

Article Classification:  
Research article

# WHEY PROTEIN ISOLATE REDUCES CORTISOL AWAKENING RESPONSE POST-EXERCISE

Michael Oldham<sup>1</sup>, Vic Ben-Ezra<sup>2</sup>, Kyle Biggerstaff<sup>2</sup>, Nate Mills<sup>2</sup>, Sarah Deemer<sup>3</sup>, Matt Sokoloski<sup>2</sup>, Chris Irvine<sup>4</sup>, Ryan Gordon<sup>5</sup>, Gena Guerin<sup>6</sup>, Manisha Rao<sup>2</sup>, Matt Brisebois<sup>7</sup>, Lauren Rhodes<sup>1</sup> & Todd Castleberry<sup>8</sup>

<sup>1</sup> East Texas A&M University, Commerce, TX, United States of America

<sup>2</sup> Texas Woman's University, Denton, TX, United States of America

<sup>3</sup> University of North Texas, Denton, TX, United States of America

<sup>4</sup> Rocky Mountain College, Billings, MT, United States of America

<sup>5</sup> Missouri State University, Springfield, MO, United States of America

<sup>6</sup> Saginaw Valley State University, University Center, MI, United States of America

<sup>7</sup> The University of South Carolina Upstate, Spartanburg, SC, United States of America

<sup>8</sup> Hanger Institute, Alexandria, VA, United States of America

## ABSTRACT

**Introduction:** Disruptions in cortisol awakening response (CAR), changes in Profile of Mood States (POMS), and decreases in heart rate variability (HRV) have been associated with fatigue and strenuous exercise. Whey protein isolate (WH) may improve stress responses. **Purpose:** To determine the effects of WH supplementation on CAR, POMS, and HRV after strenuous exercise. **Methodology:** Eleven recreationally active females ( $19 \pm 2$  yrs) completed a double-blinded, placebo-controlled crossover trial. Placebo (PL) was maltodextrin, and the intervention was 25 g of maltodextrin with 25 g of WH consumed prior to exercise. Exercise was 30 min at 70-75%  $VO_{2peak}$  ( $M = 21.7$ ,  $SD = 0.1$  ml/kg/min), 5 min rest, and 30 s Wingate anaerobic test (WAnT). HRV and POMS were recorded the following morning. Repeated measures ANOVA determined differences ( $p < .05$ ) in 60 min salivary cortisol AUCg (CAR), POMS, HRV, and WAnT fatigue index (FI). Pearson's correlation and multiple regression identified associations between CAR, POMS, HRV, and FI. **Results:** CAR was significantly different ( $p = .033$ ) between Placebo ( $33.4 \pm 2.0$  pg/dL\*hr) and WH ( $30.9 \pm 0.8$  pg/dL\*hr), with no significant differences in POMS, HRV, or FI. There was a significant correlation between POMS and FI on Day 3 during PL ( $r = -.582$ ,  $p = .030$ ). Neither CAR, POMS, nor HRV was able to predict FI (all  $p > .05$ ). **Conclusions:** Whey protein isolate may decrease CAR, but may have no effect on POMS, HRV, or FI, and no effect on short-duration sprint cycling performance. **Limitations:** WAnT performance was not affected; therefore, any association with reducing the physiological effects of central fatigue may be minimal. **Practical applications** may include a viable methodology for suppressing CAR in this type of participant. **Originality:** The current study is unique in combining nutritional supplementation, exercise, and salivary cortisol post-exercise with female participants.

## OPEN ACCESS

Submitted: 14 August 2025

Accepted: 04 November 2025

## ORCID

Michael Oldham  
<https://orcid.org/0009-0006-4054-0046>

## Cite this article as:

Oldham, M., Ben-Ezra, V., Biggerstaff, K., Mills, N., Deemer, S., Sokoloski, M., Irvine, C., Gordon, R., Guerin, G., Rao, M., Brisebois, M., Rhodes, L., Castleberry, T. (2025). Whey protein isolate reduces cortisol awakening response post-exercise. *Journal of Applied Sports Sciences*, 9(2), pp. 49 - 62. DOI: 10.37393/JASS.2025.09.02.4



This work is licensed under a Attribution-NonCommercial-ShareAlike 4.0 International (CC BY-NC-SA 4.0)

**Keywords:** whey protein isolate, CAR, POMS, HRV, fatigue, overtraining

## INTRODUCTION

Readiness, as defined by Urhausen and Kindermann (2002), refers to the state of being adequately prepared, both mentally and physically, for a specific event or activity.

Overtraining syndrome (OTS) is characterized by a performance deficit specific to the sport, accompanied by mood alterations, compromised immune function, and dysregulation of the endocrine system. Although OTS

is an undesirable outcome, it may be preceded by functional overreaching (FOR) and non-functional overreaching (NFOR), which represent stress-induced detriments in physiological and performance metrics. Significant questions persist regarding the transitions between FOR, NFOR, and OTS, as well as the thresholds for each stage. The underlying biochemical mechanisms and pathways, as well as the identification of single biomarkers for monitoring these transitions, have not been thoroughly investigated in the current scientific literature.

The cortisol awakening response (CAR) is linked to the hypothalamus-pituitary-adrenal (HPA) axis, which plays a role in how the body responds to stressors such as exercise and overtraining (Stalder et al., 2024). Prolonged increases in exercise load tend to lower CAR, while short-term increases can raise it (Anderson et al., 2018). Additionally, Lei et al. (2025) report that there may be connections between the hippocampus, exercise-induced increases in serotonin, reduced hippocampal cell activity, and increased HPA axis activity. Stalder et al. (2024) also suggest that neurological links exist between the hippocampus, hypothalamus, and amygdala, and that these are associated with changes in CAR, as measured by the Profile of Mood States (POMS). Heijnen et al. (2016) additionally found that, while chronic exercise is known to alter neurochemistry, mood, and cognitive function, there is limited research on acute exercise-related changes. Lastly, heart rate variability (HRV) is often used to measure the balance between sympathetic and parasympathetic parts of the autonomic nervous system activity (Bellenger et al., 2021). Although training can improve HRV, the relationship between HRV and CAR remains poorly understood.

This study's novelty lies in examining how whey protein supplementation affects

CAR, POMS, and HRV. Studies show that high-protein meals can improve certain aspects of well-being, as measured by POMS, compared to high-carbohydrate meals; however, research on the relationship between HRV and whey protein supplementation is scarce. Furthermore, most existing studies on whey protein's effects on CAR, POMS, and HRV focus on long-term outcomes rather than immediate effects (Huang et al., 2017; Nelson et al., 2013), while even fewer studies have attempted to connect CAR, POMS, and HRV to fatigue metrics such as fatigue index (FI) in exercise trials (Barrero, et al., 2020).

### *Specific Aims*

The effects of whey protein supplementation on CAR, POMS, HRV, and FI remain incompletely understood in the scientific literature, particularly regarding its impact on female athletes. Therefore, the primary objective of this study was to determine the effects of whey protein supplementation on CAR, HRV, POMS, and FI across two consecutive days of exercise. Building on previous findings suggesting that whey protein isolate may enhance performance on consecutive exercise days, a secondary aim of this research was to investigate the predictive capacity of CAR, POMS, and HRV for the FI, specifically the reduction in power output, within the context of a nutritional intervention. This investigation aimed to determine if the proposed supplementation regimen mitigates the neurophysiological and psychological markers of fatigue in recreationally active females, thereby offering insights into optimal recovery strategies. Given differential physiological and hormonal responses in females, particularly during the menstrual cycle and its influence on metabolic and neurological adaptations, this study aimed to bridge a critical gap in understanding how specific nutritional

interventions interact with these intrinsic factors to modulate recovery and performance (Rocha-Rodrigues et al., 2021). Additionally, given the autonomic nervous system's role in cardiac activity and its modulation by endurance exercise, understanding how whey protein influences heart rate variability, an established non-invasive measure of cardiac autonomic activity, is crucial for developing comprehensive recovery strategies for athletes (Fang & Zhang, 2024; Swart & Constantinou, 2023). The potential of whey protein to facilitate more rapid delivery of amino acids, particularly di- and tripeptides, thereby enhancing muscle protein remodeling and recovery, warrants detailed investigation in the context of acute exercise-induced fatigue (Morgan & Breen, 2021). This multifocal approach enables a comprehensive evaluation of whey protein's multifactorial influence on physiological recovery, particularly in populations where hormonal fluctuations significantly affect exercise responses (Benito et al., 2023).

### ***Hypotheses***

As the project was multi-faceted, the researchers had a number of hypotheses:

1. Whey protein isolate will lower CAR, increase HRV, and decrease POMS total mood disturbance, significantly more than placebo, suggesting attenuated physiological and psychological fatigue.
2. Whey protein isolate will have a greater effect on CAR, HRV, and POMS total mood disturbance after the second day of exercise, compared to the first day of exercise.
3. POMS, HRV, and CAR would be significantly correlated to and significantly predict FI, by greater margins in the whey protein intervention, indicating its effectiveness in mitigating exercise-induced fatigue.

## **METODOLOGY**

### ***Participant Screening and Anthropometric Measurements***

Forty-three recreationally active women, aged 18 to 35 years, were recruited for this double-blind, randomized, controlled trial. Of the initial respondents, 31 were excluded based on predefined criteria, including oral contraceptive use, training status, or concurrent use of contraindicated medications or supplements. Eligible participants completed the 2018 Physical Activity Readiness Questionnaire; any affirmative responses to the first seven questions resulted in exclusion. Weight was determined using a calibrated weigh beam scale, and height was measured with a precision stadiometer.

### ***POMS***

Participants completed a 35-item short-form POMS questionnaire (McNair et al., 1971, 1992), responding to each item on a 5-point Likert scale to indicate the intensity of their feelings. Total Mood Disturbance was calculated using the formula:  $TMD = (Vigor + Activity + Fatigue + Confusion + Depression + Anger)$ . POMS was administered post-anthropometric screening and on testing days, specifically at the 30- and 45-minute time points within the one-hour saliva collection window.

### ***Peak Oxygen Uptake Measurement***

Following screening, participants underwent a Bruce treadmill protocol (Bruce et al., 1963) to determine peak oxygen uptake using a Quinton ST65 treadmill. Heart rate was monitored with a Polar Heart Watch system, and the rate of perceived exertion was measured before each stage change. Expired gases were analyzed at 15-s intervals for indirect calorimetry using a calibrated ParvoMedics TrueOne™ 2400 Metabolic Analysis System, Model 2400.

**Dietary Intake**

Participants were instructed to maintain their usual dietary patterns. To minimize the impact of macronutrient composition on supplementation outcomes, participants consumed the same lunch meal 3-4 hours before each exercise trial, and trials were scheduled at the same time each testing day. Diet logs were kept for each trial period and analyzed using WebMD Food Calculator (<https://www.webmd.com/diet/healthtool-food-calorie-counter>) to verify consistency.

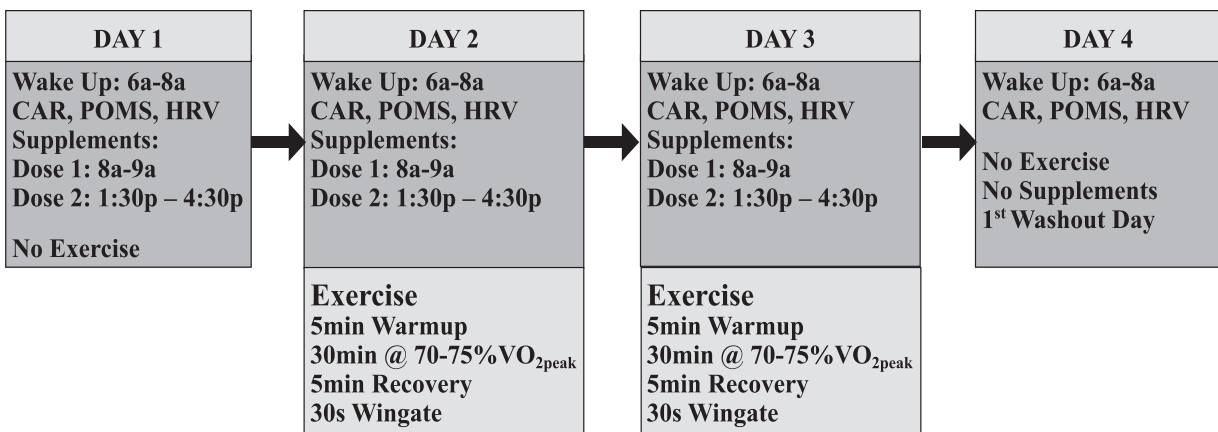
**Dosing and Timing**

The placebo consisted of 25 g of maltodextrin powder mixed with 500 ml of water (100 kcal). The whey protein dose comprised 25 g of hydrolyzed whey protein isolate powder and 25 g of unflavored maltodextrin powder,

mixed with 500 ml of water (200 kcal). Both supplements included four drops of non-caloric chocolate flavoring (Flavdrops™) for uniformity. Supplements were consumed between 08:00-09:00 and 13:30-16:30, with Dose 2 taken 30 min before the scheduled exercise. A 7-day washout period was enforced between experimental trials, starting on Day 4 of the testing phase. This washout period was doubled for both supplementation and exercise days to minimize carryover effects.

**Overview of Intervention Design**

Peak oxygen uptake ( $VO_{2peak}$ ) was completed at least 7 days prior to beginning the two exercise and supplementation cycles. Exercise and supplementation week is as indicated in Figure 1 below.



**Figure 1.** Exercise and Supplementation Cycle

**Steady-State Aerobic Exercise Trial**

Each treadmill session began with a 5-minute warm-up at 3.3 mph. Subsequently, the grade and speed were adjusted to maintain each participant’s heart rate at 70-75% of their peak oxygen uptake ( $VO_{2peak}$ ). Participants walked for 30 min, with oxygen consumption and heart rate measured during the first 5 min, between min 15 and 20, and again between min 25 and 30. Throughout the 30 minutes, treadmill adjustments were made to keep the intensity with-

in the target range. Heart rate was continuously monitored using a Polar heart rate monitor.

**Wingate Anaerobic Power Test (WAnT)**

Before the first testing session, participants were acquainted with the Velotron cycle ergometer and completed a 2-minute trial at 40 W, maintaining a cadence between 40 and 80 rpm. After the treadmill session, participants rested for 5 min before undertaking a 30-second Wingate Anaerobic Test on a Velotron®

DynaFit Pro cycle ergometer. The test involved a 20-second initial phase at 75 W, followed by a 6-second acceleration period where participants pedaled as quickly as possible, and then a load equivalent to 7.5% of body weight was applied to the flywheel. During the 30-second loaded phase, peak power (PP), mean power, and minimum power (Pmin) were recorded in watts/kg using the Velotron software. The FI was calculated using the formula:  $FI = 100 \times (PP - Pmin) / PP$ . Participants concluded the test with a 5-minute cool-down at 10 W, selecting their own cadence.

### ***Saliva Collection***

Saliva samples were collected daily for 4 days during each supplementation phase, with Days 1 and 4 as rest days and Days 2 and 3 as exercise days. Participants were instructed to collect 2 ml of saliva within 5 min of waking (between 06:00 and 08:00 hr) using the passive drool method. Additional samples were taken at 15, 30, 45, and 60 minutes after the initial wake-up sample to ensure validity. All saliva samples were immediately frozen at  $-20^{\circ}\text{C}$  for later analysis of cortisol concentrations, according to established methods for assessing physiological stress responses and autonomic nervous system activity (Ducker et al., 2020; Honceriu et al., 2023).

### ***Cortisol Awakening Response Analysis***

Saliva samples were analyzed for total cortisol using enzyme-linked immunosorbent (ELISA) assay kits (Salimetrics, LLC). CAR was calculated from the area under the curve at the time points S1 (at waking), SC15 (15 min after waking), SC30 (30 min after waking), SC45 (45 min after waking), and SC60 (60 min after waking). All samples were analyzed using an enzyme conjugate inversely proportional to the optical density of cortisol present in the samples at 450 nm (Biotek™ HT

Multimode Microplate Reader) and compared to the manufacturer's standard curve, with a sensitivity  $< 0.007 \mu\text{g/dL}$  and an assay range of  $0.012 - 3.000 \mu\text{g/dL}$ .

### ***HRV***

HRV was measured between saliva collection points SC15 and SC30 on each of the four protocol days. The smartphone application HRV4Training was used to collect HRV data via photoplethysmography. It is noted that this HRV measure is not the gold standard, but it was easily accessible to participants in a home setting. The root mean square of successive differences between R-R intervals (RMSSD) was determined from each collection using the formula:  $RMSSD = \text{sqrt}(\text{mean}(\text{RR}_{i+1} - \text{RR}_i)^2)$ , where sqrt = square root, where  $\text{RR}_i$  represents the R-R interval between heartbeats.

### ***Statistical Analysis***

A  $2 \times 4$  repeated-measures ANOVA was used to compare the Profile of Mood States, Cortisol Awakening Response, and Heart Rate Variability. A  $2 \times 2$  repeated-measures ANOVA examined differences in the fatigue index. Pearson correlation analyses assessed the relationships among the nutritional supplement intervention, FI, POMS, HRV, and CAR. Finally, a multiple regression analysis was performed to determine if POMS, HRV, and CAR were significant predictors of FI.

### ***Study Design***

The study design, results, and conclusions are derived from the dissertation work of the primary author.

## **RESULTS**

### ***Participant Characteristics***

A total of 12 women qualified for and completed the study. However, one participant was removed due to insufficient saliva volume. Ta-

ble 1 shows the average characteristics of the participants. The average peak oxygen uptake ( $VO_{2peak}$ ) was  $32.32 \pm 4.64$  ml/kg/min, indicating poor aerobic fitness. All participants were taking oral contraceptives containing  $0.12 \pm 0.1$  mg of estradiol (E2).

**Table 1.** Mean Values of Measured Parameters ( $n = 11$ )

Parameter	Mean $\pm$ SD
Age (y)	20.45 $\pm$ 1.04
Height (cm)	163.45 $\pm$ 4.06
Weight (kg)	68.44 $\pm$ 11.61
BMI (kg/m <sup>2</sup> )	25.57 $\pm$ 3.91
$VO_{2peak}$ (L/min)	2.12 $\pm$ 0.25
$VO_{2peak}$ (ml/kg/min)	32.32 $\pm$ 4.64
Exercise Intensity % of $VO_{2peak}$ 30 min – Placebo Trial	68.20 $\pm$ 3.43
Exercise Intensity % of $VO_{2peak}$ 30 min – Whey Protein Trial	68.68 $\pm$ 3.65
Average Food Intake – Placebo Trial (kcal/day)	1785.72 $\pm$ 459.62
Average Food Intake – Whey Protein Trial (kcal/day)	1656.24 $\pm$ 437.47
HR <sub>max</sub> (bpm)	193 $\pm$ 4
RER <sub>max</sub>	1.26 $\pm$ 0.13
RPE <sub>max</sub>	18 $\pm$ 2

Note: data expressed as mean  $\pm$  standard deviation

### ***VO<sub>2</sub> Exercise Ranges and Responses***

The steady-state exercising  $VO_2$  was not affected by day or supplement. Additionally, the mean respiratory exchange ratio during exercise was not significantly different between the placebo and whey trials ( $p > .05$ ). The mean RER ranged from  $.932 \pm .06$  to  $.950 \pm .04$ , indicating that participants primarily oxidized carbohydrate during the 30-minute treadmill protocol, e.g., a strenuous exercise bout was elicited.

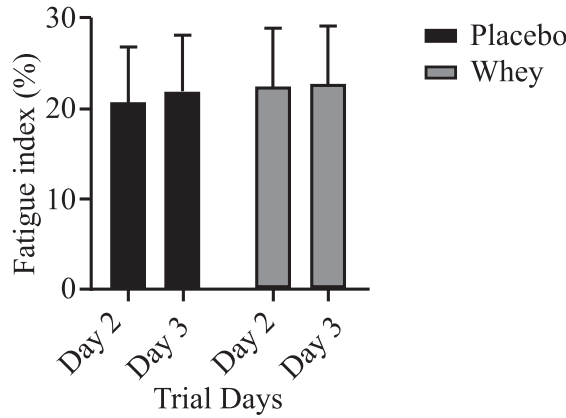
**Table 2.** Combined Mean  $VO_2$  During Treadmill Bouts ( $n = 11$ )

Condition / Trial	Mean $\pm$ SD
Day 2 Placebo (PL) (ml/kg/min)	21.78 $\pm$ 2.60
Day 3 Placebo (PL) (ml/kg/min)	21.99 $\pm$ 2.68
Day 2 Whey (WH) (ml/kg/min)	22.13 $\pm$ 2.89
Day 3 Whey (WH) (ml/kg/min)	21.95 $\pm$ 2.64
Combined Mean % $VO_{2peak}$ – PL	68.20 $\pm$ 3.43%
Combined Mean % $VO_{2peak}$ - WH	68.68 $\pm$ 3.65%

Note. Data expressed as mean + standard deviation; Combined Mean refers to the mean  $VO_2$  of 0, 5, 15, and 25 min data during the 30 min steady-state aerobic exercise trial.

### ***FI Responses***

The FI showed no statistically significant interaction between day and intervention ( $F(1,10) = 2.283$ ,  $p = .162$ ). There were also no significant main effects for day ( $F(1,10) = 4.356$ ,  $p = .063$ ) or intervention ( $F(1,10) = 2.451$ ,  $p = .149$ ). The mean FI for each exercise day and intervention is displayed in Figure 2 below. These findings suggest that neither the specific day of measurement within the experimental protocol nor the type of nutritional intervention (whey protein versus placebo) significantly influenced the participants' FI. Figure 2.



	Placebo			Whey		
	Mean	SD	N	Mean	SD	N
Day 1	20.79	6.12	11	22.52	6.45	11
Day 2	21.97	6.16	11	22.78	6.38	11

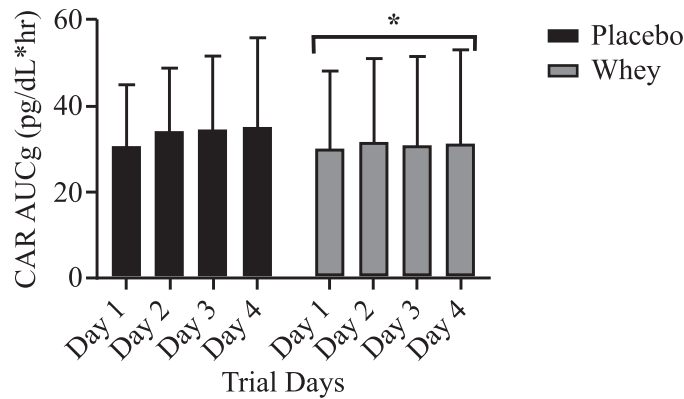
**Figure 2.** Fatigue Index

Note: Error bars represent  $\pm$  SD,  $n = 11$ .

**Cortisol Awakening Response**

Daily CAR values are presented in Figure 3. The whey protein intervention resulted in a significantly lower cortisol awakening response compared to the Placebo ( $F = 6.114$ ,  $p = .033$ ,  $\omega^2 = 0.379$ ), with CAR being higher for the Placebo trial; however, CAR did not

differ between days for either supplement or condition ( $F = 1.724$ ,  $p = .183$ ,  $\omega^2 = 0.147$ ). The reduction in CAR in the whey protein trial suggests a potential effect on physiological stress markers, possibly due to the amino acid profile supporting recovery or mitigating catabolic processes (Boutry-Regard et al., 2020).



	Placebo			Whey		
	Mean	SD	N	Mean	SD	N
Day 1	30.56	14.17	11	29.70	18.18	11
Day 2	33.59	14.77	11	31.33	19.18	11
Day 3	34.31	17.00	11	31.09	20.04	11
Day 4	34.97	20.60	11	31.40	21.42	11

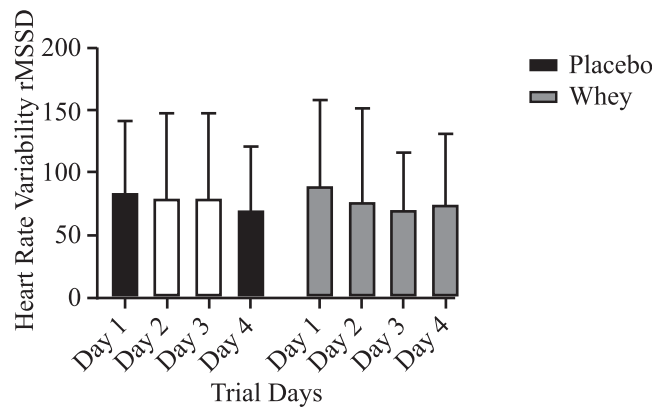
**Figure 3.** Cortisol Awakening Response Area Under the Curve (AUC)

Note. \*Placebo CAR compared to Whey CAR ( $p = .033$ ). Error bars represent  $\pm$  SD,  $n = 11$ ; Mean and SD = pg/dL \* hr.

**Profile of Mood States and Heart Rate Variability**

There were no significant differences in condition, day, or the combined effect of day and condition ( $p > .05$ ). Mean HRV, measured by rMSSD, is seen in Figure 4. Although HRV generally decreased over the four testing days for both supplements, this change was not statistically significant. This finding suggests that while physiological stress markers like

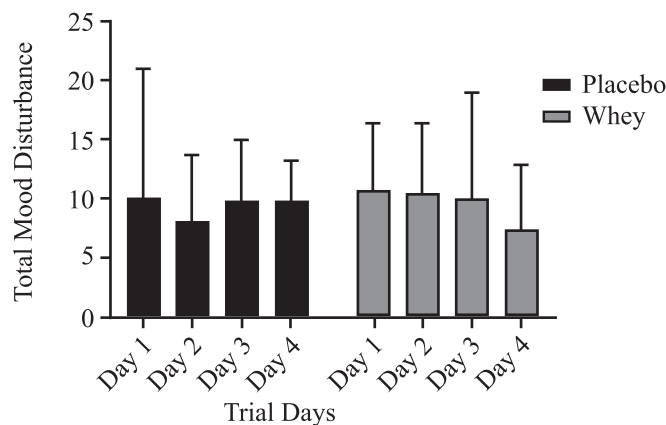
CAR was significantly affected, the physiological stress response reflected by HRV, and the psychological state assessed by POMS, did not exhibit statistically significant changes, as seen in Figure 5, potentially indicating a nuance in the interpretation, and therefore, specific effect of the whey protein on acute stress responses rather than overall recovery (Presby et al., 2023).



	Placebo			Whey		
	Mean	SD	N	Mean	SD	N
Day 1	84.15	57.02	11	89.17	69.40	11
Day 2	79.56	68.40	11	77.15	74.19	11
Day 3	79.43	68.08	11	70.38	45.88	11
Day 4	70.97	50.04	11	74.15	56.58	11

**Figure 4.** Heart Rate Variability RMSSD Difference

Note. Error bars represent  $\pm$  SD,  $n = 11$ .



	Placebo			Whey		
	Mean	SD	N	Mean	SD	N
Day 1	10.18	10.87	11	10.73	5.76	11
Day 2	8.18	5.55	11	10.55	5.94	11
Day 3	9.91	5.15	11	10.09	8.97	11
Day 4	7.91	5.32	11	7.55	5.37	11

**Figure 5.** Profile of Mood States – Total Mood Disturbance

Note. Error bars represent  $\pm$  SD,  $n = 11$ .

### ***Multiple Regression and Correlation Results***

A multiple regression analysis, in a step-wise format, aimed at determining whether CAR, POMS TMD, and HRV rMSSD could predict FI on each exercise day and during each nutritional intervention. None of the independent variables statistically predicted FI in either intervention or on any specific exercise day ( $p > .05$ ). The absence of predictive power suggests that while these physiological and psychological markers are indicative of stress and recovery, their direct relationship to the specific measure of FI used in this study may be indirect or moderated by other factors not yet understood.

Pearson correlation analyses also examined the relationship between CAR, POMS TMD, HRV rMSSD, and FI. No significant associations were found ( $p > .05$ ), except for a significant negative correlation between the Placebo exercise Day 2 FI and POMS TMD on Day 2 ( $r = -.582$ ,  $p = .030$ ), indicating that higher POMS scores were associated with a lower FI. A similar, though not statistically significant, weak negative association was observed between POMS TMD and FI during the Whey exercise day 2 ( $r = -.447$ ,  $p > .05$ ). This unexpected inverse relationship between POMS TMD and FI warrants further investigation, as it contradicts the typical understanding that increased mood disturbance correlates with heightened fatigue (Caballero-García & Córdova, 2022).

### **DISCUSSION**

This study aimed to determine whether whey protein improved recovery from exercise, specifically by examining the relationships among CAR, POMS, HRV, and FI. Researchers also sought to determine whether CAR, POMS, and HRV could predict FI. Key findings showed that whey protein reduced

CAR compared with placebo; however, POMS and HRV did not differ significantly between groups. The FI also remained undifferentiated. Furthermore, CAR, POMS, and HRV did not reliably predict FI. A weak, non-significant negative correlation was observed between POMS and FI on one of the exercise days, suggesting that mood state might have a complex relationship with fatigue.

### ***CAR***

This study found that participants in the whey protein group had a lower cortisol awakening response than those in the placebo group across the four experimental days. While researchers initially assumed a lower CAR indicated reduced stress, this was not the case here. Previous research by Anderson, Lane, and Hackney (2018) indicated a positive link between CAR and training load in athletes. Ulhôa et al. (2011) reported that shift workers could manage daily stressors with higher CAR, while those with irregular schedules used CAR to cope with the previous day's stressors. Drogos et al. (2019) observed increases in CAR over a six-month exercise program, attributing these changes to the participants' improved ability to handle physical and psychological stress, as supported by a correlation with reduced perceived stress ( $r = .523$ ,  $p = .023$ ). Therefore, while cortisol secretion may have been lower during the whey protein trial week, this study cannot establish a direct cause-and-effect relationship between whey protein and a suppressed CAR.

### ***Dietary Intake***

No significant differences in daily caloric intake were found between the Placebo and Whey Protein groups. The Placebo group consumed an average of 1786 kcal daily, while the Whey Protein group consumed 1656 kcal. Although research on dietary effects on the

CAR is limited, whey protein isolate may have blunted the CAR response compared to placebo. Sheikh et al. (2010) hypothesize that increased intake of whey protein isolate can elevate glucagon-like peptide-1 (GLP-1) secretion, which, in turn, may increase cortisol secretion. This potential increase in cortisol could create a negative feedback loop, blunting the CAR on subsequent days. Further investigation into GLP-1's role in blunting CAR is warranted, although it was not measured in this study. Additionally, the precise mechanisms through which protein supplementation influences the HPA axis warrant further exploration, particularly concerning the interaction between dietary amino acids and neuroendocrine regulation.

### **POMS**

Numerous studies link changes in the Profile of Mood States to cortisol levels, exercise intensity, and diet. These studies suggest exercise benefits mood, reducing total mood disturbance and anxiety while increasing vigor; however, this study found no significant differences in POMS TMD between the whey protein and placebo groups, nor between experimental days ( $p = .763$ ), suggesting neither the exercise nor the diet significantly altered POMS TMD. Future research could analyze POMS subscales to examine their relationships with performance indicators such as the FI. Furthermore, investigating the impact of combined exercise and nutritional interventions on individual mood states rather than just total mood disturbance could provide a more nuanced understanding of psychological recovery (Deru et al., 2023; Weinstein et al., 2007). A significant limitation of this study was the relatively low caloric intake across both groups, averaging around 1700 kcal/day, which is considerably below the typical energy requirements for active individuals (James et al., 2020).

### **HRV**

HRV is a well-established method for assessing the body's response to physical activity, particularly the parasympathetic nervous system. Factors like age, gender, fatigue, fitness level, and psychological state can influence HRV. In this study, no significant differences in HRV were observed between the placebo and whey protein trials, or across different days within each trial. Although there was a slight decrease in HRV (measured by RMSSD) in both groups, this change was not statistically significant. It is possible that the participants were moderately fit, allowing them to complete the exercise without substantial parasympathetic nervous system adjustments the following day. While the carbohydrate-dominant nature of the exercise bout suggested a strenuous acute energy demand, the HRV results suggest that the exercise stimulus might not have been sufficiently strenuous to elicit a marked HRV response indicative of physiological stress or recovery, or that the recovery period was adequate in both the control and intervention trial periods.

The higher CAR response in the Placebo group may be linked to changes in HRV, as the cardiovascular system and awakening response might similarly shift from parasympathetic to sympathetic dominance (Stalder et al., 2011). Reductions in HRV have been associated with increased negative mood and depression, potentially corresponding to parasympathetic changes. Other HRV parameters did not significantly associate with training load perception or fatigue prediction, and there were additional concerns about the measurement sensitivity of HRV and the variability in this small sample. Future studies should consider larger sample sizes and more rigorous control over extraneous variables to enhance the reliability and generalizability of HRV findings, potentially integrating other physio-

logical markers to provide a more holistic understanding of athletic recovery (Bellenger et al., 2021). In future studies, it will be critical to consider a more general physiological context when interpreting HRV data, as factors such as sleep quality, hydration status, and micronutrient availability can significantly modulate autonomic nervous system activity (Fusi et al., 2025; Gratwicke et al., 2021).

### ***Fatigue Index (FI)***

The study initially hypothesized that POMS, HRV, and CAR would correlate with and predict the FI, given their known associations with fatigue; however, the findings did not support these hypotheses. No significant differences in mean FI were found between the Placebo and Whey Protein groups on Day 2 or Day 3. Additionally, no significant correlations were observed between FI and POMS, HRV, or CAR, although the correlation between Day 3 POMS and Day 3 FI was the closest among these relationships. The FI ranges in this study were consistent with those reported in previous research. One reason for including whey protein as an intervention was to explore its potential to reduce central fatigue. Baker et al. (2006) suggested that 5-hydroxytryptamine (5-HT) activity in the brain is not altered by the transport of free tryptophan. Researchers also reported that blood levels of 5-HT returned to baseline after intense exercise bouts, despite a sharp drop immediately post-exercise, leading them to conclude that 5-HT might not be a significant factor in central fatigue. It is possible that the WAnT load used in this study was sufficient to cause a difference in FI, as hypothesized. However, the duration of the trials was too short to establish a cause-and-effect relationship with central fatigue, e.g., leaving the potential to add multiple bouts to elicit a stronger coordinated physiological and neurophysiological response.

### **CONCLUSION**

This is the first known study to investigate the potential associations between whey protein isolate and the CAR, POMS, HRV, and FI. Furthermore, few studies have included women in research comparing the interactions of CAR, HRV, POMS, and FI. The observed decrease in CAR across the four days of the whey protein isolate trial, compared with placebo, suggests a possible association among central fatigue, the hypothalamic-pituitary-adrenal axis, and strenuous exercise. Given the lack of effect on short-duration sprint cycling performance, any association with reducing the physiological effects of central fatigue may be minimal. Future studies should investigate the significance of interactions between POMS subscales (Maroulakis & Zervas, 1993) and HRV across longitudinal exercise trials, as well as the metabolic effects of whey protein on these variables. Additionally, future research should explore the effects of sleep quality on these markers, considering that sleep deprivation significantly impairs high-intensity endurance performance and alters brain activity patterns critical for motor control (Fusi et al., 2025; Sapolsky et al., 2000; Zhao et al., 2025). Lastly, examining the interrelationships between dietary interventions, such as specific macronutrient profiles, and sleep architecture could provide insight into the combined impact on physiological recovery and cognitive function (Mantantzis et al., 2022).

### **Funding**

This research received no specific grant from any funding agency in the public, commercial, or not-for-profit sectors.

### **Availability of data and materials**

The datasets generated and/or analyzed during the current study are willingly available from the corresponding author on reasonable request.

### Declaration of Conflicting Interests

The Author(s) declare(s) that there is no conflict of interest, having no commercial or financial involvements whatsoever.

### Ethical approval

Institutional Review Board (IRB) approval was obtained by both IRB-FY2019-197 and IRB 1793. Informed consent was obtained by giving written consent on IRB-approved signature forms from both institutions.

### Acknowledgements

This publication and study were derived from the dissertation work of the primary author.

### REFERENCES

- Anderson, T., Lane, A. R., & Hackney, A. C. (2018). The cortisol awakening response: Association with training load in endurance runners. *International Journal of Sports Physiology and Performance, 13*(9), 1158–1163. <https://doi.org/10.1123/ijsp.2017-0740>
- Baker, J. S., Morgan, R., Hullin, D., Castell, L. M., Bailey, D. M., & Davies, B. (2006). Changes in blood markers of serotonergic activity following high intensity cycle ergometer exercise. *Research in Sports Medicine, 14*(3), 191–203. <https://doi.org/10.1080/15438620600854744>
- Bellenger, C. R., Miller, D. J., Halson, S. L., Roach, G. D., & Sargent, C. (2021). Wrist-Based Photoplethysmography Assessment of Heart Rate and Heart Rate Variability: Validation of WHOOP. *Sensors, 21*(10), 3571. <https://doi.org/10.3390/s21103571>
- Barrero, A., Le Cunuder, A., Carrault, G., Carré, F., Schnell, F., & Le Douairon Lahaye, S. (2020). Modeling stress-recovery status through heart rate changes along a Cycling Grand Tour. *Frontiers in Neuroscience, 14*. <https://doi.org/10.3389/fnins.2020.576308>
- Benito, P. J., Alfaro-Magallanes, V. M., Rael, B., Castro, E. A., Romero-Parra, N., Rojo-Tirado, M. A., & Peinado, A. B. (2023). Effect of Menstrual Cycle Phase on the Recovery Process of High-Intensity Interval Exercise—A Cross-Sectional Observational Study. *International Journal of Environmental Research and Public Health, 20*(4), 3266. <https://doi.org/10.3390/ijerph20043266>
- Boutry-Regard, C., Vinyes-Parès, G., Breuillé, D., & Moritani, T. (2020). Supplementation with Whey Protein, Omega-3 Fatty Acids and Polyphenols Combined with Electrical Muscle Stimulation Increases Muscle Strength in Elderly Adults with Limited Mobility: A Randomized Controlled Trial. *Nutrients, 12*(6), 1866. <https://doi.org/10.3390/nu12061866>
- Bruce R.A., Blackmon J.R., Jones J.W., & Strait G. (1963) “Exercise testing in adult normal subjects and cardiac patients,” *Pediatrics, 32*(Suppl), 741–747. <https://doi.org/10.1542/peds.32.4.742>
- Caballero-García, A., & Córdova, A. (2022). Muscle Recovery and Nutrition. *Nutrients, 14*(12), 2416. <https://doi.org/10.3390/nu14122416>
- Deru, L., Chamberlain, C. J., Lance, G. R., Gipson, E. Z., Bikman, B. T., Davidson, L. E., Tucker, L. A., Coleman, J. L., & Bailey, B. W. (2023). The Effects of Exercise on Appetite-Regulating Hormone Concentrations over a 36-h Fast in Healthy Young Adults: A Randomized Crossover Study. *Nutrients, 15*(8), 1911. <https://doi.org/10.3390/nu15081911>
- Drogos, L. L., Wynne-Edwards, K., Zhou, R., Hall, S. E., Tyndall, A. V., Longman, R. S., . . . Poulin, M. J. (2019). Aerobic exercise increases cortisol awakening response in older adults. *Psychoneuroendocrinology, 103*, 241–248. <https://doi.org/10.1016/j.psyneuen.2019.01.012>
- Ducker, K. J., Lines, R. L. J., Chapman, M. T., Peeling, P., McKay, A. K. A., & Gucciardi, D. F. (2020). Validity and reliability evidence

of a point of care assessment of salivary cortisol and  $\alpha$ -amylase: a pre-registered study. *PeerJ*, 8. <https://doi.org/10.7717/peerj.8366>

Fang, M., & Zhang, P. (2024). Regulation of exercise on heart rate variability in perimenopausal and postmenopausal women. [Review of Regulation of exercise on heart rate variability in perimenopausal and postmenopausal women.]. PubMed, 49(4), 516. National Institutes of Health.

<https://doi.org/10.11817/j.issn.1672-7347.2024.230399>

Fusi, J., Scarfò, G., Silvestro, R., & Franzoni, F. (2025). Improving Sleep Quality to Enhance Athletic Activity—The Role of Nutrition and Supplementation: A Mini-Short Review [Review of Improving Sleep Quality to Enhance Athletic Activity—The Role of Nutrition and Supplementation: A Mini-Short Review]. *Nutrients*, 17(11), 1779.

<https://doi.org/10.3390/nu17111779>

Gratwicke, M., Miles, K. H., Pyne, D. B., Pumpa, K. L., & Clark, B. (2021). Nutritional Interventions to Improve Sleep in Team-Sport Athletes: A Narrative Review [Review of Nutritional Interventions to Improve Sleep in Team-Sport Athletes: A Narrative Review]. *Nutrients*, 13(5), 1586.

<https://doi.org/10.3390/nu13051586>

Heijnen, S., Hommel, B., Kibele, A., & Colzato, L. S. (2016). Neuromodulation of aerobic exercise—a review. *Frontiers in Psychology*, 6. <https://doi.org/10.3389/fpsyg.2015.01890>

Honceriu, C., Roca, M., Costache, A. D., Abalășei, B. A., Popescu, L., Puni, A. R., Maștaleru, A., Oancea, A., Drugescu, A., Adam, C. A., Mitu, O., Costache, I., Leon, M. M., Roca, I. C., Mocanu, V., & Mitu, F. (2023). Blood and Salivary Cortisol Variations in Athletes in Relation to Cardiopulmonary Exercise Testing. *Medicina*, 59(10), 1726.

<https://doi.org/10.3390/medicina59101726>

James, R., James, L. J., & Clayton, D. J. (2020). Anticipation of 24 h severe energy re-

striction increases energy intake and reduces physical activity energy expenditure in the prior 24 h, in healthy males. *Appetite*, 152, 104719. <https://doi.org/10.1016/j.appet.2020.104719>

Mantantzis, K., Campos, V., Darimont, C., & Martin, F. (2022). Effects of Dietary Carbohydrate Profile on Nocturnal Metabolism, Sleep, and Wellbeing: A Review [Review of Effects of Dietary Carbohydrate Profile on Nocturnal Metabolism, Sleep, and Wellbeing: A Review]. *Frontiers in Public Health*, 10. <https://doi.org/10.3389/fpubh.2022.931781>

Maroulakis, E., & Zervas, Y. (1993). Effects of Aerobic Exercise on Mood of Adult Women. *Perceptual and Motor Skills*, 76(3), 795-801. <https://doi.org/10.2466/pms.1993.76.3.795> (Original work published 1993)

McNair, D. M., Droppleman, L. F., & Lorr, M. (1971). *Profile of Mood States, POMS*. San Diego, CA: Educational and Industrial Testing Service.

McNair, D. M., Lorr, M., & Droppleman, L. F. (1992). *EdITS manual for the Profile of mood states*. San Diego, CA: Educational and Industrial Testing Service.

Morgan, P. T., & Breen, L. (2021). The role of protein hydrolysates for exercise-induced skeletal muscle recovery and adaptation: a current perspective [Review of The role of protein hydrolysates for exercise-induced skeletal muscle recovery and adaptation: a current perspective]. *Nutrition & Metabolism*, 18(1). BioMed Central.

<https://doi.org/10.1186/s12986-021-00574-z>

Presby, D. M., Jasinski, S. R., & Capodilupo, E. R. (2023). Wearable derived cardiovascular responses to stressors in free-living conditions. *PLoS ONE*, 18(6).

<https://doi.org/10.1371/journal.pone.0285332>

Rocha-Rodrigues, S., Sousa, M., Reis, P. L., Leão, C., Cardoso-Marinho, B., Massada, M., & Afonso, J. (2021). Bidirectional Interactions between the Menstrual Cycle, Exercise

- Training, and Macronutrient Intake in Women: A Review [Review of Bidirectional Interactions between the Menstrual Cycle, Exercise Training, and Macronutrient Intake in Women: A Review]. *Nutrients*, *13*(2), 438. <https://doi.org/10.3390/nu13020438>
- Sapolsky, R. M., Romero, L. M., & Munck, A. U. (2000). How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. *Endocrine Reviews*, *21*(1), 55–89. <https://doi.org/10.1210/er.21.1.55>
- Sheikh, H. I., Dougherty, L. R., Hayden, E. P., Klein, D. N., & Singh, S. M. (2010). Glucagon-like peptide-1 receptor gene polymorphism (Leu260Phe) is associated with morning cortisol in preschoolers. *Progress in Neuro-Psychopharmacology and Biological Psychiatry*, *34*(6), 980–983. <https://doi.org/10.1016/j.pnpbp.2010.05.007>
- Stalder, T., Evans, P., Hucklebridge, F., & Clow, A. (2011). Associations between the cortisol awakening response and heart rate variability. *Psychoneuroendocrinology*, *36*(4), 454–462. <https://doi.org/10.1016/j.psyneuen.2010.07.020>
- Stalder, T., Oster, H., Abelson, J. L., Huthsteiner, K., Klucken, T., & Clow, A. (2024). The cortisol awakening response: Regulation and functional significance. *Endocrine Reviews*, *46*(1), 43–59. <https://doi.org/10.1210/endrev/bnae024>
- Swart, A., & Constantinou, D. (2023). The effects of a 3-day mountain bike cycling race on the autonomic nervous system (ANS) and heart rate variability in amateur cyclists: a prospective quantitative research design. *BMC Sports Science Medicine and Rehabilitation*, *15*(1). <https://doi.org/10.1186/s13102-022-00614-y>
- Ulhôa, M. A., Marqueze, E. C., Kantermann, T., Skene, D., & Moreno, C. (2011). When does stress end? Evidence of a prolonged stress reaction in shiftworking truck drivers. *Chronobiology International*, *28*(9), 810–818. <https://doi.org/10.3109/07420528.2011.613136>
- Urhausen, A. & Kindermann, W. (2002). Diagnosis of overtraining: What tools do we have? *Sports Medicine*, *32*(2), 95–102. <https://doi.org/10.2165/00007256-200232020-00002>
- Weinstein, A. A., Deuster, P. A., & Kop, W. J. (2007). Heart rate variability as a predictor of negative mood symptoms induced by exercise withdrawal. *Medicine and Science in Sports and Exercise*, *39*(4), 735. <https://doi.org/10.1249/mss.0b013e31802f590c>
- Zhao, S., Alhumaid, M. M., Li, H., Wei, X., Chen, S. S.-C., Jiang, H., Gong, Y., Yun, G., & Qin, H. (2025). Exploring the Effects of Sleep Deprivation on Physical Performance: An EEG Study in the Context of High-Intensity Endurance. *Sports Medicine - Open*, *11*(1). <https://doi.org/10.1186/s40798-024-00807-4>

**Corresponding author:**

**Michael Oldham**

East Texas A&M University,  
Commerce, United States of America  
E-mail: michael.oldham@etamu.edu