

Effect of Pre-sleep Casein and Tryptophan Supplementation on Energy Expenditure Before, During, and After Exercise in Active Females

Original Research

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Abstract

Introduction: The purpose of this study was to determine the impact of pre-sleep casein protein and tryptophan supplement (SUP) ingestion on sleep quality and morning energy expenditure prior to, during, and post-exercise. **Methods:** Thirteen aerobically active females (age = 22.6 ± 1.9yrs; ht = 1.65 ± 0.06m; wt = 60.5 ± 9.6kg; %bf = 22.5 ± 4.3) ingested SUP or placebo (PLA) 30 min prior to going to bed in 2 trials. The following morning, perceived sleep quality and satiety were assessed, and resting energy expenditure (REE) was measured for 30 minutes before and after 20 min of exercise at 40-50% of VO₂max while measuring exercise energy expenditure (EEE). **Results:** Sleep quality and satiety during SUP was not significantly different than PLA trial (61.6 ± 21.5 vs 68.4 ± 20.5 mm; *p* = 0.39; 29.6 ± 8.2 vs 23.9 ± 6.6 mm; *p* = 0.43). REE before and after exercise during SUP and PLA were not significantly different *F*(1,12)=0,141, *p* = 0.71. EEE during SUP was not significantly different than PLA (112.1 ± 20.4 vs 112.2 ± 21.8 kcals; *p* = 0.91). **Conclusion:** No changes were seen when ingesting SUP prior to sleep on morning EEE and REE before and after exercise.

Key Words: protein supplementation, metabolic rate, perceived sleep

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Introduction

Protein ingestion has been shown to lead to greater thermic effect of food, which is the amount of energy expenditure above basal metabolic rate due to the cost of digesting macronutrients. It has also shown the ability to increase energy expenditure, satiety, and lower respiratory exchange ratio, indicating greater fat oxidation compared to carbohydrates¹⁻³. Adding protein to a normal mixed diet may promote weight loss and/or maintenance primarily due to increased satiety and energy expenditure^{2,4,5}.

Milk proteins, whey and casein, are digested and absorbed by the body at different rates⁶. Casein is referred to as a 'slow absorption protein' due to its slower digestion and absorptive rates compared with whey protein. Casein is commonly recommended as a night-time protein as consumption prior to sleep stimulates muscle protein synthesis overnight which results in an increase in resting energy expenditure (REE) over time⁷⁻¹³. To date, a limited amount of literature has been published on the effects of protein supplementation in the late evening (30-60 min before sleep) on next morning REE¹⁰⁻¹⁷.

In addition to pre-sleep protein consumption, sleep quality and duration may impact metabolic function. When sleep is reduced there is an observed decrease in morning resting metabolic rate (RMR), suggesting

reduced sleep leads to alterations in metabolic functioning and protein synthesis pathways in an attempt to conserve energy the following morning^{17,18}. These findings suggest improving sleep may positively influence energy expenditure and fat oxidation. As melatonin and serotonin are directly involved in sleep regulation, supplementation of their precursor, tryptophan, has been investigated and may be a simple strategy to improve sleep duration and quality^{19,20}.

Based on the available evidence of tryptophan supplementation on both sleep quantity and quality and the negative correlation seen between decreased sleep and metabolic rate, it is hypothesized that tryptophan consumption may improve metabolism and increase REE. Additionally, casein supplementation appears to positively influence metabolic rate and increase energy expenditure. However, there are limited studies investigating the effects of pre-sleep casein and tryptophan supplementation on next-morning energy expenditure prior to, during, and following exercise. Therefore, the purpose of this study was to determine the impact of pre-sleep casein and tryptophan supplement ingestion on sleep quality, perceived satiety, and morning REE prior to, during, and post-exercise in healthy, averagely fit college-aged females.

Methods

Participants

Thirteen aerobically trained (\geq three d/week, 30 min/d self-reported moderate aerobic activity for > three months) college-aged females (age: 22.6 ± 1.9 yrs; height: 1.65 ± 0.06 m; weight: 60.5 ± 9.6 kg; body fat: 22.5 ± 4.3 %; VO_{2max} : 44.07 ± 5.33 ml/kg/min) participated. Participants were excluded if they smoked, had contraindications to exercise, were considered above low risk for cardiovascular disease, or had a musculoskeletal injury that would inhibit them from participating in aerobic exercise. Additionally, participants were excluded from this study if they were taking any medications known to impact the cardiac or endocrine systems, were pregnant, or if they had a milk allergy. Participants completed one familiarization visit and two experimental trials, separated by 48 hours to one week. All procedures involving human participants were approved by the University of Mississippi Institutional Review Board. Written consent was obtained before participation.

Protocol

Height and weight were assessed using a stadiometer and standardized scale (Detecto, Webb City, MO, USA). Body composition was assessed using dual-energy x-ray absorptiometry (DXA, Hologic, Marlborough, MA, USA). After anthropometrics, resting heart rate (HR) and heart rate variability (HRV) were measured using a Polar H10 heart rate monitor chest strap and Elite HRV mobile application (Polar Electro Inc, Bethpage, NY; Elite HRV Inc, Asheville, NC) for five minutes. Resting blood pressure (BP) was then measured using an automated sphygmomanometer (American Diagnostic Company, Hauppauge, NY). A test of maximal oxygen consumption (VO_{2max}) was completed on a motor-driven treadmill (Technogym, Fairfield, NJ, USA). Participants completed a graded exercise test with increasing speed or grade each minute until the participant reached volitional fatigue. Metabolic measurements were analyzed continuously via indirect calorimetry using a ParvoMedics TrueOne 2400 metabolic cart (ParvoMedics, Salt Lake City, UT, USA). Heart rate was measured continuously and a rating of perceived exertion (RPE; Borg scale) was recorded within the last 15 seconds of each minute.

Following the graded exercise test, the participants were randomly assigned to begin the first trial taking either a non-nutritive placebo (10g; 20 kcal, 6g carbohydrate, <1g protein, 1g fat; Hershey's Cocoa, The Hershey Co., Derry Township, PA, USA) or one recommended serving of a nutritional supplement (34g; 140 kcal, 6g carbohydrate, 20g protein, 4g fat; About Time's Nighttime Recovery Formula, SDC, Pittsburgh, PA, USA). Participants were instructed to consume both mixed in 12 ounces of water 30 minutes prior to sleeping at home. The following morning, the participants arrived at the lab within 30 minutes of waking and while fasted. Participants confirmed they had consumed the supplement and both experimental trials occurred at the same hour.

The day before the first experimental trial, participants were asked to refrain from deviating from their normal diet and to complete a 24-hour dietary log. They were asked to duplicate this same diet prior to the second trial. Additionally, the participants were instructed to avoid caffeine, alcohol, and physical activity 24 hours before testing sessions.

Upon arrival to the laboratory, the participants assessed their perceived sleep quality and satiety using a visual analog scale (VAS). The VAS used was a 100mm scale with opposite feelings ('not at all' to 'extremely') of satiety and sleep quality on either end of the scale. Participants indicated their perceived satiety and sleep quality by placing a vertical line along the scale and the researchers quantified the value by measuring the marking on the line using a ruler.

Following a 5-minute seated rest, resting HR, HrV, and BP were measured. Resting energy expenditure (REE) was then assessed for 30 minutes using the metabolic cart. The first five minutes was a stabilization period, then recording began and continued for the remaining 25 minutes. Participants laid supine while expired gases were collected using a ventilated hood connected to the metabolic cart to measure REE (kcal/day), daily calories from carbohydrates and fats (kcal/day), and VO_2 (ml/kg/min) via indirect calorimetry. This protocol was repeated following the bout of exercise during both experimental trials.

After measurement of REE, participants engaged in 20 minutes of moderate exercise on a treadmill at 40-50% of their measured VO_2max , and exercise energy expenditure (EEE) was assessed. Using a Hans Rudolph two-way valve with a headpiece and nose clip (Hans Rudolph, Shawnee, KS) connected to the metabolic cart, VO_2 (ml/kg/min) and EEE (kcal) were measured throughout exercise. A heart rate monitor was worn to continuously measure exercise HR.

Statistical Analysis

Statistical analyses were conducted using SPSS 25.0 statistical software package (SPSS Inc. Chicago, IL). Paired samples t-tests were conducted to compare differences between supplement and placebo on HRV, HR, and BP, perceived satiety and sleep quality, and EEE. Two-way repeated measures ANOVA was used to compare differences in REE before and after exercise after consumption of the supplement and placebo. Results were considered significant, a priori, at $p \leq 0.05$. Data are reported as means and standard deviations.

Results

When comparing placebo and supplement trials for RHR and HrV, the standard deviation of NN intervals (SDNN) during the placebo trial was significantly greater than supplement SDNN, but all other variables were not significantly different ($p \geq 0.05$). Additionally, there were no significant differences in systolic or diastolic BP values during the supplement and placebo trials ($p \geq 0.05$). Values of resting heart rate, BP, and HrV are presented in Table 1.

Table 1 – Resting HR, HrV, and BP in both supplement and placebo trials.

	SUPPLEMENT	PLACEBO	<i>p</i> -value
RESTING HEART RATE (BPM)	61.8 ± 6.8	61.3 ± 8.1	0.803
AVG RR INTERVAL (MS)	984.3 ± 120.2	996.3 ± 135.1	0.745
RMSSD (MS)	80.9 ± 50.8	86.5 ± 51.5	0.512
LN (RMSSD) (MS)	4.2 ± 0.6	4.3 ± 0.6	0.286
SDNN (MS)	82.5 ± 47.9	102.9 ± 42.1	0.044*
NN50	128.8 ± 65.2	131.9 ± 69.0	0.788
TOTAL POWER (MS ²)	5259.9 ± 6459.8	5737.3 ± 5555.7	0.713
LF POWER (MS ²)	2496.1 ± 3181.7	2320.3 ± 1842.9	0.720
HF POWER (MS ²)	2786.0 ± 3485.7	3083.7 ± 4642.2	0.808
LF/HF RATIO	1.0 ± 0.7	1.4 ± 1.5	0.389
SYSTOLIC BLOOD PRESSURE (mmHg)	115.2 ± 6.6	115.8 ± 10.7	0.901
DIASTOLIC BLOOD PRESSURE (mmHg)	68.8 ± 6.0	68.0 ± 9.0	0.718

RMSSD = Root Mean Square of the Successive Differences; LNRMSD = natural log of Root Mean Square of the Successive Differences; SDNN = standard deviation of NN intervals; NN50 = number of adjacent NN intervals that differ from each other by more than 50 ms; LF Power = low frequency power; HF Power = high frequency power; LF/HF Ratio = ratio of low frequency to high frequency

Data are means \pm SD.

* Significantly greater in placebo than supplement trial, $p < 0.05$.

There were no significant differences in perceived satiety in the mornings following either the casein and tryptophan supplement or the placebo ($p=0.438$). Similarly, the measures of perceived sleep quality were not significantly different between the supplement and placebo trials ($p=0.395$). Measured perceived satiety and sleep quality are presented in Figure 1.

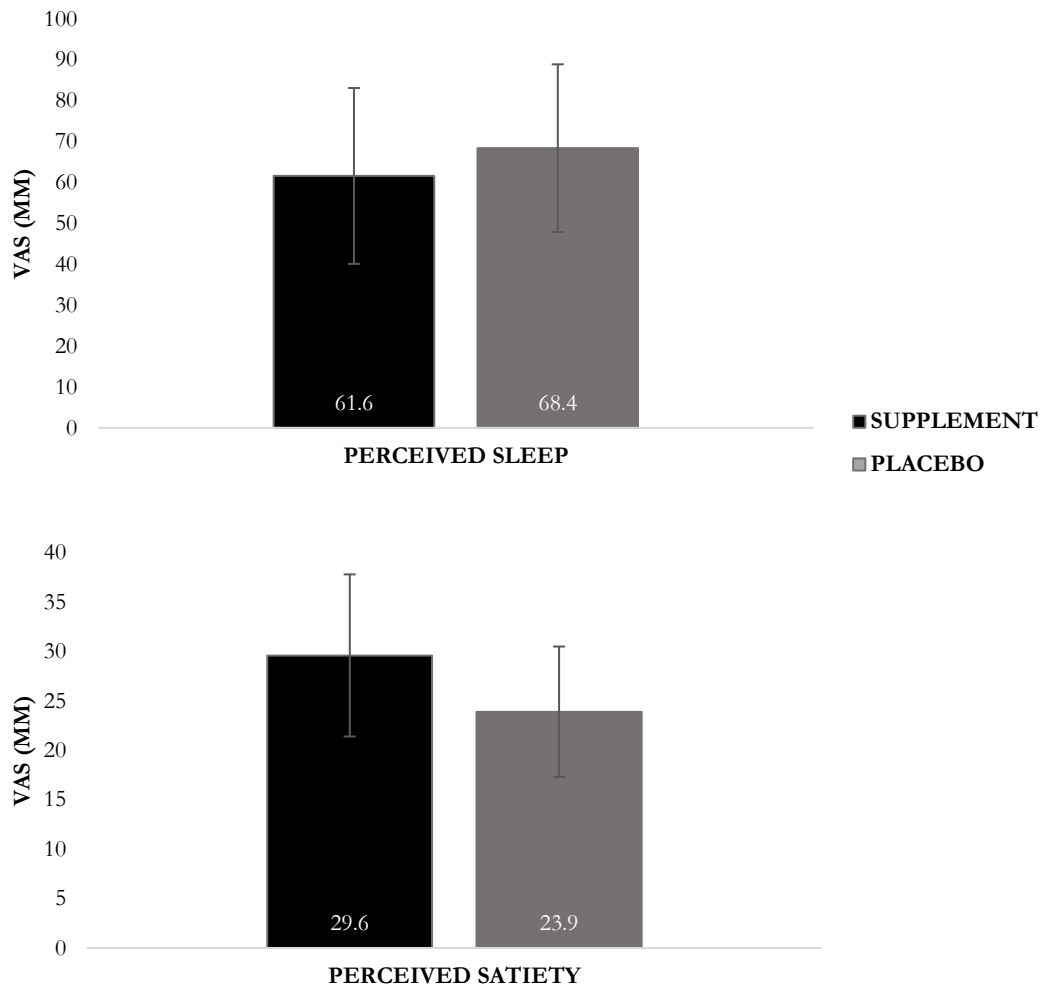


Figure 1 – Measures of perceived satiety and sleep the mornings following consumption of placebo and supplement. Mean \pm SD.

The estimated REE before and after exercise after consumption of the supplement and placebo are shown in Figure 2. There was not a statistically significant two-way interaction between supplement and placebo and time before or after exercise ($F(1,12)=0,141, p=0.714$). Figure 3 illustrates values of measured exercise energy expenditure. EEE was not significantly different between trials ($p=0.917$).

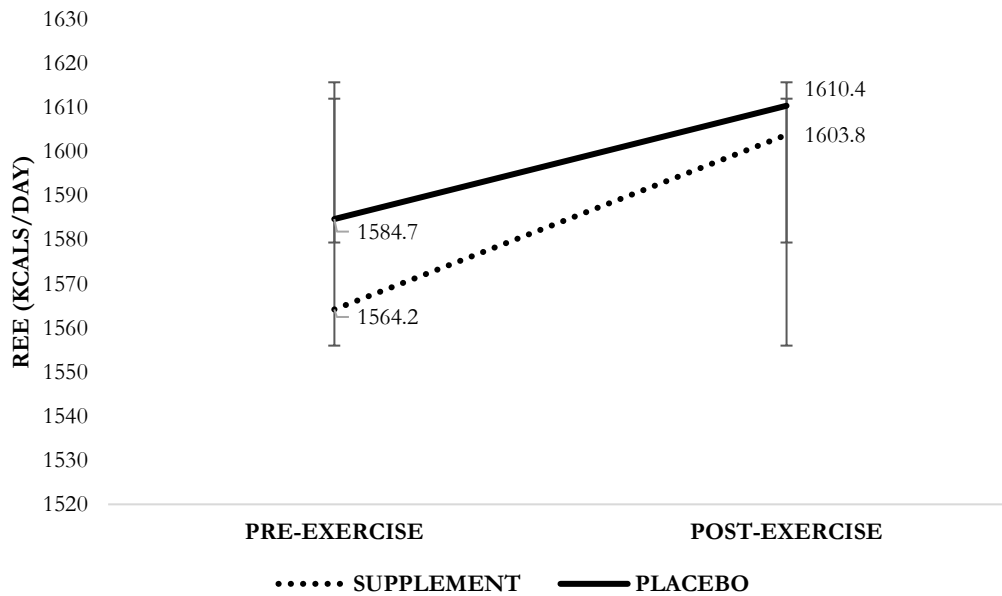


Figure 2 – Interaction between REE before and after exercise in supplement and placebo trials. Mean ± SD.

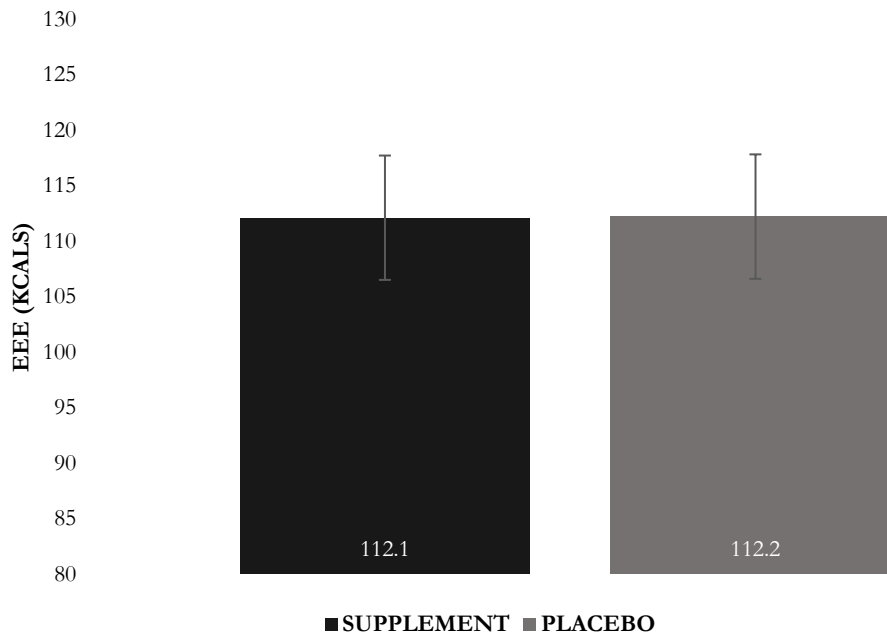


Figure 3 – Exercise energy expenditure in supplement and placebo trials. Mean ± SD.

When comparing fuel utilization before exercise the morning following consumption of either supplement or placebo, there were no significant differences in daily calories from carbohydrates ($p=0.201$) or fats ($p=0.267$). Additionally, there were no significant differences in the fuel utilization between daily calories from carbohydrates ($p=0.058$) or fats ($p=0.095$) following exercise. The daily estimated calories from carbohydrates and fats measured before and after exercise are presented in Table 2.

Table 2 – Daily calories from carbohydrates and fats before and after exercise in supplement and placebo trials.

		SUPPLEMENT (N = 13)	PLACEBO (N = 13)	<i>p-value</i>
PRE-EXERCISE (KCAL/DAY)	CARBOHYDRATES	659.9 ± 329.2	455.8 ± 558.2	0.201
	FATS	911.2 ± 372.0	1134.0 ± 816.5	0.267
POST-EXERCISE (KCAL/DAY)	CARBOHYDRATES	535.3 ± 339.1	371.3 ± 279.5	0.058
	FATS	1074.3 ± 353.1	1241.9 ± 382.4	0.095

Data are means ± SD.

Discussion

The primary finding of this study was that consumption of a supplement containing 20g casein protein and 300mg tryptophan prior to sleep did not contribute to significantly different values of energy expenditure or fuel utilization the following morning compared with consumption of a placebo. Additionally, the nighttime ingestion of a casein and tryptophan supplement did not seem to significantly influence perceived satiety or sleep quality in comparison with the placebo.

Our findings of no significant difference in estimated daily REE before and following exercise the morning after bedtime consumption of either 20g casein protein and 300mg tryptophan supplement or placebo (Figure 2) is in opposition to other studies that have also investigated the effects of nighttime casein consumption on next morning REE. This difference in findings may be due to dissimilarity in the doses of protein consumed. Based on a study conducted to compare high (48g) and low (24g) doses of casein or whey protein on next morning RMR in active women, it was found that 48g of protein from a casein supplement elicited an increase in RMR, while 24g of casein and both doses of whey protein did not¹⁰.

A notable difference in the effects on RMR and REE appears to be the protein dosage given prior to sleep, suggesting a threshold for response. Studies that saw similar results to the current study utilized smaller doses of protein, between 10 and 25 grams^{10,13,15,16,21}. The present investigation provided participants with a nighttime dose of casein containing only 20g of protein, based on manufacturer guidelines, and saw no significant differences in next morning REE before or after steady state exercise compared to the placebo. Because of the differential responses previously noted on protein dose, it has been suggested by the International Society of Sports Nutrition that 30 to 40 grams of protein should be the ideal dosage consumed prior to sleep for overnight and next morning improvements in metabolic rate¹². Therefore, lack of impact on next morning REE before and after exercise following nighttime casein supplement consumption in the present study could be credited to the relatively low dose of protein consumed.

There was not a significant difference in perceived satiety the morning following consumption of either the casein and tryptophan supplement or the placebo (Figure 1). Similarly, Madzima et al. found no significant differences in perceived satiety, hunger, and desire to eat the morning following consumption of 30g of protein from whey or casein, 33g of carbohydrates, or a placebo suggesting no differences in morning satiety despite various nighttime macronutrient consumption¹¹. Additionally, prior studies that have identified protein supplementation as a means for increasing satiety and decreasing later energy intake have observed daytime protein consumption and measured perceived satiety and intake within five hours after consumption of the supplement^{1,3,4,22}. In the present study, the time between bedtime consumption of protein and next morning measures of satiety may have been too long for any effects on appetite to be noted¹¹.

In contrast to many prior studies, the current results indicate no significant differences in perceived sleep quality after consumption of a tryptophan supplement compared to a placebo prior to sleep (Figure 1).

Prior studies have found significantly improved sleep latency, total sleep time, and an increase in perceived sleepiness following supplementation of tryptophan^{19,23–29}. In a review of the effects of tryptophan supplementation on sleep, Hartmann et al. concluded that the recommended dosage of tryptophan for beneficial effects on sleepiness and sleep latency is at least 1g^{24,30}. Additionally, Hartmann et al. also suggest that the ideal population to be studied for the effectiveness of tryptophan supplementation should be chronic insomniacs or individuals with a history of disturbed and hindered sleep^{24,30}. Although few prior studies have seen improvements in sleep latency following tryptophan supplementation in ‘normal individuals’ without sleep disturbances, only a large dose of 2.4g of tryptophan was seen to affect sleep latency two hours after supplementation²⁹. Based on manufacturer guidelines, the current study investigated the effects of only 300mg of tryptophan on individuals that were not known to have a history of chronic insomnia or sleep disturbances. Additionally, the present study measured the effects of tryptophan on sleep through the participants’ perceived sleep quality the morning following supplementation. Prior studies have measured perceived sleepiness in the hours following supplementation of tryptophan and seen a significant increase in sleepiness compared to a placebo^{23,26}. The findings of these prior studies suggest that the effects of tryptophan are more likely to be experienced with higher doses of the supplement, examined prior to sleep, and in individuals with a history of sleep difficulties.

Previous studies have found that nighttime casein consumption may increase next morning metabolic rate while consumption of a tryptophan supplement may improve sleep parameters. However, these results seem to be dependent upon the dosage of both supplements as well as the population being investigated. The suggested serving of twenty grams of protein from casein and 300mg of tryptophan does not seem to be a sufficient dose of supplementation to elicit changes in metabolic rate, energy expenditure before or after exercise, satiety, or perceived sleep quality in averagely fit college-aged females. Additionally, the supplement did not seem to have significant effects on nutrient utilization (Table 2), EEE (Figure 3), or resting HR, BP, or HrV (Table 1). A limitation of the present study is that the female participants were not tested around the same phases of their menstrual cycle. Differences in hormones could affect metabolic variables, but it is unlikely that the differences would be significant due to participants completing the two trials separated only by an average of one week. A second limitation in the current study is evident in that we did not require a standardized diet during the testing period. Although we did have participants consume the same meals 24 hours prior to both testing sessions, there is a chance that dietary intake for the two sessions was slightly different and could potentially impact metabolic variables or perceived satiety. Additionally, the present protocol had participants consume a bolus dose of protein as opposed to a dosage of protein based on body weight.

Media-Friendly Summary

In conclusion, pre-sleep consumption of 20g of casein with 300mg of tryptophan did not increase next morning metabolism prior to or following steady state exercise, exercise energy expenditure, or perceived satiety and sleep compared to a non-nutritive placebo in physically active college-aged females. However, the findings of the present study do not agree with prior studies investigating the effects of similar supplementation on other populations and with other protocols, suggesting the current results should only be applied to the confines of the participants’ characteristics and the protocol used. Because of the known negative correlation seen with reduced sleep leading to a decrease in metabolic rate and the positive correlation typically seen with nighttime protein consumption increasing metabolic rate, further investigations should be done on bedtime supplements containing both casein and tryptophan.

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