



RESEARCH ARTICLE

The Association between Dietary Protein Intake Frequency, Amount, and State of Energy Balance on Body Composition in a Women's Collegiate Soccer Team

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Abstract

Background: Although protein consumption has been documented to influence body composition in humans, the effect on fat-free mass and fat mass, as influenced by the timing, frequency, and state of energy balance during the day when protein is consumed has not been fully investigated.

Aim: The purpose of this cross-sectional study was to assess whether the amount and frequency of dietary protein intake, and the state of energy balance when consumed, are associated with body composition of collegiate women's soccer athletes.

Methods: Data from nutrition assessments conducted during off-season training were collected in 20 healthy Division 1 female soccer athletes, aged 18-21 years. At visit 1, participants were instructed on how to keep a three-day food and exercise record with hourly measures. At visit 2, food and exercise logs were reviewed, nutrition history questionnaires were completed, and the following measurements were taken: height, weight, and body composition using multi-current segmental Bioelectrical Impedance Analysis (BIA). Hourly protein intake and hourly Energy Balance (EB), a measure of whether energy intake is dynamically matching requirements (energy expenditure), were computed using nutrition analysis software.

Results: Spearman correlations were used to assess the relationships between dietary protein intake, state of energy balance when consumed, and body composition. It was

found that consuming protein in moderate amounts of between 15 to 30 g, with sufficient frequency to supply predicted daily need and while in a state of energy balance > -300 kcal, is significantly associated with lower Fat Mass Adjusted for total body weight (FM-Adj) ($r_s = -0.546$; $p = 0.013$) and greater Fat-Free Mass Adjusted for total body weight (FFM-Adj) ($r_s = 0.546$; $p = 0.013$).

Conclusion: These data suggest that collegiate female soccer players should consume their individual recommended daily amount of protein in ~15-30 g servings while in a reasonable state of energy balance (>- 300 kcal) to achieve lower fat mass and higher fat-free mass.

Keywords

Hourly energy balance, Women's soccer, Protein timing

Introduction

Dietary protein intake, and its distribution during the day, have implications for body composition and Muscle Protein Synthesis (MPS). The Recommended Dietary Allowance (RDA) for protein for average healthy adults is 0.8 g/kg of body weight per day, an amount which has been shown to prevent protein deficiency [1]. This protein recommendation is for a daily (i.e., 24-hour) period and makes no suggestion for the optimal protein distribution within the day. Prevention of

protein deficiency is important, but athletes are also interested in promoting muscular development to enhance the strength-to-body weight ratio and improve muscle recovery. It has been shown that protein intake increases MPS for up to 24 hours following resistance exercise training [2-4]. However, more research is required to fully understand the best strategies for optimizing body composition in female athletes.

Recent studies have attempted to expand on the current RDA protein recommendations for physically active populations. These studies suggest that both resistance- and endurance-based athletes may benefit from daily protein intakes that are well above the RDA, with recommended intakes in the range of 1.2-2.0 g/kg of body mass [2,5-9]. Studies also suggest that appropriate energy balance is important for maximizing muscle protein synthesis [10-12]. Moderate consumption of protein distributed with greater frequency throughout the day, as opposed to fewer but larger protein intakes, appears to enhance the maximal MPS benefit [12,13-17]. Similarly, distributing protein more evenly throughout the day, avoiding large single intakes and extended periods of time without consumed protein, has been shown to enable greater MPS and results in lower body fat percentage [13-15,18]. Studies also suggest that male athletes who consume large amounts of protein may not receive the same benefits from increasing protein frequency [19,20]. The American College of Sports Medicine, The Academy of Nutrition and Dietetics, and the Dietitians of Canada's most recent position stand recommends protein intake of 0.3 g/kg body weight immediately after exercise, as well as 0.3 g/kg body weight every 3-5 hours [6]. The most recent recommendation from the International Society of Sports Nutrition suggests that every 3-4 hours athletes should ingest 20-40 g of high-quality protein per serving or 0.25 g protein/kg body mass to maximize MPS [21].

Objectives

More information is required to clarify the association of protein intake and frequency of intake with body composition in female athletes. Having this information would support the development of sex- and sport-specific protocols for optimizing appropriate energy consumption and constituent energy substrates (including protein, carbohydrate, and fat) for enhancing sports performance, recovery, and body composition. The purpose of this study was to assess whether the amount and frequency of dietary protein intake, or state of energy balance when consumed, is associated with body fat and fat-free mass of collegiate women's soccer athletes. We hypothesized that female athletes who consume recommended amounts of total protein within a 24-hour period, and those who consume protein more frequently throughout the day while in a reasonable state of energy balance (> 300 kcal) will have lower fat mass and greater fat-free mass than those who consume

inadequate protein, consume protein less frequently, or consume protein while in a significant negative energy balance.

Methods

Study design and participants

This study was a collaborative effort among the Departments of Nutrition Science, Human Studies Exercise Science Program, and Athletics, and was approved by the Institutional Review Board (Protocol Number X150928002). Each participant received a full explanation of the study protocol and signed an approved informed consent prior to participation. Participants for the study were recruited during the offseason before beginning individual nutrition assessments. All women soccer players went through this individual nutrition assessment protocol during the offseason, regardless of their participation in the study. Cross-sectional data were collected from consented participants who completed a nutrition history form, an hourly 3-day diet and exercise record, and body composition analysis using multi-current, segmental Bioelectrical Impedance Analysis (BIA). All participants were National Collegiate Athletic Association (NCAA) Division I student athletes between the ages of 18 and 21 years and members of the women's soccer team at the University of Alabama at Birmingham (UAB). They were healthy and were provided medical clearance for athletic participation from the UAB Athletics Sports Medicine Department. Athletes who were not cleared for active sport participation were excluded from the study. A total of 21 eligible participants responded as interested, and 20 completed the full protocol.

Study protocol

Participants came to the athletic training facility for an initial nutrition visit (Visit 1) and were asked to complete an hourly 3-day food and exercise log, which included one weekend day and two weekdays, before they returned for the next visit. Participants completed the form by recording activity, duration and intensity, for each hour of the day. They also recorded food and beverage intake, with a detailed description of type and amount, for each hour of the day. To predict energy expenditure, MET-activity descriptions derived from the National Research Council Subcommittee on the Tenth Edition of the RDA were used [22]. Participants returned for Visit 2 approximately one week after the initial visit and brought completed food/activity logs. Analyses of food and activity logs were performed using established protocols [23,24]. Reported food items that were not listed in the nutrient database were added to the analysis database using information obtained from the manufacturer and/or nutrition labels. Participants providing insufficient detail on food intake and/or activity intensity were asked follow-up questions by registered dietitians specializing in sports nutrition to

enhance clarity [25]. Height was measured using a wall-mounted stadiometer. Body composition and weight were measured using a multi-current, segmental BIA device. At the end of this visit, participants completed a nutrition and health history form that contained questions regarding demographics, medical history, athletic injury history, nutrition and health goals, and food preferences.

Diet and physical activity analysis

Food and activity records were analyzed by a Registered Dietitian specializing in sports nutrition who worked with participants using NutriTiming® (NutriTiming® LLC, Atlanta, GA, USA), an online energy analysis database that is commonly used to analyze diets of athletes [26-28]. NutriTiming® provides both 24-hour and hourly Energy Balance (EB), macronutrient values, and micronutrient values derived from the USDA nutrient database. Both 24-hour and hourly protein intake (grams) were assessed. Hourly protein values were also assessed with consideration of the energy status at the time protein was consumed.

Body composition and weight analysis

Weight and body composition were measured using a Tanita MC-780U (Tanita Corp of America, Inc. Arlington Heights, Illinois, USA), an 8-mode segmental BIA system that has 3 assessment frequencies (5 kHz/50 kHz/250 kHz) and 1 measurement current (up to 90 μ A) [29]. All measurements were obtained with participants wearing light clothing with no socks or shoes. Participants were asked to step on to the scale with toes and heels placed on the electrodes of the weighing platform. This equipment allows for the estimation of whole body and segmental body composition (right leg, left leg, right arm, left arm, and trunk), and provides information on fat mass, fat-free mass (i.e. lean mass), percent body fat, and total body water.

Statistical analysis

Three quantitative variables were calculated using body composition data: Fat Mass Index (FMI) represents the BIA-derived fat mass divided by height squared (FM/Ht^2) [30]; Fat Mass Adjusted by Kg Body Mass (FM-Adj) represents BIA-derived fat mass divided by kilograms of body mass ($FM/Body\ Mass$); Fat-free Mass adjusted by Kg Body Mass (FFM-Adj) represents BIA-derived fat-free mass divided by kilograms of body mass ($FFM/Body\ Mass$).

Protein Intake (g) refers to the total daily consumption of protein in grams. Protein Intake Per Mass (Protein g/kg) refers to the total grams of protein consumed divided by kg of body mass. To assess protein servings throughout the day, several additional protein variables were created. Servings of Protein Recommended were calculated for each participant using the following method: body mass in kg was first multiplied by 1.5

of protein (representing the average recommendation, which ranges from 1.2-2.0 g/kg for soccer players [2,5,6,31]). This number was then divided by 15 g servings (the average minimum recommended serving size based on average body size [21]), resulting in Servings of Protein Recommended. For instance, in an athlete weighing 60 kg, 60 would be multiplied by 1.5 (recommended protein intake is 60×1.5), then divided by 15 (the recommended serving size of protein). This results in 6 Servings of Protein Recommended. We also attempted using 20 g and 25 g servings; however, we could not differentiate between participants due to the low number of participants who had servings at these amounts. All recommendations fell between 3-7 servings per day. Actual Servings of Protein refers to the number of servings of at least 15 g of protein that the participants consumed throughout the day. Lastly, % of Recommended Servings of Protein Achieved was calculated by dividing the number of actual servings of protein by the recommended number of servings. These variables allow for an analysis of protein frequency that considers each individual's specific protein needs.

Hourly EB measurements were computed using NutriTiming® software. This allowed for the determination of EB status at each time of protein consumption. Protein (counted when $EB > -300$) was calculated by only counting protein consumption in grams when EB was greater than -300 kcal. Protein (counted when $EB > -300$ and in max of 30 g serving) was calculated as described above, with the additional restriction that protein servings greater than 30 g were only calculated as 30 g servings. This variable was created to help capture usable grams of protein, or protein that would be used for MPS.

Descriptive statistics for participant and dietary characteristics are reported as means \pm standard deviations. A series of exploratory analyses were performed to identify the most effective model for statistical analysis. For non-normal data, Spearman correlations were performed to evaluate the relationship between protein intake and body composition. Preliminary predicted models were explored by regression analysis to obtain insight into potential prediction of higher fat-free mass and lower fat mass by protein intake strategy. Data were analyzed using SPSS (IBM® SPSS® Statistics, Version 24) and all statistical tests considered an alpha level of $p < 0.05$ for statistical significance.

Results

Twenty-one participants agreed to participate in the study, and one was excluded from the analyses because of failure to complete the diet and exercise records, resulting in a total of 20 participants included in the analyses. Three additional athletes were approached but were not consented because they were not medically cleared for athletic participation at the time of the study. Most participants were Caucasian, with one Asian and one African American participant. As described in

Table 1: Participant Characteristics (N = 20).

Characteristic	Mean	SD	Min	Max
Age (yr)	18.9	± 1.02	18	21
Height (m)	1.64	± 0.07	1.52	1.85
Weight (kg)	58.96	± 7.88	47.00	81.82
Fat Mass (kg)	11.97	± 4.58	5.27	21.55
Fat-free Mass (kg)	46.99	± 4.20	38.27	60.27
Fat Percent	19.8%	± 5.31	10.10	30.10

Table 2: Measured Dietary Intake (N = 20).

Characteristic	Mean	SD	Min	Max
Energy Intake (kcal)	2109	± 501	1335	3103
Energy Expended (kcal)	2240	± 271	1899	2923
Energy Balance (kcal)	-15.53	± 545	-1063.33	912.33
Protein (g)	100	± 34	41.12	175.05
Carbs (g)	256	± 61	157.65	375.02
Fat (g)	77	± 28	43.23	129.04
Kcals from Protein %	19	± 4	11.64	30.64
Kcals from Carbs %	49	± 6	41.23	61.14
Kcals from Fat %	32	± 5	26.42	41.78
Protein (g/kg)	1.76	± 0.733	0.72	3.72
Servings of Protein Recommended	4.7	± 0.63	3.76	6.55
Actual Servings of Protein (15 g)	2.33	± 0.70	1.00	3.67
% of Recommended Servings of Protein Achieved	0.5	± 0.18	0.18	0.88
Total Protein Intake (Maximum of 30 g counted per serving)	72.87	± 17.32	41.12	99.31
Take Protein Intake (Counted if EB >- 300)	83.78	± 39.02	27.98	167.38
Total Protein Intake (Counted if EB >- 300 and max of 30 g counted per serving)	58.10	± 24.10	16.54	99.31

Table 3: Spearman Correlations, body fat, fat-free mass, and protein intake (N = 20).

	Protein (g)		Protein (g/kg)		Actual Servings		% of Recommended Servings of Protein Achieved		Protein (counted when EB >- 300)		Protein (counted when EB >- 300 and max of 30g serving)	
	r _s	p	r _s	p	r _s	p	r _s	p	r _s	p	r _s	p
Body Mass	-0.346	0.135	-0.576	0.008*	-0.295	0.207	-0.544	0.013*	-0.462	0.042*	-0.358	0.121
Fat Mass	-0.299	0.201	-0.527	0.017*	0.374	0.104	-0.573	0.008*	-0.511	0.021*	-0.543	0.013*
%Fat	-0.215	0.362	-0.437	0.054	-0.344	0.138	-0.515	0.020*	-0.431	0.058	-0.530	0.016*
FMI	-0.287	0.221	-0.498	0.025*	-0.403	0.078	-0.566	0.009*	-0.463	0.040*	-0.546	0.013*
Fat-Adj	-0.244	0.301	-0.469	0.037*	-0.361	0.118	-0.537	0.015*	-0.459	0.042*	-0.546	0.013*
FFM-Adj	0.244	0.301	0.469	0.037*	0.361	0.118	0.537	0.015*	0.459	0.042*	0.546	0.013*

Note: %Fat = percent fat; FMI = Fat Mass Index; Fat-adj = fat mass adjusted by kg body mass; FFM-adj = Fat-Free Mass adjusted by kg body mass; *p < 0.05.

Table 1, the mean participant age was 18.9 ± 1 years. Mean body weight was 59.0 ± 7.9 kg and percent fat was 19.8 ± 5.3%. Mean fat-free mass was 46.99 ± 4 kg. Participants consumed an average of 2109 ± 501 kcals per 24 hours, and 1.76 g/kg mass of protein per 24 hours (**Table 2**). Recommended daily protein intake of 1.5 g/kg body weight was met by 55% of the athletes.

Exploratory statistical analyses demonstrated that

a non-parametric approach was most appropriate for the modeling of the data. Results from Spearman correlations related to protein intake variables and body composition are reported in **Table 3**. Total 24-hour protein (g) intake was not significantly associated with any of the body composition variables. However, when protein intake was reported as Protein Intake (g)/kg mass, it was inversely associated with FM-Adj (r_s = -0.469, P = 0.037), and positively associated with FFM-

Adj ($r_s = 0.469$, $P = 0.037$). When protein intake was reported as % of Recommended Servings of Protein Achieved, protein intake was inversely associated with FM-Adj ($r_s = -0.537$, $P = 0.015$), and positively associated with FFM-Adj ($r_s = 0.537$, $P = 0.015$). Additionally, when protein intake was reported as Total Protein Intake (counted when EB > 300 and in max of 30 g serving), protein intake was inversely associated with FM-Adj ($r_s = -0.546$, $P = 0.013$), and positively associated with FFM-Adj ($r_s = 0.546$, $P = 0.013$).

Discussion

Our results support the hypothesis that protein intake frequency, amount, and state of energy balance are associated with body composition in female collegiate soccer athletes. These data demonstrate that study participants who satisfied total protein intake requirements based on predicted physiological needs via frequent consumption patterns during the day with servings ranging from 15-30 gm/meal, and while in a state of EB > 300 kcal, had significantly greater FFM-Adj and lower FM-Adj.

To our knowledge, this is the first study that has compared 24-hour total protein intake, the frequency of protein intake, and protein intake adjusted for hourly EB with body composition in female Division I athletes. These results suggest that more emphasis should be placed on the distribution of protein intake and the state of energy balance rather than limiting the focus on total daily protein consumption based on grams of protein/kg mass. This strategy is in agreement with other recent studies that concluded that moderate consumption of protein throughout the day provides the maximal benefit for MPS [12,13-15]. Additionally, the results of our study suggest that fat-free mass is higher and fat mass is lower when protein is consumed in amounts between 15-30 g, and while in an EB > 300 kcal. Manipulating protein feeding frequency while considering EB may be a helpful addition to nutrition recommendations for female soccer players who are trying to decrease fat mass and increase fat-free mass, and is likely related to increased MPS associated with increased protein intake frequency [2,3]. However, future research is needed to further understand how increasing protein feedings while sustaining a reasonable energy balance state are associated with changes in soccer performance.

A strength of this study was that a food record was used rather than a food recall, reducing memory-associated errors with recalling both foods and food amounts consumed [24,25,32]. Another strength of the study is that hourly food (energy and nutrient) intakes and energy expenditure were estimated, enabling the analysis of both hourly protein consumption and hourly energy balance that were the basis of our primary analysis.

Limitations

This study is limited by sample size and the specificity of the sample size (collegiate female soccer players). Due to the relatively small sample size, the evaluation of our hypothesis was performed through a correlational analysis, which is non-predictive in statistical assumptions. An additional limitation is that diet records were self-reported by the participants, who may have over- or under-reported intakes, either through lack of care or through a poor understanding of portion sizes. Activity levels were self-reported, also with the possibility of inaccurate reporting of activity intensity. It is important to note that all food records were reviewed by a dietitian when brought back for Visit 2 and follow-up questions were used to diminish the possibility of inaccurate reporting.

Conclusion

In conclusion, athletes who were in a reasonably good EB at the time of protein consumption and those with more frequent protein intake had lower body fat mass and higher fat-free mass than the participants with greater energy balance deficits or with less frequent protein consumption. Based on these results, consuming protein with sufficient frequency to supply the predicted daily requirement in 15-30 g amounts appears to be a successful strategy for achieving high fat-free mass and low fat mass in collegiate female soccer players. These findings suggest the importance of considering protein amount and frequency as well as the state of energy balance when the protein is consumed when formulating dietary recommendations for female collegiate soccer players. Future studies analyzing the effects of protein frequency and EB in other groups of collegiate athletes are needed, particularly on how this strategy may impact performance and recovery.

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