/meal (compared to 45 g/meal among those consuming ≥2 meals at or above the threshold). It is possible that this may relate to the cross-sectional nature of the study and/or that we were unable to account for the potential influence of protein quality and/or co-consumption of other nutrients that affected postprandial aminoacidemia. For example, differences in the essential amino acid content and digestibility of different foods may have an impact on how many amino acids become available for skeletal muscle [28]. Regardless, our data supports the notion that a more even distribution of protein intake throughout the day, independent of daily relative protein intake, may play an important role in augmenting leg lean body mass and strength.

Limitations of this study include the cross-sectional design, rendering a conclusion on temporality not possible. However, our

Table 3
Multivariable linear regression association (β, 95% CI) between protein frequency (# meals/day above various thresholds [e.g., 15 g/meal, 20 g/meal, 25 g/meal, etc.]) and knee extensor strength and leg muscle mass.*denotes significant differences at $p < 0.05$.

	# Meals/day above evaluated protein threshold[a]		
	1 vs. 0	2 vs. 0	2 vs. 1
15 g/meal			
Strength (N)	3.0 (−20.6, 26.8)	6.0 (−19.4, 31.6)	3.0 (−9.7, 15.7)
Leg lean mass (g)	466 (−228, 1160)	1270 (534, 2006)*	804 (458, 1150)*
Sample size	n = 50 (0 meals)	n = 361 (1 meal)	n = 670 (2 meals)
20 g/meal			
Strength (N)	−1.1 (−22.5, 20.2)	6.0 (−14.8, 26.9)	7.1 (−9.5, 23.9)
Leg lean mass (g)	476 (−150, 1103)	1281 (636, 1927)*	805.1 (378, 1231)*
Sample size	n = 125 (0 meals)	n = 497 (1 meal)	n = 459 (2 meals)
25 g/meal			
Strength (N)	7.8 (−7.7, 23.3)	24.3 (7.4, 41.3)*	16.5 (3.7, 29.3)*
Leg lean mass (g)	860 (276, 1444)*	1851 (1141, 2562)*	991 (537, 1445)*
Sample size	n = 232 (0 meals)	n = 547 (1 meal)	n = 302 (2 meals)
30 g/meal			
Strength (N)	23.6 (9.5, 37.7)*	51.1 (19.3, 83.0)*	27.5 (1.3, 53.7)*
Leg lean mass (g)	1160 (678, 1643)*	2389 (1702, 3076)*	1228 (693, 1763)*
Sample size	n = 349 (0 meals)	n = 560 (1 meal)	n = 172 (2 meals)
35 g/meal			
Strength (N)	26.5 (11.9, 41.0)*	65.7 (33.3, 98.1)*	39.2 (12.8, 65.6)*
Leg lean mass (g)	1074 (712, 1437)*	2652 (1727, 3578)*	1577 (597, 2558)*
Sample size	n = 493 (0 meals)	n = 492 (1 meal)	n = 96 (2 meals)
40 g/meal			
Strength (N)	28.8 (15.6, 42.0)*	77.0 (43.1, 111.0)*	48.2 (16.5, 79.9)*
Leg lean mass (g)	1034 (585, 1483)*	2839 (1461, 4217)*	1805 (478, 3132)*
Sample size	n = 620 (0 meals)	n = 403 (1 meal)	n = 58 (2 meals)
45 g/meal			
Strength (N)	29.3 (15.3, 43.2)*	91.9 (48.4, 135.3)*	62.6 (21.0, 104.2)*
Leg lean mass (g)	1136 (712, 1559)*	3420 (1736, 5105)*	2284 (557, 4011)*
Sample size	n = 720 (0 meals)	n = 331 (1 meal)	n = 30 (2 meals)
50 g/meal			
Strength (N)	27.6 (13.3, 41.9)*	97.0 (49.5, 144.4)*	69.4 (19.4, 119.3)*
Leg lean mass (g)	1253 (901, 1606)*	3975 (2050, 5900)*	2721 (715, 4728)*
Sample size	n = 810 (0 meals)	n = 248 (1 meal)	n = 23 (2 meals)
55 g/meal			
Strength (N)	27.1 (4.8, 49.4)*	92.4 (32.8, 152.0)*	65.3 (7.0, 1235.5)*
Leg lean mass (g)	1557 (1175, 1938)*	4140 (1285, 6996)*	2583 (−273, 5440)
Sample size	n = 866 (0 meals)	n = 202 (1 meal)	n = 13 (2 meals)
60 g/meal			
Strength (N)	33.7 (9.0, 58.3)*	97.0 (20.5, 173.5)*	63.3 (−11.1, 137.8)*
Leg lean mass (g)	1613 (1204, 2022)*	4114 (3122, 5106)*	2501 (1532, 3469)*
Sample size	n = 905 (0 meals)	n = 168 (1 meal)	n = 8 (2 meals)

[a] All associations (β) were adjusted for relative protein intake (g/kg), total daily carbohydrate (g), total daily fat (g), age (continuous; y), gender (male/female), race-ethnicity (Mexican American, other Hispanic, non-Hispanic white, non-Hispanic black, and other), mean arterial pressure (continuous; mmHg), self-reported smoking status (current, former, never), and participation in moderate-to-vigorous physical activity in the past 30 days (yes/no).

findings are supported by previous experimental data suggesting that protein consumption is associated with increased lean mass [4]. In addition, dietary analysis was measured via self-report, however, the multi-pass method that the NHANES uses for diet recalls has been shown to estimate energy intake within 8–10% of actual intake [29,30]. Further, as discussed by Davy and Estabrooks, the predictive validity of dietary recall instruments has been repeatedly documented using dose-response and other predictive methods [31]. Major strengths of this investigation include employing a relatively large national sample of U.S. adults, and utilizing objective measures of lean body mass and knee extension strength. To our knowledge, this is the first nationally representative study addressing the interrelationships between protein distribution, lower extremity lean mass and lower extremity muscle strength.

Currently, the RDA for protein makes no recommendation on the per-meal distribution of dietary protein throughout the day. However, the need to define specific meal-based protein intakes that affect health indexes such as muscle mass and function has recently been stressed [32]. We propose that in a national sample of U.S. adults that eating protein more frequently within the day may be an important strategy for increasing and/or maintaining lean body mass and muscle strength. Further, a threshold of ~30–45 g of dietary protein per meal seems to produce the greatest association with lean body mass and strength when consuming more than one meal at that specific intake. Consuming dietary protein at more than one meal may be of importance for individuals seeking to optimize muscle mass and strength, but may be a particularly important strategy among individuals vulnerable to muscle mass loss including older adults and obese individuals undergoing energy-restricted diets. Future longer-term longitudinal research is required to investigate this thesis finding further.

Conflict of interest

No other authors report potential conflicts of interest.

Acknowledgments

No funding was used to prepare this manuscript. The authors' responsibilities were as follows—JPL: study design, analysis and interpretation of the data, and drafting of the manuscript; PDL: study design, performed statistical analysis, interpretation of the data, and the drafting of the manuscript; CHM: study design,

interpretation of the data, and drafting of the manuscript; and SMP: study design, interpretation of the data, and drafting of the manuscript. SMP has received grants from the following agencies: the US National Dairy Council, Dairy Farmers of Canada, and Nestle. In addition, SMP has received honoraria and travel expenses from the following: the US National Dairy Council, Dairy Farmers of Canada, and Nestle.

References

[1] Bauer J, Biolo G, Cederholm T, Cesari M, Cruz-Jentoft AJ, Morley JE, et al. Evidence-based recommendations for optimal dietary protein intake in older people: a position paper from the PROT-AGE Study Group. J Am Med Dir Assoc 2013;14(8):542–59. http://dx.doi.org/10.1016/j.jamda.2013.05.021.
[2] Deutz NE, Bauer JM, Barazzoni R, Biolo G, Boirie Y, Bosy-Westphal A, et al. Protein intake and exercise for optimal muscle function with aging: recommendations from the ESPEN Expert Group. Clin Nutr 2014;33(6):929–36. http://dx.doi.org/10.1016/j.clnu.2014.04.007.
[3] Loenneke JP, Balapur A, Thrower AD, Syler G, Timlin M, Pujol TJ. Short report: relationship between quality protein, lean mass and bone health. Ann Nutr Metab 2010;57(3–4):219–20. http://dx.doi.org/10.1159/000321736.
[4] Houston DK, Nicklas BJ, Ding J, Harris TB, Tylavsky FA, Newman AB, et al. Dietary protein intake is associated with lean mass change in older, community-dwelling adults: the Health, Aging, and Body Composition (Health ABC) Study. Am J Clin Nutr 2008;87(1):150–5.
[5] Sandoval-Insausti H, Perez-Tasigchana RF, Lopez-Garcia E, Garcia-Esquinas E, Rodriguez-Artalejo F, Guallar-Castillon P. Macronutrients intake and incident frailty in older adults: a prospective cohort study. J Gerontol A Biol Sci Med Sci 2016. http://dx.doi.org/10.1093/gerona/glw033.
[6] Volpi E, Campbell WW, Dwyer JT, Johnson MA, Jensen GL, Morley JE, et al. Is the optimal level of protein intake for older adults greater than the recommended dietary allowance? J Gerontol A Biol Sci Med Sci 2013;68(6):677–81. http://dx.doi.org/10.1093/gerona/gls229.
[7] Rafii M, Chapman K, Owens J, Elango R, Campbell WW, Ball RO, et al. Dietary protein requirement of female adults >65 years determined by the indicator amino acid oxidation technique is higher than current recommendations. J Nutr 2015;145(1):18–24. http://dx.doi.org/10.3945/jn.114.197517.
[8] Paddon-Jones D, Leidy H. Dietary protein and muscle in older persons. Curr Opin Clin Nutr Metab Care 2014;17(1):5–11. http://dx.doi.org/10.1097/MCO.0000000000000011.
[9] Cuthbertson D, Smith K, Babraj J, Leese G, Waddell T, Atherton P, et al. Anabolic signaling deficits underlie amino acid resistance of wasting, aging muscle. FASEB J 2005;19(3):422–4. http://dx.doi.org/10.1096/fj.04-2640fje.
[10] Moore DR, Churchward-Venne TA, Witard O, Breen L, Burd NA, Tipton KD, et al. Protein ingestion to stimulate myofibrillar protein synthesis requires greater relative protein intakes in healthy older versus younger men. J Gerontol A Biol Sci Med Sci 2015;70(1):57–62. http://dx.doi.org/10.1093/gerona/glu103.
[11] Moore DR, Robinson MJ, Fry JL, Tang JE, Glover EI, Wilkinson SB, et al. Ingested protein dose response of muscle and albumin protein synthesis after resistance exercise in young men. Am J Clin Nutr 2009;89(1):161–8. http://dx.doi.org/10.3945/ajcn.2008.26401.
[12] Yang Y, Breen L, Burd NA, Hector AJ, Churchward-Venne TA, Josse AR, et al. Resistance exercise enhances myofibrillar protein synthesis with graded intakes of whey protein in older men. Br J Nutr 2012;108(10):1780–8. http://dx.doi.org/10.1017/S0007114511007422.
[13] Atherton PJ, Etheridge T, Watt PW, Wilkinson D, Selby A, Rankin D, et al. Muscle full effect after oral protein: time-dependent concordance and discordance between human muscle protein synthesis and mTORC1 signaling. Am J Clin Nutr 2010;92(5):1080–8. http://dx.doi.org/10.3945/ajcn.2010.29819.
[14] Symons TB, Sheffield-Moore M, Wolfe RR, Paddon-Jones D. A moderate serving of high-quality protein maximally stimulates skeletal muscle protein synthesis in young and elderly subjects. J Am Diet Assoc 2009;109(9):1582–6. http://dx.doi.org/10.1016/j.jada.2009.06.369.
[15] Mamerow MM, Mettler JA, English KL, Casperson SL, Arentson-Lantz E, Sheffield-Moore M, et al. Dietary protein distribution positively influences 24-h muscle protein synthesis in healthy adults. J Nutr 2014;144(6):876–80. http://dx.doi.org/10.3945/jn.113.185280.
[16] Murphy CH, Churchward-Venne TA, Mitchell CJ, Kolar NM, Kassis A, Karagounis LG, et al. Hypoenergetic diet-induced reductions in myofibrillar protein synthesis are restored with resistance training and balanced daily protein ingestion in older men. Am J Physiol Endocrinol Metab 2015;308(9): E734–43. http://dx.doi.org/10.1152/ajpendo.00550.2014.
[17] USDA. Internet: http://www.ars.usda.gov/Services/docs.htm?docid=18349.
[18] CDC. Internet: http://www.cdc.gov/nchs/nhanes.htm.
[19] Chen L, Nelson DR, Zhao Y, Cui Z, Johnston JA. Relationship between muscle mass and muscle strength, and the impact of comorbidities: a population-based, cross-sectional study of older adults in the United States. BMC Geriatr 2013;13:74. http://dx.doi.org/10.1186/1471-2318-13-74.
[20] Statistics NCfH, Nutrition Examination Survey. Technical documentation for the dual energy X-ray absorptiometry (DXA) multiple imputation data files. In: Centers for disease C; 2008. Hyattsville, MD.
[21] Baron RM, Kenny DA. The moderator-mediator variable distinction in social psychological research: conceptual, strategic, and statistical considerations. J Pers Soc Psychol 1986;51(6):1173–82.
[22] Klein RJ, Proctor SE, Boudreault MA, Turczyn KM. Healthy People 2010 criteria for data suppression. Healthy People 2010 statistical notes : from the Centers for Disease Control and Prevention, 24. National Center for Health Statistics; 2002. p. 1–12.
[23] Pennings B, Groen B, de Lange A, Gijsen AP, Zorenc AH, Senden JM, et al. Amino acid absorption and subsequent muscle protein accretion following graded intakes of whey protein in elderly men. Am J Physiol Endocrinol Metab 2012;302(8):E992–9. http://dx.doi.org/10.1152/ajpendo.00517.2011.
[24] Robinson MJ, Burd NA, Breen L, Rerecich T, Yang Y, Hector AJ, et al. Dose-dependent responses of myofibrillar protein synthesis with beef ingestion are enhanced with resistance exercise in middle-aged men. Appl Physiol Nutr Metab 2013;38(2):120–5. http://dx.doi.org/10.1139/apnm-2012-0092.
[25] Paddon-Jones D, Sheffield-Moore M, Aarsland A, Wolfe RR, Ferrando AA. Exogenous amino acids stimulate human muscle anabolism without interfering with the response to mixed meal ingestion. Am J Physiol Endocrinol Metab 2005;288(4):E761–7. http://dx.doi.org/10.1152/ajpendo.00291.2004.
[26] West DW, Burd NA, Coffey VG, Baker SK, Burke LM, Hawley JA, et al. Rapid aminoacidemia enhances myofibrillar protein synthesis and anabolic intramuscular signaling responses after resistance exercise. Am J Clin Nutr 2011;94(3):795–803. http://dx.doi.org/10.3945/ajcn.111.013722.
[27] Deutz NE, Wolfe RR. Is there a maximal anabolic response to protein intake with a meal? Clin Nutr 2013;32(2):309–13. http://dx.doi.org/10.1016/j.clnu.2012.11.018.
[28] Murphy CH, Oikawa SY, Phillips SM. Dietary protein to maintain muscle mass in aging: a case for per-meal protein recommendations. J Frailty Aging 2016;5(1):49–58.
[29] Conway JM, Ingwersen LA, Moshfegh AJ. Accuracy of dietary recall using the USDA five-step multiple-pass method in men: an observational validation study. J Am Diet Assoc 2004;104(4):595–603. http://dx.doi.org/10.1016/j.jada.2004.01.007.
[30] Conway JM, Ingwersen LA, Vinyard BT, Moshfegh AJ. Effectiveness of the US Department of Agriculture 5-step multiple-pass method in assessing food intake in obese and nonobese women. Am J Clin Nutr 2003;77(5):1171–8.
[31] Davy BM, Estabrooks PA. The validity of self-reported dietary intake data: focus on the "What We Eat In America" component of the national health and nutrition examination survey research initiative. Mayo Clin Proc 2015;90(7): 845–7. http://dx.doi.org/10.1016/j.mayocp.2015.05.009.
[32] Layman DK, Anthony TG, Rasmussen BB, Adams SH, Lynch CJ, Brinkworth GD, et al. Defining meal requirements for protein to optimize metabolic roles of amino acids. Am J Clin Nutr 2015. http://dx.doi.org/10.3945/ajcn.114.084053.