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Randomized control trials

Protein intake distribution pattern does not affect anabolic response, lean body mass, muscle strength or function over 8 weeks in older adults: A randomized-controlled trial

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SUMMARY

Background & aims: In our recent acute metabolic study, we found no differences in the anabolic response to differing patterns of dietary protein intake. To confirm this in a chronic study, we investigated the effects of protein distribution pattern on functional outcomes and protein kinetics in older adults over 8 weeks.

Methods: To determine chronic effects of protein intake pattern at 1.1 g protein/kg/day in mixed meals on lean body mass (LBM), functional outcomes, whole body protein kinetics and muscle protein fractional synthesis rate (MPS) over 8-week respective dietary intervention, fourteen older subjects were randomly divided into either EVEN or UNVEN group. The UNEVEN group (n = 7) consumed the majority of dietary protein with dinner (UNEVEN, 15/20/65%; breakfast, lunch, dinner), while the EVEN group (n = 7) consumed dietary protein evenly throughout the day (EVEN: 33/33/33%).

Results: We found no significant differences in LBM, muscle strength, and other functional outcomes between EVEN and UNEVEN before and after 8-week intervention. Consistent with these functional outcomes, we did not find significant differences in the 20-h integrated whole body protein kinetics [net protein balance (NB), protein synthesis (PS), and breakdown (PB)] above basal states and MPS between EVEN and UNEVEN intake patterns.

Conclusions: We conclude that over an 8-week intervention period, the protein intake distribution pattern in mixed meals does not play an important role in determining anabolic response, muscle strength, or functional outcomes. This trial is registered at https://ClinicalTrials.gov as NCT02787889.

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1. Introduction

The benefits of increased protein intake on the age-related loss of muscle mass and strength, i.e., sarcopenia, and many related physiological functions is becoming increasingly evident [1]. The NHANES data indicates that the average protein consumption of both men and women over the age of 50 yrs is approximately 1.1 g/kg/d [2], or 77 g protein/d for 70 kg adults. NHANES data also suggests that the American pattern of dietary protein intake is typically skewed towards the evening meal, which constitutes more than half of total daily protein intake [3]. The preponderance of literature indicates that muscle protein synthesis (MPS) in resting conditions can be maximally stimulated with approximately 20–35 g of protein or 0.25 g–0.43 g/kg (based on average body weight: 80 kg) [4–7], depending on protein quality and individual age. These findings are consistent with the recent report by Moore et al. [8] showing that a maximal MPS response is achieved with 0.24 g/kg/meal and 0.4 g/kg/meal for young and older adults, respectively. The latter amount translates to the average protein intake (1.1 g/kg/d) of middle age and older American adults if an even distribution of protein intake throughout the day is assumed [2]. Thus, with the traditional pattern of meal intake (e.g., 15%/20%/65% of protein for breakfast, lunch, and dinner, respectively) in the

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United States, a maximal stimulation of MPS would theoretically occur only at the dinner meal of approximately 50 g of protein (for a 70 kg adult). In addition, this amount would theoretically exceed the protein intake required to elicit the maximal anabolic effect by ~80% (i.e., excess amount of 22 g protein). This observation led to the promulgation of a popular hypothesis that distributing total protein intake equally over three meals would result in a more frequent stimulation of MPS as compared to the traditional intake pattern [9]. Although recent acute metabolic studies in older individuals indicated no pattern effect of dietary protein intake [10,11], it has been argued that acute studies may not reflect functional changes over time [12]. Therefore, we hypothesized that 1) the 20-h integrated whole-body net protein balance and MPS would be greater with even vs. uneven distribution pattern of protein intake after the 8 week of dietary intervention; and 2) an even distribution pattern of dietary protein intake throughout the day would result in greater gains in lean mass, strength, and function after 8 weeks of dietary intervention.

2. Materials and methods

2.1. Subjects

Nineteen healthy male and female older adults [51–69 yrs] with body mass indexes between 25 and 30 kg/m^2 were enrolled in the study (February 2014 through March 2015). Subject were excluded from the study participation if subjects had any of the followings: type I or II diabetes mellitus, active malignancy within the past 6 months, history of gastrointestinal bypass surgery, lactose intolerance or allergy to milk or milk products, a chronic inflammatory or other chronic disease (e.g., HIV/AIDS), low hematocrit or hemoglobin concentration, low platelets, current use of corticosteroids, any unstable medical conditions. Also excluded were subjects who participated in regular resistance exercise (>twice per week). All subjects actively signed written informed consent, and the study was approved by the Institutional Review Board at the University of Arkansas for Medical Sciences. Subjects were then randomly assigned to EVEN or UNEVEN group. Sample size for the present study that has been estimated based on the power analysis of muscle protein synthesis rate to detect effect sizes of 0.45 or larger were sixteen older subjects (8 subjects per group). We included fourteen older adult subjects [7 subjects per group; range of age: 51–69 yrs] for the final analyses (Table 1) due to subject dropout (n = 4) and screening failures (See CONSORT Diagram; Supplemental Fig. 1).

2.2. Experimental design

During the screening visit, body composition was determined by dual-energy X-ray absorptiometry (DEXA, QDR-4500A; Hologic, Waltham, MA) (Table 1) and was repeated at 8 weeks while subjects remained on their respective diets. Eligible subjects were then randomly assigned by a study coordinator to one of two dietary pattern groups in a permuted block randomization method using a sealed envelope: UNEVEN group where subjects consumed 1.1 g protein/kg body weight/day in an uneven pattern (15/20/65% of total daily protein; breakfast/lunch/dinner, respectively); or an EVEN group where subjects consumed the same amount of protein in an even pattern (~33% of total protein with each meal) for an 8-week dietary intervention period. After the screening, a 3-d dietary record and instruction were given to all subjects. The Clinical Research Services Core (CRSC) research dietician used the information from these dietary records to estimate their habitual food intake including the amount of protein intake and food preferences. Diets were configured to provide adequate caloric intake to maintain stable body weight over the 8-week intervention period using the Harris–Benedict equation and their level of physical activity (range of physical activity factor used = 1.38–1.83), and a daily vitamin/mineral supplement was included. The study dietician prepared all diets in the Metabolic Kitchen at the CRSC (Table 2). Diets were prepared to maximize protein intake from high quality protein sources including egg, dairy, and beef (31.4 ± 0.3% of EAA in the dietary protein). Individuals adhering to a purely vegan diet were excluded from the study because of the difficulty in matching the quality of protein with the other diets. Each distribution pattern was consumed for a total of 8 weeks. Primary outcomes were studied before and after the 8-week dietary intervention, and included body composition (lean body mass) and muscle strength and functional outcomes (see Strength and functional tests). Secondary outcomes i.e., whole body protein kinetics (protein synthesis, protein breakdown, and net balance) and MPS were also determined at the beginning and end of the 8-week dietary intervention period. Subjects obtained their meal allotment from the study coordinator at the Reynolds Institute on Aging (RIOA) twice each week. Prior to dietary intervention, subjects were provided a dietary record and point-and-shoot digital camera to record all the information regarding their food intake including the time of meal consumption and the amount of food leftover [10], which helped the study dietician ascertain calorie/protein intake as well as study compliance. This trial is registered at http://ClinicalTrials.gov under NCT02787889.

Table 1
Group characteristics before and after 8-week dietary intervention.

Groups	EVEN		UNEVEN	
Intervention period	Pre	Post	Pre	Post
N (M/F)	7 (4/3)		7 (2/5)	
Age, yrs	58.1 ± 2.4		60.3 ± 2.4	
Height, cm	170.6 ± 3.3		170.9 ± 4.5	
Total mass, kg	80.4 ± 2.4	78.9 ± 2.1	79.7 ± 4.7	76.9 ± 4.8
BMI, kg/m^2	27.7 ± 0.6	27.2 ± 0.7	27.2 ± 0.7	26.3 ± 0.9
LBM, kg	50.5 ± 2.7	50.3 ± 3.1	47.7 ± 4.2	46.9 ± 4.1
Body fat mass, %	31.8 ± 2.6	31.5 ± 2.9	35.3 ± 2.0	34.3 ± 2.4

Values are expressed as means ± SEM; M/F is the no. of male and female subjects in each group; BMI, body mass index; LBM, lean body mass.

Table 2
Interventional diet during the entire study period.

Groups		EVEN	UNEVEN
Daily energy intake, kcal	Total	2390 ± 139	2194 ± 162
	Per kg	29.7 ± 1.4	27.5 ± 0.9
Protein, g	B	29.3 ± 0.9	13.1 ± 0.8***
	L	29.3 ± 0.8	17.7 ± 1.1***
	D	29.2 ± 0.9	55.6 ± 3.6***
	Total	87.8 ± 2.6	86.4 ± 4.9
Fat, g	B	25.8 ± 1.5	12.2 ± 0.8***
	L	38.0 ± 2.1	25.1 ± 2.0***
	D	29.0 ± 2.7	46.4 ± 4.0**
	Total	92.8 ± 6.3	83.6 ± 6.6
Carbohydrate, g	B	100.0 ± 6.6	88.5 ± 5.2
	L	99.1 ± 3.1	84.9 ± 6.0
	D	109.4 ± 10.8	106.9 ± 11.5
	Total	308.5 ± 19.6	280.3 ± 21.8
Fiber, g	B	5.7 ± 0.4	5.2 ± 0.3
	L	9.8 ± 0.4	8.0 ± 0.6*
	D	10.1 ± 0.7	9.9 ± 0.8
	Total	25.6 ± 1.3	23.0 ± 1.5

Values are expressed as means ± SEM; B, breakfast; L, lunch; D, Dinner. Independent student t-test revealed no differences in daily total intakes of energy, protein, fat, carbohydrate, and fiber. Significant differences in B, L, or D existed between the EVEN and the UNEVEN as the study was designed for; $^{*}p < 0.05$, $^{**}p < 0.01$, and $^{***}p < 0.001$.

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