Title: Effects of dietary protein patterning on weight loss and resistance training-induced changes in body composition, skeletal muscle, and indices of metabolic syndrome

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I. Project Title: Effects of dietary protein patterning on weight loss and resistance training-induced changes in body composition, skeletal muscle, and indices of metabolic syndrome

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II. Industry Summary

The purpose of this study was to assess whether redistributing equivalent total daily protein intakes from one high protein-containing meal into multiple moderately-high protein-containing meals leads to greater retention of muscle mass in exercising adults undergoing weight loss. Adults consuming fewer calories than needed for body weight maintenance while eating high quality proteins such as pork, dairy, egg, and beef effectively lost weight, improved body composition, and improved cardio-metabolic health. The within-day protein distribution did not confer additional improvements in body composition, skeletal muscle, and health outcomes.

III. Keywords

Dietary protein, weight loss, exercise, heart-health, muscle mass

IV. Scientific Abstract (to be presented at EB2017)

Background: Emerging research suggests that re-distributing total protein intake from one high-protein meal daily, to multiple moderately-high protein meals, improves 24 h muscle protein synthesis. Over-time, this may promote positive changes in body composition and indices of cardio-metabolic health.
Objective: We sought to assess the effects of within-day protein intake distribution on dietary energy restriction and resistance training-induced changes in body composition and clinical cardio-metabolic health indicators.
Design: In a randomized, parallel-design study, 41 men and women (mean ± SEM; age: 35 ± 2 y; BMI: 31.5 ± 0.5 kg/m2) consumed an energy-restricted diet (750 kcal/d below requirement) for 16 wk while performing resistance training 3 d/wk. Subjects consumed 90 g protein/d (1.0 ± 0.03 g•kg-1•d-1, 125% of the recommended dietary allowance, at intervention week 1) in either a skewed (10 g breakfast, 20 g lunch, 60 g dinner; n=20) or even (30 g breakfast, lunch, and dinner; n=21) distribution pattern. Body composition and cardio-metabolic health indices were measured pre- and post-intervention.
Results. Over time, body mass (-7.9 ± 0.6 kg, LSmean ± SE), lean mass (-1.0 ± 0.2 kg), fat mass (-6.9 ± 0.5 kg), serum glucose (-2.9 ± 1.2 mg/dL), HOMA-IR (-1.3 ± 0.2), total cholesterol (-21 ± 4 mg/dL), and triglycerides (-26 ± 7 mg/dL) each decreased. Mid-thigh muscle area (cm2) did not change over time. Within-day protein distribution did not differentially affect these body composition and cardio-metabolic health index responses.
Conclusions: The effectiveness of dietary energy-restriction combined with resistance training to improve body composition, including fat loss and muscle retention, and clinical indices of cardio-metabolic health is not influenced by the within-day distribution of protein when adequate total protein is consumed.

V. Introduction

About two-thirds of adults in the United States are overweight or obese (BMI ≥25 kg/m2) (1). Obesity is associated with an increased risk of chronic disease and metabolic syndrome (MetS) and a reduced physical functioning capacity, all of which contribute to disproportionately high healthcare expenditures and premature mortality (2-4). Purposeful moderate dietary energy restriction (500-750 kcal/d energy deficits) is an effective means for overweight/obese adults to reduce body weight and fat mass and improve their health profile (5-7). However, ~25% of the weight lost by energy restriction only consists of lean body mass (LBM), which includes skeletal muscle (8, 9). A moderate dietary energy restriction with increased protein intake of 1.0 g protein/(kg•d) has been recommended for weight loss to prevent or improve medical complications associated with obesity (7). In addition, high total dietary protein intakes (1.2-1.5 g protein/(kg•d)) are reported to preserve LBM and improve body composition during weight loss in young, middle-aged, and old adults compared to normal protein intakes (0.8 g protein/(kg•d)) (5, 10, 11). The higher total protein intakes in these studies were achieved by increasing the amount of protein consumed at breakfast and lunch, as well as dinner. Thus, the subjects consumed multiple high-protein meals daily, in contrast to the typical distribution of consuming lower quantities of protein at breakfast and lunch (12). Emerging research indicates that the consumption of multiple high protein meals daily may be superior to only consuming one meal (typically dinner) to stimulate muscle protein synthesis throughout the day (1, 13, 14). This concept is based on research showing that the patterning of energy and protein intake influences muscle protein synthesis (15) and whole body composition and protein retention (16, 17). Paddon-Jones D et al. has reported that adults who consumed a 90 g protein/d diet with protein intake evenly distributed across meals (30 g each at breakfast, lunch, and dinner) exhibited a 25% higher rate of muscle protein synthesis over 24 hours compared to adults who consumed an iso-nitrogenous diet with a skewed protein distribution (10 g breakfast, 15 g lunch, 65 g dinner) (18). Since 25-30 g of high quality protein per meal is needed to maximally stimulate post-prandial muscle protein synthesis (15), the dinner meal typically contains excess protein and breakfast and lunch contain insufficient protein. Very limited research exists regarding the effects of protein intake on skeletal muscle size after weight loss (8), and currently, no longitudinal studies have evaluated the effectiveness of consuming an even vs. skewed distribution of protein intake across meals on phenotypic changes in skeletal muscle size over the longer-term. Recent studies have also suggested that even distributed protein patterning may promote satiety and improve blood glucose response in healthy adult men and women (18, 19). However, there is a lack of controlled, longer-duration trials that seek to investigate the effects of daily protein distribution on appetite, glucose response and MetS parameters after weight loss in overweight or obese population.

VI. Objectives

Section 1: Includes objectives included in the manuscript being prepared for submission to AJCN in March, 2017.

Primary Aim: To assess the effects of within-day dietary protein intake distribution on dietary energy-restriction and resistance training-induced changes in lean body mass and mid-thigh muscle area.

Hypothesis: Lean body mass, mid-thigh muscle area, and muscle strength would decrease less in the even versus skewed within-day dietary protein distribution group.

Section 2: Includes objectives declared in the grant that are not included in the upcoming manuscript submission but have been assessed and are to be included in upcoming manuscripts.

Primary Aim: To assess the effects of within-day dietary protein intake distribution on dietary energy-restriction and resistance training-induced changes in appetite, glucose response, and MetS parameters.

Hypothesis: Appetite and glucose response parameters will be improved in the even vs. skewed protein distribution group.

VII. Materials and Methods

Table 1.		
	Goal	**Completed**
Subjects (n)	40	41

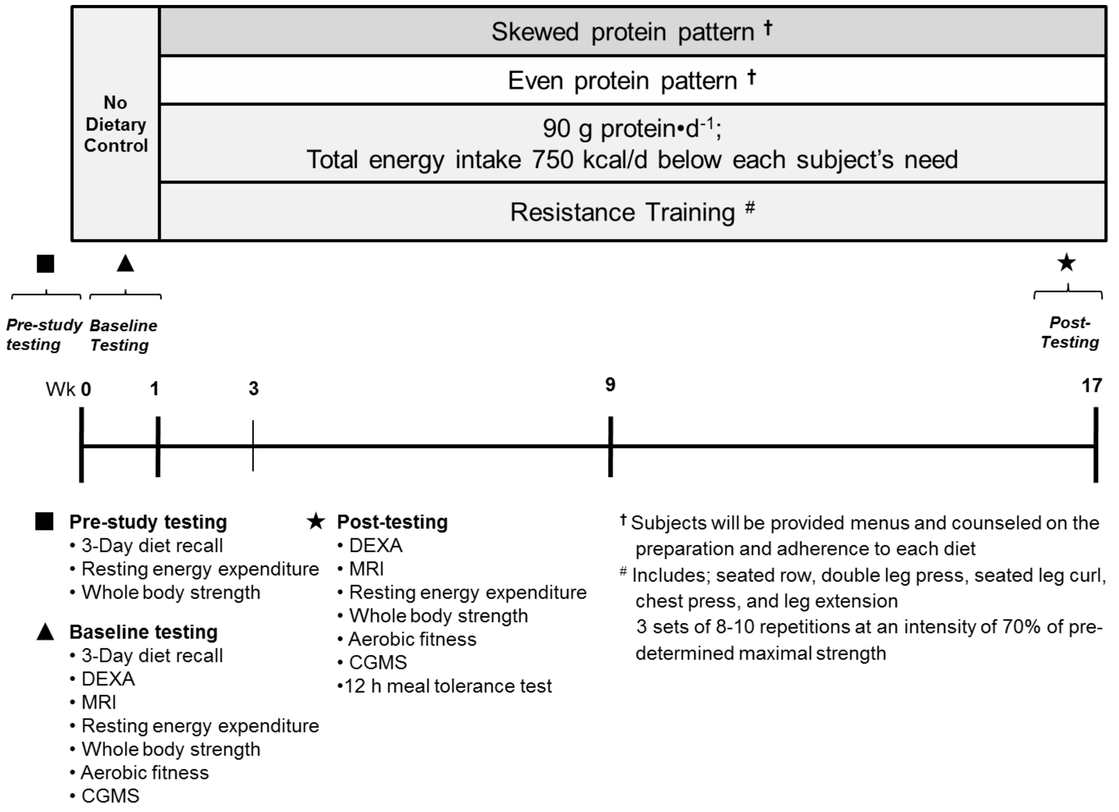
Table 2. Data report			
Variable	**Entry status**	**Accuracy check**	**Statistical analysis**
Weekly body weight	Completed	Completed	Completed
Hip and waist circumference	Completed	Completed	Completed
DXA	Completed	Completed	Completed
MRI	Completed	Completed	Completed
Blood (baseline and post)	Completed	Completed	Completed
Blood pressure	Completed	Completed	Completed
Whole body strength	Completed	Completed	Completed
Menu checklists	Completed	Completed	Completed
Meal compliance pictures	Completed	Completed	Completed
Exercise training log	Completed	Completed	Completed
24 hour dietary recall	Completed	Completed	Completed
Resting energy expenditure	Completed	Completed	Completed
Appetite questionnaire	Completed	Completed	Pending
Glucose and insulin 12 h MTT	Completed	Completed	Pending
Continuous glucose monitoring	Completed	Completed	Pending
Aerobic Fitness	Completed	Completed	Pending

Section 1: Includes methods used to collect data that will be included in the manuscript for submission to AJCN in March, 2017.

Experimental Design: This 18-week study included a 2-week baseline testing period, followed by a 16-wk randomized, controlled intervention period (Table 1). During the intervention period all subjects consumed a controlled, energy-restricted diet and participated in a progressive overload resistance training program. Each subject was randomly assigned (using an online randomization plan generator; http://www.randomization.com/) to one of two dietary groups and instructed to consume meal-specific foods and beverages to achieve either an even (EVEN) or skewed (SKEW) within-day protein distribution. Post-intervention testing was completed during intervention week 16. All subjects were instructed to maintain their habitual types and levels of physical activities aside from the prescribed resistance training.

Subjects: Fifty-eight adults recruited from the greater Lafayette, IN, community provided written consent prior to participation. Study inclusion criteria were: age 19-50 y; BMI 27.0-34.9 kg/m2; weight stable (± 4.5 kg during previous 3 months); non-smoking; not diabetic; no acute illness; not pregnant or lactating; ability to exercise; not claustrophobic and able to complete magnetic resonance imaging (MRI) testing; willing and able to travel to testing facilities. The study protocol and all study documents were approved by the Purdue University Biomedical Institutional Review Board. The study is registered at clinicaltrials.gov as NCT02066948. Fifty-four subjects completed all baseline testing, while four subjects (EVEN, n=2; SKEW, n=2) dropped out at this time because of personal conflicts with the menu (n=3) or a scheduling conflict (n=1). Thirteen subjects left the study during the intervention period (EVEN, n=7; SKEW, n=6) for personal reasons (n=6), time commitment (n=2), noncompliance with menu (n=2), noncompliance with exercise (n=1), medication change (n=1), or pregnancy (n=1). Forty-one subjects (EVEN, n=21, 6 male, 15 female; SKEW, n=20, 9 male, 11 female) completed all study procedures and their data were analyzed (Figure 1).

Figure 1. Experimental design



Diet Intervention: Each subject's total energy requirement was estimated pre-study using sex-specific equations for overweight or obese adults (20). Throughout the intervention period, each subject consumed a diet providing 750 kcal/d less than their energy requirement. The diets consisted of a 1400 kcal/d base diet that contained 90 g of protein, 40 g of fat, and 170 g of

carbohydrate (i.e., a 35% to 65% ratio of non-protein energy intake from fat and carbohydrate). Additional fat and carbohydrate were added to each subject's daily intakes, as necessary, to achieve their individualized energy allowance while maintaining the 35% to 65% fat to carbohydrate ratio. For all subjects, the prescribed within-day distribution of total energy intake was ~20% breakfast, 30% lunch, 36% dinner, and 14% snacks, respectively. The prescribed within-day protein distributions of the two groups were: 1) EVEN, 30, 30, and 30 g protein/meal consumed at breakfast, lunch, and dinner, respectively, and 2) SKEW, 10, 20, and 60 g protein/meal, respectively. Foods consumed as snacks contained minimal protein. Dietary protein sources are listed in Supplemental Table 1. The individualized menus were developed using Pronutra software (Viocare, Inc. Princeton, NJ). The within-day energy and protein distributions of the SKEW pattern were consistent with NHANES distributions (12).
Body mass was measured at baseline and once weekly during the intervention period using a digital platform scale (model ES200L, Ohaus Corporation, Pine Brook, NJ). If body mass loss was <0.5 kg/wk for two consecutive weeks the subject's energy intake was lowered by reducing non-protein energy intake. At baseline, each subject's 24-h food intakes were assessed for energy and macronutrient contents using a 3-d food record (Nutrition Data System for Research software (Version 2014, Nutrition Coordinating Center, University of Minnesota). Salting and herbal seasoning of food, non-energy, caffeine-containing beverages, and water were allowed ad libitum during the intervention. Subjects were provided with a digital food scale (Salter Microtronic Electronic Kitchen Scale, Boca Raton, FL, USA) to aid in measuring out portion sizes. All diet-related activities and assessments were performed in conjunction with the Indiana Clinical Research Center bio-nutrition facility at Purdue University.

Dietary compliance: During the intervention, dietary compliance was assessed using daily menu checklists and periodic pre- and post-meal date and time identified photography. The study dietitian and other research staff members also contacted subjects weekly in person, though email, and by phone, to encourage compliance to the prescribed menus. See Supplemental Table 2 for more information about dietary compliance measures.

Resistance Training: All subjects were instructed to perform, with supervision and coaching, 3 sets of resistance exercises 3 non-consecutive days per week. The exercises included seated chest press, seated upper back row, seated bilateral leg extension, seated bilateral angled leg presses and seated bilateral leg curl (core exercises) and 2 additional auxiliary exercises. The hip abductor and seated shoulder press exercises were alternated every other training session with the latissimus dorsi pull down and hip adductor exercises. The first set for each exercise was completed in 8-10 repetitions with the last two sets completed to volitional fatigue. In weeks 1, 2, and 3-16 of the intervention, each exercise was performed at 60, 70, and 80% of their most recently measured 1-repetition maximum (1RM), respectively. Each training session lasted ~1 h and included 10-minute warm-up and cool-down periods consisting of low-intensity aerobic exercise and stretching.

During baseline and every fourth week, 1RM testing was performed to measure subject's maximal strength on the 5 core exercises. Measurements were taken on the same machines used for training. Whole body strength was considered the sum of the 1RMs for 4 core exercises. Seated bilateral angled leg presses were removed from the analysis of composite whole body strength due to subject's maximizing the available weight stack.

Resistance Training Compliance: Resistance training compliance was calculated as the percentage of resistance training sessions attended out of 48 total sessions.

Body Composition: Within-day dietary protein distribution did not influence responses over time for any of the whole body, mid-thigh, and mid-calf composition outcomes (Table 4). Over time,

whole body mass, BMI, lean mass, fat mass, fat mass percent, lean and fat mass indexes, appendicular lean and fat masses, waist and hip circumferences, and waist to hip ratio each decreased and lean mass percent increased (main effects of time, $P < 0.05$).
Mid-thigh cross section, subcutaneous fat, and IMAT areas decreased, independent of within-day dietary protein distribution (main effects of time, $P < 0.05$). Mid-thigh muscle area did not change from pre- to post-intervention (main effect of time, $P = 0.797$). Mid-calf cross section, muscle, subcutaneous fat, and IMAT areas decreased from pre- to post-intervention (main effects of time, $P < 0.05$).

Biochemical Analyses: Fasting blood samples were collected as previously described (21) at baseline and intervention week 16, at least 1-2 days after a resistance training session. Blood samples were collected into serum-separator tubes, processed to obtained serum, and aliquots were either stored in cryovials at -80ºC until thawed for insulin analysis or sent to a commercial analytical laboratory (Mid America Clinical Laboratories, Indianapolis, IN, USA) for measurements of glucose, blood urea nitrogen (BUN), and clinical lipid-lipoprotein profile. The insulin aliquots were analyzed by electroilluminescence immunoassay on a COBAS e411 analyzer (Roche Diagnostic Systems, Indianapolis, IN). The homeostatic model assessment of insulin resistance (HOMA-IR) was calculated: fasting glucose (mg/dL) X fasting insulin (µU/mL)/405) (24).

Statistical Analysis: Our primary outcomes were the changes in lean mass and right mid-thigh muscle area from pre- to post-intervention. The lack of published data limited our ability to perform appropriate power calculations. Sample size of 20 subjects/group was chosen to provide >85% power to statistically confirm differential responses between EVEN and SKEW within-day dietary protein distribution groups equal to one standard deviation of the within-group variability for each primary outcome.

Analyses were completed using the data from the 41 subjects who completed the intervention. All data are presented as LSmeans ± SE of the LSmean unless otherwise stated. The main effect of time and group-by-time interactions were assessed using a 2 X 2 factor repeated-measures ANOVA (MIXED procedure; group: EVEN, SKEW; time: pre- and post-intervention). Main effects of time were the pooled LSmeans change in the variable from pre- to post-intervention. Group-by-time interactions were the effect of within-day dietary protein distribution (group) from pre- to post-intervention (time). An unpaired, two-tailed t-test (TTEST procedure, SAS version 9.3; SAS Institute) was used to test for differences in pre-intervention age and height and post-intervention menu checklist compliance percent, meal picture compliance percent, and resistance training compliance percent. Statistical significance was determined at $P < 0.05$. Statistical analysis was performed using SAS software (Version 9.3; SAS Institute).

Section 2: Includes methods used to collect the data that will be included in future manuscripts.

Appetite: Subjective appetitive ratings (hunger, fullness, and desire to eat) were collected every waking hour for 3 non-consecutive days during pre- and post-intervention using a 100 mm visual analog scale.

12 h meal tolerance test: A meal tolerance test, which provided 3 meals over 12 h, was conducted on 1 day during the last two weeks of the intervention. Serum glucose and insulin were collected using standard techniques and analyzed on a COBAS e411 analyzer (Roche Diagnostic Systems, Indianapolis, IN).

Continuous glucose monitoring: A continuous glucose monitor (CGM; iPro2 Profession CGM, Medtronic MiniMed) was worn by each subject during the pre- and post-intervention testing week. Each subject arrived at the testing facilities in a fasted state and a trained technician placed a CGM into interstitial tissue on their lower abdomen. The subjects were then instructed to use a manual blood glucose monitor prior to consuming each meal and before bed for every day of the week they wore the CGM. After testing was complete, the manual blood glucose data were entered into the iPro2 software to calibrate the CGM data.

Resting Energy Expenditure: Resting energy expenditure was measured using indirect calorimetry during pre- and post-intervention testing. Subjects would lay in a supine position for 45 min underneath a testing hood. The hood was enclosed tightly around the subjects to ensure a seal between the hood and subject. Lights were dimmed and the subjects were asked to remain still and awake during the testing period.

Aerobic fitness: Subjects VO_2 maximum was estimated using a sub-maximum procedure during pre- and post-intervention testing.

VIII. **Results**

Section1: Results reported in manuscript for AJCN submission in March, 2017.

Subjects: Subject characteristics at baseline were not significantly different between EVEN and SKEW groups (Table 3).

Dietary intervention: At baseline, habitual protein intake in the EVEN (15 ± 4, 30 ± 3, 31 ± 5 g protein/meal; 82 ± 4 g/d) and SKEW (15 ± 2, 31 ± 4, 39 ± 5 g protein/meal; 90 ± 4 g/d) were not different. Based on body mass at week 1, subjects consumed 1.0 ± 0.04 g•kg-1•d-1 in the EVEN group and 1.0 ± 0.04 g•kg-1•d-1 in the SKEW group (Supplemental Table 4). Based on the 3-day dietary analysis of menu checklists and body mass at week 16, subjects consumed 1.1 ± 0.04 and 1.1 ± 0.04 g•kg-1•d-1 in the EVEN and SKEW groups, respectively (Supplemental Table 4). Week 16 menu checklists indicated that the EVEN group consumed 31 ± 0, 29 ± 0, and 29 ± 0 g of protein at breakfast, lunch, and dinner, respectively (Supplemental Table 5). The SKEW group consumed 11 ± 0, 20 ± 0, and 59 ± 0 g of protein at breakfast, lunch, and dinner, respectively (Supplemental table 5). Subjects in the EVEN and SKEW groups were deemed compliant to within-day protein distribution 80 ± 4 and 82 ± 3 %, respectively, of 54 meals visually assessed using photography and 92 ± 2 and 88 ± 2 %, respectively, of 294 meals assessed using menu checklists (Supplemental table 3).

Resistance training intervention: Resistance training compliance was > 85% for both groups, but statistically lower for the EVEN versus SKEW groups: averaging 41 (86.0 ± 1.6%) and 43 (91.0 ± 1.5%) of 48 resistance training sessions, respectively (Supplemental Table 3). Whole body strength increased by ~20% in both groups, independent of protein distribution (Table 4).

Body Composition: Within-day dietary protein distribution did not influence responses over time for any of the whole body, mid-thigh, and mid-calf composition outcomes (Table 4). Over time, whole body mass, BMI, lean mass, fat mass, fat mass percent, lean and fat mass indexes, appendicular lean and fat masses, waist and hip circumferences, and waist to hip ratio each decreased and lean mass percent increased (main effects of time, $P < 0.05$).

Mid-thigh cross section, subcutaneous fat, and IMAT areas decreased, independent of within-day dietary protein distribution (main effects of time, $P < 0.05$). Mid-thigh muscle area did not change from pre- to post-intervention (main effect of time, $P = 0.797$). Mid-calf cross section, muscle, subcutaneous fat, and IMAT areas decreased from pre- to post-intervention (main effects of time, $P < 0.05$).

Cardio-metabolic Indexes: Within-day dietary protein distribution did not influence responses over time for any of the cardio-metabolic indexes measured (Table 5). There were reductions in fasting serum glucose, insulin, HOMA-IR, total cholesterol, HDL, LDL, CHOL:HDL ratio, triglyceride, and reclining systolic and diastolic blood pressures (main effects of time, $P < 0.05$).

Table 3
Subject's changes in anthropometrics, body composition, and whole body strength in the EVEN and SKEW groups after consuming an energy-restricted diet and performing resistance training for 16 wk [1]

	EVEN			SKEW				
Parameters	Pre	Post	Δ	Pre	Post	Δ	Time	G X T[*]
Age[1,2] (y)	33 ± 2	—	—	36 ± 2	—	—	—	—
Height[1,2] (cm)	170.5 ± 1.7	—	—	173.1 ± 1.9	—	—	—	—
Body mass[4] (kg)	95.8 ± 2.3	87.2 ± 2.3	-8.6 ± 0.9	92.7 ± 2.2	85.4 ± 2.2	-7.3 ± 0.9	0.000	0.320
BMI[4] (kg/m^2)	31.8 ± 0.5	28.9 ± 0.5	-2.9 ± 0.3	30.7 ± 0.5	28.3 ± 0.5	-2.4 ± 0.3	0.000	0.274
Waist circumference[5] (cm)	104.6 ± 1.8	95.4 ± 1.8	-9.1 ± 1.0	102.5 ± 1.7	94.1 ± 1.8	-8.4 ± 1.0	0.000	0.601
Hip circumference[6] (cm)	114.5 ± 1.5	107.6 ± 1.5	-6.8 ± 0.8	112.5 ± 1.4	106.2 ± 1.4	-6.2 ± 0.8	0.000	0.599
Waist:Hip[5]	0.91 ± 0.01	0.88 ± 0.01	-0.02 ± 0.00	0.91 ± 0.01	0.88 ± 0.01	-0.02 ± 0.00	0.000	0.996
Whole body[4]								
Lean mass (kg)	55.7 ± 1.2	54.2 ± 1.2	-1.5 ± 0.3	54.0 ± 1.1	53.6 ± 1.1	-0.4 ± 0.4	0.000	0.067
Lean mass (%)	57.8 ± 1.0	61.9 ± 1.0	4.0 ± 0.5	58.1 ± 1.0	61.9 ± 1.0	4.4 ± 0.5	0.000	0.576
Fat mass (kg)	36.9 ± 1.6	29.8 ± 1.6	-7.0 ± 0.7	35.7 ± 1.5	28.9 ± 1.5	-6.7 ± 0.7	0.000	0.789
Fat mass (%)	38.8 ± 1.1	34.4 ± 1.1	-4.3 ± 0.5	38.6 ± 1.1	33.9 ± 1.1	-4.7 ± 0.5	0.000	0.640
Lean mass index (kg/m^2)	18.4 ± 0.2	17.9 ± 0.2	-0.5 ± 0.1	17.7 ± 0.2	17.6 ± 0.2	-0.1 ± 0.1	0.000	0.066
Fat mass index (kg/m^2)	12.4 ± 0.5	10.0 ± 0.5	-2.4 ± 0.2	11.9 ± 0.4	9.6 ± 0.4	-2.2 ± 0.2	0.000	0.695
Lean mass:fat mass	1.6 ± 0.1	1.9 ± 0.1	0.3 ± 0.0	1.6 ± 0.1	2.0 ± 0.1	0.4 ± 0.0	0.000	0.281
Right leg[4] (kg)								

Leg mass	16.3 ± 0.4	14.9 ± 0.4	-1.3 ± 0.1	16.0 ± 0.4	14.7 ± 0.4	-1.2 ± 0.1	0.000	0.594
Lean mass	10.0 ± 0.2	9.6 ± 0.2	-0.3 ± 0.1	9.7 ± 0.2	9.5 ± 0.2	-0.1 ± 0.1	0.001	0.186
Fat mass	5.7 ± 0.3	4.7 ± 0.3	-0.9 ± 0.1	5.7 ± 0.3	4.7 ± 0.3	-1.0± 0.1	0.000	0.691
Right mid-thigh area[7] (cm^2)								
Cross section	307 ± 10	276 ± 10	-31 ± 3	288 ± 10	263 ± 10	-24 ± 4	0.000	0.253
Muscle	157 ± 4	155 ± 4	-2 ± 1	145 ± 4	147 ± 4	1 ± 1	0.797	0.136
Subcutaneous fat	131 ± 10	103 ± 10	-28 ± 3	123 ± 10	98 ± 10	-24 ± 3	0.000	0.515
IMAT	10 ± 0	9 ± 0	-1 ± 0	10 ± 0	9 ± 0	-1 ± 0	0.000	0.829
Right mid-calf area[7] (cm^2)								
Cross section	12.6 ± 0.3	11.7 ± 0.3	-0.8 ± 0.1	12.2 ± 0.3	11.5 ± 0.3	-0.7 ± 0.0	0.000	0.452
Muscle	7.9 ± 0.2	7.5 ± 0.2	-0.3 ± 0.0	7.8 ± 0.2	7.5 ± 0.2	-0.2 ± 0.0	0.000	0.444
Subcutaneous fat	3.2 ± 0.2	2.8 ± 0.2	-0.4 ± 0.0	3.1 ± 0.2	2.7 ± 0.2	-0.4 ± 0.0	0.000	0.806
IMAT	1.3 ± 0.1	1.0 ± 0.1	-0.3 ± 0.0	0.9 ± 0.1	0.8 ± 0.1	-0.2 ± 0.0	0.000	0.375
Strength (kg)								
Whole body[8]	511 ± 22	625 ± 24	114 ± 17	535 ± 22	630 ± 24	95 ± 19	0.000	0.470

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[1] Data are presented as means ± SEM; An unpaired t-test (TTEST procedure, SAS version 9.3; SAS Institute) was used to test for differences in pre-intervention age and height.

[2] EVEN: n= 6 males and 15 females, SKEW: 9 males and 11 females

[3] Data are presented as LSmean ± SE of the LSmean; A repeated-measure ANOVA (MIXED procedure, SAS version 9.3; SAS Institute) was used to test for main effect of time and group-by-time interaction. No significant group-by-time interactions for the measured variables were observed between the EVEN and SKEW groups. Waist:hip, waist to hip ratio; IMAT, intramuscular adipose tissue; Whole body strength, sum of the 4 core exercises.

[4] EVEN: n= 6 males and 15 females, SKEW: 9 males and 11 females

[5] Pre: EVEN: n= 6 males and 15 females, SKEW: 9 males and 11 females; Post: EVEN: n= 6 males and 15 females, SKEW: 7 males and 11 females

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[6] Pre: EVEN: n= 6 males and 15 females, SKEW: 9 males and 11 females; Post: EVEN: n= 6 males and 15 females, SKEW: 8 males and 11 females

[7] Pre: EVEN: n= 6 males and 15 females, SKEW: 9 males and 10 females; Post: EVEN: n= 6 males and 14 females, SKEW: 7 males and 10 females

[8] Pre: EVEN: n= 5 males and 15 females, SKEW: 7 males and 11 females; Post: EVEN: n= 2 males and 12 females, SKEW: 4 males and 8 females

*Group-by-time interaction

Table 4
Changes in cardio-metabolic indexes in the EVEN and SKEW groups after consuming an energy-restricted diet and performing resistance training for 16 wk[1]

Cardio-metabolic indexes	EVEN			SKEW			*P*	
	Pre	Post	Δ	Pre	Post	Δ	Time	G X T*
Fasting glucose[2] (mg/dL)	91 ± 2	88 ± 2	-3 ± 1	93 ± 2	90 ± 2	-2 ± 1	0.025	0.729
Fasting insulin[3] (µIU/mL)	14.4 ± 1.4	7.0 ± 1.3	-7.4 ± 1.6	11.9 ± 1.3	8.3 ± 1.2	-3.5 ± 1.6	0.000	0.108
HOMA-IR[3]	3.4 ± 0.3	1.5 ± 0.3	-1.8 ± 0.4	2.7 ± 0.3	1.8 ± 0.3	-0.8 ± 0.4	0.000	0.123
Total cholesterol[2] (mg/dL)	190 ± 6	170 ± 6	-20 ± 5	200 ± 6	178 ± 6	-21 ± 5	0.000	0.846
HDL[2] (mg/dL)	42 ± 2	40 ± 2	-2 ± 1	44 ± 1	41 ± 1	-2 ± 1	0.038	0.941
LDL[2] (mg/dL)	121 ± 6	110 ± 6	-10 ± 4	129 ± 6	113 ± 6	-15 ± 4	0.000	0.409
Chol:HDL[2] (mg/dL)	4.8 ± 0.2	4.4 ± 0.2	-0.3 ± 0.1	4.7 ± 0.2	4.4 ± 0.2	-0.2 ± 0.1	0.015	0.666
Triglycerides[2] (mg/dL)	134 ± 10	98 ± 10	-35 ± 10	133 ± 10	116 ± 10	-17 ± 10	0.000	0.217
BUN[2] (mg/dL)	12 ± 0	12 ± 0	0 ± 0	12 ± 0	13 ± 0	1 ± 0	0.158	0.382
Reclining SBP[2] (mm Hg)	117 ± 1	110 ± 1	-6 ± 1	119 ± 1	113 ± 1	-5 ± 1	0.000	0.627
Reclining DBP[2] (mm Hg)	79 ± 1	73 ± 1	-6 ± 1	80 ± 1	75 ± 1	-5 ± 0	0.000	0.752

[1] Data are presented as LSmean ± SE of the LSMEAN ; A repeated-measure ANOVA (MIXED procedure, SAS version 9.3; SAS Institute) was used to test for main effect of time and group-by-time interaction. No significant group-by-time interactions for the measured variables were observed between the EVEN and SKEW groups. Chol: HDL, total cholesterol to high density lipoprotein cholesterol ratio; BUN, blood urea nitrogen; SBP, systolic blood pressure; DBP, diastolic blood pressure.

[2] EVEN: n= 6 males and 15 females, SKEW: 9 males and 11 females

[3] Pre: EVEN: n= 6 males and 12 females, SKEW: 8 males and 10 females; Post: EVEN: n= 5 males and 15 females, SKEW: 9 males and 11 females

*Group-by-time interaction

Supplemental Table 1
Dietary protein source quantity

Protein source	% protein source quantity/wk	Protein source quantity (servings/wk)
Pork	18	5 (3 oz)
Beef	18	5 (3 oz)
Dairy	18	14*
Egg	10	8 (1 whole)
Fish	4	1 (3 oz)
Non-animal	30	—

*1 serving = 8 oz milk, 6 oz yogurt, or 1 oz cheese

Supplemental Table 2
Detailed procedures used during testing

Methods	Procedures
Dietary compliance	Completed menu checklists were collected and new menus distributed at the beginning of each week when the subjects performed the first of three weekly resistance training sessions. After receiving the checklists, research staff would calculate compliance. Menu checklist compliance is calculate as the total number of compliant meals out of 294 meals (14 wk). The percent of non-compliant meals were further categorized by whether the subjects deviated from the menu, under consumed for that meal (missing food item), or did not record the eating occasion. Starting on the second or third week of intervention, subjects were also counseled to take pictures with their smart phones before and after consuming their meals. Pictures were typically taken on Sunday, Monday, and Wednesday, every other week until week 14 (54 meal pictures total). Meal pictures were not taken during intervention weeks 15 and 16 because of the high burden of post-study experimental testing. The pictures needed to include all study foods prescribed in the menu and a time card and watch to document the date and eating duration of their meals. Using the time-stamp on the digital picture, the timing of the meal was crosschecked with the time on the card and watch provided in the picture. Pictures were sent electronically to the study dietitian whereupon they were assessed for compliance to the meal by identifying the consumption of protein containing meals in the before and after meal pictures. Meal pictures were specifically used to assess compliance to within-day protein distribution. As such, a subject was compliant to that eating occasion when they accurately sent a before and after picture of the meal and they consumed all of the prescribed protein containing foods.
Resistance Training	Prior to the start of the intervention, subjects were familiarized with the training protocol and exercise equipment. During the intervention, subjects reported to the A. H. Ismail Center for Health, Exercise and

	Nutrition, Purdue University, at either 0600 or 1700 for use of weight stack resistance training equipment (TechnoGym, Fairfield, NJ, USA). Subjects completed a readiness-for-exercise questionnaire prior to each training session to assess their current health status and ability to safely complete a training session. Each training session lasted ~1 h and included 10-minute warm-up and cool-down periods. During baseline and every fourth week, 1-repetition maximum (1RM) testing was performed to measure subject's maximal strength on the following 5 core exercises: chest press, seated upper back row, seated bilateral leg extension, seated bilateral angled leg presses and seated bilateral leg curl. Subject's 1RMs were assessed with multiple single-repetition attempts with defined ranges of motion separated by 2-3 minutes. Measurements were taken on the same machines used for training. Whole body strength was considered the sum of the 1RMs for 4 core exercises. Seated bilateral angled leg presses were removed from the analysis of composite whole body strength due to subject's maximizing the available weight stack. During the intervention, subjects completed 3 non-consecutive days of resistance training in which they individually performed 3 sets on each of the 5 core exercises along with the seated angled bilateral leg press and 2 additional auxiliary exercises. The hip abductor and seated shoulder press exercises were alternated every other training session with the latissimus dorsi pull down and hip adductor exercises. The first set for each exercise was completed in 8-10 repetitions with the last two sets completed to volitional fatigue. In weeks 1, 2, and 3-16 of the intervention, each exercise was performed at 60, 70, and 80% of their most recently measured 1RM, respectively. All exercises were performed using defined ranges of motion with equal eccentric and concentric contraction time so that each repetition lasted 4-6 seconds. Subjects rested for 60-90 seconds between each set.
Anthropometry	The subjects wore loose fitting clothing and remained in a supine position for the entirety of the DXA procedure. Height was measured without shoes at pre-intervention using a wall-mounted stadiometer (Holtain Ltd, Crymych, Wales, United Kingdom). BMI were calculated at pre- and post-intervention using whole body mass from DXA and baseline body height. Body circumference was measured in triplicate at the natural waist and hip using a spring tape measure.
MRI	Subjects arrived at the Purdue University MRI facility after an overnight fast and 48 h of limited physical activity prior to scanning. Upon arrival, the subject's calf and thigh lengths were measured from the bony protrusion on the lateral side of the knee to the distal-most location below the ankle bulge and from the lateral and medial bony protrusions of the knee to the inguinal crease, respectively. Sixty-six percent of the calf length starting from below the ankle bulge and 50% of the thigh length starting from the bony protrusions were marked using a vitamin E capsule taped to skin at the designated site. Forty-five minutes prior to scanning, subjects laid in the supine position on a gurney to control for the influence of posture-related fluid shifts on muscle size. After transferring to the scanner table from the gurney while remaining supine, the subject's heels were fixed on a nonmetallic support in order to control joint and scan angle and to minimize compression of the legs against each other and the MRI gurney. Specific foot angles were recorded to create a reproducible position for the post-intervention scans. Subjects were positioned in the

	MRI scanner in a feet-first direction using the vitamin E capsule as the external landmark. The scanning sequence was positioned to image the thigh by placing the inferior-most slice at the top of the tibial notch and calf by placing the superior-most slice at the bottom of the tibial notch on the subject's right leg. Axial T1-weighted scans were acquired using an 8-channel nee array coil (calf) and an 8-channel torso array coil (thigh) (Invivo Corporation, Gainesville, FL)k. A three-plane fast gradient echo scout scan established the location of the tibial notch. Scan parameters for the thigh were: repetition time/echo time (TR/TE) = 5/1.6ms; field-of-view (FOV) = 48cm; acquisition matrix = 256x128; slice thickness = 5mm; 20 axial slices, interslice spacing = 20mm; 40 coronal slices, interslice spacing = 0mm; 2 sagittal slices, interslice spacing = 10mm. Those for the calf are: repetition time/echo time (TR/TE) = 6/2.5ms; field-of-view (FOV) = 30cm; acquisition matrix = 256x128; slice thickness = 3mm; 15 axial slices, interslice spacing = 0mm; 30 coronal slices, interslice spacing = 0.5mm; 30 sagittal slices, interslice spacing = 0.5mm. The localizer scans were followed by high-resolution imaging of the thigh and calf regions. In each case, a fast spin echo sequence [TR/TE = 600/12 ms; FOV = 16cm for calf and 22cm for thigh; acquisition matrix = 384x224 for calf and 384x192 for thigh; slice thickness = 8mm; 24-36 axial slices, interslice spacing = 0mm; number of excitations (NEX) = 3] were performed. Image files were transferred to a personal computer and analyzed using Medical Image processing, Analysis and Visualization (MIPAV) from NIH. For all subjects, 4-7 slices (dependent upon height) were chosen to be analyzed for the thigh (superior slice is where the gluteus muscle disappears, inferior slice is before the rectus muscle appears, every third slice was traced), and 6-7 slices were analyzed for the calf (superior slice is where peroneus longus appears, inferior slice is where the gastrocnemius disappears, every third slice was traced). All images were corrected using inhomogeneity N3 correction. Cross-sectional area (CSA), IMAT, muscle, and subcutaneous fat (SubQ) contents of the thigh and calf were quantified using Fuzzy C-means algorithm. Data were reported using average area of all traced slices for mid- thigh and calf areas.

Supplemental Table 3
Diet and exercise compliance in the EVEN and SKEW groups while consuming an energy-restricted diet and performing resistance training for 16 wk[1]

Parameter	EVEN	SKEW
Menu checklist compliance (%)	92 ± 2 (94)	88 ± 2 (92)
Menu deviation (%)	8 ± 2 (0)	17 ± 5 (6)
Missing food item (%)	33 ± 7 (24)	31 ± 7 (17)
Not recorded (%)	54 ± 7 (62)	47 ± 8 (48)
Meal picture compliance (%)	80 ± 4 (87)	82 ± 3 (85)
Missing pictures (%)	85 ± 4 (94)	82 ± 6 (97)
Missing protein containing foods (%)	9 ± 3 (0)	10 ± 4 (0)
Other (%)	6 ± 3 (0)	3 ± 2 (0)
Total non-compliant pictures (#)	11 ± 2 (7)	10 ± 2 (8)
Missing pictures (#)	9 ± 2 (5)	8 ± 2 (7)
Missing protein containing foods (#)	1 ± 0 (0)	1 ± 0 (0)
Other (#)	1 ± 1 (0)	0 ± 0 (0)
Exercise	86 ± 2[a] (85)	91 ± 2[b] (93)

[1] Data are presented as mean ± SEM (median); An unpaired t-test (TTEST procedure, SAS version 9.3; SAS Institute) was used to test for differences in intervention meal and exercise compliance. Different letters indicate a significant difference between groups within rows, *P* < 0.05.

Supplemental Table 4
Subject's habitual, prescribed, and week 16 nutrient intakes[1,2]

Nutritional information	EVEN			SKEW		
	Habitual	Prescribed	Week 16	Habitual	Prescribed	Week 16
Energy (kcal/d)	2131 ± 116^{a}	1779 ± 110^{ab}	1571 ± 113^{b}	2210 ± 116^{a}	1794 ± 113^{ab}	1556 ± 116^{b}
Carbohydrate (%En)	48 ± 2^{a}	53 ± 2^{ab}	48 ± 2^{a}	49 ± 2^{a}	52 ± 2^{ab}	59 ± 2^{b}
Protein (%En)	16 ± 1^{a}	21 ± 1^{b}	23 ± 1^{b}	16 ± 1^{a}	21 ± 1^{b}	24 ± 1^{b}
Fat (%En)	36 ± 1^{a}	26 ± 1^{b}	30 ± 1^{bc}	35 ± 1^{ac}	26 ± 1^{b}	26 ± 1^{b}
Carbohydrate (g/d)	255 ± 16^{ac}	238 ± 16^{ab}	192 ± 16^{b}	266 ± 16^{a}	238 ± 16^{ab}	198 ± 16^{bc}
Protein (g/d)	82 ± 4	90 ± 4	89 ± 4	90 ± 4	91 ± 4	91 ± 4
Fat (g/d)	86 ± 6^{a}	52 ± 5^{b}	52 ± 6^{b}	87 ± 6^{a}	54 ± 6^{b}	45 ± 6^{b}
Protein (g/kg/d)	0.89 ± 0.05^{a}	0.99 ± 0.04^{ab}	1.07 ± 0.04^{b}	0.98 ± 0.04^{ab}	1.00 ± 0.04^{ab}	1.09 ± 0.04^{ab}

[1] Data are presented as LSmean ± SE; A repeated-measure ANOVA (MIXED procedure, SAS version 9.3; SAS Institute) was used to test for differences between groups in habitual, prescribed, and week 16 for nutrient intakes. Carbohydrates were reduced during the intervention to maintain a constant weight loss of approximately 0.5 kg/wk. Values within the same row with different superscript letters indicate significant difference, Tukey adjusted $P < 0.05$. %EN, percentage of energy
[2] Habitual: EVEN: n= 5 males and 14 females, SKEW: 9 males and 10 females, Prescribed: EVEN: n= 6 males and 15 females, SKEW: 9 males and 11 females; Week 16: EVEN: 5= males and 15 females, SKEW: 9 males and 10 females.

Supplemental Table 5
Subject's reported within-day nutrient intake pattern[1,2]

Nutritional information	EVEN				SKEW			
	Total (d)	Breakfast	Lunch	Dinner	Total (d)	Breakfast	Lunch	Dinner
Energy (kcal)	1567 ± 46	407 ± 19	566 ± 16	600 ± 21	1562 ± 60	327 ± 10*	529 ± 18	660 ± 35
Energy (%)	100	26 ± 1	36 ± 1	38 ± 1	100	21 ± 1*	34 ± 1	42 ± 1*
Carbohydrate (g)	191 ± 11	45 ± 4	65 ± 4	81 ± 5	199 ± 12	55 ± 3	75 ± 4	59 ± 1*
Fat (g)	52 ± 1	11 ± 1	22 ± 1	20 ± 1	45 ± 2	8 ± 0*	17 ± 0*	19 ± 1
Protein (g)	89 ± 1	31 ± 1	29 ± 0	29 ± 0	91 ± 1*	11 ± 0*	20 ± 1*	59 ± 1*
Protein (%)	100	35 ± 0	33 ± 1	33 ± 0	100	12 ± 0*	22 ± 0*	65 ± 1*
Protein (g/kg)	1.08 ± 0.03	0.38 ± 0.01	0.35 ± 0.01	0.35 ± 0.01	1.09 ± 0.03	0.13 ± 0.01*	0.24 ± 0.01*	0.71 ± 0.02*

[1] Data are presented as mean ± SEM; An unpaired, two-tailed t-test (TTEST procedure, SAS version 9.3; SAS Institute) was used to test for differences between group total, breakfast, lunch, and dinner nutrition information from 3-d menu checklists during the final week of the intervention. Menu check-off sheets included any deviations from the prescribed menu.

[2] EVEN: 5= males and 15 females, SKEW: 9 males and 10 females

*Indicates a significant difference between groups within outcome variable, *P* < 0.05.

Section 2: These data are collected and entered. However, statistical analyses are pending. This part of the research project will be completed after the manuscript reporting results from section 1 is submitted for peer review (estimated mid-March, 2017).

IX. Discussion

Section 1:

Theoretically, over time, evenly distributing protein intake should improve lean mass and skeletal muscle size more so than a skewed distribution by consistently maximizing MPS rates after each meal of the day. This study demonstrates distributing daily protein intake evenly among three meals (30 g at breakfast, lunch, and dinner) compared to consuming protein in a more typical skewed distribution (10 g at breakfast, 20 g at lunch, and 60 g at dinner) does not influence body composition responses in adults undergoing purposeful weight loss and resistance training. Protein distribution did not affect changes in lean mass (measured at whole body and right leg) or skeletal muscle groups that either maintained (mid-thigh) or reduced (calf) muscle size after the intervention. Our results demonstrate that protein distribution does not influence whole body, regional, and muscle specific regions/areas of the body.

These lean mass and skeletal muscle results bring into question the appropriateness to extrapolate results from short-term MPS studies (18, 22) to predict chronic lean soft tissue responses. Prior to initiating the current study, only one study assessed the effects of within-day protein distribution on 24 h MPS rate. This study was completed in young adults in energy balance and demonstrated that an evenly consuming protein (30 g at breakfast, lunch, and dinner) resulted in a 25% greater 24 h MPS rate than consuming a skewed distribution (10 g at breakfast, 15 g at lunch, and 60 g at dinner) with the same amount of total daily protein (18) However, since initiating the current study, subsequent research has shown inconsistent results (22, 23). One study in older adults, also in energy balance, showed that consuming an even protein distribution did not have a differential effect on daily MPS rate (23). The authors (23) attributed the inconsistent results primarily to a difference in the study population (older adults vs. younger adults) and the age-associated blunting of the postprandial MPS response to protein ingestion (24). These results (23) were supported by a subsequent study that also reported no effect of protein distribution on MPS rates in older adults in energy balance (22). Perhaps more applicable to the current study, this same group (22) also measured daily MPS rates in energy restriction with and without resistance training. They reported that a balanced protein distribution (25 g at breakfast, lunch, and dinner) resulted in a 19% greater 13 h MPS rate than a skewed protein distribution (10 g at breakfast, 15 g at lunch, and 50 g at dinner) irrespective of resistance training (22). While relating these acute MPS results to the current null lean mass and muscle area results is problematic, one possibility is that faster MPS is not an important determinant of long-term lean mass and muscle area homeostasis with energy restriction and resistance training. A second possibility is that even protein distribution did not promote faster MPS with concurrent dietary energy restriction and resistance training.

A third possibility may be that the quantity of protein prescribed at each meal between the EVEN and SKEW groups was not sufficiently different to affect daily MPS. High-quality protein sources including pork, egg, beef, and dairy were prescribed at breakfast, lunch, and dinner during the intervention. When these types of high-quality protein sources are consumed, the maximal MPS response is estimated to be reached at doses of 0.24 g protein/kg body mass (25). According to the menu checklists, the SKEW group consumed ~20 and ~60 g of high quality protein at lunch and dinner, respectively. This is equivalent to ~0.24 g/kg at lunch and ~0.71 g/kg at dinner, both of which are hypothesized to be adequate quantities of protein to maximize the MPS response in young adults. Consequently, the SKEW group likely consumed two meals/d (lunch and dinner) that provided sufficient protein to maximize MPS. It may be that a slightly slower MPS rate at one meal (breakfast) does not substantially impact whole body lean mass, right leg lean mass, and right leg muscle area especially when the subjects were consuming greater than the RDA for protein (~1.0 $g•kg^{-1}•d^{-1}$). Regardless, within the context of a typical U.S. protein distribution, our results are ecologically valid based on the skew and quantities of protein consumed at each meal (26).

Perhaps importantly, premises of the protein distribution concept are that fasting-state MPS is comparable between treatments and over time, and that the differential postprandial MPS responses between EVEN and SKEW ultimately are responsible for enhanced lean mass and muscle size responses. These premises might not apply to weight loss. Before weight loss, as expected, the rate of MPS is faster in the postprandial vs. post-absorptive state (15). However, after weight loss, while postprandial MPS does not change, post-absorptive MPS increases to a rate faster than postprandial MPS (27). The weight loss-induced increase in post-absorptive MPS would contribute to higher daily MPS and potentially reduce the impact of meal-to-meal differences in postprandial MPS between EVEN and SKEW distributions

To our knowledge, only observational assessments on the influence of within-day protein distribution on lean soft tissue exist in humans. In support of the even protein distribution concept, an analysis of NHANES data, 1999-2002, showed that more frequent consumption of meals ≥ 30 g was associated with greater leg lean mass (28). However, the reference group was consuming 0.64 g protein/kg body mass, which is less than the RDA for protein (0.8 g protein/kg body mass). Conversely, the comparator groups (groups consuming 1 and 2 meals/d containing ≥ 30 g protein) had a relative protein intake of 1.06 and 1.4 g protein/kg body mass, respectively. These results (28) perhaps more accurately reflect the consequences of consuming less than the RDA on lean mass quantity and not the benefits of evenly distributing protein intake. The ensuing NuAge study (Quebec Longitudinal Study on Nutrition as a Determinant of Successful Aging), a longitudinal cohort study, also characterized the effect of protein distribution on lean mass after a 2-y follow up in both older men and women (29). The NuAge study showed that an even protein distribution was associated with greater lean mass in both older men and women at baseline and after follow-up. However, changes in lean body mass over the 2-y observational period were not different between the even and skewed distributions groups and, in fact, support the results from the current study (29). In contrast to the observational study designs discussed previously (28, 29), the current study used a randomized controlled feeding design to assess differential effects of protein distribution on lean soft tissue changes. Our study was designed to compare an even protein distribution pattern as outlined in the literature (18, 22, 23) against a pattern that is representative of how protein is consumed meal-to-meal in the U.S. (skewed) (26). Results from the current study indicate that an even protein distribution does not differentially effect body composition when compared to a typical U.S. skewed pattern of protein consumption (26) and that evenly redistributing meal-to-meal protein quantity may not be an effective strategy to increase lean mass or skeletal muscle retention while achieving weight loss with resistance training.

Outcomes of cardio-metabolic health were not an *a priori* outcome, however, improvements in body composition as a result of consuming an energy restricted diet are often associated with improvements in cardiovascular health and metabolic syndrome (5-7). Consequently, since we hypothesized that an even distribution would improve body composition more effectively than a skewed distribution, measuring changes in cardio-metabolic health was warranted. Incidentally, null results from the body composition responses between groups negates testing this hypothesis. However, cardio-metabolic results further support that protein distribution does not influence improvements in overall health with weight loss and resistance training.

This study had multiple strengths including the use of a randomized study design, which resulted in reductions in body mass comparable to those of similar weight-loss paradigms (5, 6, 30). Both intervention groups had >86% compliance with resistance training (attending ≥ 41 of

48 exercise sessions), >88% compliance to the prescribed diets (from menu checklists), and >80% compliance to the within-day protein distribution (from meal pictures).

Section 2:

Pending writing a manuscript reporting results from this section.

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