

Morning Versus Evening Short-Term Whey Protein Supplementation in Collegiate Athletes

Original Research

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Abstract

Introduction: Benefits of protein consumption are established, yet athletes often consume insufficient protein. The effect of protein supplementation timing on self-reported wellness measures (SRWM) is unknown. The purpose was to examine the effect of protein supplementation timing on overall protein intake and SRWM.

Methods: Collegiate athletes (men: n=13; body mass: 76.1 ± 6.6 kg; body fat %: 14.8 ± 2.3%) (women: n=16; body mass: 72.5 ± 10.8 kg; body fat %: 24.9 ± 4.6%), defined as protein-insufficient (daily intake <1.5 g/kg body weight) participated. Protein supplementation occurred over two 2-week periods (morning, evening) separated by a 2-week washout. Daily SRWM (fatigue, soreness, sleep, stress, mood, energy, recovery, satiety) were collected. ANOVA assessed differences in total protein intake and SRWM measures across conditions. Spearman correlations assessed relationships between protein intake and SRWM.

Results: No sex difference existed in protein intake based on supplementation timing. Compared to baseline, morning and evening supplementation led to an increase (p<0.05) in absolute and relative protein intake for men and women. Satiety was increased during morning and evening conditions compared to washout for men (p=0.004) and women (p=0.012), but other SRWM did not differ. Correlations existed for relative protein intake and satiety (r=0.499, p<0.001) and stress (r=-0.321, p=0.019).

Conclusions: Protein supplementation enabled participants to achieve the recommended protein intake and provided a greater feeling of satiety. Satiety did not differ between morning and evening, providing flexibility as to when to ingest a daily supplement.

Key Words: women athletes; protein timing; sleep; fatigue; soreness; stress; mood; satiety

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Introduction

As a result of rigorous training and academic schedules, collegiate student-athletes encounter barriers that hamper healthy food choices, including limited time for food preparation, insufficient financial resources, inadequate meal planning and preparation skills, and travel schedules that require “eating on the road.”

^{1,2} Prior research has reported that both men and women collegiate athletes under consume total energy, carbohydrates, and protein^{3,4} which may be explained by their lack of nutrition knowledge.^{5,6} Further,

men and women athletes have different energy requirements, and their eating behaviors may differ as a result. Adequate nutrition not only promotes good health and nutritional status, but also improves exercise performance and recovery. While each macronutrient offers a specific benefit for performance, adequate protein balance is essential for anabolism and recovery.⁷

Protein aids the recovery process following muscle damage, caused by repeated muscle actions during practice and game play, through facilitating muscle repair, muscle remodeling, and immune function.⁸ Yet, despite the established importance of dietary protein, insufficient intake persists among collegiate athletes.⁹ It has been demonstrated that collegiate athletes from a variety of sports (i.e. soccer, basketball, track, softball, volleyball, swimming, golf, baseball) consume inadequate protein.^{10–12} Insufficient protein consumption may create a negative nitrogen balance, leading to increased catabolism, and impaired recovery from exercise. Adequate protein intake may improve exercise performance by enhancing energy utilization and stimulating increases in muscle mass; therefore, protein supplementation is beneficial to the college athlete's diet.¹³ Further, protein supplementation is a convenient and easy way to enable athletes to reach adequate protein levels. However, it is unknown how daily dietary protein intake changes with protein supplementation.

It is recommended that endurance and resistance trained athletes consume a minimum of 1.2-1.4 g/kg bodyweight (BW) and 1.7-2.0 g/kg BW of protein, respectively.¹³ Overall, 1.2 g/kg BW is considered to be the minimum amount of protein required for adequate recovery and tissue repair for any athlete.¹⁴ Additional research has suggested that 1.2 g/kg BW may be too low, and notes 1.5 g/kg BW to be a more optimal dosage.^{15,16} Therefore, protein supplementation in addition to the consumption of protein-containing food may be necessary.¹⁷

Previous protein timing studies have focused upon the type of protein supplemented, such as the use of a "fast" (~3hr) digesting protein (e.g., whey) versus a "slow" (~7hr) digesting protein (e.g., casein).¹⁸ It has been recommended that whey protein be consumed during rest¹⁹ and post-exercise due to increased whole body protein synthesis and leucine oxidation.²⁰ Conversely, casein has a limited impact on whole body protein synthesis, and instead inhibits whole body protein breakdown,²⁰ making it likely more suitable for pre-sleep or prolonged fasting conditions.²¹ However, research has shown that pre-sleep whey may improve sleep quality and alertness, and reduce morning sleepiness,^{22,23} and be as effective as casein in increasing morning metabolism.²⁴ Further, it has been reported that whey provides greater benefit to skeletal muscle protein synthesis when compared to casein due to its higher leucine content (12.5% versus 8.5% of total protein, respectively) and faster absorption rate.⁹ Also, whey protein has a superior protein efficiency ratio (3.2), biological value (104), and net protein utilization (92) when compared to casein (2.5, 77, and 76, respectively).²¹ Thus, the favorable effects of whey protein on protein synthesis resulted in its selection for use in the current study. While the timing of protein is important for stimulating muscular growth and recovery, no studies have explored the effect of timing on perceived wellness markers.

Daily protein supplementation is associated with increased protein synthesis,^{25–31} which may be related to reduced fatigue and soreness,³² and a potential increase in satiety hormones and amino acids.³³ Previous studies have reported increased protein synthesis and the attenuated decline in postprandial protein synthesis with just two weeks of protein supplementation.³⁴ In addition, four weeks of protein supplementation was associated with enhanced muscle recovery and reduced muscle damage.³⁵ Therefore, short-term protein supplementation is likely to elicit various benefits. While the benefits of protein consumption for protein synthesis and recovery have been clearly delineated,¹³ the effects of protein quantity and timing on athletes' perceptions of wellness and quality of life have not been examined. These self-reported wellness measures are likely of interest to those working with athletes since sleep,^{36–40} fatigue,⁴¹ soreness,^{42,43} stress,⁴⁴ mood,³⁹ and satiety⁴⁵ can negatively impact athletic performance.^{46,47}

Therefore, the aim of the current study was to investigate the effects of short-term whey protein supplementation timing on overall protein intake and self-reported wellness measures when supplemented to protein-insufficient collegiate athletes. We hypothesized that whey protein supplementation would enable protein-insufficient athletes to achieve optimal protein intake (>1.5g/kg BW) and would positively impact self-reported wellness measures irrespective of supplementation timing.

Methods

Participants

Collegiate male (n=13) and female (n=16) athletes (i.e., National Collegiate Athletic Association Division I) from a variety of sports participated in this study. Of the 35 athletes screened for insufficient protein intake (i.e., <1.5 g/kg BW), six were deemed ineligible, resulting in a sample size of 29 athletes. Characteristics of participants are presented in Table 1. Participants maintained consistent daily training schedules, following sport-specific training regimens with neuromuscular demands particular to their sport, while under the direction of a Certified Strength and Conditioning Specialist® (NSCA-CSCS®). Prior to participation in the study, athletes completed a medical history form and were cleared for intercollegiate athletic participation by the sports medicine staff. Risks and benefits were explained to athletes and an institutionally approved consent form was signed prior to participation. The Institutional Review Board for Human Subjects approved all procedures (IRB# 1147609-3).

Table 1. Characteristics of Participants (n=29).

	Men (n=13)	Women (n=16)
Age (yrs)	19.9 ± 0.9	20.1 ± 0.7
Height (cm)	177.4 ± 2.2	170.9 ± 8.9
BF%	14.8 ± 2.3	24.9 ± 4.6
FM (kg)	11.2 ± 1.6	18.5 ± 5.8
FFM (kg)	62.0 ± 5.9	51.9 ± 6.0
BM (kg)	76.1 ± 6.6	72.5 ± 10.8
Sport	n (%)	n (%)
Lacrosse	0(0)	5(31)
Soccer	3(23)	4(25)
Track & Field	6(46)	4(25)
Baseball	1(8)	0(0)
Swimming	2(15)	0(0)
Volleyball	1(8)	3(19)

Values are mean ± SD, n: number; %: percent; expressed as n(%).
yrs: years; cm: centimeters; BF%: body fat percent; kg: kilograms; FM: fat mass; FFM: fat free mass; BM: body mass.

Protocol

Prior to the start of this randomized block, crossover study, potential participants completed a seven-day baseline screening period to determine protein sufficiency status. During the baseline period, participants were instructed to take pictures and record all foods and beverages consumed daily. Dietary intake was analyzed by a trained research assistant using dietary software (Nutritionist Pro; Axxya System, First Databank, Inc., San Bruno, CA, USA). Individuals with protein consumption <1.5 g/kg BW were deemed eligible for the study.^{15,16} The baseline period included assessment of body composition and familiarization with the self-reported wellness measures that would be used during the course of the study. After completion of the baseline period, those classified as protein-insufficient were randomly assigned to a two-week phase of daily morning or evening protein supplementation. Following a two-week washout period, participants crossed over and completed a two-week phase of the other daily protein supplementation condition (i.e., morning or evening). Participants completed self-reported wellness measures and food logged daily throughout the duration of the study. The timeline of procedures is provided in Figure 1.

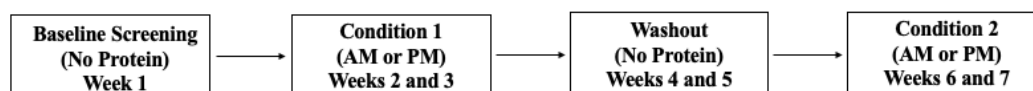


Figure 1. Timeline of Procedures.

Body Composition

Dual energy x-ray absorptiometry (DXA) (Hologic, Horizon A model, Hologic Inc., Waltham, MA, USA) was used to assess body composition. Participants were instructed to wear standardized clothing with no metal parts (i.e., drawstring pants and t-shirt) and were scanned using the whole-body scan mode (Hologic APEX software, ver. 5.5.3.1, Bedford, MA, USA). Calibration and procedures were performed to manufacturer specifications. All DXA scans were overseen by an International Society for Clinical Densitometry (ISCD) Certified Bone Densitometry Technologist (CBDT®). Radiation (~3.4mSV) did not exceed limits for x-ray exposure. Weekly calibration for the body composition measures was performed using a whole-body phantom (Hologic #1104).

Protein Supplementation

Protein supplementation consisted of daily servings of whey protein isolate (Dymatize ISO-100, Dallas, TX) that was measured, weighed on a digital food scale, and packed into individually labeled plastic sealed bags. Participants reported to the laboratory on a weekly basis and were given seven pre-measured servings of protein according to their body weight (25 g protein for <69 kg BW and 30 g for 69-90 kg BW), as commonly recommended by registered dietitians. Protein was administered for two weeks of morning supplementation and two weeks of evening supplementation, separated by a two-week washout period.⁴⁸ Following the two-week washout period, participants crossed over and completed the other daily protein supplementation condition (i.e., morning or evening). Participants were instructed to consume the protein within 90 minutes of waking for the morning condition, and within 90 minutes of going to sleep for the evening condition.

Food Log

Participants were given an electronic spreadsheet file to record daily food intake and asked to take pictures of all food and beverages consumed each day during the baseline, morning, evening, and washout conditions. Further, participants were encouraged to maintain consistent dietary behaviors. Dietary intake was analyzed by a trained research assistant using dietary software (Nutritionist Pro; Axxya System, First Databank, Inc., San Bruno, CA, USA).

Self-reported Wellness Measures

Participants were instructed to complete daily self-reported wellness measures thirty to sixty minutes following the ingestion of protein and send them to the researcher by the end of each day. Participants rated their fatigue, sleep quality, soreness, stress, mood, and sleep quantity on Likert scales of 1-5⁴⁹, satiety on a Likert scale of 1-11,⁵⁰ and recovery on a Likert scale of 0-10.⁵¹ During the washout period, participants were instructed to complete the self-reported wellness measures following a mid-afternoon snack.

Statistical Analysis

Data are expressed as mean \pm standard deviation. A natural log-transformation was applied prior to analysis for any non-normally distributed variables (i.e. fatigue, sleep, hunger). Data was split by gender and a one-way analysis of variance (ANOVA) was used to analyze the effects of protein supplementation and protein timing on self-reported wellness and satiety measures across conditions (i.e., morning, evening, and washout). A secondary ANOVA, in which morning and evening supplementation were combined and compared to no supplementation, was also performed. When appropriate, the Bonferroni test was used for post-hoc analysis. A Spearman correlation was run to assess relationships between protein intake and self-reported wellness measures. The alpha level was set at $p < 0.05$. Statistical analysis software was used for all analyses (SPSS version 25.0; IBM, Armonk, NY).

Results

Protein Intake

Table 2. contains absolute and relative protein intake across conditions. For men and women, there was no difference in protein intake based upon the timing of the protein supplementation (morning vs evening, $p=1.0$). Compared to baseline, both morning and evening supplementation led to a significant increase in absolute (men: $p=0.032$ morning, $p=0.001$ evening; women: $p<0.001$ morning and evening) and relative (men: $p<0.001$ morning, $p=0.027$ evening; women: $p=0.021$ morning, $p=0.002$ evening)

protein intake. During the washout period protein intake was not different from baseline in men or women. A total of seven individuals (women: n=5; men: n=2) remained protein-insufficient throughout the entire study.

Table 2. Absolute and Relative Protein Intake (95% Confidence Intervals) Across Conditions.

Sex	Absolute (g)				Relative (g/kg BW)			
	BASE	AM	WASH	PM	BASE	AM	WASH	PM
Men (n=13)	97.8±18.7 (82.6-107.6)	125.2±14.6** (114.2-135.6)	94.8±26.7 (76.4-114.6)	133.8±19.1** (119.4-147.3)	1.3±0.2 (1.14-1.40)	1.7±0.2* (1.6-1.8)	1.2±0.3 (1.0-1.3)	1.7±0.3** (1.6-1.9)
Women (n=16)	72.5±10.8 (71.2-85.7)	107.9±17.8** (98.4-117.4)	75.7±17.1 (66.9-84.5)	112.9±15.6** (104.0-121.9)	1.1±0.2 (1.0-1.2)	1.5±0.3** (1.4-1.7)	1.1±0.2 (0.9-1.2)	1.5±0.2** (1.3-1.6)

BASE: baseline; AM: morning supplementation; WASH: washout; PM: night supplementation. Values represent mean ± SD, *p<0.01 compared to BASE, **p<0.001 compared to BASE.

Caloric and macronutrient intake during non-supplementation and supplementation are included in Table 3. When collapsed into supplementation versus non-supplementation phases, protein intake was significantly higher during supplementation in women (110.9 ± 16.4 g vs 76.5 ± 15.9 g, p<0.05, Cohen's d=2.130) and men (133.2 ± 25.8 g vs 99.0 ± 24.4 g, p<0.05, Cohen's d=1.363). Additionally, men showed significantly higher caloric intake during supplementation (2495.5 ± 579.5 kcal vs 2143.4 ± 462.4 kcal, p=0.019, Cohen's d=0.672). There were no differences between supplementation and non-supplementation phases for caloric intake in women. Further, carbohydrate and fat consumption did not differ for men or women throughout the study.

Table 3. Daily Caloric and Macronutrient Intake (95% Confidence Intervals) During Non-supplementation and Supplementation.

	Men (n=13)		Women (n=16)	
	NPS	PS	NPS	PS
Calories kcal	2143.4±462.4 (1956.6-2330.2)	2495.5±579.5* (2261.4-2729.6)	1752.9±445.0 (1586.7-1919.0)	1897.4±341.9 (1772.0-2022.8)
Carbohydrates g	246.4±57.6 (223.2-269.7)	272.7±66.4 (245.9-299.5)	219.2±77.1 (190.4-248.0)	215.7±55.7 (195.2-236.1)
Fat g	90.2±36.0 (75.7-104.8)	93.7±23.2 (84.3-103.0)	64.8±18.7 (57.8-71.8)	67.4±17.8 (60.8-73.9)
Protein g	99.0±24.4 (89.2-108.9)	133.2±25.8** (122.8-143.6)	76.5±15.9 (70.6-82.3)	110.9±16.4** (104.9-116.9)

NPS: non-supplementation; PS: protein supplementation; Kcal: kilocalories; g: grams
Values represent mean ± SD, *p<0.01 compared to NPS, **p<0.001 compared to NPS.

Self-reported Wellness Measures

No differences were observed across time (morning, washout, evening) in self-reported wellness measures of fatigue, sleep quality, soreness, stress, mood, sleep quantity, and recovery (Table 4). However, satiety was increased during morning and evening conditions compared to washout for men (p=0.004) and women (p=0.012) (Table 4). Further, secondary analysis of supplementation vs. no supplementation indicated a higher level of satiety in the supplement condition (p=0.002) while other self-reported wellness measures remained unchanged.

Table 4. Self-reported Wellness Measures (95% Confidence Intervals) Across Conditions.

Wellness	Men (n=13)			Women (n=16)		
	AM	WASH	PM	AM	WASH	PM
Fatigue	3.5±0.6 (3.1-3.9)	3.4±0.5 (3.0-3.7)	3.4±0.6 (3.0-3.7)	3.6±1.1 (2.4-4.8)	3.1±0.2 (2.9-3.3)	3.2±0.7 (2.6-3.2)
Sleep Quality	3.7±0.7 (3.2-4.5)	4.0±0.5 (3.7-4.3)	3.9±0.4 (3.6-4.2)	3.7±0.7 (3.4-4.0)	4.0±0.5 (3.4-4.1)	3.9±0.4 (2.7-4.4)
Soreness	3.4±0.4 (3.1-3.7)	3.2±0.5 (2.9-3.6)	3.4±0.5 (3.1-3.7)	3.5±0.4 (3.2-4.0)	3.4±0.4 (3.0-3.8)	2.8±0.6 (1.7-3.8)
Stress	3.3±0.7 (2.9-3.8)	3.4±0.6 (3.0-3.8)	3.3±0.5 (3.0-3.6)	3.6±0.6 (3.1-4.2)	3.6±0.5 (3.0-4.1)	3.0±0.7 (1.8-3.8)
Mood	4.1±0.2 (3.9-4.3)	4.0±0.5 (3.7-4.3)	4.0±0.5 (3.6-4.3)	4.0±0.5 (3.5-4.7)	3.9±0.5 (3.5-4.3)	3.9±0.7 (2.8-4.6)
Sleep Hours	3.3±0.7 (2.8-3.8)	3.1±0.6 (2.7-3.5)	3.0±0.7 (2.5-3.4)	3.2±0.3 (3.0-3.5)	3.2±0.5 (2.7-3.7)	3.0±0.4 (2.2-3.8)
Satiety	6.7±0.9* (6.0-7.4)	5.7±1.02 (5.1-6.3)	7.0±1.0** (6.3-7.7)	7.1±1.3* (5.4-8.6)	5.8±0.7 (5.2-6.7)	6.6±1.0* (4.2-7.3)

AM: morning supplementation; WASH: washout; PM: evening supplementation

Values represent mean ± SD, *p<0.01 compared to WASH, **p<0.001 compared to WASH

Discussion

The purpose of the current study was to examine the effects of whey protein supplementation timing (morning vs evening) on total protein intake and self-reported wellness measures in protein-insufficient collegiate athletes (<1.5 g/kg BW). Our results provide insight into whether or not protein timing should be considered for athletes in order to improve their diet and self-reported wellness measures. It was hypothesized that whey protein supplementation would enable protein-insufficient athletes to achieve optimal protein intake (>1.5 g/kg BW) and would positively impact self-reported wellness measures irrespective of supplementation timing. Notable findings included: 1) whey protein supplementation, regardless of timing, enabled athletes to increase their protein intake without compromising carbohydrate or fat consumption, and 2) whey protein supplementation was associated with greater feelings of satiety and perhaps lower levels of stress.

Insufficient protein intake for athletes can lead to negative nitrogen balance, resulting in a loss of fat free mass⁵², which adversely affects sport performance. In the present study, baseline levels of consumed protein were inadequate for men (1.3±0.2 g/kg BW/day) and women (1.1±0.2 g/kg BW/day) athletes. Such baseline insufficiency in collegiate athletes may be a result of limited nutrition education, busy schedules, limited protein availability, and lack of convenience. Of note, protein insufficiencies have been reported previously in collegiate women athletes from soccer, basketball, cross-country, and track and field (1.2 g/kg BW),¹⁰ as well as softball (1.0 g/kg BW)¹¹ and volleyball (0.9 g/kg BW).¹² Further, collegiate men athletes from track and field, basketball, golf, swimming, football, baseball, and wrestling, were reported to have an average protein intake of 1.2±0.5 g/kg BW.³ Since protein insufficiency in collegiate athletes is common, yet there is an increased protein demand in this population due to the high exercise intensity associated with their sport training,⁵³ protein supplementation is likely beneficial. In the current study, irrespective of timing, supplementation increased protein intake above baseline by 30% for men and 36% for women athletes to ~1.7 g/kg BW and 1.5 g/kg BW, respectively.

There was no difference in protein intake between morning and evening supplementation. As expected, protein intake during morning and evening was significantly greater than baseline and washout periods. Therefore, when provided supplementation most athletes were able to meet and exceed the minimum requirements for protein intake. Further, our results are in support of those reported previously with morning vs evening casein protein supplementation, in which significantly more protein was consumed with supplementation despite the time of day.⁵⁴

While the majority of athletes were able to reach the designated protein requirement via supplementation, seven athletes remained below the minimum requirements (1.5 g/kg BW) throughout the study. This finding points to the importance of taking an individualized approach for athlete nutrition and providing routine assessments to monitor protein status.⁵⁵ The lack of difference between the timing of protein supplementation permits a flexible timeline of daily protein intake for athletes. The current findings show supplementation, despite time of day, is necessary for protein-insufficient athletes.

Although protein requirements were met with both morning and evening supplementation conditions, it is interesting to note that athletes reverted to baseline levels of protein consumption during the washout period (men: 1.3 ± 0.2 ; women: 1.1 ± 0.2 g/kg BW). This may suggest that athletes do not have sufficient knowledge of protein needs or the selection of high protein foods. Therefore, nutrition education may be necessary in conjunction with protein supplementation.¹² In fact, access to a sports dietitian clearly provides benefit toward athlete nutrition knowledge and dietary habits. Results from previous research showed that providing women volleyball players with an educational intervention led to significant improvements in nutrition knowledge and protein intake.¹² Further, when a sports dietitian is available, athletes demonstrate a greater understanding of nutrient periodization and make overall healthier nutrition choices throughout the day.^{56,57} However, it has been reported that many athletes rely on nutritional recommendations provided by their sport coaches, strength coaches, and athletic trainers, of which 30% of athletic trainers and 17% of strength coaches lacked appropriate nutrition knowledge.⁵⁸ In addition to placing focus on educating individual athletes, coaches and athletic trainers would likely benefit from general sports nutrition education.

Protein supplementation enabled athletes to increase protein intake without compromising carbohydrate or fat intake. Men significantly increased caloric intake during supplementation, but women did not, and neither group reported a change in carbohydrate or fat consumption. Review of the participant food logs confirmed that increases in protein intake, while on supplementation, were due to consumption of the supplement mixed with a variety of liquids. Twenty-five participants mixed the protein powder with milk products (i.e., almond (6), skim (4), 2% (10), whole (4), chocolate (1)), which represented the total protein difference with supplementation. Additionally, we confirmed that men consumed the higher calorie milk products, accounting for most of the observed increase in caloric intake while on supplementation.

In terms of self-reported wellness measures, satiety is of interest. While ratings of satiety between morning (men: 6.7 ± 0.9 ; women: 7.1 ± 1.3 AU) and evening (men: 7.0 ± 1.0 ; women: 6.6 ± 1.0 AU) supplementation did not differ, they were higher than the washout period for both timing conditions. Further, the correlation between protein intake and satiety was a positive relationship (absolute protein: $r=0.399$; relative protein: $r=0.499$), a finding which is consistent with previous literature that supports higher protein diets, regardless of carbohydrate source⁵⁹ led to decreased hunger due to an increase in satiety hormones^{33,60,61}. This relationship is promising, as it has been previously shown that 50% of collegiate athletes report feelings of hunger during training, practice, or competition.⁵⁶ The aforementioned reported greater feeling of fullness (satiety) is of interest because it might deter athletes from making unhealthy food choices throughout the day. No other differences were observed across time in self-reported wellness measures.

Interestingly, there was an inverse relationship between protein intake and perception of stress (absolute protein: $r=-0.287$; relative protein: $r=-0.321$) implying that greater protein consumption may be linked to reduced perceptions of stress. Protein has been identified as one specific nutrient that may reduce cortisol and adrenaline levels for the purposes of stress management.⁶² In contrast, a previously published study showed no relationship between protein consumption and salivary cortisol (a biomarker of stress) in the general population.⁶³ Overall, more research is needed to explore the relationship between protein consumption and perception of stress in the athletic population.

The strength of this seven-week study is the implementation of a whey protein supplement in protein-insufficient collegiate men and women athletes from a variety of sports over two separate timing conditions. No previous studies have examined protein intake and self-reported wellness measures in athletes, despite wellness markers playing an important role in sports performance. However, some limitations do exist. A larger sample size may be needed to provide adequate statistical power and produce additional significant findings. Additionally, no placebo supplement was provided for athletes, thus we

were unable to assess the placebo effect of protein consumption. Further, protein was administered according to body weight ranges (25 g protein for <69 kg BW and 30 g for 69-90 kg BW), rather than relative to individual body weight (~0.3 – 0.4 g/kg), which contributed to variability in protein intake. Lastly, the self-reported wellness measures could have been influenced by athletes' training loads, which were not monitored in the current study. However, it should be noted that athletes maintained strict training regimens consistent with neuromuscular demands particular to their sport. Lastly, self-reported wellness measures were used for familiarization at baseline and not regularly administered during this screening period, thus supplementation conditions can only be compared to the washout condition when athletes were not receiving protein supplementation.

Media-Friendly Summary

Changes were observed in short-term protein ingestion in athletes identified as protein-insufficient (<1.5 g/kg BW/day) when supplementing in either the morning or evening. Consuming one whey protein supplement per day enabled the majority of athletes to achieve the recommended protein intake and provided greater satiety. Since no differences in satiety were observed between morning and evening conditions, athletes have flexibility as to when they choose to supplement each day. Since many collegiate athletes do not consume sufficient protein relative to their activity level, it is recommended that nutritional education for athletes, athletic trainers, and coaches focus upon the benefits and requirements of protein. Further, in order to achieve optimal results, it is likely necessary to individualize protein supplementation recommendations relative to an athlete's baseline nutritional intake and activity level.

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References

1. Malinauskas B, Overton R, Carpenter A, Corbett A. Summer league college baseball players: do dietary intake and barriers to eating healthy differ between game and non-game days? *SMART J.* 2007;3(2):23-34.
2. Palumbo C, Clark N. Case problem: nutrition concerns related to the performance of a baseball team. *J Am Diet Assoc.* 2000;100:704-705.
3. Hinton P, Sanford T, Davidson M, Yakushko O, Beck N. Nutrient intakes and dietary behaviors of male and female collegiate athletes. *Int J Sport Nutr Exerc Metab.* 2004;14:389-405.
4. Jagim AR, Zabriskie H, Currier B, Harty PS, Stecker R, Kerksick CM. Nutrient Status and perceptions of energy and macronutrient intake in a Group of Collegiate Female Lacrosse Athletes. *Journal of the International Society of Sports Nutrition.* 2019;16(1):43. doi:10.1186/s12970-019-0314-7
5. Werner EN, Guadagni AJ, Pivarnik JM. Assessment of nutrition knowledge in division I college athletes. *Journal of American College Health.* 2020;0(0):1-8. doi:10.1080/07448481.2020.1740234
6. Holden S. Nutritional Knowledge of Collegiate Athletes. 2018;33:65-77.
7. Kerksick CM, Wilborn CD, Roberts MD, et al. ISSN exercise & sports nutrition review update: research & recommendations. *J Int Soc Sports Nutr.* 2018;15(1):38. doi:10.1186/s12970-018-0242-y
8. Heaton L, Davis J, Rawson E, et al. Selected in-season nutritional strategies to enhance recovery for team sport athletes: a practical overview. *Sports Med.* 2017;47:2201-2218.
9. Witard OC, Wardle SL, Macnaughton LS, Hodgson AB, Tipton KD. Protein Considerations for Optimising Skeletal Muscle Mass in Healthy Young and Older Adults. *Nutrients.* 2016;8(4):181. doi:10.3390/nu8040181
10. Shriver L, Betts N, Gena W. Dietary intakes and eating habits of college athletes: are female college athletes following the current sports nutrition standards? *J Am Coll Health.* 2013;61(1):10-16.
11. Nepocatysh S, Balilionis G, O'Neal E. Analysis of dietary intake and body composition of female athletes over a competitive season. *MJSSM.* 2017;6(2):57-65.
12. Valliant M, Emplaincourt H, Wenzel R, Garner B. Nutrition education by a registered dietitian improves dietary intake and nutrition knowledge of a NCAA female volleyball team. *Nutrients.* 2012;4:506-516.
13. Jager R, Kerksick C, Campbell B, et al. International Society of Sports Nutrition Position Stand: protein and exercise. *J Int Soc Sports Nutr.* 2017;14.
14. Phillips S, Van Loon L. Dietary protein for athletes: From requirements to optimum adaptation. *J Sports Sci.* 2011;29(S1):S29-S38.

15. Delk-Licata A, Behrens C, Benardot D, et al. The association between dietary protein intake frequency, amount, and state of energy balance on body composition in a women's collegiate soccer team. *Int J Sports Exerc Med.* 2019;5(3).
16. Parnell J, Wiens K, Erdman K. Dietary intakes and supplement use in pre-adolescent and adolescent Canadian athletes. *Nutrients.* 2016;8(9).
17. Crittenden R, Buckley J, Cameron-Smith D, et al. Functional dairy protein supplements for elite athletes. *Aust J Dairy Technol.* 2009;64(1).
18. Boirie Y, Dangin M, Gachon P, Vasson M-P, Maubois J-L, Beaufrère B. Slow and fast dietary proteins differently modulate postprandial protein accretion. *PNAS.* 1997;94(26):14930-14935. doi:10.1073/pnas.94.26.14930
19. Tang JE, Moore DR, Kujbida GW, Tarnopolsky MA, Phillips SM. Ingestion of whey hydrolysate, casein, or soy protein isolate: effects on mixed muscle protein synthesis at rest and following resistance exercise in young men. *J Appl Physiol.* 2009;107.
20. Dangin M, Guillet C, Garcia-Rodenas C, et al. The rate of protein digestion affects protein gain differently during aging in humans. *J Physiol.* 2003;549(Pt 2):635-644. doi:10.1113/jphysiol.2002.036897
21. Hoffman J, Falvo M. Protein- Which is Best? *Journal of Sports Science and Medicine.* 2004;3:118-130.
22. Markus CR, Jonkman LM, Lammers JHCM, Deutz NEP, Messer MH, Rigtering N. Evening intake of alpha-lactalbumin increases plasma tryptophan availability and improves morning alertness and brain measures of attention. *Am J Clin Nutr.* 2005;81(5):1026-1033. doi:10.1093/ajcn/81.5.1026
23. Milagres MP, Minim VPR, Minim LA, Simiqueli AA, Moraes LES, Martino HSD. Night milking adds value to cow's milk. *J Sci Food Agric.* 2014;94(8):1688-1692. doi:10.1002/jsfa.6480
24. Madzima TA, Panton LB, Fretti SK, Kinsey AW, Ormsbee MJ. Night-time consumption of protein or carbohydrate results in increased morning resting energy expenditure in active college-aged men. *British Journal of Nutrition.* 2014;111(1):71-77. doi:10.1017/S000711451300192X
25. West DWD, Abou Sawan S, Mazzulla M, Williamson E, Moore DR. Whey protein supplementation enhances whole body protein metabolism and performance recovery after resistance exercise: a double-blind crossover study. *Nutrients.* 2017;9(7):735. doi:10.3390/nu9070735
26. Camera DM, West DWD, Phillips SM, et al. Protein ingestion increases myofibrillar protein synthesis after concurrent exercise. *Medicine & Science in Sports & Exercise.* 2015;47(1):82-91. doi:10.1249/MSS.0000000000000390
27. Alghannam AF, Templeman I, Thomas JE, et al. Effect of carbohydrate-protein supplementation on endurance training adaptations. *Eur J Appl Physiol.* 2020;120(10):2273-2287. doi:10.1007/s00421-020-04450-1
28. Areta J, Burke L, Ross M, et al. Timing and distribution of protein ingestion during prolonged recovery from resistance exercise alters myofibrillar protein synthesis. *J Physiol.* Published online 2013:2319-2331.
29. Areta JL, Burke LM, Camera DM, et al. Reduced resting skeletal muscle protein synthesis is rescued by resistance exercise and protein ingestion following short-term energy deficit. *American Journal of Physiology-Endocrinology and Metabolism.* 2014;306(8):E989-E997. doi:10.1152/ajpendo.00590.2013
30. Mollahosseini M, Shab-Bidar S, Rahimi MH, Djafarian K. Effect of whey protein supplementation on long and short term appetite: A meta-analysis of randomized controlled trials. *Clinical Nutrition ESPEN.* 2017;20:34-40. doi:10.1016/j.clnesp.2017.04.002
31. Ridge A, Devine A, Lyons-wall P, Conlon J, Lo J. The impact of whey protein supplementation in older adults on nutrient intakes and satiety over an 11-week exercise intervention. *Food Quality and Preference.* 2018;68:72-79. doi:10.1016/j.foodqual.2018.01.013
32. Pasiakos S, Lieberman H, McLellan T. Effects of protein supplements on muscle damage, soreness and recovery of muscle function and physical performance: a systematic review. *Sports Med.* 2014;44:655-670.
33. Veldhorst M, Smeets S, Soenen S, et al. Protein-induced satiety: effects and mechanisms of different proteins. *Physiol Behav.* 2008;94(2):300-307.
34. Hector A, Marcotte G, Churchward-Venne T, et al. Whey Protein Supplementation Preserves Postprandial Myofibrillar Protein Synthesis during Short-Term Energy Restriction in Overweight and Obese Adults | The Journal of Nutrition | Oxford Academic. *The Journal of Nutrition.* 2014;145(2):246-252.

35. Shenoy S, Dhawan M, Singh Sandhu J. Four Weeks of Supplementation With Isolated Soy Protein Attenuates Exercise-Induced Muscle Damage and Enhances Muscle Recovery in Well Trained Athletes: A Randomized Trial. *Asian J Sports Med.* 2016;7(3). doi:10.5812/asjasm.33528
36. Mah C, Mah K, Kezirian E, Dement W. The effects of sleep extension on the athletic performance of collegiate basketball players. *Sleep.* 2011;34(7):943-950.
37. Marshall G, Turner A. The importance of sleep for athletic performance. *Strength Cond J.* 2016;38(1):61-67.
38. Copenhaver E, Diamond A. The value of sleep on athletic performance, injury, and recovery in the young athlete. *Pediatric Annals.* 2017;46(3):106-111.
39. Andrade A, Bevilacqua G, Coimbra D, Pereira F, Brandt R. Sleep quality, mood and performance: a study of elite brazilian volleyball athletes. *J Sports Sci and Med.* 2016;15(4):601-605.
40. Watson A. Sleep and athletic performance. *Curr Sports Med Rep.* 2017;16(6):413-418.
41. Tavares S, Smith T, Driller M. Fatigue and recovery in rugby: a review. *Sports Med.* 2017;47:1515-1530.
42. Lewis P, Ruby D, Bush-Joseph C. Muscle soreness and delayed-onset muscle soreness. *Clin Sports Med.* 2012;31:255-262.
43. Cheund K, Hume P, Maxwell L. Delayed onset muscle soreness: treatment strategies and performance factors. *Sports Med.* 2003;33(2):145-164.
44. Kerr G, Leith L. Stress management and athletic performance. *The Sport Psychologist.* 1993;7(3):221-231.
45. Hickett A, Shields D, Henning M. Perceived hunger in college students related to academic and athlete performance. *Educational Sciences.* 2019;9(3).
46. MacKinnon L. Special feature for the Olympics: Overtraining effects on immunity and performance in athletes. *Immunol Cell Biol.* 2000;78:502-509.
47. Govus A, Coutts A, Duffield R, Murray A, Fullagar H. Relationship between pertaining subjective wellness measures, player load, and rating of perceived exertion training load in American college football. *Int J Sports Physiol Perform.* 2017;13(1):95-101.
48. Kraemer WJ, Solomon-Hill G, Volk BM, et al. The Effects of Soy and Whey Protein Supplementation on Acute Hormonal Responses to Resistance Exercise in Men. *Journal of the American College of Nutrition.* 2013;32(1):66-74. doi:10.1080/07315724.2013.770648
49. Hooper SL, Mackinnon LT. Monitoring overtraining in athletes. Recommendations. *Sports Medicine (Auckland, NZ).* 1995;20(5):321-327. doi:10.2165/00007256-199520050-00003
50. Flint A, Raben A, Blundell JE, Astrup A. Reproducibility, power and validity of visual analogue scales in assessment of appetite sensations in single test meal studies. *International Journal of Obesity and Related Metabolic Disorders: Journal of the International Association for the Study of Obesity.* 2000;24(1):38-48. doi:10.1038/sj.ijo.0801083
51. Laurent CM, Green JM, Bishop PA, et al. A practical approach to monitoring recovery: development of a perceived recovery status scale. *Journal of Strength and Conditioning Research.* 2011;25(3):620-628. doi:10.1519/JSC.0b013e3181c69ec6
52. Phillips S. Dietary protein requirements and adaptive advantages in athletes. *Br J Nutr.* 2012;108:S158-S167.
53. Wooding D, Packer J, Hiroyuki K, et al. Increased protein requirements in female athletes after variable-intensity exercise. *Med Sci Sports Exerc.* 2017;49(11):2297-2304. doi:10.1249/MSS.0000000000001366
54. Antonio J, Ellerbroek A, Peacock C, Silver T. Casein protein supplementation in trained men and women: morning versus evening. *Int J Exerc Sci.* 2017;10(3):479-486.
55. Larson-Meyer DE, Woolf K, Burke L. Assessment of nutrient status in athletes and the need for supplementation. *International Journal of Sport Nutrition and Exercise Metabolism.* 2018;28(2):139-158. doi:10.1123/ijsnem.2017-0338
56. Hull MV, Neddo J, Jagim AR, Oliver JM, Greenwood M, Jones MT. Gender differences and access to sports dietitian influence dietary habits of collegiate athletes. *J Int Soc Sports Nutr.* 2016;13(38).
57. Hull MV, Neddo J, Jagim AR, Oliver JM, Greenwood M, Jones MT. Availability of a sports dietitian may lead to improved performance and recovery of NCAA Division I baseball athletes. *J Int Soc Sports Nutr.* 2017;14(29).
58. Torres-McGehee TM, Pritchett KL, Zippel D, Minton DM, Cellamare A, Sihila M. Sports nutrition knowledge among collegiate athletes, coaches, athletic trainers, and strength and conditioning specialists. *J Athl Train.* 47(2):205-211.

59. Gentile CL, Ward E, Holst JJ, et al. Resistant starch and protein intake enhances fat oxidation and feelings of fullness in lean and overweight/obese women. *Nutrition Journal*. 2015;14(1):113. doi:10.1186/s12937-015-0104-2
60. Bilsborough S, Mann N. A review of issues of dietary protein intake in humans. *Int J Sport Nutr Exerc Metab*. 2006;16:129-152.
61. Journel M, Chaumontet C, Darcel N, Fromentin G, Tome D. Brain responses to high-protein diets. *Advan Nutr*. 2012;3(3):322-329.
62. Singh K. Nutrient and stress management. *J Nutr Food Sci*. 2016;6(4). doi:10.4172/2155-9600.1000528
63. Lemmens S, Born L, Martens E, Westerterp-Plantenga M. Influence of consumption of a high-protein vs. high carbohydrate meal on the physiological cortisol and psychological mood response in men and women. *PLOS One*. 2011;6(2).

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