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Dietary Protein Distribution Positively Influences 24-h Muscle Protein Synthesis in Healthy Adults[1–3]

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Abstract

The RDA for protein describes the quantity that should be consumed daily to meet population needs and to prevent deficiency. Protein consumption in many countries exceeds the RDA; however, intake is often skewed toward the evening meal, whereas breakfast is typically carbohydrate rich and low in protein. We examined the effects of protein distribution on 24-h skeletal muscle protein synthesis in healthy adult men and women (n = 8; age: 36.9 ± 3.1 y; BMI: 25.7 ± 0.8 kg/m^2). By using a 7-d crossover feeding design with a 30-d washout period, we measured changes in muscle protein synthesis in response to isoenergetic and isonitrogenous diets with protein at breakfast, lunch, and dinner distributed evenly (EVEN; 31.5 ± 1.3, 29.9 ± 1.6, and 32.7 ± 1.6 g protein, respectively) or skewed (SKEW; 10.7 ± 0.8, 16.0 ± 0.5, and 63.4 ± 3.7 g protein, respectively). Over 24-h periods on days 1 and 7, venous blood samples and vastus lateralis muscle biopsy samples were obtained during primed (2.0 μmol/kg) constant infusion [0.06 μmol/(kg·min)] of L-[ring-$^{13}C_6$] phenylalanine. The 24-h mixed muscle protein fractional synthesis rate was 25% higher in the EVEN (0.075 ± 0.006%/h) vs. the SKEW (0.056 ± 0.006%/h) protein distribution groups (P = 0.003). This pattern was maintained after 7 d of habituation to each diet (EVEN vs. SKEW: 0.077 ± 0.006 vs. 0.056 ± 0.006%/h; P = 0.001). The consumption of a moderate amount of protein at each meal stimulated 24-h muscle protein synthesis more effectively than skewing protein intake toward the evening meal. J. Nutr. 144: 876–880, 2014.

Introduction

The current RDA for protein describes the minimum quantity of protein that should be consumed daily to prevent deficiency. Although there is increasing evidence that modestly increasing dietary protein intake beyond 0.8 g protein/(kg·d) has beneficial effects on health-related outcomes such as the regulation of muscle mass, body composition, and function in all adults (1–5), results from longer-term feeding studies remain somewhat inconsistent, with several reporting little to no benefit of increased protein intake for these specific outcomes.

Although total daily protein intake is relatively easy to standardize and compare across different trials, most research efforts have not addressed the distribution of protein across multiple daily meals. Data from the NHANES demonstrate that adults in the United States skew protein (and energy) consumption toward the evening meal (6). For example, mean protein consumption for adults aged ≥19 y is ~3-fold greater at dinner (38 g protein) compared with breakfast (13 g protein) (6).

Although the ability of dietary protein to stimulate muscle growth and repair may be influenced by factors including habitual physical activity, health status, body mass and composition, and age, it appears that a single meal containing ~30 g of high-quality protein maximally stimulates muscle protein synthesis in healthy adults (4,7–10). In some individuals, the consumption of greater amounts of dietary protein per meal (i.e., ≥40 g) may further improve net protein anabolism, a function of both synthesis and breakdown (11). However, care must be taken to avoid exceeding daily energy requirements (5,12).

Meals containing <30 g of protein attenuate the postprandial muscle protein synthesis (13,14). This dose-response may be especially deleterious for older adults experiencing anabolic resistance, or an exaggerated reduction in muscle protein synthesis in response to meals with a lower protein content (15,16).

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[3] Supplemental Tables 1 and 2 and Supplemental Figures 1 and 2 available from the ''Online Supporting Material'' link in the online posting of the article and from the same link in the online table of contents at http://jn.nutrition.org.

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The question remains whether simply manipulating the distribution pattern of protein consumption over a 24-h period can meaningfully affect outcome-focused measures (e.g., body composition, muscle strength, functional capacity) in healthy adults whose diets are not overtly deficient. Age-related conditions such as osteoporosis and sarcopenia do not develop acutely. Rather, they are insidious processes precipitated by suboptimal lifestyle practices in early middle age (17,18). The conceptual model for this study contends that establishing a dietary framework containing moderate amounts of high-quality dietary protein at each meal represents an efficient and feasible dietary strategy to optimize 24-h muscle protein synthesis while being mindful of issues such as protein cost and daily energy consumption.

We hypothesized that an even protein distribution (~30 g for breakfast, 30 g for lunch, and 30 g for dinner) would result in a greater 24-h muscle protein synthetic response than the same total amount of protein delivered in a skewed format (~10 g for breakfast, 15 g for lunch, and 65 g for dinner).

Participants and Methods

Participants. This study was approved by the University of Texas Medical Branch Institutional Review Board and independently reviewed by a data safety and monitoring board. Volunteers were recruited through flyers and newspaper advertisements. Medical screening for study eligibility included a medical history, complete blood count, plasma electrolytes, blood glucose concentration, and liver and renal function tests. Participants were excluded for the following: BMI >30 kg/m^2, metabolic disease, low hematocrit or hemoglobin, vascular disease, hypertension, cardiac abnormalities, renal disease, recent weight loss or gain, participation in a weight-loss diet/exercise program, smoking, and anabolic steroid usage. Eight healthy male (n = 5) and female (n = 3) volunteers between the ages of 25 and 55 y qualified for and participated in this study (**Table 1**). All participants were physically active but not athletically trained.

Study design. Individuals participated in a randomized 7-d crossover feeding study with a 30-d washout period. The general study design is described in **Supplemental Figure 1**. On days 1 and 7 of each diet, participants completed a 24-h metabolic study (**Supplemental Fig. 2**). A second metabolic study (day 7) was included to determine if there was habituation to the study diets over time. After the medical screening, participants were asked to maintain their normal diets and to avoid strenuous activity 72 h prior to each metabolic study. Participants were admitted to the Institute for Translational Science–Clinical Research Center (ITS-CRC)[8] at ~1500 h the day before each metabolic study. At 0530 h the next morning, after a standardized evening meal and overnight fast, an 18-gauge polyethylene catheter (Insyte-W; Becton Dickinson) was inserted into a forearm vein for blood sampling. A second 18-gauge polyethylene catheter was inserted into a forearm vein of the opposite arm for infusion of the stable isotope tracer. Background blood samples were drawn for analysis of phenylalanine enrichment and concentration (serum separator tubes; BD Vacutainer SST). A primed (2 μmol/kg), constant infusion [0.06 μmol(kg·min)] of L-[ring-$^{13}C_6$]phenylalanine (Cambridge Isotope Laboratories) was started at 0700 h and maintained for 26 h. Three muscle biopsies (100–150 mg) were obtained at 0900 h, 1230 h, and at 24 h (0900 h) from the lateral portion of the vastus lateralis (19). Peripheral venous blood samples were obtained before ingestion of each meal and every 20 min for 2–3 h after meal consumption. To reduce any potential complications associated with reduced physical activity (i.e., 36 h of bed rest) during the metabolic studies, participants completed a 30-min bout of moderate-intensity treadmill walking (~60% of age-predicted maximum heart rate) at 1600 h on days 1 and 7.

[8] Abbreviations used: EVEN, even daily protein distribution; FSR, fractional synthesis rate; ITS-CRC, Institute of Translational Science–Clinical Research Center; SKEW, skewed daily protein distribution.

TABLE 1 Physical characteristics of the healthy adult study participants[1]

Characteristic	Value
Age, *y*	36.9 ± 3.1
Height, *m*	1.72 ± 0.03
Body mass, *kg*	76.8 ± 2.9
BMI, *kg/m^2*	25.7 ± 0.8
Body fat, %	32.2 ± 2.1
Lean mass, *kg*	50.1 ± 2.8

[1] Values are means ± SEMs; n = 8.

Study diets. All meals were prepared and cataloged by ITS-CRC bionutrition staff to control macronutrient and energy intake of the 2 study diets. The Harris-Benedict equation with a standardized activity factor of 1.6 was used to estimate daily energy requirements. The diets were designed to exceed the RDA and to broadly reflect daily protein consumption in the United States [i.e., 1.2 g protein/(kg·d)] (6). The study diets were isoenergetic and isonitrogenous, and provided a total of ~90 g protein/d, but differed in the distribution pattern. Diet 1 provided an even protein distribution (EVEN) at each meal: ~30 g (breakfast), 30 g (lunch), and 30 g (dinner). Diet 2 provided an exaggerated skewed protein distribution (SKEW): ~10 g (breakfast), 15 g (lunch), and 65 g (dinner). Carbohydrate intake was held constant, whereas dietary fat intake was manipulated to ensure that the total daily energy consumption remained similar for each diet.

During inpatient metabolic studies (days 1 and 7), meals were provided at 0930, 1300, and 1700 h. The meals on days 1 and 7 were identical. On days 2–6, volunteers were given the option of returning to the ITS-CRC for meals or receiving prepackaged take-home meals. An outpatient dietary log book was provided to document meal times, uneaten or (nonapproved) additional food items, and miscellaneous notes. All meals contained a variety of high-quality plant- and animal-based protein sources. Although 100% dietary compliance was encouraged, uneaten food items from the metabolic study days and midweek meals (frozen and stored) were returned to the ITS-CRC metabolic kitchen and analyzed by using Nutrition Data System for Research software version 2006, developed by the Nutrition Coordinating Center, University of Minnesota. Examples of the EVEN and SKEW study menus provided to participants during metabolic study days are presented in **Supplemental Tables 1** and **2**.

Mixed muscle protein fractional synthesis rate. Plasma and bound and intracellular mixed muscle L-[ring-$^{13}C_6$]phenylalanine enrichments were determined as previously described (7,20,21). Mixed muscle protein fractional synthesis rate (FSR) was calculated by measuring the direct incorporation of L-[ring-$^{13}C_6$]phenylalanine into protein, using the precursor-product model:

$$\mathrm{FSR} = \left[\left(E_{p2} - E_{p1}\right) / \left(E_m \cdot t\right)\right] \cdot 60 \cdot 100,$$

where E_{p1} and E_{p2} were the enrichments of bound L-[ring-$^{13}C_6$]phenylalanine for sequential biopsies, t was the time interval between biopsies, and E_m represented the mean L-[ring-$^{13}C_6$]phenylalanine enrichment in the muscle intracellular precursor pool.

Statistical analysis. Mixed-effects linear regression techniques were used to analyze FSR in response to breakfast and over 24 h. The "xtmixed" command in Stata 12.1 (StataCorp LP) was used to generate a model for group (EVEN vs. SKEW) and time (day 1 vs. day 7) as fixed effects and participant as a random effect. A Shapiro-Wilk W test for residual normality was also performed. Residuals were plotted and examined for fit. Outlying values were excluded if they failed to meet model assumptions and were ≥2 SDs from the mean. The final model output included main effects for group and time and an interaction effect (group × time). Between-group contrasts with a Bonferroni correction were performed at each time point (day 1 and day 7). Mixed-effects linear regression was used to analyzed mean plasma L-[ring-$^{13}C_6$]phenylalanine enrichments with group (EVEN vs.

SKEW) and time (day 1 vs. day 7) as fixed effects and blood draw time point as a random effect. Muscle intracellular L-[ring-$^{13}C_6$]phenylalanine enrichments are part of the FSR calculation and are presented descriptively. Data are presented as means ± SEs; $P < 0.05$ was accepted as being significant.

Results

Dietary intake

Identical meals were provided to participants during the metabolic studies (days 1 and 7). Seven-day mean energy and macronutrient intake is presented in **Table 2**. Total 24-h protein, carbohydrate, and fat consumption in the SKEW and EVEN conditions was not different. Both diets exceeded the RDA for protein [0.8 g/(kg·d)] by ~50%. The SKEW diet met the RDA for protein during the evening meal alone. In all versions of the EVEN and SKEW menus used in this study, the animal-to-vegetable protein ratio was ~2:1 (Supplemental Tables 1 and 2).

Plasma and muscle enrichments

Mean plasma L-[ring-$^{13}C_6$]phenylalanine enrichments did not differ between study days (i.e., day 1 vs. day 7) for either group ($P > 0.05$). Plasma and muscle intracellular L-[ring-$^{13}C_6$]phenylalanine enrichments are presented in **Fig. 1** and **Table 3**.

Mixed muscle protein synthesis

Breakfast meal: 30 vs. 10 g protein. During the initial metabolic study (day 1), muscle protein synthesis in response to the breakfast meal containing 30 g protein was ~30% higher than with the 10-g protein meal ($P = 0.006$). A similar response was observed on day 7 after dietary habituation ($P = 0.002$) (**Fig. 2**).

Twenty-four-hour even vs. skewed protein distribution. Both 24-h diets contained a similar amount of protein (90–94 g). On study day 1, mixed muscle protein synthesis was ~25% higher when dietary protein was evenly distributed across 3 meals compared with the SKEW condition ($P = 0.003$). A similar muscle protein synthetic response was observed after 7 d of habituation to each diet ($P = 0.001$) (**Figs. 3** and **4**).

TABLE 2 Seven-day mean energy and macronutrient intake in healthy adults consuming diets with an EVEN or SKEW protein distribution[1]

Meal	Energy	Protein	Protein	Carbohydrate	Fat
	kcal	*g*	*g/kg*	*g*	*g*
Breakfast					
EVEN	848 ± 47.8	31.5 ± 1.3	0.41 ± 0.01	83.8 ± 2.8	43.0 ± 2.2
SKEW	537 ± 34.1	10.7 ± 0.8	0.14 ± 0.01	79.3 ± 7.4	19.7 ± 1.5
Lunch					
EVEN	820 ± 28.6	29.9 ± 1.6	0.39 ± 0.02	116 ± 11.1	26.0 ± 1.1
SKEW	683 ± 33.6	16.0 ± 0.5	0.21 ± 0.01	113 ± 9.5	18.4 ± 1.0
Dinner					
EVEN	727 ± 47.6	32.7 ± 1.6	0.42 ± 0.01	112 ± 7.3	16.4 ± 1.1
SKEW	1100 ± 49.3	63.4 ± 3.7	0.82 ± 0.03	115 ± 9.3	43.6 ± 1.4
Daily total[2]					
EVEN	2400 ± 121	94.1 ± 3.7	1.22 ± 0.02	312 ± 17.8	85.4 ± 4.0
SKEW	2320 ± 113	90.1 ± 4.6	1.17 ± 0.04	307 ± 24.1	81.7 ± 3.0

[1] Values are means ± SEMs; $n = 8$. EVEN, even daily protein distribution; SKEW, skewed daily protein distribution.
[2] There were no differences between total daily macronutrient and energy intakes, $P > 0.05$.

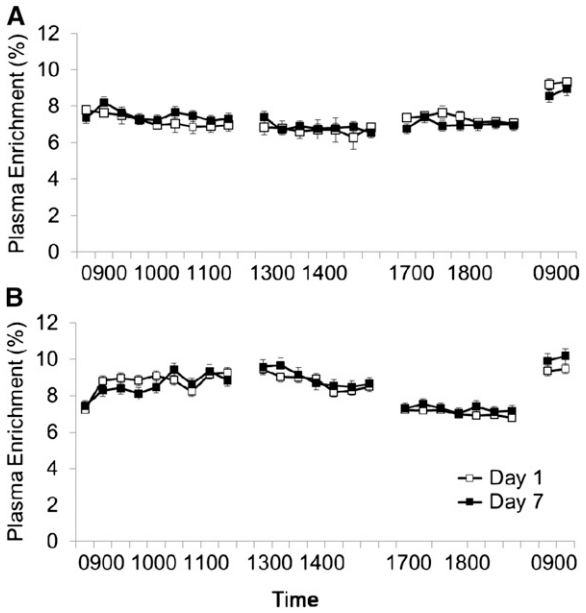

FIGURE 1 Plasma L-[ring-$^{13}C_6$]phenylalanine enrichments in healthy adults on days 1 and 7 of diets with an EVEN (*A*) or SKEW (*B*) protein distribution. Values are means ± SEMs; $n = 8$. EVEN, even daily protein distribution; SKEW, skewed daily protein distribution.

Discussion

This study demonstrates that consuming a moderate amount of high-quality protein 3 times a day stimulates muscle protein synthesis to a greater extent than the common practice of skewing protein consumption toward the evening meal. Specifically, 24-h muscle protein synthesis was ~25% greater when protein intake was evenly distributed, compared with the SKEW diet. This result was not altered by several days of habituation to either protein distribution pattern.

There is broad agreement among many protein researchers that the RDA for protein [0.8 g protein/(kg·d)], although sufficient to prevent deficiency, is insufficient to promote optimal health, particularly in populations exposed to catabolic stressors such as illness, physical inactivity, injury, or advanced age (4,22–25). Several recent consensus statements have suggested that a protein intake between 1.0 and 1.5 g/(kg·d) may confer health benefits beyond those afforded by simply meeting the current RDA (4,26,27). In the current study we provided diets that exceeded the RDA for protein by 50% but were consistent with the average daily protein intake of the U.S. adult population [i.e., 1.2 g protein/(kg·d)] (6). Our data demonstrate that the quantity

TABLE 3 Muscle L-[ring-$^{13}C_6$]phenylalanine intracellular enrichments in muscle biopsy samples of healthy adults on days 1 and 7 after the ingestion of diets with an EVEN or SKEW protein distribution[1]

	Day 1		Day 7	
	EVEN	SKEW	EVEN	SKEW
Bx 1	0.048 ± 0.004	0.063 ± 0.005	0.061 ± 0.004	0.067 ± 0.005
Bx 2	0.057 ± 0.003	0.068 ± 0.005	0.065 ± 0.003	0.072 ± 0.004
Bx 3	0.071 ± 0.003	0.078 ± 0.007	0.079 ± 0.004	0.077 ± 0.004

[1] Values are means ± SEMs tracer-to-tracee ratios; $n = 8$. Bx 1–3, muscle biopsy samples 1–3; EVEN, even daily protein distribution; SKEW, skewed daily protein distribution.

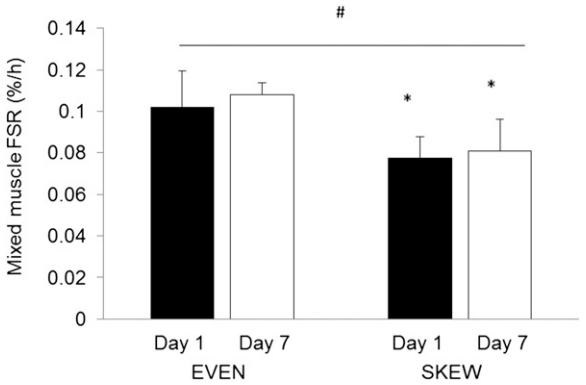

FIGURE 2 Mixed muscle protein FSRs in healthy adults on days 1 and 7 after the ingestion of a breakfast meal containing 30 g (EVEN) or 10 g (SKEW) of protein. Values are means ± SEMs; n = 8. *Different from EVEN at that time point, $P < 0.05$. #Main effect of group between 30- and 10-g meals, $P < 0.05$. EVEN, even daily protein distribution; FSR, fractional synthesis rate; SKEW, skewed daily protein distribution.

of protein consumed over a 24-h period is not the sole determinant of its potential to stimulate muscle protein synthesis.

Our data are also consistent with the contention that there is no protein synthetic advantage gained by consuming increasingly large servings of protein in a single meal (7,16). Unlike fat or carbohydrate, the human body has limited capacity to transiently store "excess" dietary protein from a single meal to acutely stimulate muscle protein anabolism at a later time. For example, we have previously demonstrated that a single large serving of a high-quality protein (12 ounces lean beef, 90 g protein) has no greater effect on muscle protein synthesis than a more modest 4-ounce (30 g protein) meal (7). In the current study, muscle protein synthesis after the EVEN breakfast meal (31.5 ± 1.3 g protein) was ~40% higher than after the SKEW breakfast (10.7 ± 0.8 g protein). Although not directly assessed, it is likely that lunch followed a similar pattern, favoring the EVEN protein distribution (EVEN vs. SKEW: 29.9 ± 1.6 vs. 16.0 ± 0.5 g protein). Reconciling total daily protein intake by end-loading protein during the SKEW evening meal (EVEN vs. SKEW: 32.7 ± 1.6 vs. 63.4 ± 3.7 g protein) failed to make up the difference in 24-h muscle protein synthesis.

One of the limitations of this, and many, muscle metabolism studies is our inability to concurrently measure muscle protein synthesis and breakdown. Consequently, the question remains whether 24-h net muscle protein anabolism could be improved by adding even more protein to either group. Recent commentary suggests that larger protein meals (≥30 g protein/meal) may provide a greater net anabolic effect by maximally stimulating muscle protein synthesis while progressively inhibiting protein breakdown (11). Protein breakdown is difficult to measure in non–steady state conditions (i.e., after a meal or exercise) without resorting to complex and invasive 3-pool modeling techniques. Nevertheless, the theory that larger protein meals provide a greater net anabolic effect is applicable and perhaps beneficial in situations in which an individual's meal plan and total energy requirements can accommodate additional protein. However, great care must be taken when translating these theories for dietary prescription. A daily protein distribution of 40-40-40 g may be advantageous for some individuals; however, a protein distribution of 10-10-100 g will likely not offer a similar net anabolic advantage.

Although the results of our current study support the potential of dietary protein distribution to ultimately influence outcomes such as muscle mass and function, there is clearly a need for longer-duration feeding trials or retrospective examination of daily dietary protein intake patterns in previous nutrition studies. In healthy individuals, modest, acute differences in 24-h muscle protein synthesis cannot be expected to precipitate changes in muscle mass or function over a period of weeks or perhaps months. Nevertheless, a subtle, chronic depression of muscle protein synthesis is entirely consistent with the gradual onset and progression of sarcopenia, an insidious condition that takes many years to manifest (28).

Few studies to date have examined the longer-term outcomes associated with protein distribution patterns. The ones we are aware of have focused on an older population who are more likely to experience anabolic resistance after a meal and are arguably more likely to benefit from optimizing protein intake and distribution than their younger peers. In a recent cross-sectional study in free-living elderly aged ≥75 y, Bollwein et al. (29) found that although frail, prefrail, and nonfrail individuals did not differ in absolute or relative protein consumption (all participants exceeded the RDA), nonfrail participants displayed a more even protein distribution pattern across all daily meals, whereas frail and prefrail participants skewed protein consumption toward the noon meal.

Using a clinical trial to explore a similar theme, Bouillanne et al. (30) conducted a 6-wk randomized "protein distribution" trial in hospitalized older adults (n = 66; age: 85 y; BMI: 21 kg/m^2). Patients were provided with "spread" (0800 h: 12.2 g; 1200 h: 21 g; 1600 h: 13.5 g; 1900 h: 21.2 g) or "pulsed" (0800 h: 4.5 g; 1200: 47.8 g; 1600 h: 2.3 g; 1900 h: 10.9 g) protein distribution diets that provided a commendable 1.31 g protein/(kg·d). Although no changes in handgrip strength or activities of daily living were noted, the pulsed diet did produce a modest, but significant improvement in lean mass compared with the "spread" protein diet.

Although the results from the study by Bouillanne et al. (30) superficially conflict with our results, many of the broader themes and conclusions are consistent with our data. Specifically, in this hospitalized and potentially anabolic-resistant older population, the quantity of protein consumed at each meal in the "spread/distributed" protein group (i.e., 12–21 g/meal) was likely insufficient to optimally stimulate muscle protein synthesis across all meals. Moving forward, it may be of considerable clinical relevance to continue to explore and refine protein distribution patterns in patients and populations at increased risk of losing muscle mass and function (16,23,31).

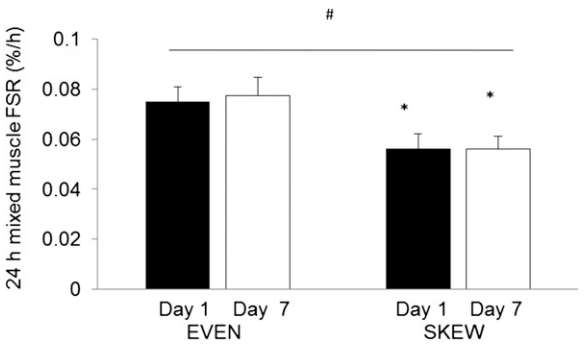

FIGURE 3 Twenty-four-hour mixed muscle protein FSRs in healthy adults on days 1 and 7 after the ingestion of diets with an EVEN or SKEW protein distribution. Values are means ± SEMs; n = 8. *Different from EVEN at that time point, $P < 0.05$. #Main effect of group between EVEN and SKEW, $P < 0.05$. EVEN, even daily protein distribution; FSR, fractional synthesis rate; SKEW, skewed daily protein distribution.

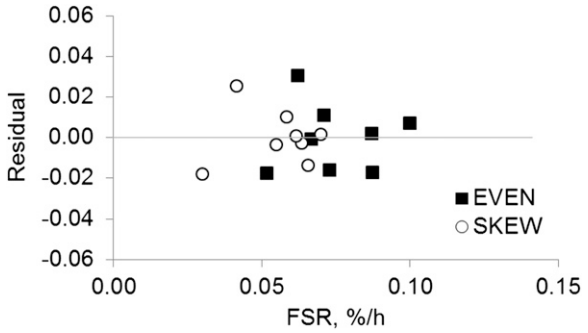

FIGURE 4 Residual plot of 24-h protein synthesis in healthy adults on days 1 and 7 after the ingestion of diets with an EVEN or SKEW protein distribution. EVEN, even daily protein distribution; FSR, fractional synthesis rate; SKEW, skewed daily protein distribution.

In conclusion, the consumption of a moderate amount of high-quality protein 3 times a day provides a more effective means of stimulating 24-h muscle protein synthesis than the common practice of skewing protein intake toward the evening meal. We recommend a moderate, meal-driven approach to daily protein consumption that is mindful of the interplay of issues such as protein anabolism, cost, and daily energy consumption.

Acknowledgments

D.P.-J. and D.K.L. designed the research; M.M.M., J.A.M., K.L.E., S.L.C., and D.P.-J. conducted the research; M.M.M., E.A.-L., M.S.-M., and D.P.-J. analyzed the data; M.M.M., E.A.-L., and D.P.-J. wrote the manuscript; and D.P.-J. had primary responsibility for final content. All authors read and approved the final manuscript.

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