THE EFFECT OF WHEY PROTEIN ISOLATE SUPPLEMENTATION ON CORTISOL AWAKENING RESPONSE, PROFILE OF MOOD STATES, AND HEART RATE VARIABILITY IN RECREATIONALLY ACTIVE WOMEN

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ABSTRACT

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Decreases in heart rate variability (HRV), disruptions in cortisol awakening response (CAR), and changes in Profile of Mood States (POMS), have been associated with fatigue due to repetitive strenuous physical exercise. Whey protein isolate components can potentially affect stress responses. PURPOSE: To determine the effects of whey protein isolate supplementation on CAR, POMS, and HRV after strenuous exercise. METHODS: Eleven recreationally active women (19 ± 2 yrs) completed a double blinded, cross-over placebo and supplement regimen (25 g of maltodextrin (PL) or 25 g of maltodextrin plus 25 g of whey protein isolate (WH)) on Day 1, 2, 3, twice a day. Exercise on Day 2 and 3 was 30 min on a treadmill at 70-75% VO_{2peak} (21.7 ± 0.1 ml/kg/min), 5 min rest, then a 30 s Wingate anaerobic test (WAnT). Saliva (2ml) was collected on Days 1 – 4, immediately upon waking and every 15min for the next hour. HRV and POMS were recorded after waking as well. Repeated measures ANOVA were used to determine differences ($p < .05$) in cortisol AUCg, POMS, HRV, and WAnT fatigue index (FI). Pearson correlation and multiple regression models were implemented to determine associations between CAR, POMS, and HRV to FI. RESULTS: Cortisol AUC were significantly different ($p = 0.033$) between PL (33.4 ± 2.0 µg*hr/dL) and WH

(30.9 ± 0.8 µg*hr/dL). There were no significant differences in POMS, HRV, or FI ($p > .05$). Significant Pearson correlation existed on Day 3 during the PL trial, between POMS and FI ($r = -.582$, $p = 0.030$). Neither CAR, POMS, nor HRV was able to predict FI through multiple regression equations (all $p > .05$). CONCLUSIONS: Whey protein isolate may decrease CAR on post-exercise days, but may have no effect on POMS, HRV, or FI. Additionally, with no effect on the short-duration sprint cycling performance, the physiological effect of the reduction in central fatigue may be minimal.

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CHAPTER I

INTRODUCTION

Readiness

Webster's Dictionary defines ready as "prepared mentally or physically for some experience or action" (Merriam-Webster, 2019, n.p.). How then can an athlete be ready for training or competition? The readiness of an athlete, for the next day of training, is a multi-factor puzzle with a foundation that rose out of studies on overtraining syndrome (OTS). Urhausen and Kindermann (2002) describe overtraining as a sport specific decrease in performance coupled with disturbances in mood state, immune system function, and endocrine system regulation.

While OTS is an undesirable condition, it is often preceded by, and sometimes noticeably so, stress-induced detriments in physiological and performance measures referred to as functional overreaching (FOR) and non-functional overreaching (NFOR) (Urhausen & Kindermann, 2002). Many questions remain regarding transitions between FOR, NFOR, and OTS, and thresholds for each. Explanations regarding biochemical mechanisms and pathways, and single biomarkers to monitor these transitions have not been fully explored in the current scientific literature.

Cortisol Awakening Response

The hypothalamus-pituitary-adrenal (HPA) axis, an endocrine system that plays a major role during stress responses, is commonly associated with the secretion of the hormone cortisol (Sapolsky, Romero, & Munck, 2000). Cortisol targets most tissues in the body, including the liver, where it increases gluconeogenesis, thus acting in

conjunction with glucagon, to increase glucose concentration. This is especially noted at night when a person is essentially fasting. Cortisol concentration rises overnight to keep blood glucose at the homeostatic baseline (Rodwell, Bender, Botham, Kennelly, & Weil, 2018); however upon awakening, a 50 - 156% increase over normal circadian concentration may occur (Stadler et al., 2016). This large increase in cortisol concentration, which is superimposed over the circadian concentration increase and peaks between 30 min and 45 min post awakening, is termed as the cortisol awakening response (CAR). CAR can be highly variable, where no clear picture of dysfunction can be associated with an increase or decrease in CAR (Anderson and Wideman, 2017).

Long-term increases in training load typically result in a decrease in CAR, whereas short-term increases in training load tended to increase CAR. If the training load was applied appropriately, then the CAR response tended to decrease, whereas if the training load increased too sharply, i.e., too short of recovery time and or too great an increase in load in short time period, then the CAR response tended to increase (Anderson & Wideman, 2007).

Potential connections between the receptors in the hippocampus where increases in serotonin happen, e.g., from the physical stress of exercise, reduce cellular activity in the hippocampus, which in turn increases HPA activity (Basso and Suzuki, 2017). If this pattern is chronic, then HPA axis dysregulation may occur. Additionally, neurological connections between the hippocampus, hypothalamus, and the amygdala, as measured by Profile of Mood States (POMS), being associated with changes in CAR can be identified

(Basso and Suzuki, 2017). While these associations may appear tangential, studies that measure CAR and POMS in response to acute exercise have not been widely conducted.

Profile of Mood States

Exercise has been well documented to cause changes in neurochemistry, both acutely and longitudinally, over a variety of intensities and modalities, and across ages in both males and females, but scientific literature is still lacking in areas of acute exercise changes associated with affective state and cognitive functioning (Basso and Suzuki, 2017). Purvis, Gonsalves, and Deuster (2010) agree that using diagnostic tools, such as POMS, can help to provide a clearer picture of dose-related mood disturbances, especially when combined with performance data, to identify athlete responses to changes in training volumes and intensities.

POMS has been widely used over longitudinal studies to identify changes in mood state over a week, a longer training cycle, or over a competitive season (Broodryk, Pienaar, Edwards, & Sparks, 2017; Kenttä, Hassmén, & Raglin, 2006; Klaperski, Dawans, Heinrichs, & Fuchs, 2014). Filaire, Bernain, Sagnol, and Lac (2001) used POMS to determine the relationship between mood state, salivary testosterone to salivary cortisol ratio, and team performance. After an intense 7-week training cycle, game winning percentage declined over 16 wks from 71.4% to 47.3%, while vigor subscale score significantly decreased from 62.0 at baseline to 46.2 ($p < 0.01$). Additionally, salivary testosterone to salivary cortisol ratio significantly decrease ($p < 0.05$) from 25.7 to 19.9 in the same time period, indicating a potential catabolic state (Filaire et al., 2001).

Heart Rate Variability

Parasympathetic modulation of heart rate is typically expressed, for heart rate variability (HRV) analysis, as a variety of variables, including R-R intervals using Fast Fourier transformation and root mean square of successive R-R interval differences (RMSSD; Currie, Thomas, & Goodman, 2009). Positive adaptations in HRV to training and physical exercise exist in both R-R intervals and HF domains, but most markedly exhibited by decreased vagal tone and a resting bradycardia in sedentary populations (Sandercock, Bromley, & Brodie, 2005). Salivary α - amylase is positively correlated ($r = -0.212$, $p < 0.05$) correlated as increased stress markers to decreased HRV (Edmonds, Burkett, Leicht, & McKean, 2015). The psychophysiological state of 'well-being,' as measured by POMS, and beat to beat parasympathetic modulation of HRV have been associated with to OTS, NFOR, and FOR; however associations between HRV and CAR are still lacking in the scientific literature (Stadler, Evans, Hucklebridge, and Clow, 2011; Purvis et al., 2010). However, awakening-induced activation of both cardiovascular (HRV) and endocrine systems (CAR) may have a temporal relationship in shared positive functionality of arousal (Stadler et al., 2011).

Whey Protein Isolate Supplementation

Whey protein, especially whey protein isolate, supports muscle protein synthesis, protein balance, and recovery from strenuous exercise (West, Sawan, Mazzulla, Williamson, and Moore, 2017). High protein compared to high carbohydrate meals have no difference in tryptophan/branched chain amino acid (BCAA) ratio increases, i.e., no difference in fatigue, and cortisol secretions throughout the day, but do increase well-

being subcategories in POMS (Sihvola et al., 2013). Huang et al. (2107) administered 33.5 g/day of whey protein isolate or a maltodextrin placebo for 3 wks, to elite male marathon runners, prior to a marathon challenge. After 1 week post-marathon, participants in the whey protein group were able to cover significantly more distance compared to the placebo group (3776 ± 58 m vs 3566 ± 112 m, $p < 0.001$).

Protein administered post-exercise can also affect recovery. Male cyclists (n = 12) were given a carbohydrate/fat (CON) or an isocaloric protein/leucine/carbohydrate/fat (PRO-LEU) intervention, for 6 days of high intensity cycle training. After 6 days, blood cortisol levels in the PRO-LEU group was 21% lower than the CON group prior to exercise ($p < 0.05$), indicating a decline in stress response to upcoming exercise (Nelson et al., 2013). Minimal connections in the scientific literature between HRV and whey protein have been established. Charlot, Pichon, Richalet, & Chapelot (2013) gave either high carbohydrate or high protein meals 60 min prior to being exposed to hypoxia for 15 min, followed by 30 min of exercise in hypoxia at 60% VO_{2max}. A 3.1 ± 0.4% increase in saturation in the high carbohydrate group compared to the high protein group, but no difference in HRV between the groups for heart rate or low-frequency R-R intervals to high-frequency R-R intervals.

Problem Statement and Specific Aims

The ability of a person to recover from stress related to physical exercise, and perform physical exercise on consecutive days, has been measured by CAR, HRV, POMS, and Fatigue Index (FI) as individual markers, but never all together. The current study measured CAR, HRV, POMS, and FI in recreationally active women, age 18 to 35

yrs. *Recreationally active*, as defined by the American College of Sports Medicine (Riebe, Ehrman, Liguori, & Magal, 2018) is participating in aerobic exercise less than twice a week or more and or less than 80 min per week, and a minimum of 30 min per week. Additionally, the study determined the effect of placebo and whey protein isolate supplementation on CAR, HRV, POMS, and FI. Participants completed 30 min of walking on the treadmill at 70-75% VO_{2peak}, followed by 5 min of rest, then a maximal exertion Wingate cycle ergometer trial, on 2 consecutive days. CAR, HRV, and POMS were sampled or surveyed the morning following each exercise day. The primary purpose of this study was to determine the effect of whey protein supplementation on CAR, HRV, POMS, and FI. In addition, since whey protein isolate supplementation has been shown to have positive effects on performance on consecutive days of physical exercise, a secondary purpose of the study examined the ability of CAR, POMS, and HRV to predict FI in either nutritional intervention.

Hypotheses

1) Whey protein isolate will lower CAR, increase HRV, and decrease POMS total mood disturbance, significantly more than placebo trial.
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 will be significantly correlated to fatigue index on the second day of exercise.
4) CAR, POMS, and HRV will be able to significantly predict fatigue index.

Terms

Cortisol

Cortisol Awakening Response (CAR)

Tryptophan

5-hydroxytryptamine (5-HT)

17-beta – estradiol (E2)

Profile of Mood States (POMS)

Heart Rate Variability (HRV)

Root Mean Square of Successive R-R Interval Differences (RMSSD)

R-R Intervals

Normal Sinus R-R Intervals for 5-min Segments (SDNN)

Average Normal Sinus R-R Intervals for 5-min Segments (SDANN)

Cycle ergometer

VO_{2max}

AUC_g

Hypothalamus-Pituitary-Adrenal (HPA) Axis

Sympathetic Nervous System (SNS)

Diurnal

Overtraining

Overtraining syndrome (OTS)

Non-Functional Overreaching (NFOR)

Functional Overreaching (FOR)

Branched Chain Amino Acid

Whey Protein Isolate

Training

Training Load

Assumptions

The assumptions of this study were:

1. All participants will refrain from caffeine or alcohol ingestion 48 hr prior to and during testing, refrain from sexual activity 48 hr prior to and during testing days, and refrain from training or exercise 48 hr prior to and during testing days.
2. All participants have no gastrointestinal problems with whey protein or maltodextrin
3. All participants have access to a smartphone with Wi-Fi capabilities,
4. All participants exercise more than 30 min a week but less than 80 min a week, e.g. less than or equal to 2 times per week in aerobic exercise, and will keep the same pattern of food intake and exercise during all testing phases.
5. All participants have a VO_{2max} less than 45 ml/kg/min.
6. All participants are pre-menopausal women.

Limitations

1. Results can only be applied to aerobic protocols on two consecutive days at 70-75% VO_{2max}, followed by a single Wingate. Alternating recovery days or greater than 2 consecutive exercise days may alter the results of the stress responses.

2. Dosing of supplements is an equal dose regardless of body weight. Doses relative to body weight may alter HRV, CAR, and POMS results.

Significance of the Study

Few studies have investigated the CAR, POMS, HRV, and FI in the context of a comprehensive comparison. Additionally, the effect of whey protein supplementation on CAR, POMS, HRV, and FI has not been fully explained in the scientific literature. Furthermore, even fewer studies have included women as a research subject comparing the interactions of cortisol stress responses, HRV, POMS, and FI in women. This study will address the stress responses of CAR, HRV, POMS, and FI with whey protein supplementation in recreationally active females. This study aims to provide insight to methods of reducing stress responses and allowing for a more recovered, more "ready to perform" athlete on days after exercise.

CHAPTER II

REVIEW OF LITERATURE

Readiness

Exercise can be described as a multivariate disturbance in the homeostatic state, either acutely or chronically. This change in homeostasis can be influenced by physiological factors such as energy status of the cells, dietary factors, age, gender, biological rhythms, and cardiovascular properties, as well as psychological factors contributing to neurophysiological interactions (Kreider, Fry, & O'Toole, 1998). Since exercise should be considered a daily lifestyle event, the ability to recover and exercise the next day surfaces as a concern for the average person and athlete alike. Measures of readiness and/or recovery have included sport-specific performance, Borg-Scale, heart rate, creatine kinase, testosterone, cortisol, and mood profile to list only a few of the available means (Saw, Main, & Gastin, 2016; Urhausen and Kindermann, 2002). Gabbett et al. (2017) describe a step-by-step strategy for monitoring and interpreting data from an athlete's initial exposure to training to subsequent stimuli. Researchers describe a model that monitoring external and internal work load, e.g., how the athlete responds to the load, followed by evaluating the athlete's perceived well-being, and finally objective physical readiness measures of cycle ergometer, counter-movement jumps, and or submaximal hear rate recovery tests.

The combination of both physical and perceptual readiness provides a more comprehensive interpretation of the readiness of the athlete for subsequent stimuli. Crewther, Potts, Kilduff, Drawer, and Cook (2018) used morning to pre-testing, i.e., 2 x

20 m shuttle runs, and pre-test to post-test salivary cortisol and testosterone changes in relation to match outcomes in male rugby players. Salivary cortisol showed the highest ability to discriminate the outcomes of rugby matches (d = 1.6, *90% CI* = 1.3-1.9), albeit using a small sample size of matches. Resnick (2017) indicated HRV could characterize changes in parasympathetic modulation that occur with normal training and distinguish those changes from abnormal shifts in vagal tone due to overtraining, e.g. a shift toward sympathetic dominance. Sandercock et al. (2005) reported positive changes in HRV and vagal tone as a result of training in healthy individuals, when rest periods were applied appropriately. Hautala, Kiviniemi, and Tulppo (2009) reported HRV values similar in pre-race and post-race days, but significantly lower HRV on day two post-race, indicating a delayed effect physical exertion on HRV and athlete readiness. Rowell, Aughey, Hopkins, Stewart, and Cormack (2017), however, used countermovement jump (CMJ) performance in conjunction with salivary testosterone and cortisol concentrations as a measure of athlete readiness in elite Australian rules football players (age 23.3 ± 4.1 yrs). Researchers reported both CMJ and hormonal concentrations were sensitive to the match load, as measured through accelerometry, but flight time to contraction time in the CMJ was the most sensitive as a measure of recovery post-match.

Watkins (2017) coupled vertical jump (VJ) with the Brunel Mood Assessment (BAM) in healthy resistance trained males (age 24.5 ± 1.51 yrs), measuring before and after two separate resistance workouts. VJ decrement correlated with back squat volume (r = 0.648) prior to the second workout, with significant increases in fatigue scores delayed until after workout two (+9.60 ± 3.40 pts). Watkins (2017) concluded the VJ is a

viable measure of current fatigue levels. Longitudinally, CMJ has been compared to a daily well-being (DWB) questionnaire and a perceived recovery status scale (PRS) in youth athletes (Sawczuk, Jones, Scantlebury, & Till, 2018). Researchers reported correlations with PRS to training load (r = .28, ± 0.05), but not the DWB (r = -.05, ±.06) or CMJ (r = -.09 ± .06), indicating the PRS, and not the CMJ, may provide greater understanding of response to training. Determining the readiness of an athlete before, during, and after a training cycle can be crucial information for coaches and trainers. Gabbett et al. (2017) suggests a multi-modal approach to monitoring of athlete readiness.

Exercise Fatigue

Fatigue is state where the individual experiences a sensation of tiredness and or weakness, which is often accompanied by physiological factors in the nervous, vascular, muscular, and energy systems (Gladden, 2016). Such factors as skeletal muscle acidosis have been reported as having a critical role in overall body fatigue but cannot be considered the sole causative factor. Noakes (2000) indicated that there are additional models for fatigue inducement through exercise: (1) energy supply and depletion; (2) muscle power to muscle recruitment; (3) biomechanical properties of the system; and (4) psychological. The original work done by Hill (1925) stipulated maximum cardiac output as the limiting factor and fatigue inducing agent, whereby blood supply is limited to the skeletal muscles in regard to demand, causing inefficient metabolism to produce lactic acidosis, and thus fatigue. Dill (1938) suggested the limited cardiac output would in fact cause ischemia in the heart, induced by exercise, thus limiting the heart itself. The theory was widely overlooked as a central factor, but was again championed by Rowell

(1993), indicating limitations to cardiac output probably determine a participant's VO_{2max}.

However, a paradox exists at altitude, i.e. the lactate paradox, where peak cardiac output falls with increasing altitude, acting as a protective mechanism where lactate levels are the same at altitude as at sea level, even in a lower oxygen saturation condition (Noakes, 1998). Rowell (1993) concluded that a central "governor" must exist in the central nervous system functioning to prevent myocardial ischemia. Dr. Anthony Hackney, at University of North Carolina – Charlotte, has focused on this central governor postulate, identifying the neuroendocrine connections to stress and exercise as a modifier of stress within the neuroendocrine system. Acutely, hormones such as adrenocorticotropic hormone (ACTH), arginine vasopressin, cortisol, testosterone, cytokines, prolactin, epinephrine (Epi), and norepinephrine (NE), have been identified as serving a role in response to stress in general and induced by exercise (Hackney, 2006). Factors such as mode of the physical activity, anaerobiosis of the exercise, environmental conditions, age, gender, nutritional status, circadian rhythms, and genetics have also been cited as influencers on how the neuroendocrine system responds to acute exercise bouts. Hackney (2006) concedes that neuroendocrine adaptation to exercise training is a fluctuating target to pin down, making any one conclusion regarding a central cause to fatigue almost impossible. The current study designed to address a multi-faceted approach to the response to physical stress and the effect of supplementation on those systems.

Numerous markers have been identified for monitoring exercise fatigue both acutely and longitudinally. Verde, Thomas, and Shephard (1992) cited acute cortisol concentrations post exercise, resting lymphocyte proliferation, ratio of helper to suppressor T cell changes, serum creatine kinase concentrations, as well as IgG and IgM synthesis rates as potential markers for sustained training paradigms. While the participants in the study were not clinically ill nor lagging in performance, e.g., not over-trained, only POMS showed significant changes sensitive enough to detect effects of heavy training loads. Djaoui, Haddad, Chamari, and Dellal (2017) reported changes in heart rate (HR), resting heart rate (HR_{rest}), and HRV, used in conjunction with blood lactate, creatine kinase, urea, as well as salivary IgA, cortisol and testosterone may provide a broad scale picture of physiological responses to increased training load and therefore fatigue in soccer players. Djaoui et al. (2017) concluded that while the list is by no means fully comprehensive, HR markers may provide the most cost effective way of day to day athlete fatigue monitoring. Wallace et al. (2014) reported using HRV as being particularly helpful in monitoring large groups of athletes and/or a large volume of training sessions, in addition to session rating of perceived exertion (sRPE), regarding training load and athlete fatigue. Using endurance trained athletes, 1500 m time trial performance was correlated to the training load quantification models training impact (TRIMP), running training stress score (rTSS), and change in HR ratio, as well as comparisons to POMS. Wallace et al. (2014) concluded rTSS ($r = .70 \pm 0.11$), sRPE ($r = 0.60 \pm 0.10$), and TRIMP ($r = 0.65 \pm 0.13$) as being particularly interesting in monitoring athlete fatigue.

Central Fatigue

Fatigue caused by physical exertion has been discussed in two main areas of focus, central fatigue, which involves the central nervous system roles, and peripheral fatigue involving the dysfunction of muscular contraction mechanisms (Davis & Bailey, 1997). As discussed earlier, pinning fatigue down to a single causative agent is difficult. Chaudhuri and Behan (2004) cited internal and external sources of motivation, i.e., the limbic system and incentives, respectively, environmental factors such as heat and homeostatic state, and feedback from a multitude of systems including motor, sensory, and cognitive. Central fatigue has also been associated with increased mental fatiguing loads, which in turn reduce motivation and potentially motor weakness (Tanaka, Mizuno, Tajima, Sasabe, and Watanabe, 2009). Tanaka et al. (2009) subjected 20 healthy male participants to a mental fatiguing task for 30 min and reported significant reductions in vagal nerve activity, concluding that reduced vagal tone seems to be a clear sign of central fatigue. Davis and Bailey (1997) discussed the roles of nutrition, brain dopamine, choline and acetylcholine, cytokines, and ammonia, but all centering on the central role of 5-hydroxytryptamine (5-HT). McMorris, Barwood, and Corbett (2018) describe further neural connections between the lateral prefrontal cortex (LPFC) and limbic/hypothalamic areas with 5-HT receptors. The LPFC then may enter a feedback awareness state, integrating the information, and “makes a decision” to stop exercising or continue. Cordeiro et al. (2014) hypothesized that 5-HT itself may not be the limiting factor, but rather a factor associated with 5-HT production, e.g., tryptophan hydroxylase. Cordeiro et al. (2014) ran male adult Wistar rats (250-300 g) on a constant motion

treadmill at an 18m/min, 5% grade, until they fatigued. Rats were either injected with a saline solution or parachlorophenylalanine (p-CPA). The p-CPA solution was intended to inhibit the tryptophan hydroxylase conversion of tryptophan to 5-HT, thus delaying fatigue, and as a side benefit, facilitate greater cutaneous heat loss. Additionally, rats were injected with 20.3 μM tryptophan to speed up fatigue, e.g. reduce time to fatigue (TTF) on the treadmill. Cordeiro et al. (2014) reported a significantly higher TTF in p-CPA rats compared to saline rats (70.7 ± 20.8 min vs. 22.2 ± 2.5 min, $p = 0.03$), when tryptophan was administered, concluding that p-CPA did in fact inhibit tryptophan hydroxylase activity.

Central fatigue has also been associated with hyperthermic conditions, where cardiovascular changes in stroke volume, cardiac output and heart rate, to maintain homeostasis in body temperature, conflicts with the motive drive to continue exercise (Rowell, 1974). Nybo and Nielsen (2001) reported similar findings regarding maximal voluntary contraction (MVC) of skeletal muscles declined in hyperthermic conditions. Participants completed cycle ergometer trials in hyperthermic (40°C) and thermoneutral (18°C) conditions at 60% of VO_{2max} for 60 min, then 2 min of MVC for leg extensors (exercised muscle group) and handgrip (non-exercised muscle group). In both muscle groups, MVC declined more rapidly and to a greater extent, 82 ± 6% thermoneutral vs. 54 ± 7% hyperthermia, in leg extensors; thus it was concluded that the hyperthermic conditions had the greatest effect on muscles that were already fatigued, thus impairing performance. Amann (2011) identified a peripheral to central link in Nybo and Nielsen's (2001) work, where subjects completed two consecutive days of 10 min bouts at 83% and

67% W_{peak}, followed by a 5 km time trial cycling bout. The greatest decline (298 ± 14 W vs 332 ± 18 W) in power output in the 5km time trial was exhibited after the 83% Wpeak fatiguing bout, compared to the 67% W_{peak} fatiguing bout. Additionally, subjects had lower central motive drive (CMD) after the 83% W_{peak} fatiguing bout (-36% vs -20%), as measured by vastus lateralis normalized EMG activity. Nybo and Nielsen (2001) were able to identify the feedback loop to CMD as Group III and Group IV afferent fibers by applying an opioid analgesic (fentanyl) to the vastus lateralis during the 5 km time trial. Group mean power output and CMD for the fentanyl group was significantly higher under both fatiguing conditions. Nybo and Nielsen (2001) concluded that the fentanyl effective at eliminating the inhibitory influence of the afferent fibers to CMD.

Baker et al. (2006) identified adrenaline (A), noradrenaline (NE), free tryptophan (f-Trp), prolactin (PRL), nonesterified fatty acids (NEFA), and blood lactate, as being potential markers of fatigue following 30 s of high-intensity cycle ergometer exercise. Acutely high post-exercise plasma concentrations of A, NA, blood lactate returned to baseline levels 24 hrs later. Free-tryptophan decreased immediately post-exercise, but returned to near normal concentrations 24 hrs later. Baker et al. (2006) concluded that decreased f-TRP concentration was likely caused by a decrease in plasma NEFA concentration, causing no change in PRL concentration, thus serotonin activity was not affected. The researchers therefore make the case that central fatigue from a single 30 s bout of exercise is not a limiting factor on exercise performance. Carroll, Taylor, and Gandevia (2017) review literature regarding the ability of both central and peripheral factors to recover from fatiguing exercise. The researchers noted that short (< 2-3min)

duration exercise bout recovery can be linked to motor neuron responsiveness or sensitivity as a central mechanism, and metabolite accumulation, i.e. K^+, as a peripheral mechanism. In longer (> 6 min) exercise bouts, peripheral mechanism of fatigue may be linked to [Ca^{2+}] and glycogen depletion, where central mechanisms are as yet not clearly defined. The latter of these is part of the aim of the current study, whereby BCAA and whey protein isolate may provide necessary metabolites to both delay fatigue and enhance recovery for subsequent exercise bouts.

Overtraining

While FOR, NFOR, and OTS are not experimental variables or even aims of the current study, it is important to note that many of the same variables regarding athlete readiness to train and those associated with specifically NFOR and OTS are of interest in the current study. Urhausen and Kindermann (2002) describe OTS as a sport specific decrease in performance couple with mood state disturbances. Functional overreaching is characterized by a desirable increase in training load, followed by a short-term decrease in performance. If the athlete is given adequate rest intervals time, i.e., 1 to 3 minutes between exercise sets and 1 to 2 days between exhaustive exercise days, training results in an increase in skill and performance (Faigenbaum et al., 2009). Through periodization of workouts, it is common for athletes to follow consecutive day routines in multiple exercise modalities, e.g., endurance and resistance training (Cissik, 2002). NFOR is characterized by inadequate rest intervals between training load increases, followed by a decrease in adherence to technique and athletic performance, and associated with negative POMS and physiological responses (Pankanin, 2018). Training load in both

FOR and NFOR is calculated by multiplying training volume (minutes) and training intensity (rating of perceived exertion – RPE; Impellizerri, Rampinini, Coutts, Sassi, and Marcora, 2004).

Most notably, OTS, NFOR, and FOR have been associated with dysfunction of the HPA axis, as manifested in changes to POMS, typically in relation to changes in cortisol concentrations. Broodryk, Pienaar, Edwards, and Sparks (2017) subjected 47 female collegiate soccer players to repeated five-meter shuttle runs for 15 min. The researchers measured POMS, salivary cortisol, State-trait anxiety inventory questionnaire (STAI), and rating of perceived exertion (RPE) 15 min following the anaerobic fatiguing trial (AFT). A significant correlation between increased cortisol concentration, total mood disturbance (TMD), and fatigue subscales was reported ($r = 0.63$, $p < 0.05$). Broodryk et al. (2017) concluded that anaerobic fitness may have influences in both psychological and hormonal markers of stress. Anderson, Haake, Lane, & Hackney (2016) reported no difference in CAR over a 4-week training period in collegiate endurance athletes, when compared to training load. However, when the researchers controlled for distance in the training load, a strong positive correlation between CAR AUC_g ($r = 0.753$, $p = 0.012$), was reported. The researchers concluded that CAR may be variable in endurance athletes, as a training adaptation, in acute measurements, but when compared to the overall training load, may be valuable in monitoring accumulating physiological stress. Anderson, Lane, and Hackney (2018) extended the study to recreational endurance runners over a shorter 2-week protocol and reported a negative correlation between CAR and training load ($r = -.352$, $p = 0.025$). The researchers

concluded CAR is affected by regular exercise training, and therefore, fitness level and exercise training load must be controlled for when measuring CAR. Sanchez, Romero, and Ortis (2013) identified correlations between HRV and POMS in Spanish professional soccer, basketball, and field hockey players (age = 26.7 ± 3.43 yrs). Players exhibiting a lower vigor subscale score correlated to significant reductions in HRV ($r = -.77$, $p < 0.05$). Sanchez et al. (2013) concluded those players with mood disturbances may be monitored using HRV in conjunction with POMS for evaluating OTS and training load repercussions.

Cortisol Awakening Response

Cortisol is a glucocorticoid steroid hormone produced in the zona fasciculata of the cortical region in the adrenal gland, and is typically secreted in a diurnal pattern, peaking between 5 am to 7 am, with a low point between 3 pm to 5 pm. Functions of cortisol also include facilitating bioenergetic pathways for proteins and carbohydrates, reducing the inflammatory response by preventing the production of eicosanoids and interleukins, reducing amino acid uptake by cells, and enhancing protein catabolism (Melmed, 2015).

The CAR is a vital component of the transition from physiological patterns associated with sleep to those for waking and alert day processes. In conjunction with diurnal patterns of cortisol secretion throughout the day and night, CAR is usually recognized as a sharp rise immediately following waking, peaking between 30 – 45 min later, and gradually declining over the next 15 – 30 min (Anderson and Wideman, 2017). Additionally, Clow, Hucklebridge, and Thorn (2010) associated normal functioning of

the HPA axis and CAR with the hippocampal region of the brain. The researchers identified cortisol secretory patterns in relation to adrenocorticotropic hormone (ACTH) secretory patterns in subjects just prior to waking. The apparent separation of CAR and ACTH was interpreted as an anticipatory rise in ACTH, while CAR lagged slightly behind temporally. Frokjaer et al. (2013) and Roa, Elias, Castro, and Moreira (2013) further connect CAR to the HPA axis where decreased HPA axis inhibitory control, e.g., hippocampus suppression, and HPA axis dysregulation are often associated with mood disorders, blunted CAR, and potentially Cushing's Syndrome.

Anderson and Wideman (2017) reported that CAR can be highly variable, where no clear picture of dysfunction can be associated with an increase or decrease in CAR. Lesser trained individuals were also reported have a larger CAR in response to changes in training load, indicating some training adaptation by HPA axis. CAR tends to be influenced by state factors, i.e., time of awakening, prior day experiences, and anticipation of the day ahead, rather than trait-like factors, i.e., age, gender, and body mass index (BMI). Clow, Hucklebridge, and Thorn (2010) discussed the connection of CAR, as a discreet and distinct component of the circadian cycle, to the suprachiasmatic nucleus (SCN) of the hypothalamus as being the major regulatory mechanism. The hippocampus, which may be switched off and mediated by the SCN as a negative feedback loop, was cited as playing a central role in CAR regulation. Cortisol receptors, including glucocorticoid and mineralocorticoid receptors, are located in a variety of areas including the hippocampus and prefrontal cortex.

As cortisol is acutely associated with single or multiple exercise bouts, CAR is now being associated with stress related to exercise on days before, during, and following exercise trials. Anderson et al. (2018) recruited 15 recreational endurance athletes and monitored training loads over a 2 wk period. The researchers reported a positive relationship between both CAR and CAR% (the slope of increase from 0 to 30 min) and training impulse ($r = .352$, $p = .025$; $r = .373$, $p = .012$). Anderson et al. (2018) concluded that CAR could be affected by changes in training load in recreational athletes. Drogos et al. (2019) investigated the effect of 6 months of aerobic exercise programming on older adults regarding CAR, stress, and cognitive performance in older adults. The researchers used the Center for Epidemiological Studies, Depression Scale (CES-D), Beck Anxiety Inventory (BAI), the Anxiety Sensitivity Index (ASI), and the Perceived Stress Scale (PSS) to assess depressive, anxiety, anxiety sensitivity, and subjective stress levels. A positive increase in CAR was associated with lower stress scores over the trial period.

Labsy et al. (2013) reported no differences in next day CAR after exercising in the morning or afternoon, even after a significant ($p < .01$) rise in acute cortisol after exercise, indicating timing of exercises was not a mitigating factor in CAR. Participants were recreational soccer players that completed 90 min of aerobic exercise either in the morning or afternoon. Labsy et al. (2013) additionally identified dehydroepiandrosterone (DHEA) secretory patterns related to HPA axis function. While there was a rise in CAR, there was no significant change in diurnal cortisol area under the curve (AUC) nor DHEA concentrations as a result of the acute exercise bout, regardless of time of day. Ulhoa, Marqueze, Kantermann, Skene, and Moreno (2011) identified CAR patterns in shift

workers compared to irregular-shift workers. Although not exercise-based physical stress, the study presented an interesting perspective regarding recovery and anticipatory connections between CAR and fatigue. Ulhoa et al. (2011) compared 21 shift workers, e.g., 8:00 to 18:00 h, Monday through Friday truck drivers, to 23 irregular-shift workers, e.g., arriving to work at 20:30 h, loading the truck, and begin driving at approximately 22:30 h, and finishing their duty in the morning at approximately 08:00 h. The irregularity came from a non-regulated end time, where traffic conditions and weather could have played a part at shortening or lengthening the work day, compounded by the issue of working through the night. The most applicable data to the current study revolves around CAR values for both work days and off days. Ulhoa et al. (2011) reported irregular-shift workers had higher CAR during off days compared to shift workers, where shift workers had significantly lower CAR during off days compared to working days. The researchers concluded that shift workers were able to compensate on work days, but needed little recovery on off days, where irregular-shift workers needed extra compensation on off days for recovery, as evidenced by elevated CAR accompanied by increased stress scores.

While stress associated with physical exercise is a factor involved in modulation of CAR, dietary influences can also play an important role in CAR, especially on days after exercise. Hucklebridge, Clow, Abeyguneratne, Huezo-Diaz, and Evans (1999) examined the effects of higher and lower fasting glucose concentrations on CAR in healthy male (n = 14) and female (n = 13) adults, age 20 – 66 yrs. Low fasting glucose (FG) mean ± SEM was 3.8 ± 0.18 mmol/L, where the high fasting glucose mean ± SEM

was 5.1 ± 0.08 mml/L immediately after waking. Initial cortisol concentration for the low FG group was 11.7 nmol/L and increased to 19.6 nmol/L at the 30 min time-point. The high FG group started at 7.89 nmol/L and increased to 19.6 nmol/L at 30 min as well. Hucklebridge et al. (1999) concluded the difference in CAR slopes was due to the difference in fasting glucose initial values, e.g., a compensatory shift as a result of the overnight fast. Participants with lower fasting glucose then would have a higher compensatory response from the HPA axis, resulting in a higher slope to CAR. If mean energy intake and/or macromolecule distribution across the intake are disrupted, as in disordered eating, then potential dysfunction of HPA axis activity and CAR can arise. Filaire, Massart, Hua, and Le Scanff (2015) reported that within the 26 professional female tennis players, including those with reported disordered eating (DE) patterns (n = 12) and those without (n = 14), participants with DE exhibited overall higher diurnal cortisol secretion (11.3 ± 1.2 nmol/L/min) and higher CAR (10.1 ± 1.3 nmol/L) compared to non-DE participants (8.1 ± 1.4 nmol/L/min; 5.4 ± 0.7 nmol/L). The researchers concluded that the presence of DE caused CAR and diurnal cortisol patterns similar to those reported in the literature concerning Anorexia Nervosa, leading to dysregulation of the HPA axis and potential migrations of proinflammatory cytokines such as interleukin-6, which can hyperstimulate the HPA axis.

Specific macromolecules have also been investigated to determine interactions with CAR and HPA axis activity. Soltani, Keim, and Laugero (2019) hypothesized that dietary intake based on the Dietary Guidelines of America (DGA), e.g., 56% carbohydrates, 18% protein, and 26% fat, would significantly affect salivary cortisol

concentrations throughout the day and in response to a Trier Social Stress Test (TSST), compared to the typical American diet (TAD), e.g., 52% carbohydrates, 15% protein, and 34% fat. Participants were overweight or obese women, age 20-64 yrs, with a BMI of 25 – 39.9 kg/m^2. Soltani et al. (2019) concluded that individuals with a higher carbohydrate (CHO) intake over the 8 wks had significantly lower CAR compared to low or average CHO intake, in either DGA groups and or the TAD-based groups.

CAR has also been associated with changes in neurotransmitter concentrations that have physiological commonalities with the HPA axis, e.g., tryptophan. Tryptophan (Trp) has also been linked as a serotonergic pathway associated with fatigue due to exercise (Hall, Raaymakers, Saris, and Wagenmakers, 1995). The researchers hypothesized that when BCAAs compete for the same blood brain transporters as Trp, the Trp/BCAA ratio can be changed enough to reduce serotonin production, and therefore reduce melatonin accumulation, thereby reducing fatigue. The researchers recruited 10 endurance trained male athletes were to cycle at 70 - 75% of maximal power output and ingest drinks containing either 6% sucrose (placebo), 6% sucrose plus 3 g of Trp, 6 g of BCAA, or 18 g of BCAA. The BCAA supplements reduced Trp uptake by as much as 8 – 12%, where the Trp solution increased Trp uptake from 7 to 20 times normal uptake values. Hall et al. (1999) concluded that Trp supply did not affect serotoninergic activity nor time to exhaustion, e.g., central fatigue. Vielhaber et al. (2005) used a Trp depletion (TD) compared to a sham depletion (SD) in patients (n = 11 control; n = 15 insomnia; n = 12 obsessive compulsive disorder). The CAR AUC in the TD group (38.22 ± 16.01 mmol/L*hr) were significantly lower than the SD group (63.55 ± 44.70 mmol/L*hr), p =

.033. Therefore, the SD group that received the Trp had higher CAR. Vielhaber et al. (2005) concluded the decrease in morning cortisol, CAR AUC, was due to the central antisertoninergic effect of the TD, alluding to a potentially important interaction between levels of Trp and CAR. Capello and Markus (2014) reported similar results after Trp administration. The researchers recruited 118 college age men and women (24 ± 2 yrs) and performed genotyping of the 5-HT transporter linked polymorphic region, e.g., a 5-HT transporter and availability gene. Participants were categorized as S-allele (n = 60) and L-allele (n = 58), and each group was given 7 days of Trp or placebo to intake. POMS, salivary cortisol, and appetite were assessed. The Trp group had significantly lower CAR (6.26 ± 0.42 mmol/L) compared to the placebo group (12.47 ± 1.03 mmol/L), p = .023, especially in the S/S genotype group.

Assessing Psychological Stress through Profile of Mood States

The use of POMS to investigate mood disturbances was developed in 1971, and was composed of 65 items (McNair, Droppleman, & Lorr, 1971), and has been more recently been adopted in multiple languages and adapted to a shortened form POMS-SF (Curran, Andrykowski, & Studts, 1995; McNair, Lorr, & Droppleman, 1992; Shacham, 1983). Investigators typically ask the participant, during administration of POMS, how he or she has been feeling at a particular time, i.e., right now, over the last week, over the last month (Purvis et al., 2010), covering six subscales of questions regarding tension-anxiety, depression, anger-hostility, vigor-activity, fatigue, and confusion-bewilderment, and totaled into a composite score as total mood disturbance. Participants respond using a Likert scale from 0 to 4, ranging from *not at all* to *extremely* (McNair et al., 1992).

POMS has been associated with changes in biomarkers such as cortisol, changes in training intensity or training load, and both acute and longitudinal dietary interventions. Corrado (2017) reviewed interactions between biomarkers and psychological indices of mood and concluded psychological outcomes can be caused by physiological processes, as measured by hormonal changes. Maroulakis and Zervas (1993) reported similar findings for 99 women who underwent aerobic exercise bouts either in the morning or afternoon. Exercise participants reported lower POMS Total Mood Disturbance (TMD) scores when compared to controls, and that the effects persisted 24 hrs later. Maroulakis and Zervas (1993) concluded that participation in aerobic exercise can improve mood.

Training volume and training load changes have also been associated with changes in POMS. O'Connor et al. (1989) investigated the association of changes in training load and POMS Global Mood and salivary cortisol. The researchers concluded that as training load increased to the point of overtraining, POMS Global Mood increased, e.g., an increase in mood disturbance, with concomitant increases in cortisol, where those changes were reversed when training load went into a lower volume tapering phase. Purvis et al. (2010) reported that along increasing training intensity, the soccer players presented more favorable POMS scores when performance was successful. However, when performance was unsuccessful and training intensity was still very high, soccer players reported decreases in vigor and increases in tension and depression. Verde et al. (1992) identified biomarkers of a heavy increase in training in 10 highly trained distance runners (M = 29.8; SD = 1.7 yrs), who increased training load by 38% over a 3-

week period. The researchers concluded POMS was the best single marker of dysfunction among the runners, where increased fatigue and decreased vigor sub-scale scores was coupled with a lack of improvement in running performance. Oshima et al. (2017) reported stability of POMS TMD over a 9-day high volume training camp for college age Japanese football athletes. While the researchers reported significant increases in basal cortisol levels as the camp proceeded ($p = .003$), none of the POMS scores were significantly different ($p > .05$). Oshima et al. (2017) did however report reductions in significant decreases in acylated ghrelin as the camp progressed, indicating a potential mood disturbance not evaluated by POMS TMD during the intensive training load.

The association with an increase in catabolic physiological processes, e.g., a decrease in salivary testosterone to cortisol ratio, did not significantly correlate with changes in POMS or performance. Acute bouts of exercise have also been investigated in association with POMS. Tartar, Salzmann, Pierreulus, and Antonio (2018) reported significant increase in vigor, decrease in depression, and decrease in total mood disturbance, after a 30 min aerobic session at 75-85% of maximum heart rate. The researchers concluded that an acute bout of aerobic exercise may have positive effects on mood state, immediately post-exercise. However, if the intensity is very high, e.g., a competition, results on subscales may be different. Chiodo et al. (2011) reported lower values of depression, tension-anxiety, anger-hostility, fatigue, and bewilderment-confusion, with higher vigor scores prior to competition in youth Taekwondo athletes.

After competition, the opposite was reported, where anger-hostility and depression increased, and vigor decreased, both independent of match outcome.

In relation to repetitive acute bouts of training, Kenttä et al. (2006) reported significant decreases in energy index (vigor minus fatigue), even after a single day of training, in elite kayakers. The trend of decrease in the energy index continued over 3 weeks of training, showing a continued decline. All participants reported increases in the energy index scores after both short and long recovery cycles, indicating the effects of training intensity on mood state subscales can be reversed with adequate recovery. Broodryk et al. (2017) reported similar results in female soccer players after repeated anaerobic fatiguing tests. The researchers recruited 47 semi-professional female soccer players (22.0 ± 2.7 yrs) to undergo an aerobic fatiguing trial (AFT), e.g., 5 m shuttle runs, and collect salivary cortisol samples and psychological state and trait data. Cortisol, psychological fatigue and TMD increased from baseline and or pre- to post-AFT ($p < 0.05$); however, vigor and confusion sub-scale scores decreased from baseline and or pre- to post-AFT ($p < 0.05$). Broodryk et al. (2017) reported a positive relationship was seen between state-anxiety and TMD ($r \geq 0.63$, $p < 0.05$). The researchers concluded an AFT can be considered to have both physiological and psychological results in female soccer players.

CAR and POMS have also been associated with changes in physiological functioning due to stressors, both psychological and physical. Powell and Schlotz (2012) recruited 23 participants ($M = 24.9$; $SD = 3.5$ yrs) to monitor stresses of everyday life and compare that data to CAR and changes in stress levels based on Anticipatory Stress

Questionnaire (ASQ) data. The researchers reported no association (b = 46.8; SE = 58.8; p = .44) between CAR and ASQ and concluded that no significant associations between stress anticipation and same-day CAR was a result of lack of association between anticipation of stress and actual stress incurred during the day. Schulz, Kirschbaum, Prüßner, and Hellhammer (1998) reported CAR could be used as a "biological correlate" for chronic stress. The researchers recruited one-hundred college age students (51 females and 49 males; M = 25.7 yrs) to provide Trier Inventory for the Assessment of Chronic Stress (TICS) and morning cortisol samples. Participants who were categorized as high stress (above the median score of 22 / 40) had significantly higher cortisol concentrations at 30, 45 and 60 min after awakening (30 min: $F = 7.7$, $p < 0.01$; 45 min: $F = 10.3$, $p < 0.01$; 60 min: $F = 10.8$, $p < 0.01$). Schulz et al. (1998) concluded that a higher chronic workload resulted in higher cortisol concentrations after waking compared to lower chronic workload participants.

Casolino et al. (2012) investigated the viability of using a psychological response profile, in conjunction with performance data, as a means of discriminating between sub-groups of national team level taekwondo athletes. The researchers recruited 20 elite black belt athletes considered "selected," and 5 established Italian national team, considered "established," taekwondo athletes (9 women, 16 men; age 23.0 ± 3.1 yrs) were included in the 3-week training camp. While all athletes experience an "iceberg" profile for POMS, e.g., an elevated vigor sub-scale, only the "established" athletes were significantly higher in vigor prior to camp ($p < .05$). Casolino et al. (2012) concluded that athletes may have given "desirable" answers to POMS questions, fearing negative

repercussions of undesirable POMS profiles. Additionally, the session RPE and blood lactate may be more sensitive to discriminating between levels of national team athletes, rather than psychological indices. Shibuya et al. (2014) investigated the association between CAR and POMS subscales in adolescent females age 13 – 16 yrs, as a means of monitoring psychosomatic symptoms due to emotional stress in everyday living. The researchers reported significant positive correlations between CAR and the POMS subscales Tension-Anxiety ($r = 0.418$; $p < 0.05$), Depression-Dejection ($r = 0.467$; $p < 0.05$), Fatigue ($r = 0.482$; $p < 0.05$), and Confusion ($r = 0.572$; $p < 0.01$), concluding that HPA axis activation may have a strong association with somatic effective symptoms as evidenced by changes in POMS subscale scores.

Hough, Corney, Kouris, and Gleeson (2013) recruited 12 recreationally active males (*M* = 25; *SD* = 4 yrs) to complete 11 days of increased training load, e.g., 7 hrs/week to 17 hrs/week; a 143% increase. The researchers reported a suppressed acute cortisol response, both pre- and post-exercise at either 55% W_{max} or 70% W_{max} (*M* = -166%), coupled with increased burnout and fatigue scores on the Recovery-Stress Questionnaire (REST-Q) compared to pre-training scores. Hough et al. (2013) concluded that the increased training load was too severe as evidenced by a blunted cortisol response and negative psychological indices. MacDonald and Wetherell (2019) reported similar effects regarding blunting of cortisol, more specifically CAR, in association with anticipation of upcoming competition, as evidenced by changes in self-reported cognitive and somatic anxiety levels. Participants were eight elite male university rowers (*M* = 20.62, *SD* = 1.30 yrs), who underwent four consecutive competition weekends, including

training days and competition days. Cortisol samples were collected on practice days as well as competition days. MacDonald and Wetherell (2019) reported a statistically significant increase in the CAR during the training phase compared with the competition phase (F (1,6) = 20.1, p = 0.004, η^2 = 0.77), couple with greater cognitive anxiety on the competition day compared with the preparation day (F (1,6) = 20.61, p = 0.004, η^2 = 0.78). The researchers concluded the blunted CAR response in conjunction with elevated anxiety levels was not the psychological or physiological state for optimal performance.

Diaz et al. (2013) reported similar data between CAR and POMS in national level swimmers, comparing control days to competition days. Day 2 of competition CAR AUC_g post- competition levels (142.7 ± 40.2) were significantly higher than pre-competition levels (65.1 ± 37.7), as well as Day 1 pre-competition levels (93.3 ± 30.8) and post-competition levels (156.2 ± 3). Associated Day 1 and Day 2 POMS TMD at CAR time-points were 21.0 and 17.2, respectively, with significant correlations between Day 1 and Day 2 CAR and POMS (Day 1 r = .71, p < .05 and Day 2 r = .69, p < .05), respectively. Diaz et al. (2013) concluded that the changes in CAR were positively associated with changes in POMS TMD; however, physiological and psychological changes during the day may or may not correspond to variation in CAR the next day. Markus et al. (2008) investigated the role of hydrolyzed protein on plasma tryptophan (TRP) to large neutral amino acid (LNAA) ratio and mood state changes. Participants (n = 9 men; n = 9 women; 18 - 30 yrs) were given a 312-ml drink containing different TRP or LNAA concentrations: 20 g casein protein (Con) plus 0.4 g TRP and 10 g LNAA, and test condition drinks including 15 g intact alpha-lactalbumin (ALAC) whey protein plus

0.8 g TRP and 9.4 g LNAA, protein hydrolysate or HPROT plus 0.8 g TRP and 4 g LNAA, 0.8 g pure tryptophan plus 1.2 g synthetic peptide plus 0.8 g TRP. POMS questionnaires were administered before, 60 min after consumption, and 210 min after consumption. A significant increase in plasma TRP to LNAA ratio was reported 60–210 min after ALAC ($F(6,96) = 72$; $p < 0.0001$), peaking at a 67% increase 120 min after intake ($p < 0.0001$); however, ALAC was significantly lower than HPROT ($F(4,60) = 60,4$; $p < 0.0001$) or TRP ($F(4,64) = 30,83$; $p < 0.0001$). Mood improvements were reported at 60 min after HPROT ($p = 0.015$) and TRP ($p = 0.010$). Additionally, the improvements in mood were sustained at the 210 min mark for HPROT. Markus et al. (2008) concluded the use of a hydrolyzed protein may increase TRP levels, and subsequently 5-HT levels in the brain, accompanied by improvements in related mood and affective behavior.

Sihvola et al. (2013) assessed NASA Task Load Index (NASA-TLI) performance compared to salivary cortisol, plasma TRP, and POMS, given a high protein, high carbohydrate, or control breakfast drink. While no significant cortisol concentrations were reported ($p > .05$), a significantly higher sense of well-being compared to placebo and high carbohydrate drinks was reported for the high protein drink ($p = .028$). Sihvola et al. (2013) concluded the increase in TRP to LNAA ratio accounted for the difference in mood state but was not associated with greater performance on the NASA-TLI.

Heart Rate Variability

HRV has also been measured at both acute and longitudinal time intervals with varying changes and adaptations related to psychological stress and stress induced by

physical exercise. Longitudinally, sports teams have often measured HRV to identify changes in parasympathetic modulation as related to OTS and performance. Kingsley and Figueroa (2016) reviewed a number of studies, in resistance training and aerobic modalities, where there was no change in resting HRV, but rather changes in recovery HRV, citing possibilities of training adaptions in healthy young subjects. Djaoui et al. (2017) reviewed studies regarding tracking training load adaptations in soccer players and concluded HR before training, HR reserve during training, HRV during rest days, blood lactate, as well as blood and salivary immunological status would be valuable for coaches, trainers, and players to understand and monitor to ensure optimal training load design and execution. Athletes are able to easily use HRV on a daily basis either with chest strap heart rate monitors or smartphone. In either case a Kubios analysis is used for R-R interval analysis, either by rear-facing camera flash of a smartphone via photo plethysmography or the smartphone application output. To date, root mean square of successive difference between R–R intervals (rMSSD) remains the gold standard for tracking daily HRV change and parasympathetic adaptations (Resnick, 2017; Sandercock, 2005).

Mourot et al. (2004) reported only changes to standing HRV at 24 hrs and 48 hrs post-exercise, regardless of the exercise being continuous or interval in mode. James, Barnes, Lopes, and Wood (2002) reported similar results wherein R-R intervals were the same at pre-exercise and 72 hrs post but were lower 1 hr post-exercise. Hautala et al. (2009) and Leti and Bricout (2013) agree, independently, that HRV HF domains can significantly change the day after competition. Soares-Miranda et al. (2009) recruited 84

healthy university students to undergo free-living physical activity (PA) assessment as well as monitoring HRV R-R intervals during the testing phase. Participants were divided into 3 Tertiles based in PA. The researchers reported that participants who were the most active (Tertile 3) had higher vagal modulation compared to less active participants (Tertile 1 & 2), and that there was a positive dose response relationship between vigorous physical activity and vagal modulation, e.g., vigorous physical activity is necessary to gain the parasympathetic outflow benefits. Hackney (2006) reported more specifically that the exercise stimulus must be of a high enough intensity to elicit changes in acute and or longitudinal markers of stress. The researcher reported a critical threshold of 50-60% of VO_{2max} for at least 30 min to elicit an acute cortisol response and longer durations would further augment the response. Therefore, it was concluded that the level of physical activity during the two days of exercise each week could be vigorous enough to elicit a response from the HPA axis and or HRV modulations. Stanley, Peake, and Buchheit (2013) reported however that the greatest changes in HRV would be seen in participants with the lowest level of fitness, e.g., sedentary, prior to testing.

Similar to the current study's aims of improving HRV modulation, Lee, Wood, and Welsch (2002) reported increases in natural log high frequency (lnHF) power between short aerobic training cycles. McCartney, MacDonald, Millar, and Rakobowchuk (2009) recruited 10 young healthy endurance trained males to complete a single and four consecutive WAnT bouts. HRV was assessed for time and frequency domain at time interval before, 5-20 min post (Post1), 45-60 min post (Post2), and 105-120 min post exercise (Post3). After the single WAnT bout, all HR measures were at

baseline levels after 60 min of recovery. The standard deviation of all normal sinus R-R intervals (SDNN) was significantly reduced in Post 1 ($p < 0.01$) in both conditions and at Post 2 (45–60 min) after the 4 Wingate protocol only. McCartney et al. (2009) concluded the more intense the WAnT bout, referring to the number of consecutive bouts, the greater the change in R-R intervals for HRV. Additionally, higher intensity anaerobic exercise have been reported to increase vagal outflow in young adults, where low intensity did not change R-R intervals or RMSSD (Soares-Miranda et al., 2009). Although not salivary cortisol, the positive relationship indicated potential for the current study and aims.

Dietary influences on HRV have had limited specific discussion in the current literature. Lima-Silva et al. (2010) investigated the effects of carbohydrate (CHO) availability on HRV after moderate and severe exercise bouts to exhaustion. The researchers recruited 6 healthy males (age 26.5 ± 6.7 yrs) to undergo moderate intensity cycling bouts at 50% of the difference between the first (LT1) and second (LT2) blood lactate breakpoints, and at a severe intensity, e.g., 25% of the difference between the maximal power output and LT2. Exercise bouts were to be completed to exhaustion, followed by a 5 min rest period, and then a 1 min 125% VO_{2max} cycling bout. Dietary interventions consisted of a 10% CHO and 65% CHO intake. Lima-Silva et al. (2010) reported no difference in rMSSD between short-term low carbohydrate and high carbohydrate diets; however, there was significantly higher low-frequency to high-frequency ratio in the low carbohydrate diet, concluding low CHO availability can modulate sympathetic activity, e.g., HRV, during severe-intensity exercise. Jaatinen et

al. (2014) recruited high trait anxiety individuals (n = 67) between 18 – 63 yrs to receive either active yogurt cultures or standard yogurt cultures. The researchers monitored POMS and HRV, as well as inflammatory markers. HRV (M = 49.1, SD = 2.3 ms) and recovery index (M = 106.6, SD = 33.4) were higher in the active group than in controls (42.5 ± 2.2 ms and 80.0 ± 29.3), p = 0.046 and p = 0.02, respectively. Jaatinen et al. (2014) concluded that daily intake of yogurt may have positive health benefits including reduction of stress and improved physiology. As yogurt is a milk-based product, the data from Jaatinen et al. (2014) may support the aims of the current study regarding the effects of whey protein isolate on HRV.

Young, Cousins, Watkins, and Benton (2017) investigated the connection between HRV, POMS, and dietary intake. The researchers surveyed 156 medication-free adults, age 18 – 34 yrs. Young et al. (2017) reported a depressed mood significantly influenced diet quality (β = 0.169, *95% CI* = 0.016 - 0.793), and indirectly affected HRV R-R intervals (β = 0.011, *95% CI* = 0.001 - 0.002), accounting for 17% of the effect on HRV. The researchers concluded the link between disinhibited eating, e.g., poor eating habits, could be linked to chronically low HRV and poor mood states. Weinstein, Deuster, and Kop (2007) associated reductions in HRV with increased negative mood and depression. The researchers recruited 40 participants (M = 31.3, SD = 7.5 yrs) to either continue their regular aerobic exercise pattern or to withdraw from their regular exercise pattern for 2 wks. In the exercise withdrawal group, participants had higher negative mood scores after 2 wks, compared with control (16.0 ± 28.5 vs −1.8 ± 18.8; p = 0.03). Baseline LF/HF HRV ratios correlated with the increases in symptoms (r > 0.4; p

< 0.05) in the exercise-withdrawal group. Weinstein et al. (2017) concluded that the mood changes may corroborated by parasympathetic changes. Wallace et al. (2014) reported trivial correlations in POMS (r = -0.12 ± 0.31) with fatigue modeling in trained runners (age 38.6 ± 9.4 years) after 15 wks of endurance running training, but high beat to beat correlation to predicted fatigue (r = -0.54 ± 0.31). No other HRV parameters had significant association to training load perception or fatigue prediction. Wallace et al. (2014) concluded HRV, but not POMS, may be useful in monitoring fatigue in trained athletes.

CAR, i.e., a sympathetic response, and HRV, i.e., a parasympathetic response, have also been associated as markers of stress. Stalder, Evans, Hucklebridge, and Clow (2011) recruited 38 healthy participants (age 18-40 yrs) for peri-awakening analysis of CAR and HRV on 2 consecutive weekdays. Additionally, the researchers surveyed the participants using a PSS to measure stress levels over the prior month. While CAR responses and HRV responses increase post-awakening, Stadler et al. (2011) reported no significant association between changes in CAR or cortisol AUC and HRV, LF HRV, and or HF HRV. The researchers concluded that while there seems to be a relationship between CAR, measures of cardiovascular modulation, and HPA axis modulation, the lack of association between factors could have been due to individual variability in all three analytes.

As a primary focus, females were the subjects for this study; therefore, the impact of menstrual phase on HRV must be considered. Yazar and Yazici (2016) reported standard deviation of average normal sinus R-R intervals for all 5-min segments

(SDANN) were significantly lower in the luteal phase than during the follicular phase. However, heart rate recovery (HRR), rMSSD, and percentage of successive normal sinus R-R intervals longer than 50 ms (pNN50) were not different between luteal and follicular phases. The researchers concluded that markers of HRV and HRR are unaffected by menstrual phase, where changes in SDNN and SDANN for all 5-min segments could have been the result of sympathetic stimulation during the luteal phase. Rebelo et al. (2011) investigated oral contraceptive usage and associations with HRV changes in 125 women. Participants were grouped as physically active and taking oral contraceptives (active-OC), physically active but not taking oral contraceptives (NOC), sedentary and taking oral contraceptives (sedentary-OC), and sedentary and not taking oral contraceptives (sedentary-NOC). Rebelo et al. (2011) reported no significant difference in HRV components comparing OC users to non-users; however, differences were present comparing activity levels of the groups, where more active participants had higher dynamic variability of HRV compared to sedentary participants ($p < .05$).

Whey Protein Isolate

The effects of whey protein supplementation on biomarkers of muscle protein synthesis, muscle damage, and stress have also been investigated; however, little research exists regarding connections with HRV, POMS, and CAR. West et al. (2017) reported 25g of whey protein (PRO), when compared to an energy matched CHO placebo, yielded better net protein balance overnight, after a single bout of resistance exercise. The researchers recruited 12 trained men (M = 24.4, SD = 4 yrs) to perform resistance exercise prior to consuming the whey protein intervention. Protein balance was

monitored over 10 hours at night, and again 24 hrs post exercise. Net protein balance improved significantly in PRO (p = 0.064; effect size (ES) = 0.61) during overnight recovery from exercise. Net protein balance was still significantly higher in PRO (p = 0.036, ES = 0.69) 24 hrs post-exercise. West et al. (2017) concluded that whey protein promoted whole body anabolism, e.g., reduced protein breakdown, and may improve recovery from strenuous acute exercise. Forbes, McCargar, Jelen, and Bell (2014) investigated the effect of 2 different types of whey protein on amino acid profiles and hormones such as insulin, cortisol, testosterone, and growth hormone. The researchers recruited 9 males (M = 29.6, SD = 6.3 yrs) for a repeated measures controlled trial consisting of a typical mixed diet containing 10% protein (0.8 g/kg), 65% carbohydrate, and 25% fat (Control – C); an energy matched with carbohydrate to the whey protein conditions (Placebo – P); 0.8 grams of whey protein isolate (W1) per kg body mass per day (g/kg/d) added to the participant's typical daily diet; and 1.6 g/kg/d of supplemental whey protein (W2) added to the participant's typical daily diet. As expected, total amino acid concentration, essential amino acid concentration, BCAA concentration, and leucine concentration all increased post-prandial in W1 and W2, with no changes in C or P. There were, however, no significant differences in glucose, insulin, testosterone, or cortisol post-prandial between the groups.

When proteins are mixed with carbohydrates, the majority of studies report increases in performance, with no difference in cortisol concentrations, when compared to carbohydrate placebos (Hansen et al., 2015; Hansen et al., 2016). Hansen et al. (2016) recruited 16 elite cyclists for a randomized double-blind study comparing the effects of

protein supplementation consumption during intense training sessions on both performances, e.g., a 10 s peak power test and a 5 min all out anaerobic bout, and markers of recovery. Participants were given an isocaloric control beverage of carbohydrate (CHO, 84 g/h) and a mixed protein and carbohydrate beverage (PRO-CHO, 14 g protein/h and 69 g CHO/h) each day during the 6-day training bout. As expected, performance declined in both groups' 5 min bouts, but no significant difference was reported between the groups. No change was reported in peak power for either group. No significant differences between the groups were reported for cortisol, lactate dehydrogenase, or myoglobin. Creatine kinase (CK) did have a slightly different pattern of progression across the 6 days, ultimately though on Day 7, there was no significant difference in concentration between the groups. Additionally, Hansen et al. (2015) reported a decrease in mental performance capacity in the placebo group compared to the PRO-CHO mixture dose, regardless of dosing prior to or after exercise bouts.

Dietary intake of carbohydrate meals and protein meals has also been associated with changes in HRV. Charlot et al. (2013) investigated whether carbohydrate loading during a meal would reduce arterial oxygen saturation during exercise. The researchers recruited 11 male subjects (M = 20.1, SD = 1.8 yrs) to consume either a high carbohydrate meal (70% CHO, 12% PRO) or an isoenergetic high protein meal (35% CHO, 48% PRO) 60 min prior to being exposed to 15 min of hypoxia and 30 min of exercise in hypoxia at 60% VO_{2max}. Charlot et al. (2013) monitored oxygen saturation and HRV continually through the trials. The researchers reported no differences in HRV when subjects consumed either high protein or high carbohydrate meals 60 min prior to a

15 min hypoxia condition preceding a 30min exercise bout. The high carbohydrate condition tended to reduce oxygen desaturation compared to the high protein condition.

As a part of whey protein, BCAA, have been hypothesized to have an effect on CAR. Trp in the brain acts as a precursor to serotonin (5-HT). Intake of dietary Trp increases 5-HT activity and has been linked to central fatigue, and the ingestion of BCAAs, which compete for Trp brain transporters, may help mitigate the fatigue (Hall et al., 1995). Prolonged exercise, i.e., over 2 hrs, tends to decrease BCAA and increase free Trp in the blood, where free Trp is not bound to albumin. Hall et al. (1995) cited increases in free fatty acid mobilization and transport acting to displace Trp from albumin, thereby increasing the Trp /BCAA ratio. Short-term or acute exercise, i.e., 30 min to 2 hrs, tends to increase BCAA in the blood; with minimal to no changes in free tryptophan concentrations (Blomstrand & Saltin, 2001). Therefore, the researchers have investigated whether sufficient BCAA intake could balance the increase of the Trp/BCAA ratio. Blomstrand, Hassmen, Ek, Ekblom, and Newsholme (1997) administered 6-8 g of BCAA to male cyclists during a 60 min cycling bout at 70% VO_{2max}, and again during a 20 min maximum effort cycling bout. Subjects reported a lower RPE and lower mental fatigue during the BCAA trial compared to the placebo trial. Additionally, Blomstrand and Saltin (2001) cited ingestion of carbohydrates and BCAA mixtures delayed central fatigue due to increased insulin response and decreased free fatty acid mobilization, thereby lowering the Trp/BCAA ratio. Wilckens, Schweiger, and Pirke (1992) reported similar connections of 5-HT and glycogen status, whereby exercise

induced changes to basal ganglia, limbic system, hippocampus, and hypothalamus 5-HT receptors, indicating a link in 5-HT transport, neuroendocrine and behavioral functions.

VO_{2max} Treadmill Protocol

Bruce and Hornsten (1969) made recommendations, for exercise stress tests on patients with cardiac ischemia, that stages of increase start lower and increase at graded linear rate that is different from stress tests being implemented at the time. The necessity for a ramp protocol was introduced and since that time has taken on many different forms and iterations. The modified Bruce treadmill protocol was used for the current study based on the ability to produce a reliable and valid measurement of VO_{2peak} in recreationally active women. Myers et al. (1991) compared 10 coronary artery disease patients who were asymptomatic during exercise to 11 patients with coronary heart disease and exercise induced angina, and to 10 age-matched normal participants. Myers et al. (1991) used both treadmill and cycle ergometer protocols, in both step-wise increase and ramp increase methodologies. Overall, cycle ergometer protocols VO_{2max} were 16% lower than treadmill protocols VO_{2max} (18.7 ± 7 ml/kg/min vs. 21.4 ± 8 ml/kg/min). Great variance was reported across the six protocols; however, the slope of the treadmill ramp and cycle ergometer ramp were closely associated with each other (0.80 vs 0.78), indicating the ramp protocol, regardless of method yielded the most consistent slope results. Myers and Bellin (2000) reviewed a number of studies in clinical and cardiopulmonary research, and concluded ramp protocols produced (1) linearity of VO_2 response, either above or below the ventilatory threshold; (2) a reproducible ventilatory threshold; (3) time constant VO_2 kinetics and peak VO_2

compared to 15 W/min incremental tests. Boone and Bourgois (2012) explained the linear nature of VO_2 response to ramp protocols may be linked to the initial lag of VO_2 response to increases in work load. The researchers reported that even training adaptation differences may be spread across a ramp protocol, where a clearer aerobic capacity may be determined due to mean response time (MRT) of aerobic systems being a non-factor when increments of increased work load are smaller.

Treadmill Protocol

In order to elicit a cortisol response, e.g., a stressful event occurring, the treadmill protocol must be at a high enough intensity and a long enough duration to do so. Hackney (2006) reported a minimum of 50-60% of VO_{2max} as a critical threshold for eliciting an acute cortisol response and holding that intensity constant for a longer duration bout will further augment the response. Hill et al. (2008) supported this by reporting an 83.1 ± 18.5% increase in cortisol for exercise sessions at 80% of VO_{2max}, compared to 39.9 ± 11.8% increase for 60% VO_{2max} and 5.7 ± 11.0% increase for 40% VO_{2max} bouts. Snegovskaya and Viru (1993) reported a distinction between intensity, workload, and duration in 16 male rowers. The researchers reported higher cortisol levels in response to 40 min of rowing at anaerobic threshold compared to 7 min of rowing at supramaximal effort level. Snegovskaya and Viru (1993) concluded that hormonal reaction to the duration of the exercise bout may be due to physiological lag in the endocrine systems involved. Garrett and Kirkendall (2000) specifically refer to this lag as the time it takes to stimulate ACTH secretion, either through catecholamine or corticotropic releasing hormone stimulation, and finally to cortisol secretion at the

adrenal glands. The current study was designed to reach the cortisol threshold, at 70-75% VO_{2max}, and sustain that for 30 min (Hackney, 2006).

Cycle Ergometer Protocol

The Wingate Anaerobic Test (WAnT) as referenced by Bar-Or, Dotan, and Inbar (1977) and Katch, Weltman, Martin, & Gray (1977) was used to measure the supramaximal effort of an individual, measuring power output every 5 s. The WAnT has been used in innumerable studies to determine anaerobic capacity of an individual, but fewer studies have investigated the role of central and peripheral fatigue in conjunction with WAnT performance. Fernandez-del-Olmo et al. (2013) recruited 10 recreationally active males (age 25 ± 3 yrs) to complete 5 sets of maximal voluntary contractions (MVC) of knee extensors prior to and after a 30sec WAnT. A significant decrease (83.77 ± 3.2% to 82.76 ± 3.97%) in MVC after the second set ($t = 4.08$, $p = 0.027$). Fernandez-del-Olmo et al. (2013) pointed to reductions in motor nerve amplitude (-36%) as a central fatigue indicator, and a blood lactate exceeding 13mM/L, although not statistically significant different between the control and intervention trial, as a peripheral indicator of fatigue. Baker et al. (2006) further explored the concept of central fatigue, biomarkers, and the 30s WAnT. The researchers recruited 18 physically active males (23 ± 2 yr; 75.3 ± 11.0 kg; 11.6 ± 2.7% body fat) to complete a 30 s WAnT. Baker et al. (2006) collected blood samples pre and post WAnT to measure concentrations of adrenaline, noradrenaline, prolactin, nonesterified fatty acids (NEFA), and plasma-free Trp. Plasma-free Trp decreased post-exercise by 23.5% ($p < 0.05$), as well as a 46% ($p < 0.05$) decrease in NEFA, but no significant change in prolactin concentrations. Baker et al.

(2006) concluded that because no change in prolactin occurred, no appreciable change in tryptophan transport across the blood brain barrier occurred as well, e.g., the single 30 sec WAnT bout was not sufficient enough to cause central fatigue.

The current study was designed as to not rely on a single modality of fatigue, but rather a successive aerobic bout and anaerobic bout to induce fatigue. Naharudin and Yusof (2013) consider fatigue index as the preferred measure of a decline in anaerobic performance, especially over successive days of exercise. Stuckey et al. (2012) reported connections between repeated WAnT bouts and a single WAnT bout to heart rate, blood pressure variability, HRV, and baroreflex sensitivity (BRS). After the four WAnT bout, HRV high frequency and low frequency measures were decreased at both 60 min and 120 min post-exercise, where the single WAnT bout values for the same variables had returned to pre-exercise levels by the 60 min time point. The researchers concluded the additional bouts of WAnT shifted central control of HR from parasympathetic control to sympathetic control to a greater extent when compared to the single WAnT bout, e.g., a greater level of central fatigue.

Saliva Collection for Cortisol Concentration Measurement

Collection of cortisol samples in either a laboratory-based or home-based collection design is both easy and convenient for researchers and participants. However, while sampling is easy, questions of intra-individual stability and the temporal variability are addressed by using a multisampling approach rather than a single time-point sample. Pruessner et al. (1997) were among the first to quantify cortisol concentrations peri-awakening comparing single to multi-day protocols as well as the influence of age on

cortisol concentrations. Study 1 included boys (n = 21) and girls (n = 21) age 11.16 ± 1.99 yrs, over 3 consecutive days, sampling at 0, 10, 20, and 30 min post awakening. Study 2 included men (n = 35) and women (n = 35) age 26.5 ± 6.31 yrs, over 3 weeks, e.g., 1 day per week, sampling at 0, 15, 30, and 60 min post awakening. Study 3 included men (n = 20) and women (n = 20) age 70.4 ± 5.72 yrs, over 2 consecutive day, sampling at 0, 15, 30, 60 min post awakening. No significant difference was reported between baseline concentrations across the three studies. Girls and women tended to have overall higher cortisol concentrations across the temporal aspect, compared to boys and men, regardless of the age group. Within Study 2, the female participants were further divided into those taking OC and those not taking OC. Females taking OC had slightly lower cortisol responses temporally, with an effect size f^2=.05, explaining approximately 4% of variability in the free cortisol levels peri-awakening. Pruessner et al. (1997) concluded data showed good intra-individual stability across days and weeks, varying between r = .39 and r = .70, and therefore, can be useful in diagnosing both changes in individual patterns as well as diagnosing healthy from unhealthy individuals. Almeida, Piazza, and Stawski (2009) urged caution when evaluating CAR responses across multiple days, especially in older adults, reporting a high degree of intra-individual variability at stability correlations ranging from 0.13 to 0.26 (p < .01).

Estradiol

The inclusion of female participants in research studies involving physiological pathways has typically been during the follicular phase of the menstrual cycle, which as a purely scientific controllable variable seems both logical and prudent. It is, however, not

practical nor representative of the hormonal concentrations in which women exercise and or compete in sport (Frisén, 2016). The researcher recruited 48 physically active women between ages 18 - 35 yrs, (n = 29 with OC use; n = 19 without OC use). Frisén (2016) reported significantly higher serum cortisol levels in OC athletes across all seasons of training and competition; however, the pattern of increase and decrease was not different from non-OC athletes. The researcher concluded OC use and seasonal variances must be considered when evaluating the effects of training programs on cortisol concentrations. Oosthuyse and Bosch (2010) reviewed the effect of the menstrual cycle on exercise metabolism, citing possible interactions between estradiol and a decline in carbohydrate metabolism kinetics, promotion of free fatty acid availability and oxidation, and possible ties to reduced protein oxidation. The researchers concluded that menstrual phase study's findings can ultimately be confounded by individual variability between subjects and day to day variations within subjects.

While the question of hormone variability may have been addressed, or at least partially addressed, interactions with CAR, HRV, and POMS warrants attention. Gozansky, Lynn, Laudenslager, and Kohrt (2005) reported the relationship between salivary cortisol concentrations, in response to exercise, and serum cortisol concentrations are statistically strengthened when participants were on OC (r = .75, p < .001) compared to participants not taking OC (r = .67, p < .001). The data from Gozansky et al. (2005) are validating for the use of salivary cortisol as an indirect measure of total serum cortisol, as well as indicating the need to control for OC use among participants. As discussed earlier in this review, Pruessner et al. (1997) reported

that subjects taking OC had smaller CAR (p = .10), however the Cohen's d effect size (f2 = .05, ω2 = .04) indicated OC only accounted for 4% of the early morning rise in cortisol. Wolfram, Bellingrath, and Kudielka (2011) investigated variance in CAR across the menstrual cycle in 29 normally cycling women (M = 26.3, SD = 3.9 yrs). Participants completed 4 sampling sessions: (1) during menses; (2) during follicular phase; (3) at ovulation, i.e., verified by an ovulation test kit; and (4) during the luteal phase, e.g., 6 – 9 days post ovulation. Wolfram et al. (2011) reported there was no significant change in CAR ($F(3,84) = 1.15$, $p = 0.33$) or POMS ($F < 1.58$, all $p > 0.20$) across the four-cycle phases, with the exception of a slightly higher CAR during ovulation (11.51 ± 9.45 nmol/L; p = .05).

Teixeira, Ramos, Vianna, and Ricardo (2015) investigated the role of oral contraceptives, across the menstrual cycle, on HRV. The researchers recruited 17 healthy women (M = 24.4, SD = 3.7 yrs) using monophasic OC between 0.020 – 0.035 mg of ethinyl estrogen and low dose progesterone. Participants were randomly studied twice during individually timed menstrual cycles: (1) approximately 4 days after starting the placebo pill (M = 3.6, SD = 1.5 days) for low hormone (LH) values; and (2) approximately 21 days (M = 21.5, SD = 1.0) days after the onset of menstruation for high hormone (HH) values. Teixeira et al. (2015) reported stable HRV across low LH and HH phases when participants were taking OC. Researchers concluded the constant estrogen levels were responsible for the stability. The only exception to the stability was in rMSSD where levels dropped from 67.8 ± 8.6 during LH to 57.9 ± 4.9 during LH (p = .08), indicating a potentially clinically relevant variable. Since the current study did not

directly measure estradiol concentrations, the current researcher assumed based on past literature data that OC would likely have little impact on the experimental variables, especially in a repeated measures experimental design. However, it must be noted that the current study did not collect data regarding what menstrual phase participants were in during any of the testing protocol weeks or during baseline screening.

CHAPTER III

METHODS

Purpose

The purpose of this study was to determine the effect of whey protein supplementation on CAR, POMS, HRV, and FI. The double-blinded, randomized, control trial was designed with both aerobic and anaerobic exercise bouts across 2 consecutive days for each condition, e.g., placebo and whey protein, in recreationally active women age 18 to 35 yrs (M = 20.45, SD = 1.04 yrs).

Participant Screening and Anthropometric Measurements

All participants signed an informed consent document approved by the University Institutional Review Board (IRB). To ensure enough participants finish the entire testing protocol, 12 to 15 participants were recruited for participation. A power analysis using the G-power computer program (Faul, Erdfelder, Lang, & Buchner, 2007) indicated that a total sample of 12 people would be needed to detect large effects (d = .8) with 95% power using an F-test between means with an alpha at 0.05. Participants were given a 2018 Physical Activity Readiness Questionnaire (PAR-Q Plus; Riebe et al., 2018). If participant's answered *yes* to any of the first seven question on the PAR-Q Plus, 2018, they were excluded from study eligibility. Data from participants can be found in Appendix L.

The current researcher assumed that all participants refrained from caffeine or alcohol ingestion 48 hr prior to and during testing, refrained from sexual activity 48 hr prior to and during testing days, refrained from training or exercise 48 hr prior to and

during testing days, had no gastrointestinal problems with whey protein or maltodextrin, had access to a smartphone with Wi-Fi capabilities, exercised more than 30 min a week but less than 80 min a week, e.g., less than or equal to 2 times per week in aerobic exercise, and kept the same pattern of food intake and exercise during all testing phases.

Exclusion criteria were any cardiovascular, pulmonary, and or metabolic diseases, outside the age range of 18 to 35 yrs, not taking physician prescribed oral contraceptives, currently taking anti-depressant or anti-hyperactivity medication, were currently pregnant, were clinically diagnosed as hyperthyroid, had irregular, i.e. outside a 28 day range, menstrual cycle patterns within the last 3 months, and answered in the affirmative to any of the first seven questions on the PAR-Q Plus.

Weight was measured with a weigh beam stainless steel scale (Cardinal/Detecto, Webb City, MO, USA) while wearing shorts, socks, undergarments, and a shirt. The researcher set the large and small poises at the approximate weight of the participant. The participant stepped onto the scale platform. The researcher moved the small poise along the slide until the pointer balances at zero. The indicator arrows on the small and large poises pointed to a number indicating pounds. The researcher added the numbers from both small and large poises and recorded the participant's weight. The scale was calibrated to a zero weight prior to all testing (Cardinal/Detecto, Webb City, MO, USA). Height was measured using a precision stadiometer (Perspective Enterprises; Kalamazoo, MI), which measured height at the highest point on the cranium in relation to the ground. The mean of three measurements, precision 0.1 cm, range 70–200 cm, was calculated. The participant stood straight in an upright position; feet together; knees straight; heels,

buttocks, and back touching the back part of the stadiometer. The head was positioned in the Frankfurt plane. The arms hung relaxed at the side of the body, the inner part of the hand facing the thigh. The mobile, horizontal part of the stadiometer touched the head of the subject, with light pressure on the hair. Participants were instructed to perform an inspiration, and the final value was read. Participants were asked for brand/dose of oral contraceptives information during screening. All screening was done by female researchers.

POMS

Prior to peak oxygen uptake (VO_{2peak}) measurement, each participant completed a 35-item Profile of Mood States (POMS) short-form (SF) questionnaire, as referenced in Appendix K (Curran, et al., 1995; McNair et al., 1971, 1992). Participants self-reported answers to the 35 items using a 5-point Likert Scale to describe the intensity of feelings (i.e., *not at all = 0*; *somewhat = 1*; *moderately so = 2*; *very much so = 3*; *very very much so = 4*). POMS TMD was calculated using the formula TMD = (((depression-dejection) + (tension-anxiety) + (anger-hostility) + (fatigue-inertia) + (confusion-bewilderment)) – (vigor-activity)). POMS was administered after to anthropometric screening, and on testing days between the 30 min and 45 min timepoints during the one-hour waking/saliva collection cycle.

Peak Oxygen Uptake Measurement

After screening for participation in the experimental trial, participants performed a Bruce treadmill protocol (Bruce & Hornsten, 1969) for determination of peak oxygen uptake (VO_{2peak}), on a Quinton ST65 motor driven treadmill (Quinton Instruments

Company, Bothell, WA). The Bruce Treadmill Protocol was administered in the stages indicated in Table 1.

Table 1

Bruce Treadmill Protocol

Stage	Speed (mph)	Grade (%)
1	1.7	10
2	2.5	12
3	3.4	14
4	4.2	16
5	5.0	18
6	5.5	20
7	6.0	22

Note: mph = miles per hour. Each stage is 3 min in duration.

The speed and grade were increased to the next stage every 3 min. Termination was signaled by the subject dismounting with the aid of arm rails and straddling the treadmill belt at the point of intolerance. VO_{2peak} was deemed to have been reached if at least three of the following four criteria are achieved (Day, Rossiter, Coats, Skasick, & Whipp, 2003): (1) 90% of age predicted heart rate maximum (220-age); (2) respiratory exchange ratio (RER) > 1.15; (3) a plateau of oxygen uptake (≤ 150 ml/kg/min in VO_2 over the last 30 s of test); and (4) Borg's rating of perceived exertion (RPE) of ≥ 18, on a 6-20 scale. Optimal time for the VO_{2peak} protocol duration was 8 to 10 min (Yoon, Kravitz, & Robergs, 2007). HR was monitored with a Polar Heart Watch system (Polar

Electro Inc., Lake Success, New York). The RPE was measured prior to each stage change (Borg, 1982). Breath-by-breath expired gases were collected and continuously sampled, and averaged every 1 min, for indirect calorimetry with a metabolic cart (Parvo Medics True One 2400, Sandy, UT, USA). Expired gas passed through a Hans Rudolph (Hans Rudolph Inc., Dallas, TX, USA) two-way non-rebreathing valve, a saliva collector trap, a Creative Biomedics Inc. (San Clemente, CA, USA) CB-1501-2 filter, a Hans Rudolph series-3813 heated pneumotachometer, and into a 4 L mixing chamber. The mixed expired gas was then be continuously sampled using a 61 cm Nafion tube (Permapure, Toms River, NJ, USA) by a paramagnetic oxygen analyzer (0–25 % range with 0.1 % accuracy) and an infrared carbon dioxide analyzer (0–10 % range, with 0.1 % accuracy) to measure fractions of expired oxygen (FEO_2) and carbon dioxide ($FECO_2$) (McFarland and Wu, 2012). The metabolic cart flow meter (Hans Rudolph Inc., Dallas, TX, USA) was calibrated with a 3 L syringe, and the gas analyzer was calibrated with known gas mixtures (O_2: 16.0%; CO_2: 4.0%; MacFarlane & Wu, 2012).

Participants were also be familiarized with the Velotron cycle ergometer (RacerMate, Inc., Seattle, Washington USA). The seat was adjusted so that the participant's leg was near complete extension at the bottom of the pedal stroke. Participants pedaled at a self-selected cadence between 40 and 80 revolutions per min (rpm) at 40W for the 2 min familiarization (Zuniga et al., 2012).

Saliva Collection

Saliva samples were collected on a 4-day cycle during each supplementation phase. Days 1 and 4 were no-exercise days, and Days 2 and 3 were exercise days. Each

day, participants set an alarm to wake between 0600 and 0800 hr, followed by a 1 hr sampling phase. Immediately upon waking (S1), participants collected 2 ml of saliva, using the passive drool technique (see Appendix I), into the collection vial provided. Saliva samples collection were completed within 5 min of waking to ensure S1 validity (Stadler et al., 2016). Being awake was determined by the participant according to the following criteria: when participants were conscious, aware of who and where they were, and can clearly differentiated between states of sleeping and waking (Stadler et al., 2016). Participants recorded the actual collection time. Participants were also be instructed to follow saliva collection guidelines (see Appendix J).

Participants collected 2 ml saliva samples at 15, 30, 45, and 60 min after S1, again recording collection times on the data sheet. Once all samples are collected for the day, samples were placed in a -20°C freezer at the participant's home until all trials for the phase were completed. If the participant had no freezer at the home location, the samples for each day were to be returned to the Exercise Physiology Lab at Texas A&M University – Commerce within 5 hr of sampling. Upon completion of each 4-day experimental trial (placebo or whey protein), participants returned the frozen samples to Exercise Physiology Lab at Texas A&M University – Commerce. Frozen samples were be transported to the Exercise Physiology Lab in insulated containers with freezer blocks to prevent thawing. Samples were then be stored at -80°C for future analysis.

Cortisol Awakening Response Analysis

The CAR, was calculated using area under the curve with respect to the ground (AUC_g) using the formula: $AUC_g = (((S1 + SC_{15})/2)*15) + (((SC_{15} + SC_{30})/2)*15) +$

$(((SC_{30} + SC_{45})/2)*15) + (((SC_{45} + SC_{60})/2)*15)$, where S1 = salivary cortisol concentration (pg/mL) upon waking, SC_{15} = cortisol concentration (pg/mL) 15 min after waking, SC_{30} = cortisol concentration (pg/mL) 30 min after waking, SC_{45} = cortisol concentration (pg/mL) 45 min after waking, and SC_{60} = cortisol concentration (pg/mL) 60 min after waking (Pruessner, Kirschbaum, Meinlschmid, & Hellhammer, 2003).

HRV

HRV was measured between the 15 min and 30 min saliva collection timepoints each of the 4 protocol days. Participants were instructed to collect HRV data after collecting the 15 min saliva sample. HRV data collection instructions were as follows: participants would sit quietly and breathe naturally for 4 min, and then again for 1 min during the measurement of HRV, whereby participants placed the index finger of the dominant hands over the smartphone camera and LED flash. Participants were familiarized with the measurement technique prior to testing. HRV measurement was collected on the participant's smartphone using photoplethysmography (PPG), through an application (app) called HRV4Training (HRV4T), available at http://www.hrv4training.com/. HRV4T was available on multiple smartphone operating system platforms. Participants' raw data were automatically exported, through the smartphone app, to the password protected research team platform on http://www.hrv4training.com/, when the app was closed at the end of measurement during each day of the experimental trial.

PPG was measured via reflection through the illumination of the skin using an LED (e.g. the smartphone's flash) and through detection of the amount of light that was

reflected by a photodetector or a camera located next to the light source. HRV4T acquired the video stream at a frame rate of 30 Hz, where red, green, and blue (RGB) channels were averaged over the entire frame, before converting between the RGB and the hue, saturation, and value (HSV) color space. A Butterworth band pass filter of order 4 and frequency pass band between 0.1 and 10 Hz was used by the app to remove the direct current component (DC) of the signal, as well as any high-frequency noise while maintaining the alternating current component (AC). Correlation of PPG to both electrocardiogram and Polar H7 chest strap was very high ($R = 0.99$, $p < 0.05$) in a recent comparison study (Plews et al., 2017). The root mean square of successive difference between R–R intervals (rMSSD) was determined from each collection. Raw data from the app were transformed into rMSSD data using the following formula: RMSSD = sqrt (mean $(RRi+1 - RRi)^2$), where sqrt = square root; RRi = R-R interval between beats (Dey et al., 2014).

Exercise Protocol Summary

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.

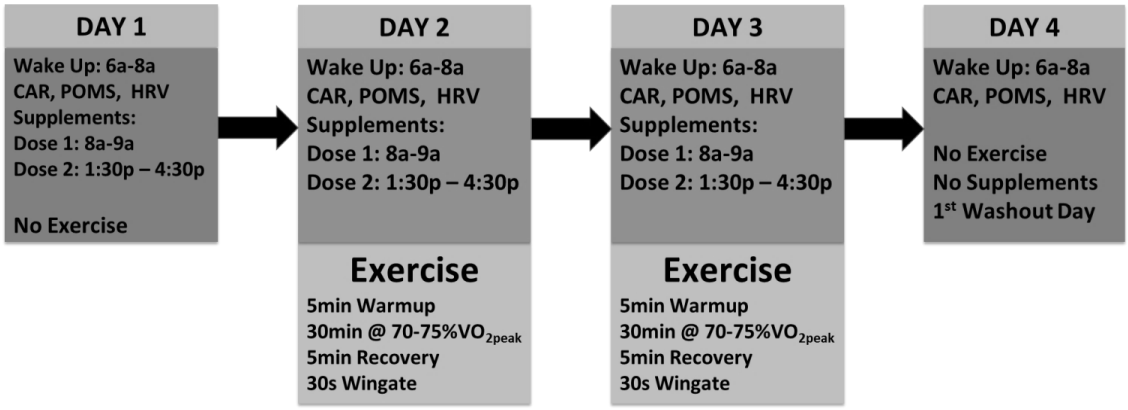


Figure 1. Summary of Exercise and Supplementation Cycle

Treadmill

Each exercise session consisted of 5 min of walking on a treadmill at of 3.3 mph with a grade and speed adjustments to achieve 70-75% VO_{2peak} for each participant, followed by 30 min of walking at that intensity range (e.g., 70-75% VO_{2peak}). VO_2 was measured and recorded during the first 5 min of exercise, 15-20 min, and the last 5 min of each exercise session. If the participant was not within the desired intensity range, the treadmill grade and/or speed was increased or decreased to achieve the desired intensity range. During exercise, HR was monitored and recorded at the same time intervals using a Polar heart rate monitor (Lake Success, NY).

Cycle Ergometer

After the treadmill protocol completion, a 5 min recovery period was initiated. Participants walked around the laboratory facility at a slow, self-selected pace to allow the HR to drop below 120 bpm. After the HR has dropped below 120 bpm, participants sat quietly for the remainder of the recovery phase. At the end of the 5 min recovery period, participants completed a 30 s WAnT (Bar-Or et al., 1977; Katch et al. 1977)

protocol on a cycle ergometer by Velotron® DynaFit Pro (Racer-Mate, Seattle, WA). The Velotron® is an electronically braked cycle ergometer through computer-controlled software (Velotron® Coaching Software, Version 1.5, RacerMate Inc., USA), and an eddy current braking system built around a heavy (55lb / 25 kg), large diameter flywheel. The Velotron® cycle was operated in ergometer mode via the software and required no calibration. Greater trochanter height was measured by a 60" woven tape measure (American Diagnostic Corporation, Hauppauge, NY, USA), from the palpated greater trochanter to the floor, along a plumb line. This distance was then multiplied by 1.02 to determine the seat height. Peak power output capability on the Velotron has been documented at 102% of greater trochanter height from the floor (Rankin & Neptune, 2010). Seat height was adjusted to the calculated 102% greater trochanter height for each participant. Toe clips were used to decrease foot slippage during the WAnT. All trials were completed in a seated position on the saddle of the cycle ergometer. During each WAnT, participants were verbally encouraged.

A 20 s initiation phase at a work rate equal to 75 W was completed, followed by a 6 s acceleration phase, during which participants pedaled as fast as possible. Immediately at the end of this phase, the load equal to 7.5% body weight was applied to the flywheel, and subjects pedaled 'all out.' Across the 30 s load period, peak power (PP), the highest power output recorded during exercise; mean power (MP), the average power recorded during exercise; and minimum power (Pmin), the lowest power output recorded, were expressed in Watt/kg and recorded from all trials using the Velotron software. FI (expressed in %), where FI = (PP – Pmin)/PP) * 100, was also be recorded.

During exercise, power output was sampled at a frequency equal to 10 times per second. Near the end of the trial, participants were encouraged to finish, with clear instruction and countdown from three to zero. Participants then began a 5 min cool-down at 10W and at a self-selected cadence.

Supplementation

Participants were instructed to follow normal dietary patterns and maintain body weight for the duration of the study. In order to reduce the effect of macronutrient composition on supplementation outcomes, participants consumed the same lunch meal between 3 and 4 hr prior to each exercise trial. To verify consistency, each participant recorded a four-day diet log for each trial period. Dietary intake was analyzed using WebMD.com Food Calculator.

A double-blinded, randomized, control trial was employed. All supplements were mixed and distributed to participants one day prior to testing in each experimental trial. Participants were instructed to keep the supplements refrigerated below 40°C. If participants did not have access to refrigeration, the supplementation drink were to be picked up between 0800 and 0900, and between 13:30 and 16:30 each day during the testing cycle at the research facility. Each supplement was contained in a Thermo Scientific Nalgene DS2185-0032 Wide-Mouth Amber Economy Bottle (Cole-Parmer North America, Vernon Hills, IL, USA). The placebo dose consisted of 25g of maltodextrin powder (Hard Eight Nutrition LLC, Henderson, NV USA), mixed with 500ml of distilled water, totaling 100kcal. The whey protein dose consisted of 25g of hydrolyzed whey protein isolate powder and 25g of maltodextrin powder (Hard Eight

Nutrition LLC, Henderson, NV USA), mixed with 500ml of distilled water, totaling 200kcal. All supplement doses had four drops of chocolate flavored, non-caloric, Flavdrops™ (Myprotein.com, Meridian House, CW9 7RA, United Kingdom) added to each 500ml for flavor uniformity across the conditions. Supplements were consumed between 0800 and 0900 for Dose 1, and between 13:30 and 16:30 for Dose 2 (Kerksick et al., 2006). Dose 2 was consumed 30 min prior to the arranged exercise bout time. On Day 1 of the cycle, dosing time were to be the same as exercise days, i.e., Day 2 and Day 3 of the experimental trial.

Washout Period

A 7-day washout period was enforced between experimental trials. The washout period began on Day 4 of the testing phase because no supplementation was given on that day, and continued for 6 consecutive days, totaling 7 days. While washout periods vary from 3 days to 7 days between experimental trials (Hays, Kim, Wells, Kajkenova, & Evans, 2009; West et al., 2017), the current study was designed where the washout time was double both nutritional intervention supplementation days and exercise days.

Biomarker Analysis

All saliva samples were stored at the participant's home or returned within 5 hrs of sampling daily during each experimental trial, and then returned to Exercise Physiology Lab at Texas A&M University – Commerce. Samples were thawed only once for ELISA analysis (Daly, White, Varnum, Anderson, & Zangar, 2005; Fry, Kraemer, & Ramsey, 1998). Each time point saliva sample was analyzed using enzyme linked immunosorbent assay (ELISA) protocols for total cortisol, using pre-produced kits

from Salimetrics, LLC (Carlsbad, CA, USA). Cortisol were prepared and analyzed according to manufacture directions (see Appendix H).

Statistical Analysis

All statistical analyses were performed using SPSS 25 (SPSS, Chicago, IL). Multiple statistical techniques were used to analyze the data. A one-way ANOVA was performed to describe anthropometric data variance within the group during the screening and baseline phase. Dependent variables included age (yrs), height (m), weight (kg), BMI (kg/m^2), VO_{2peak} (ml/kg/min), average VO_2 during 30 min treadmill protocol (ml/kg/min), and mean caloric intake over 4-day dietary recall.

A 2 (Condition) x 4 (Day) repeated measures ANOVA, comparing Supplement Intervention to Day on dependent variables of POMS, CAR AUC_g, and HRV, was implemented to determine differences in variance. A two-way repeated measures ANOVA comparing Day to Supplement Intervention for Fatigue Index was implemented to determine differences in variance between placebo and whey/maltodextrin interventions. Two-way repeated measures ANOVA comparing Day to Supplement Intervention for CAR AUCg, HRV, and POMS was implemented to determine differences in variance between placebo and whey/maltodextrin interventions. Pearson's correlation (*r*) analyses of nutritional supplement intervention on fatigue index, POMS, HRV, and CAR AUC_g was implemented to determine if POMS, HRV, and or CAR AUC_g were linearly associated with fatigue index. Lastly, a Multiple Regression analysis of nutritional supplement intervention on fatigue index, POMS, HRV, and CAR AUC_g

was implemented to determine if POMS, HRV, and CAR AUC_g were significant predictors of fatigue index.

CHAPTER IV

RESULTS

Participant Characteristics

Emails were sent to honors college sponsors and sorority house presidents at a mid-size university in the Southwest on May 8, 2019 and August 26, 2019. From these two emails, 43 women responded with interest in participation. A follow-up screening meeting that also included more detailed study information as well as the time commitment was held for the 43 women, of which 31 were excluded due to not taking oral contraceptives, training status (< 30 min or > 80 min per week), and or taking contraindicated medicines or supplements (e.g., anti-depressants). Out of the total number of responders, 12 women qualified for participation in this study, and were scheduled for a first visit to the lab, 4 in the summer and 8 in the fall of 2019. While 12 women completed the study, one subject was ultimately removed from the study group due to lack of saliva volume for ELISA analysis. All 12 women signed University approved Informed Consent. Table 2 presents the mean descriptive characteristics of the study participants. The raw data for each participant can be found in Appendix L. All data are presented as mean ± standard deviation (SD) unless otherwise stated.

A recreationally active participant was defined as someone participating less than or equal to twice a week in aerobic activity for a total of 80 min at moderate intensity (~5-6 METS) and having a maximal oxygen uptake (VO_{2max}) that is classified at or below the average for age, ≤ 35.2 ml/kg/min (Riebe et al., 2018). As seen in Table 2, the average VO_{2peak} for participants was 32.32 ± 4.64 ml/kg/min. Participants self-reported

their aerobic activity per week, however no actual measurement of activity was conducted in the study.

Table 2

Participant Descriptive Characteristics

	Mean ± SD
Age (y)	20.45 ± 1.04
Height (cm)	163.45 ± 4.06
Weight (kg)	68.44 ± 11.61
BMI (kg/m^2)	25.57 ± 3.91
VO_{2peak} (L/min)	2.12 ± 0.25
VO_{2peak} (ml/kg/min)	32.32 ± 4.64
% of VO_{2peak} 30 min TM – Placebo Trial	68.20 ± 3.43
% of VO_{2peak} 30 min TM – Whey Trial	68.68 ± 3.65
Daily Caloric Intake – Placebo Trial w/PL	1785.72 ± 459.62
Daily Caloric Intake – Whey Trial w/WH	1656.24 ± 437.47
Maximum Heart Rate during VO_{2max} (beats per min)	193.73 ± 4.34
Maximum Respiratory Exchange Ratio during VO_{2max}	1.26 ± 0.13
Maximum Rating of Perceived Exertion during VO_{2max}	18.36 ± 1.86

Note: *n* = 11. *SD* = Standard Deviation; BMI = Body Mass Index; VO_{2peak} = peak oxygen consumption;

Aerobic activity was verified with relative VO_{2peak}, where screening would ideally be less than 35.2 ± 2 ml/kg/min. It is believed that all participants achieved VO_{2peak} during the Bruce treadmill protocol by achieving at least three of the four following criterion: (1) women achieved a plateau in oxygen uptake within the final minute of the test (< 150 mL/min change in VO_2 with increasing work rate) using 30 s averages (Howley, Bassett, and Welch, 1995); (2) all women achieved an RER > 1.10; (3) all

women achieved a HR within 10 bpm of age-predicted maximum heart rate; and (4) Borg's Rating of Perceived Exertion (RPE) Scale $_{6\text{-}20} \geq 17$ (Beltz et al., 2018). All participants were actively taking prescribed oral contraceptives, containing 0.12 ± .1 mg of estradiol (E2).

VO_2 Exercise Ranges and Responses

A two-way repeated measures ANOVA was conducted that examined the effect of exercise day and intervention trial on VO_2 exercise range during the 30 min treadmill exercise bout. Treadmill grade and speed were adjusted during the exercise bout to exercise between 70 – 75% of VO_{2peak}. There was no statistically significant interaction between day and intervention on VO_2 exercise range, $F(1,10) = 1.299$, $p = .281$. Additionally, there was no statistically significant main effect of either day, $F(1,10) = 1.181$, $p = .303$, or intervention, $F(1,10) = .006$, $p = .941$. The mean VO_2 exercise range data and percentage of VO_{2peak} are reported in Table 3. Combined mean reflects the mean of VO_2 data taken at 0, 5, 15, and 25 min during the 30 min exercise bout.

Table 3

VO_2 Data During 30min Treadmill Exercise Bouts

	Mean ± SD
Day 2 Placebo (PL)	21.78 ± 2.60
Day 3 Placebo (PL)	21.99 ± 2.68
Day 2 Whey (WH)	22.13 ± 2.89
Day 3 Whey (WH)	21.95 ± 2.64
Combined Mean % VO_{2peak} – PL	68.20 ± 3.43%
Combined Mean % VO_{2peak} - WH	68.68 ± 3.65%

Note: *n* = 11. *SD* = Standard Deviation; VO_{2peak} = peak oxygen consumption in ml/kg/min; Combined Mean refers to the mean VO_2 of 0, 5, 15, and 25 minute data during the 30 minute treadmill bout.

Respiratory Exchange Ratio During Exercise Bouts

The mean respiratory exchange ratio (RER), expressed as carbon dioxide production ÷ oxygen uptake (Pendergast, Leddy, & Venkatraman, 2000) during the exercise bouts for the placebo and whey trials were not statistically significantly different, as seen in Table 4. Mean RER ranged from .932 ± .06 to .950 ± .04 suggesting participants were oxidizing primarily carbohydrate during the 30 min treadmill protocol.

Table 4

ANOVA Table - Respiratory Exchange Ratio

Effect	MS	df	F	p	Greenhouse-Geisser	Huynh-Feldt
Day	.002	1,10	1.964	.191	.191	.191
Condition	.0003	1,10	.195	.668	.668	.668
Day*Condition	.000008	1,10	.013	.910	.910	.910

Note: n = 11. Variables. Day: Exercise Day 1 and Exercise Day 2; Condition: Placebo and Whey Trials.

*Statistically significant at $p < .05$.

Fatigue Index Responses

A two-way repeated measures ANOVA was conducted that examined the effect of exercise day and intervention trial on 30 s WAnT Fatigue Index, (FI expressed in %), where FI = (PP – Pmin)/PP) * 100. There was no statistically significant interaction between day and intervention on FI, $F(1,10) = 2.283$, $p = .162$. Additionally, there was no statistically significant main effect of either day, $F(1,10) = 4.356$, $p = .063$, or intervention, $F(1,10) = 2.451$, $p = .149$. Mean FI for each exercise day and intervention are reported in Figure 2 and Table 5.

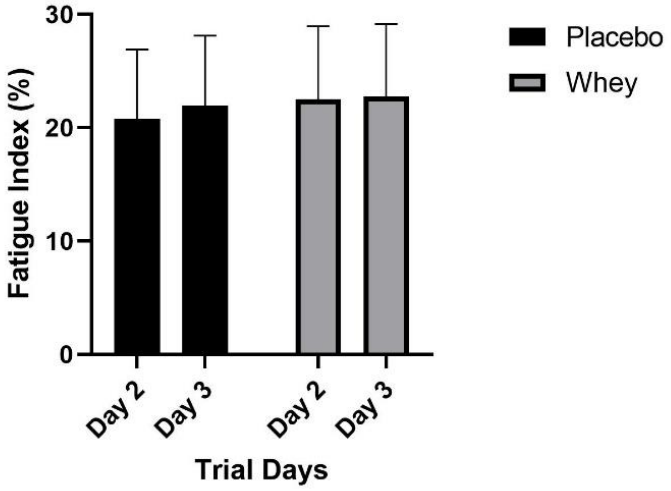
Table 5

ANOVA Table - Fatigue Index

Effect	MS	df	F	p
Day	17.691	1,10	4.365	.063
Condition	5.746	1,10	2.451	.149
Day*Condition	2.318	1,10	2.283	.162

Note: *n* = 11. Variables. Day: Exercise Day 1 and Exercise Day 2; Condition: Placebo and Whey Trials.

*Statistically significant at $p < .05$



	Placebo			Whey		
	Mean	SD	N	Mean	SD	N
Day 2	20.79	6.12	11	22.52	6.45	11
Day 3	21.97	6.16	11	22.78	6.38	11

Figure 2. Fatigue Index. Note: Fatigue index (FI) as calculated FI = (Peak Power – $Power_{min}$)/Peak Power) * 100. Error bars represent ± SD, n = 11.

Cortisol Awakening Response

A two-way (Day x Condition) repeated measures ANOVA was conducted to examine the effect of trial day and intervention condition on CAR as measure by salivary

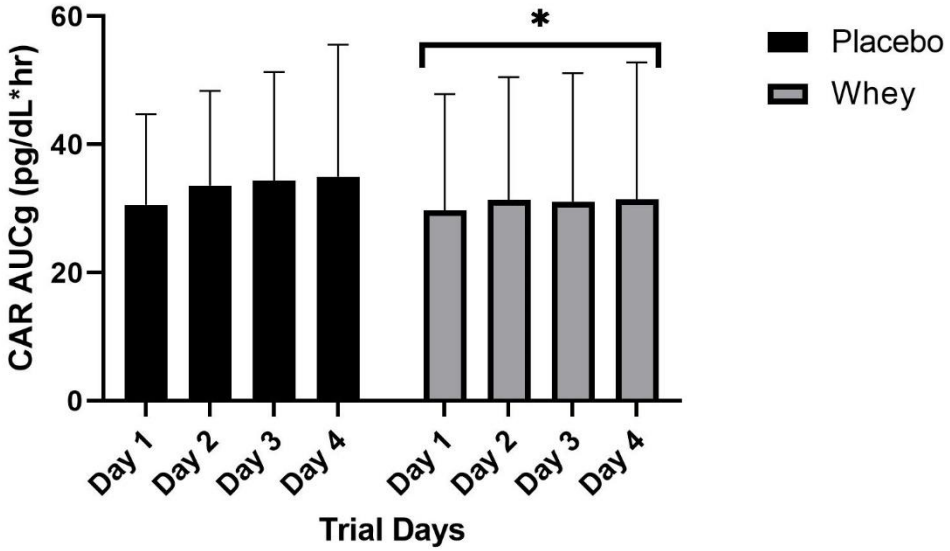
cortisol concentrations. Salivary cortisol concentrations were aggregated and analyzed as a calculated area under the curve with the respect to the ground (AUC_g). Mean CAR AUC_g are shown in Table 6. Mauchly's test indicated the assumption of sphericity did not apply to condition as there were only 2 intervention levels, however, sphericity was upheld for both Day (X^2 (5) = 2.721, p = .744) and Day * Condition (X^2 (5) = 2.840, p = .726). The mean CAR AUCg was significantly affected by condition (F(1,10) = 6.114, p = .033, ω^2 = .379), but no main effect of day on mean CAR AUC_g (F(3,30) = 1.724, p = .183, ω^2 = .147) or a combined effect of day and condition on CAR AUC_g (F(3,30) = 1.470, p = .243, ω^2 = .128). CAR AUC_g for the PL trial was significantly higher than the CAR AUCg for the WH trial, as reported in Figure 3. In Figure 3, CAR AUC_g data are reflected in pg/dL * hr. This is not to indicate a cortisol concentration per hour, rather the cortisol AUC_g within the hour of sample collection.

Table 6

ANOVA Table – Cortisol Awakening Response

Effect	MS	df	F	p	Greenhouse-Geisser	Huynh-Feldt
Day	71.1	3,30	1.724	.183	.195	.183
Condition	43.7	1,10	6.114	.033*	.033*	.033*
Day*Condition	8.1	3,30	1.470	.243	.248	.243

Note: Variables. Day: Trial days 1-4; Condition: Placebo and Whey. n = 11. *Statistically significant at p < .05.



	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: Cortisol awakening response (CAR) during the first 60 minutes after waking of each day. * Placebo CAR compared to Whey CAR (p = .033). Error bars represent ± SD, n = 11; AUCg is in reference to ground or X-axis.

Profile of Mood States

A two-way (Day x Condition) repeated measures ANOVA was conducted to examine the effect of trial day and intervention condition on POMS as measured by TMD. Mean TMD was calculated as (tension/anxiety + depression + anger/hostility + fatigue + confusion/bewilderment) – vigor. Mean TMD data are shown in Table 7. Mauchly's test indicated the assumption of sphericity did not apply to condition as there were only two intervention levels; however, sphericity was not upheld for both Day (X^2 (5) = 12.739, p = .027), and therefore, Greenhouse-Geisser was used for comparison.

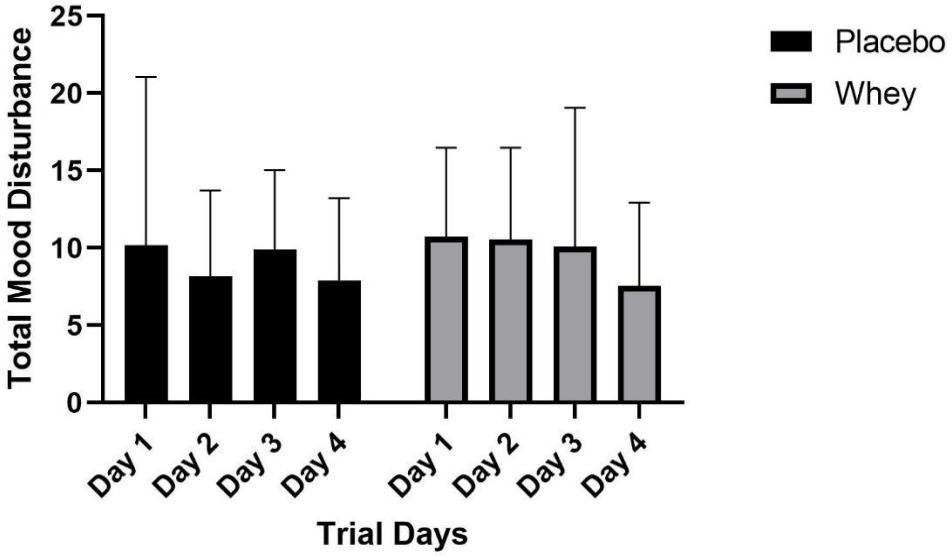
Sphericity was upheld for Day * Condition (X^2 (5) = 10.158, p = .072). The mean TMD was not significantly affected by condition (F(1,10) = .343, p = .571, ω^2 = .033), and additionally no main effect of day on mean TMD (F(3,30) = .244, p = .763, ω^2 = .118) or a combined effect of day and condition on TMD (F(3,30) = 1.470, p = .243, ω^2 = .024). TMD for the PL trial was not significantly higher than the TMD for the WH trial, as reported in Figure 4.

Table 7

ANOVA Table – Profile of Mood States Total Mood Disturbance

Effect	MS	df	F	p	Greenhouse-Geisser	Huynh-Feldt
Day	31.3	3,30	1.336	.281	.285	.284
Condition	10.2	1,10	.343	.571	.571	.571
Day*Condition	7.7	3,30	.244	.865	.763	.802

Note: Variables. Day: Trial days 1-4; Condition: Placebo and Whey. 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 4. Profile of Mood States – Total Mood Disturbance. Note: Profile of Mood States Total Mood Disturbance (POMS TMD) was taken between 30 – 45 min after waking each day. Error bars represent ± SD, n = 11.

Heart Rate Variability

A two-way (Day x Condition) repeated measures ANOVA was conducted to examine the effect of trial day and intervention condition on HRV as measured by rMSSD. Mean rMSSD data are shown in Table 8. Mauchly's test indicated the assumption of sphericity did not apply to condition as there were only two intervention levels; however, sphericity was not upheld for both Day (X^2 (5) = 19.691, p = .002) and Day * Condition (X^2 (5) = 11.800, p = .039), therefore, Greenhouse-Geisser was used for comparison. The mean rMSSD was not significantly affected by condition (F(1,10) =

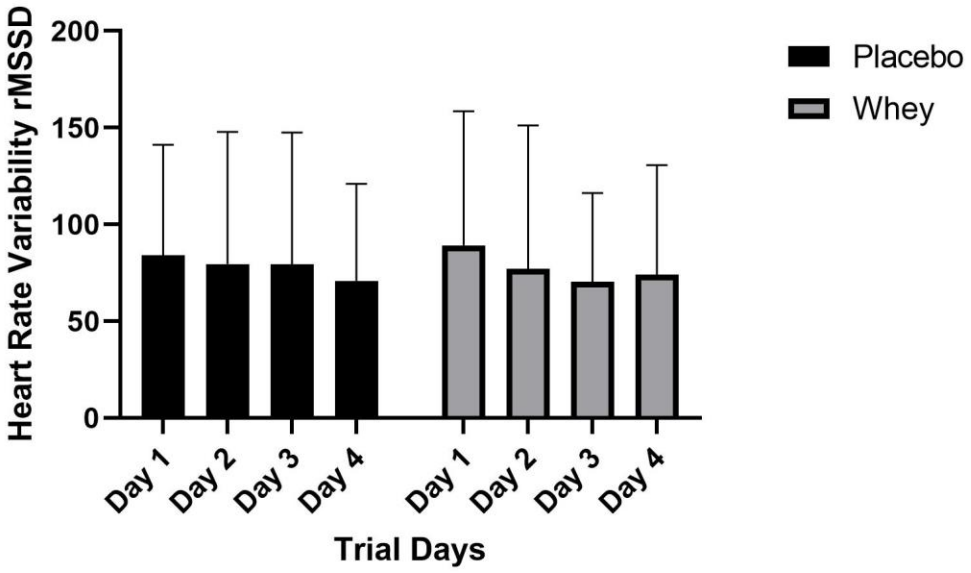
1.345, p = .273, ω^2 = .119), and additionally no main effect of day on mean rMSSD (F(3,30) = .489, p = .552, ω^2 = .047) or a combined effect of day and condition on rMSSD (F(3,30) = .356, p = .716, ω^2 = .034). HRV for the PL trial was not significantly lower than the TMD for the WH trial, as reported in Figure 5.

Table 8

ANOVA Table – Heart Rate Variability rMSSD

Effect	MS	df	F	p	Greenhouse-Geisser	Huynh-Feldt
Day	1336.2	3,30	.489	.692	.552	.569
Condition	624.4	1,10	1.345	.273	.273	.273
Day*Condition	358.9	3,30	.356	.785	.716	.764

Note: Variables. Day: Trial Days 1-4 for each week; Condition: Placebo and Whey. n = 11.



	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 5. Heart Rate Variability Root Mean Sum of Square Difference. Note: Heart Rate Variability root mean square of successive difference (HRV rMSSD) was taken between 15 – 30 min after waking each day. Error bars represent ± SD, n = 11.

Multiple Regression and Correlation Results

A multiple regression analysis was conducted to determine if CAR AUC_g, POMS TMD, and HRV rMSSD could be used to predict FI for each of the two exercise days in each nutritional intervention trial. The multiple regression was conducted in a stepwise manner as Model 1: CAR AUC_g; Model 2: CAR AUC_g + POMS TMD; Model 3: CAR AUC_g + POMS TMD + HRV rMSSD with a dependent variable as FI. Tables 9-12 reflect the four separate multiple regression analyses, e.g., Day and Condition combinations. None of the independent variables reached statistical significance

regarding predictive ability toward FI in either nutritional intervention or on any exercise day specifically.

Table 9

Exercise Day 2 Placebo Multiple Regression Table

Variable	*B*	*SE B*	*β*	*p*	*95% CI*
Step 1					
CAR AUCg	-.097	.134	-.234	.489	[-.401,.207]
Step 2					
CAR AUC_g *	-.069	.178	-.166	.710	[-.480,.343]
POMS TMD	-.123	.475	-.112	.802	[-1.219,.972]
Step 3					
CAR AUCg *	-.049	.191	-.118	.806	[-.501,.404]
POMS TMD*	-.071	.509	-.065	.893	[-1.275,1.133]
HRV rMSSD	.018	.036	.201	.627	[-.066,.102]

Note: Exercise Day 1 Placebo Fatigue Index (FI): Cortisol Awakening Response Area Under the Curve (CAR AUC_g): Profile of Mood States Total Mood Disturbance (POMS TMD): Heart Rate Variability Root Mean Square Successive Differences (HRV rMSSD). R^2 = .06 for Step 1: ΔR^2 = .01 for Step 2: ΔR^2 = .03 for Step 3. CI = confidence interval.

Table 10

Exercise Day 3 Placebo Multiple Regression Table

Variable	*B*	*SE B*	*β*	*p*	*95% CI*
Step 1					
CAR AUC_g	-.119	.120	-.313	-.348	[-.390,.153]
Step 2					
CAR AUC_g *	-.067	.110	-.176	.561	[-.321,.187]
POMS TMD	-.672	.364	-.537	.102	[-1.512,.167]
Step 3					
CAR AUC_g *	-.097	.119	-.254	.445	[-.378,.185]
POMS TMD*	-.755	.388	-.603	.093	[-1.672,.162]
HRV rMSSD	-.024	.030	.253	.458	[-.094,.047]

Note: Exercise Day 2 Placebo Fatigue Index (FI): Cortisol Awakening Response Area Under the Curve (CAR AUC_g): Profile of Mood States Total Mood Disturbance (POMS TMD): Heart Rate Variability Root Mean Square Successive Differences (HRV rMSSD). R^2 = .10 for Step 1: ΔR^2 = .27 for Step 2: ΔR^2 = -.10 for Step 3. CI = confidence interval.

Table 11

Exercise Day 2 Whey Multiple Regression Table

Variable	B	*SE* B	β	p	95% CI
Step 1					
CAR AUC_g	-.094	.102	-.293	.382	[-.326,.138]
Step 2					
CAR AUC_g *	-.096	.110	-.298	.408	[-.349,.157]
POMS TMD	-.037	.354	-.036	.919	[-.853,.779]
Step 3					
CAR AUC_g *	-.102	.121	-.318	.427	[-.389,.185]
POMS TMD*	-.032	.378	-.031	.935	[-.926,.862]
HRV rMSSD	-.009	.046	-.074	.849	[-.119,.100]

Note: Exercise Day 1 Whey Fatigue Index (FI): Cortisol Awakening Response Area Under the Curve (CAR AUC_g): Profile of Mood States Total Mood Disturbance (POMS TMD): Heart Rate Variability Root Mean Square Successive Differences (HRV rMSSD). R^2 = .09 for Step 1: ΔR^2 = .001 for Step 2: ΔR^2 = .005 for Step 3. CI = confidence interval.

Table 12

Exercise Day 3 Whey Multiple Regression Table

Variable	B	*SE* B	β	p	95% CI
Step 1					
CAR AUC_g	-.076	.103	-.239	.479	[-.309,.157]
Step 2					
CAR AUC_g *	-.070	.098	-.221	.492	[-.295,.155]
POMS TMD	-.312	.218	-.438	.191	[-.815,.191]
Step 3					
CAR AUC_g *	-.067	.108	-.210	.554	[-.321,.187]
POMS TMD*	-.310	.233	-.437	.225	[-.861,.241]
HRV rMSSD	-.004	.029	.045	.899	[-.065,.073]

Note: Exercise Day 1 Whey Fatigue Index (FI): Cortisol Awakening Response Area Under the Curve (CAR AUC_g): Profile of Mood States Total Mood Disturbance (POMS TMD): Heart Rate Variability Root Mean Square Successive Differences (HRV rMSSD). R^2 = .06 for Step 1: ΔR^2 = .19 for Step 2: ΔR^2 = .002 for Step 3. CI = confidence interval.

Additionally, Pearson correlation analyses were conducted to determine the association between CAR AUC_g, POMS TMD, and HRV rMSSD and FI on each of the two exercise days during both nutritional intervention trials. Results of the Pearson

correlation indicated that there was no significant association between PL exercise day 1 fatigue index and CAR AUC_g (r = -.234, p = .245), POMS (r = -.212, p = .265), or HRV rMSSD (r = .271, p = .210). Results of the Pearson correlation indicated that there was no significant association between PL exercise Day 2 fatigue index and CAR AUC_g (r = -.313, p = .174) or HRV rMSSD (r = .047, p = .446); however, a significant moderate negative association with POMS (r = -.582, p = .030) was determined. An r = -.582 indicated as POMS increased, FI decreased. Results of the Pearson correlation indicated that there was no significant association between WH exercise Day 1 fatigue index and CAR AUC_g (r = -.293, p = .191), POMS (r = .006, p = .493), or HRV rMSSD (r = .012, p = .486). Results of the Pearson correlation indicated that there was no significant association between WH exercise day 2 fatigue index and CAR AUC_g (r = -.239, p = .239) or HRV rMSSD (r = .118, p = .365); however, while not statistically significant POMS (r = -.447, p = .084) exhibited a weak negative association with FI similar to Day 2 exercise in the PL trial.

CHAPTER V

DISCUSSION AND SUMMARY

Major Findings

The ability of a person to recover from stress related to repeated strenuous physical exercise has been measured independently by CAR, POMS, HRV, and FI, but never all four variables at once. Additionally, whey protein has been shown to affect recovery. The purpose of this study was to (1) determine if whey protein supplementation would positively affect CAR, POMS, HRV, and FI, and (2) determine the ability of CAR, POMS, and HRV to predict FI performance. Disruptions in HPA feedback pathways have been linked to changes in variability of HRV (Spyer, 1989), changes in attention and affect, e.g., POMS (Masterman & Cumming, 1997), and changes in CAR (Stadler et al., 2011).

The major finding of this study were: (1) whey protein supplementation (WH) reduced CAR AUC_g compared to placebo (PL); (2) POMS and HRV responses were not significantly different in either PL or WH; (3) FI was not significantly different in either PL or WH; neither CAR, POMS, nor HRV had the ability to significantly predict FI; and (4) only POMS on exercise Day 3 had a statistically insignificant strong negative correlation to changes in FI.

CAR

The physiological purpose of cortisol is to increase availability of metabolic substrates that will allow increased capacity both during a physical or psychological stress, recovery from said stressors, and as a preparatory boost to the metabolic demands

of waking (Anderson and Wideman, 2017; Clow et al., 2010). The hippocampus has been associated with helping to regulate CAR, as well as acute cortisol responses to stress (Anderson and Wideman, 2017). The association with the hippocampus, which might normally play an inhibitory role regarding HPA axis functioning, may actually play more of a role just prior to waking (Clow et al., 2010). Clow et al. (2010) identified a separation of ACTH secretion and cortisol secretion in subjects that had been forewarned of an early waking time versus those whom were awakened early by surprise. The anticipatory rise in ACTH was not accompanied by a rise in cortisol in the forewarned group. Frokjaer et al. (2013) tied the serotonin transporter gene (SERT) in the pre-frontal cortex to changes in CAR, indicating increases in CAR may be associated with decreased HPA axis inhibitory control, therefore hippocampus suppression, in individuals with mood disorders. Roa et al. (2013) agreed with Frokjaer et al. (2013) indicating a HPA dysregulation associated with Cushing's Syndrome was highly negatively correlated (r = -0.8, $p < .0001$) to a blunted or suppressed CAR.

The results of the current study indicate a lower CAR response across the 4 days of the experimental trial in the WH group compared to the PL group. The original assumptions by the researchers were that a reduced CAR would be an indication of reduced stress, however this is not the case. Literature regarding shifts in CAR as a response to exercise ranges from CAR suppression to CAR elevation to no changes in CAR. Anderson et al. (2018) reported a significant increase in CAR ($r^2 = .352$, $p = .025$) when training load increased in recreational athletes. Ulhoa et al. (2011) reported a higher CAR in shift workers during work days compared to off days. Additionally,

irregular-shift workers (time and length of shifts were unpredictable), had higher CAR during off days. Ulhoa et al. (2011) concluded that shift workers were able to compensate for same day stressors with elevated CAR, whereas irregular-shift workers were compensating for the previous day stressors. Even longitudinal studies, such as Drogos et al.'s (2019), reported increases in CAR over a 6-month exercise intervention. The researchers concluded that the changes in CAR pre- versus post-intervention were due to the ability of subjects to handle the physiological stress as well as the psychological stress of the exercise session, as evidenced by a strong correlation to reduced perceived stress ($r = .52$, $p = .023$), acting as a stress buffer. Labsy et al. (2013) reported no differences in next day CAR after exercising in the morning or afternoon, even after a significant ($p < .01$) rise in acute cortisol after exercise, indicating timing of exercises was not a mitigating factor in CAR. Additionally, Jacobson and Sapolsky (1991) reported a higher hippocampal type II corticosteroid receptor occupancy after a high degree of physiological stress, e.g., exercise, leading to a negative feedback loop with the HPA axis, thereby suppressing cortisol secretion. It can be speculated, therefore, that glucocorticoid secretion was higher somehow during the WH trial week; however, no cause and effect relationship between WH and a suppressed CAR can be directly measured by the current study.

If CAR can increase, decrease, or show no change due to exercise bouts or physical stressors on the prior day, then dietary intake may play a role in differences in CAR responses. In the current study, no significant differences in mean daily caloric intake, including the addition of the CHO placebo or the CHO and WH was present.

Mean daily intake for the PL was 1786 kcal and 1656 kcal for the WH group. However, research regarding dietary effects on CAR is limited, where the majority of work focuses on acute supplementation and acute cortisol responses. A few studies however address associations with CAR and diet. Hucklebridge et al. (1999) reported that subjects with higher fasting glucose (5.1 ± 0.1 mmol/L) had significantly lower CAR ($p < .001$) than the lower fasting glucose group (3.8 ± 0.2 mmol/L). The researchers concluded that the compensatory suppression of CAR was tied to the higher resting blood glucose levels upon waking. Soltani et al. (2019) supported Hucklebridge et al. (1999) indicating 8 wks of increased carbohydrate intake resulted in lower CAR compared to low or average CHO intake in either DGA groups and or the TAD. Soltani et al. (2019) concluded that the increased dietary CHO was significant in reducing CAR as well as psychological stress. These findings would seem to be in line with common convention as to the interaction between blood glucose levels, CAR, and the waking cycle, where acute cortisol and CAR are inversely related to blood glucose concentrations (Anderson et al., 2016; Hucklebridge et al., 1999; Melmed, 2015). Additionally, a higher CAR has been associated disordered eating patterns, where blood glucose concentrations were elevated, in young women (Filaire et al., 2015).

It is possible then that the intake of whey protein isolate had a specific effect of blunting the CAR compared to the placebo group. Research in this area is limited to a few studies. Sheikh, Dougherty, Hayden, Klein, and Singh (2010) point to a potential connection between glucagon-like peptide-1 receptor (GLP-1) where increased levels of GLP-1 secretion, caused by increased whey protein isolate intake (Chen & Reimer, 2009)

can lead to increased cortisol secretion. This increased cortisol secretion upon dosing with WH could have elevated cortisol concentrations during the day, and potentially creating a negative feedback loop for blunting CAR the next day (Mondelli, et al., 2010). Another hypothesis is that Trp played a role in reducing the CAR. Trp in the brain acts as a precursor to serotonin (5-HT). Intake of dietary Trp increases 5-HT activity and has been linked to central fatigue, and the ingestion of BCAAs, which compete for Trp brain transporters, may help mitigate the fatigue (Hall et al., 1995).

Since whey protein isolate contains BCAAs, ingestion of WH should reduce Trp uptake into the brain by increasing concentrations of BCAAs in the blood. Although not measured in the current study, the Trp hypothesis has some support. Vielhaber et al. (2005) used a Trp depletion (TD) treatment compared to a sham depletion (SD) treatment in patients with a history of obsessive compulsive disorder. CAR AUC in the TD group (38.22 ± 16.01 mmol/L*hr) were significantly lower than the SD group (63.55 ± 44.70 mmol/L*hr), $p = .033$. Therefore, the SD group that received the Trp had higher CAR. In the current study, if BCAAs were actually transported in the brain, a possible reduction in Trp in the brain may have resulted in lower CAR. Capello and Markus (2014) reported similar results after 7 days of Trp administration to 118 college age men and women (24 ± 2 yrs) where the Trp group had significantly lower CAR (6.26 ± 0.42 mmol/L) compared to the placebo group (12.47 ± 1.03 mmol/L), $p = .023$. Again, while not measured, Trp may have reduced CAR in the WH trial compared to PL group in the current study.

POMS

Many studies have identified the associations between acute changes in POMS and acute changes in cortisol concentrations, changes in exercise intensity and or training load, and acute or prolonged dietary interventions (Corrado, 2017; Maroulakis & Zervas, 1993; O'Connor et al., 1989; Verde et al., 1992). The cumulative findings of these studies purport that exercise has positive physiological and psychological effects as evidenced by a reduction in TMD, decreases in anxiety and tension sub-scales of POMS, and increases in vigor sub-scales of POMS. Verde et al. (1992) specifically note that when training load increases, even by as little as 38% increase over 3-weeks in trained distance runners, an increase in global (total) mood disturbance is seen as a negative psychological outcome, specifically in areas of increased fatigue. Therefore, it was important for the current study to identify any deleterious effects of the aerobic and WAnT exercise bouts on the recreationally active female participants POMS TMD. Statistical analysis revealed no significant difference between the two intervention trials in either day (p = .763) or in experimental condition (p = .243). Since no significant differences occurred, it may be concluded that the exercise trials themselves and the dietary interventions, did not cause significant changes in POMS TMD, either positively or negatively. It should be noted that the individual sub-scales were not analyzed in the current study and are of interest for future research to identify possible associations between POMS subscales and performance indicators such as FI.

One way of exploring the results for POMS TMD in the current study is to compare results of CAR and POMS TMD and identify a common relationship. Although

the current study did not run statistical analyses of correlation between the two variables, which may be warranted in future studies, patterns emerged that are congruent with current research data. Oshima et al. (2017) reported similar stability of POMS TMD to the current study results over a 9-day high volume training camp for college age Japanese football athletes. While the researchers reported significant increases in basal cortisol levels as the camp proceeded ($p = .003$), none of the POMS scores were significantly different ($p > .05$). Broodryk et al. (2017) reported similar results in female soccer players after repeated anaerobic fatiguing tests. Even though POMS TMD increased significantly post-exercise compared to pre-exercise ($p < .01$) and acute cortisol increased significantly ($p < .001$), the two variables showed no relationship ($r = -0.1$, $p > .05$). More closely related to the current study data regarding CAR, Powell and Schlotz (2012) reported no association ($b = 46.8$; $SE = 58.8$; $p = .44$) between CAR and the Anticipatory Stress Questionnaire (ASQ), a similar psychological index to POMS. The researchers concluded that a higher CAR was a physiologically favorable response to attenuate distress. Another explanation regarding no change in POMS TMD centers on the participants' provision of "desirable" answers on the POMS questionnaire. Casolino et al. (2012) reported no significant difference in mood in taekwondo athletes during a selection camp, indicating a positive iceberg profile, whereby athletes show low levels of tension, depression, anger, fatigue, and confusion, but elevated levels of vigor. While not specifically measured in the current study, this hypothesis of "desirable" answers on the POMS questionnaire could explain the lack of difference between the intervention trials and days.

While no change in the POMS TMD was a common result, decreases and increases in POMS TMD and POMS sub-scales have been associated with changes in CAR. Hough et al. (2013) reported a suppressed acute cortisol response, after a 143% increase in training load over 11 days of intense cycling bouts, coupled with increased burnout and fatigue scores on the Recovery-Stress Questionnaire (REST-Q). The researchers concluded that the increased training load was too severe as evidenced by a blunted cortisol response and negative psychological indices. MacDonald and Wetherell (2019) reported that increased cognitive anxiety on competition days in elite rowers resulted in reduced or blunted CAR. The researchers concluded that the increase in anxiety regarding competition manifested physiologically in a blunted CAR. Shibuya et al. (2014) reported positive correlations between increased CAR and POMS sub-scales. Tension ($r = 0.418$, $p < .05$) and fatigue ($r = .482$, $p < .05$) sub-scales both increased as CAR values increased during exposure to everyday junior high and high school settings. Diaz et al. (2013) reported similar connections between CAR and POMS in swimmers comparing control days to competition days, where Day 2 of competition CAR AUCg post-competition levels (142.7 ± 40.2) were significantly higher than pre-competition levels (65.1 ± 37.7), as well as Day 1 pre-competition levels (93.3 ± 30.8) and post-competition levels (156.2 ± 3.0). Associated Day 1 and Day 2 POMS TMD at CAR time-points were 21.0 and 17.2, respectively. Day 1 and Day 2 Pearson correlation data were $r = .71$, $p < .05$ and $r = .69$, $p < .05$, respectively. The researchers concluded that the changes in CAR were positively associated with changes in POMS TMD. Additionally, Schulz et al. (1998) concluded that chronically stressed individuals had

significantly higher cortisol after waking compared to individuals with low stress environments.

There is some evidence, as presented earlier, that dietary intake may play a role in changes in POMS and mood states. Markus et al. (2008) reported significant improvements in TMD at 60 min and 210 min after ingesting hydrolyzed protein compared to pure Trp, casein protein, Trp-containing synthetic peptide, or alpha-lactalbumin. The most durable improvements in TMD were reported at 210 min. Sihvola et al. (2013) assessed NASA Task Load Index (NASA-TLI) performance compared to salivary cortisol, plasma Trp, and POMS, given a high protein, high carbohydrate, or control breakfast drink. While no significant cortisol concentrations were reported ($p > .05$), a significantly higher sense of well-being compared to placebo and high carbohydrate drinks was reported for the high protein drink ($p = .028$). The researchers concluded the increase in Trp to large neutral amino acid ratio accounted for the difference in mood state, but was not associated with greater performance on the NASA-TLI.

HRV

HRV is a widely accepted means of assessing parasympathetic reactivity to physical exercise, which can be affected by age, gender, central fatigue, physical fitness, and psychological states (Djaoui et al., 2017). Resnick (2017) indicated HRV could characterize changes in parasympathetic modulation that occur with normal training and distinguish those changes from abnormal shifts in vagal tone due to overtraining, e.g., a shift toward sympathetic dominance. Sandercock et al. (2005) reported positive changes

in HRV and vagal tone as a result of training in healthy individuals, when rest periods were applied appropriately. The results of the current study revealed no significant difference between the PL or WH trials, nor any significant differences between days of each trial. While there was a decline in HRV as measured by rMSSD, from 84.15 ± 57.02 to 70.97 ± 50.04 during the PL week and from 89.17 ± 69.40 to 74.15 ± 56.58 during the WH week, the decline was not statistically significant (all $p > .05$). Additionally, the SD for each day and week was very high, indicating the inherent variability within HRV analysis. Possible explanations for the lack of significant change in HRV are as varied as the individual responses themselves.

Level of physical fitness of the participants and exercise intensity level are two areas that have been explored in the literature. Soares-Miranda et al. (2009) reported that participants who were active generally had higher vagal modulation compared to less active participants, and that there was a positive dose response relationship between vigorous physical activity and vagal modulation, e.g., vigorous physical activity is necessary to gain the parasympathetic outflow benefits. The current study employed an aerobic treadmill bout averaging 68.20 ± 3.4% and 68.68 ± 3.6% of VO_{2peak} during the PL and WH weeks, in addition to an "all-out" WAnT bout. Hackney (2006) reported a critical threshold of 50-60% of VO_{2max} to elicit an acute cortisol response and longer durations would further augment the response. Therefore, it was concluded that the level of physical activity during the two days of exercise each week was vigorous enough to elicit a response from the HPA axis. McCartney et al. (2009) support the methodology, indicating the more intense the WAnT bout, referring to the number of consecutive bouts,

the greater the change in R-R intervals for HRV. Had the current study employed a multiple bout WAnT protocol, it is possible a change in FI could have resulted. Stanley et al. (2013) reported, however, that the greatest changes in HRV would be seen in participants with the lowest level of fitness, e.g., sedentary, prior to testing. It is possible then that the participants in the current study were of moderate fitness from a parasympathetic standpoint, and therefore, were able to complete the exercise bouts without much parasympathetic modulation the next day; however, with an average VO_{2max} of 32.32 ± 4.64 ml/kg/min, the ACSM *Guidelines for Exercise Testing and Prescription* (Riebe et al., 2018) would classify participants as having poor cardiovascular health.

The use of whey protein isolate and carbohydrates as an interaction with HRV is somewhat limited. Lima-Silva et al. (2010) reported no difference in rMSSD between short-term low carbohydrate and high carbohydrate diets; however, there was significantly higher low-frequency to high-frequency ratio in the low carbohydrate diet. The researchers concluded a possible modification of autonomic control during the low carbohydrate diet, as evidence by lower catecholamine concentrations, e.g., epinephrine, norepinephrine, and dopamine were all lower in the low-CHO versus high-CHO group. Jaatinen et al. (2014) reported higher baseline HRV, e.g., more variable, when participants consumed casein and B vitamin supplementation compared to controls. Young et al. (2017) reported consuming an unhealthy diet was associated with a reduced HRV and shorter R-R intervals. It is possible therefore that the dietary supplementation could have played a role in the HRV, but not likely as reported in the statistical analyses.

The significantly higher CAR response by participants in the PL group could have been associated with changes in HRV. Stalder et al. (2011) reported that it is possible that the cardiovascular system can be associated with the awakening response as a shift from parasympathetic to sympathetic dominance. The researchers reported however no significant association between CAR and HRV at either S1 or AUC concentrations. These findings are congruent with the findings of the current study. POMS has also been associated with changes in HRV. Weinstein et al. (2007) associated reductions in HRV with increased negative mood and depression in participants who were asked to withdraw from exercise after being regular exercise participants for 6 months. The researchers concluded that the mood changes may corroborated by parasympathetic changes. Wallace et al. (2014) reported small correlations in POMS ($r = -0.12 \pm 0.31$, $p > .05$) with fatigue modeling in trained runners, but high beat-to-beat correlation to predicted fatigue ($r = -0.54 \pm 0.31$, $p < .05$). No other HRV parameters had significant association to training load perception or fatigue prediction. Wallace et al. (2014) concluded HRV, but not POMS, may be useful in monitoring fatigue in trained athletes.

Lastly, OC usage could have played a role in HRV; however, Rebelo et al. (2011) reported no significant difference in HRV components comparing OC users to non-users. These findings align with the current study data.

Fatigue Index (FI)

The WAnT has been used in many studies to determine anaerobic capacity of an individual, but fewer studies have investigated the role of central and peripheral fatigue in conjunction with WAnT performance. The current study hypothesized that POMS, HRV,

and CAR would be significantly correlated to FI, as these variables have been associated with fatigue (Baker et al., 2006). The results of the current study failed to support the hypothesis, where there was no significant difference in the PL or WH mean FI for Day 2 or Day 3, respectively (PL: 20.79 ± 6.12%; 21.97 ± 6.16% and WH: 22.52 ± 6.45%; 22.78 ± 6.38%). Additionally, there was no significant correlation between FI and POMS, HRV, or CAR; however, Day 3 POMS compared to Day 3 FI (r_s = -.552, p = .078), was the closest correlation. Ranges for the FI fell within ranges reported in other studies. Zupan et al. (2009) reported mean FI at 42 ± 7.9%, with a range of 16% to 61%, in NCAA collegiate female athletes. Yet although ranges for FI were within somewhat comparable data sets, it does not explain why there was no significant difference between the PL and WH trials. One of the main reasons for using WH as an intervention was to help identify the role whey protein isolate may play in reducing central fatigue. Baker et al. (2006) reported that 5-HT activity in the brain was not changed by the transport of free tryptophan into the brain. After intense cycle ergometer bouts, blood levels of 5-HT had returned to near baseline, when they had fallen sharply immediately post-exercise. Baker et al. (2006) concluded that 5-HT may not in fact play a role in central fatigue. Additionally, Davies, Carson, and Jakeman (2018) reviewed the effects of whey protein on temporal recovery after resistance training. The researchers concluded whey protein doses administered pre-exercise, as in the case of the current study, may enhance recovery when compared to post-exercise ingestion. Rindom et al. (2016) compared the effect of whey protein (WP) and collagen protein (CP) on peak anaerobic power in

resistance trained males. Rindom et al. (2016) reported no difference in FI based on WP or CP.

Similarly, Hansen et al. (2016) reported no significant improvements in performance, as measured by peak power, between CHO and CHO-PRO, in elite cyclists. Jaafar, Rouis, Attiogbe, Vandewalle, and Driss (2016) reported that a 10% of body weight load would be more appropriate in recreationally trained individuals. Some of the earliest work on WAnT load optimization reported optimal loads around 8.5% body weight for female participants (Dotan & Bar-Or, 1983). Jaafar et al. (2016) also reported that changes in FI were independent of load when expressed relative to PP. It is also possible to conclude that the WAnT load applied in the current study was not high enough to elicit an FI deficit difference as hypothesized.

Estradiol

The inclusion of female participants in research studies involving physiological pathways has typically been during the follicular phase of the menstrual cycle, which as a purely scientific controllable variable seems both logical and prudent. It is, however, not practical nor representative of the hormonal concentrations in which women exercise and or compete in sport (Frisén, 2016). Oosthuyse and Bosch (2010) reviewed the effect of menstrual cycle on exercise metabolism and concluded that menstrual phase can ultimately be confounded by individual variability between subjects and day-to-day variations within subjects. It is for this very reason the current study included estradiol OC as an inclusion criterion for participants; however, it is conceded by Oosthuyse and

Bosch (2010) that the actual menstrual phase of participants was not recorded, and therefore, could have had some confounding effect on dependent variables.

While the question of hormone variability may have been addressed, or at least partially addressed, the interaction with the critical variables in the current study warrants attention. Gozansky et al. (2005) reported the association between salivary and serum cortisol concentrations, after exercise, were statistically strengthened when participants were on OC ($r = .75$, $p < .001$) compared to participants not taking OC ($r = .67$, $p < .001$). Pruessner et al. (1997) reported that subjects taking OC had smaller CAR (p = .10); however, the Cohen's *d* effect size ($f^2 = .05$, $\omega^2 = .04$) indicated OC only accounted for 4% of the early morning rise in cortisol. Wolfram et al. (2011) reported there was no change in CAR and POMS across the four cycle phases, with the exception of a slightly higher CAR during ovulation (11.51 ± 9.45 nmol/L; $p = .05$). Teixeira et al. (2015) reported stable HRV across low hormone (LH) and high hormone (HH) phases when participants were taking OC. Teixeira et al. (2015) concluded the constant estrogen levels were responsible for the stability. The only exception to the stability was in rMSSD where levels dropped from 67.8 ± 8.6 during LH to 57.9 ± 4.9 during LH ($p = .08$), indicating a potentially clinically relevant variable. Since the current study did not directly measure estradiol concentrations, the current researcher assumed based on past literature data that OC would have little impact on the experimental variables, especially in a repeated measures experimental design. The lack of menstrual phase information could have a contributing factor in the statistical similarities of the 2 intervention weeks.

Without confounding variable data for statistical analysis, the current study failed to follow through with this potential statistical issue.

Summary

This is the first study to investigate CAR, POMS, HRV, and FI is the context of the effects of whey protein isolate. Furthermore, even fewer studies have included women as a research subject comparing the interactions of CAR, HRV, POMS, and FI. As demonstrated by a decrease in CAR across the 4 days of the whey protein isolate trial when compared to placebo, a possible association between central fatigue, HPA axis, and strenuous exercise may have some interaction. However with no effect on the short-duration sprint cycling performance, any association with reducing the physiological effects of central fatigue may be minimal.

Limitations

The current study findings can only be applied to aerobic protocols on 2 consecutive days at 70-75% VO_{2max}, followed by a single WAnT. Alternating recovery days or greater than 2 consecutive exercise days may alter the results of the stress responses. Additionally, since participants were not able to maintain the 70-75% VO_{2max} range during the 30 min treadmill bouts, the exercise bout may not have been significant enough to induce fatigue related to exercise. Dosing of supplements is an equal dose regardless of body weight. Doses relative to body weight may alter HRV, CAR, and POMS results. Additionally, since the PL and WH doses were not isocaloric, as utilized in numerous studies, some variability may have been introduced regarding dietary intake of macronutrients. All participants underwent exercise bout between 2 pm and 5 pm as a

controlled time for typically low levels of cortisol. However, no baseline cortisol levels were obtained immediately prior to exercise, nor were diurnal patterns of cortisol established to verify the cortisol trough. No menstrual phase data were collected, nor was there a non-OC control group for comparison, potentially leading to confounding variables not accounted for statistically.

Recommendations for Future Study

Studies in the future may address some of the limitations of the study. One such study may utilize a diurnal cortisol pattern to tease out effects of exercise across the cortisol secretory patterns of the day. Additionally, since the current study only involved female participants, addressing male participant response pattern may serve to compare gender differences in response to the exercise bouts. As the current study also limited female participants to those actively taking prescribed OC, the use of a non-OC control group could prove interesting. Lastly, as Trp was not measure in this study, determination of concentrations immediately post-exercise may clarify the interaction of BCAA and Trp, and their role in fatigue and readiness.

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Appendix A

Institutional Review Board Approval Letters

Texas Woman's University
Institutional Review Board (IRB)
irb@twu.edu
https://www.twu.edu/institutional-review-board-irb/

May 2, 2019

Michael Oldham

Health Promotion & Kinesiology

Re: Initial - IRB-FY2019-197 The Effect of Branched Chain Amino Acid and Whey Protein Isolate Supplementation on Cortisol Awakening Response, Profile of Mood States, and Heart Rate Variability in Recreationally Active Women

Dear Michael Oldham,
The above referenced study has been reviewed at a fully convened meeting by the TWU IRB - Denton operating under FWA00000178 and approved on May 1, 2019. If you are using a signed informed consent form, the approved form has been stamped by the IRB and uploaded to the Attachments tab under the Study Details section. This stamped version of the consent must be used when enrolling subjects in your study.

Note that any modifications to this study must be submitted for IRB review prior to their implementation, including the submission of any agency approval letters, changes in research personnel, and any changes in study procedures or instruments. Additionally, the IRB must be notified immediately of any adverse events or unanticipated problems. All modification requests, incident reports, and requests to close the file must be submitted through Cayuse.
Approval for this study will expire on April 30, 2020. A reminder of the study expiration will be sent 45 days prior to the expiration. If the study is ongoing, you will be required to submit a renewal request. When the study is complete, a close request may be submitted to close the study file.

If you have any questions or need additional information, please contact the IRB analyst indicated on your application in Cayuse or refer to the IRB website at http://www.twu.edu/institutional-review-board-irb/.

Sincerely,

TWU IRB – Denton

Texas Woman's University
Institutional Review Board (IRB)
irb@twu.edu
https://www.twu.edu/institutional-review-board-irb/

June 18, 2019

Michael Oldham
Health Promotion & Kinesiology

Re: Modification - IRB-FY2019-197 The Effect of Whey Protein Isolate Supplementation on Cortisol Awakening
Response, Profile of Mood States, and Heart Rate Variability in Recreationally Active Women

Dear Michael Oldham,

The modifications listed below have have been reviewed and approved on June 15, 2019 by theTWU IRB - Denton. If you made changes to your consent form, the newly approved form has been restamped by the IRB and uploaded to the Attachments tab under the Study Details section. This stamped version of the consent must be used when enrolling subjects in your study.

Modifications:

1. Change in Title : FROM: "The Effect of Branched Chain Amino Acid and Whey Protein Isolate Supplementation on Cortisol Awakening Response, Profile of Mood States, and Heart Rate Variability in Recreationally Active Women" TO: "The Effect of Whey Protein Isolate Supplementation on Cortisol Awakening Response, Profile of Mood States, and Heart Rate Variability in Recreationally Active Women"

2. Primary Changes:
(1) Remove branched chain amino acid condition from the nutritional supplementation intervetion to simplify the effects of the supplementation and exercise trial interactions.
(2) Whey protein isolate supplement changed to whey protein isolate (25g) + maltodextrin (25g) at 200 kcal to lessen the insulin response on participants.
(3) Placebo supplement changed to 25g of maltodextrin at 100 kcal to reduce overall caloric effect of placebo.

3. Secondary Changes:
(1) Screening protocol / baseline VO2max testing changed from a modified Bruce ramp protocol to a standard Bruce protocol to reduce maximal testing time on participants.

(2) Changed inclusion criteria:
(a) Participants MUST be taking physician prescribed oral contraceptives to ensure standardized estradiol levels

(b) Removal of ability to consume BCAA + maltodextrin due to analyte being removed from the study

(3) Changed exclusion criteria:
(a) Participants not taking physician prescribed oral contraceptives may not participate - to ensure standardized estradiol levels
(b) Removal of ability to consume BCAA + maltodextrin due to analyte being removed from the study
(4) Estradiol removed as an analyte variable / statistical covariate due to participants being on prescription OC.
(5) Time commitment change: Only 2 1/2 weeks of testing totaling 14 hours.
(6) Changed requirement of refraining from caffeine, sexual activity, and exercise (other than testing protocol) from 24 hours prior to 48 hours prior AND during the 4 day testing cycle to further eliminate influence of these activities and behaviors on experimental data.
(7) Statistical techniques change to:
(a) Two-way repeated measures MANOVA comparing Day to Supplement Intervention for POMS, CAR, and HRV
(b) Two-way repeated measures ANOVA comparing Day to Supplement Intervention for Fatigue Index
(c) Multiple Regression analysis of nutritional intervention on fatigue index, then POMS, HRV, and CAR added in a stepwise manner.
(8) Change research questions/hypotheses -
(a)Does whey protein isolate, given prior to repetitive day exercise bouts, effect CAR, HRV, and or POMS total mood disturbance, compared to a placebo?
Hypothesis: Whey protein isolate will lower CAR, increase HRV, and decrease POMS total mood disturbance, significantly more than placebo trial.
(b) Will the effect of whey protein isolate effect CAR, HRV, and or POMS total mood disturbance, to a greater extent on the day after the first or second day of exercise?
Hypothesis: 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.
(c) How will POMS, HRV, and CAR predict fatigue index on either the first or second day of exercise? Hypothesis: POMS, HRV, and CAR will be significant predictors of fatigue index on the second day of exercise.

If you have any questions or need additional information, please contact the IRB analyst indicated on your application in Cayuse or refer to the IRB website at http://www.twu.edu/institutional-review-board-irb/.

Sincerely,

TWU IRB - Denton

Appendix B

Participant Recruitment Material - Email Recruitment Script

The Effect of Whey Protein Isolate Supplementation on Cortisol Awakening Response, Profile of Mood States, and Heart Rate Variability in Recreationally Active Women

Good Morning/Afternoon,

My name is Michael Oldham and I'm inviting you to take part in a research study. I am an Ad Interim Instructor here at Texas A&M University-Commerce, in the Health and Human Performance Department, and a doctoral student at Texas Woman's University. The purpose of this study is to determine your body's next day response to exercise stress, by analyzing a hormone related to stress called cortisol, and determine if nutritional supplements help with that response. I'm looking for females, who don't exercise very much currently, to participate in the study.

Your part in the study will be to run or walk on a treadmill for 30 minutes, rest 5 minutes, and then perform a 30 second all-out cycling trial to measure your peak power, at the Exercise Physiology Lab – Texas A&M University - Commerce. You will collect a small amount of saliva on the day before, on 2 exercise days, and the day after the exercise bout, at your home. Saliva collection will be at 5 time points: immediately when you wake up, 15, 30 and 45 minutes after waking up, and lastly 60 minutes after waking up. I will also provide you with nutritional supplements, at no cost to you during the study, for you to consume on specific days during the exercise trial periods.

It will take you about 1 hour on the pre-exercise day, 2 hours on each exercise day, and 1 hour on the post-exercise day, for a total of 4 hours each week. The study will last approximately three and one-half weeks. For participating in the study, you will be compensated $75 in gift cards, distributed as $25 at the beginning of the study, and $50 for completing the study. Participating in this study will help us get a better understanding of your ability to perform exercise on repeated days. Your participation in this study is VOLUNTARY, and you may leave the study at any time.

You may stop by the Health and Human Performance office, Field House 100, to setup a time to discuss your participation or email me directly at michael.oldham@tamuc.edu. You may also contact Dr. Vipa Bernhardt with questions about the study at vipa.bernhardt@tamuc.edu.

Thank you for your time today!
Michael Oldham
Doctoral Candidate – Texas Woman's University
Ad Interim Instructor – Texas A&M University – Commerce

Confidentiality Risk Statement:
There is a potential risk of loss of confidentiality in all email, downloading, electronic meetings, and internet transactions.

Appendix C

Participant Screening Form

Subject Number:_____ Age:_____
Height (cm):_____ Weight (kg):_____
Informed consent signed? Yes No
ACSM PAR-Q+ Completed

Cardiovascular, pulmonary, or metabolic diseases? Yes No
ACSM diseases or disabilities? Yes No

Are you able to refrain from caffeine during, and 48 hours prior to, testing days?
Yes No
Are you able to refrain from alcohol during, and 48 hours prior to, testing days?
Yes No
Are you able to refrain from sex during, and 48 hours prior to, testing days?
Yes No
Are you able to refrain from exercising during, and 48 hours prior to, testing days?
Yes No
Are you taking or prescribed any anti-Depressant medications? Yes No

Are you taking or prescribed anti-hyperactivity medications? Yes No
Are you pregnant? Yes No

Are you clinically diagnosed as having hypothyroidism? Yes No

Do you exercise more than 80 minutes a week or less than 30 minutes, and or are a trained athlete?
Yes No
Are you allergic to whey protein or experience gastrointestinal distress (i.e. bloating, flatulence, stomach cramps, or diarrhea) when you consume whey protein?
Yes No
Are you allergic to maltodextrin or experience gastrointestinal distress (i.e. bloating, flatulence, stomach cramps, or diarrhea) when you consume maltodextrin?
Yes No
Are you currently prescribed and taking oral contraceptives (birth control)?
Yes Brand/dose:_____
No
Have you had more than two x-rays taken on any body part in the last 12 months?
Yes No

Date: _____
TIME:_____ am / pm
Qualified:_____ Disqualified:_____

Appendix D

Informed Consent Form

TEXAS WOMAN'S UNIVERSITY (TWU)
CONSENT TO PARTICIPATE IN RESEARCH

Title: The Effect of Whey Protein Isolate Supplementation on Cortisol Awakening Response, Profile of Mood States, and Heart Rate Variability in Recreationally Active Women

Principal Investigator: Michael Oldham, MS…… moldham1@twu.edu 972/951-1591
Co-Investigator: Vipa Bernhardt, PhD.....vipa.bernhardt@tamuc.edu 903/886-5549
Faculty Advisor: Vic Ben-Ezra, PhD……..vbenezra@twu.edu 940/898-2597
Kyle Biggerstaff, PhD……..kbiggerstaff@twu.edu 940/898-2596
Anthony Duplanty…….aduplanty@twu.edu 940/898-2591

Summary and Key Information about the Study

You are being asked to participate in a research study conducted by Mr. Michael Oldham, a doctoral student at Texas Woman's University and Ad Interim Instructor at Texas A&M University - Commerce, as a part of his dissertation. The purpose of this study is to determine your body's next day response to exercise stress, by analyzing a hormone related to stress called cortisol, and determine if nutritional supplements help with that response. Following the completion of first trial week, you will be given a $25 gift card, and you will receive a $50 gift card after completing the entire study, for your participation. The greatest risks of this study include discomforts associated with moderate to heavy exercise, such as strained muscles and/or muscle soreness. We will discuss these risks and the rest of the study procedures in greater detail below.

Your participation in this study is completely voluntary. If you are interested in learning more about this study, please review this consent form carefully and take your time deciding whether or not you want to participate. Please feel free to ask the researcher any questions you have about the study at any time.

Description of Procedures

As a participant in this study you will be asked to undergo a screening day, lasting approximately 2 hours to measure your height, weight, age, and ask some health-related questions. You will also complete a maximum oxygen uptake treadmill protocol to determine your ability to consume oxygen during exercise. During the treadmill test, you will experience changes in treadmill speed and grade, occurring every 3 minutes. You will initially start out walking but may need to start running if the test lasts longer than 12 minutes. You can make the decision to stop the test at any time, and you will be verbally encouraged by the research team to achieve your maximal effort. We will measure oxygen uptake using a metabolic cart, while you breath through a breather hose and wear a nose clip, to collect and monitor all gases. During screening you will also be scanned with a dual x-ray absorptiometer (DEXA) to determine your percent of fat free mass, i.e. muscle mass.

Once you have been screened for the study and completed your maximal oxygen uptake treadmill test, your time commitment is as follows:

Week 1: Day 1 - Collection of Samples (1hr); Days 2 & 3 – Collection of Samples (1hr) & Exercise (2hrs); Day 4 – Collection of Samples (1hr) → You will wait 7 days (including Day 4),

where no supplements or testing exercise is given. You may exercise as you have in past, following your normal routine.

_____

Initials

Week 2: Day 1 - Collection of Samples (1hr); Days 2 & 3 – Collection of Samples (1hr) & Exercise (2hrs); Day 4 – Collection of Samples (1hr) → You will wait 7 days (including Day 4), where no supplements or testing exercise is given. You may exercise as you have in past, following your normal routine.

Your main role in the study is divided into 3 main parts: sample collection at your home, exercise protocols, and taking nutritional supplements. Sample collection involves 3 types of samples over 4 days during each of the 3 testing cycles. You will collect saliva samples by a method called passive drool, which you will be able to practice with the research team. You will collect about 2ml of saliva immediately when you wake up, between 6:00am – 8:00am, and also at 15, 30, 45, and 60 minutes after waking up. You will also record what time those samples are collected. Between the 15-minute and 30-minute collection, you will record your heart rate using a smartphone app, at no charge. Between the 30-minute and 45-minute collection, you will take a short 35 question survey to measure your mood called Profile of Mood States. All collection of samples will be done at your home. You will store the saliva samples in your freezer until the end of the testing week, and then return them in the styrofoam cooler, with freezer blocks to the Exercise Physiology Lab at Texas A&M University – Commerce.

The exercise protocol, which occurs the 2nd AND 3rd day of each testing cycle involves walking on a treadmill for 5 minutes at an increasing intensity to achieve a level between 70-75% of your maximum oxygen uptake level, which we established during the screening day. When you reach the desired range, you will walk for 30 minutes. During that 30 minutes we will measure your oxygen uptake, every 10 minutes, to make sure you are staying in the range. After the 30 minutes, you will rest for 5 minutes, and then complete a maximal effort, 30-second, cycle ergometer test to measure your anaerobic exercise ability.

You will also be provided with nutritional supplements, at no cost to you during the study, for you to consume on specific days during the trial periods. Supplements will be consumed on the 1st, 2nd, and 3rd day of each testing cycle, but NOT on the fourth day. Samples will be distributed to you by the research team. You will be consuming either a maltodextrin (sugar) placebo, totaling 100 calories or a whey protein isolate / maltodextrin beverage, totaling 200 calories, each time. You will not know which drink you are drinking, nor will Mr. Oldham. Only members of the research team responsible for dosing will know. You will drink the supplement on each of the designated day between 8:00am -9:00am, and 30 minutes before your designated exercise time, e.g. between 1:30pm – 4:30pm. Each dose is will contain 200 calories, totaling 400 calories each dosing day (1st – 3rd).

In order to qualify for participation, you must be at least 18 years old and no older than 35 years old, not currently pregnant, free from any cardiovascular, pulmonary, and or metabolic diseases,

be physically fit enough to perform moderate to heavy exercise, are not clinically diagnosed as having hypothyroidism, and are able to consume maltodextrin (sugar) or whey protein without allergic reaction, digestive difficulty, and or side-effects. You must also be currently taking prescribed oral contraceptives.

Potential Risks

1) During the exercise trial, you may experience discomforts similar to those associated with any type of moderate-to-heavy exercise, such as strained muscles and/or soreness. Every effort will be made to minimize these risks by careful observations during testing by the trained research personnel. You will be led through an adequate and uniform warmup routine before starting the exercise trial.

_____

Initials

2) In the rare case of a medical emergency or injury, medical emergency response plans are posted near the treadmill and cycle ergometer. Emergency equipment, such as an AED, is available in the Field House at Texas A&M University - Commerce. Both Mr. Oldham and Dr. Bernhardt
are Red Cross AED and CPR certified, both have at least a master's degree in Exercise Physiology, and are experienced researchers in exercise physiology, exercise prescription, and exercise testing protocols. All
other additional personnel are AED and CPR certified. All researchers are experienced in recognizing and handling signs of physical stress and emergency health situations.

3) Maximal exercise testing may produce light-headedness, fatigue, nausea, and delayed-onset muscle soreness. These side effects are usually minimal in fit subjects. They are also minimized by having a gradual warm-up as well as cool-down (at least 5 minutes each) and by having the subject refrain from eating for at least 2-3 hours before their test. For treadmill testing, there is also a small risk of falling. This risk is managed by having at least one spotter at the subject's side.

There is also a risk of a cardiac event, such as a heart attack, cardiac arrest, or dangerous arrhythmia. This risk is less than 1 occurrence in 12,000 tests of healthy subjects, and approximately 1-2 occurrences in 10,000 tests of higher risk and diseased subjects. Again, both Mr. Oldham and Dr. Bernhardt
are Red Cross AED and CPR certified, both have at least a master's degree in Exercise Physiology, and are experienced researchers in exercise physiology, exercise prescription, and exercise testing protocols. All other additional personnel are AED and CPR certified. All researchers are experienced in recognizing and handling signs of physical stress and emergency health situations.

4) Dry mouth and dehydration - You will be allowed to drink water during the exercise trials as desired, and enough water to alleviate dry mouth after exercise trials. You can drink water

between saliva collection time points up to 5 minutes prior to sample collection.

5) DEXA Scan - Procedures such as CT scans, X-rays and/or radioactive drugs will be used during this research study to see how you are doing. The cumulative radiation exposure from these tests is considered small and is not likely to adversely affect you or your disease. The dosage for a DEXA scan is 0.001 millisievert (mSv). As a comparison, 0.001 mSv is equivalent to approximately 3 hours of naturally occurring radiation you may encounter in the environment, such as sunlight. A chest x-ray would be 100x stronger than a DEXA scan. However, the effects of radiation add up over a lifetime. It is possible that having several of these tests may add to your risk of injury or disease. When deciding to enter this study, think about your past and future contact with radiation. Examples of contact with radiation include x-rays taken for any reason or radiation therapy for cancer treatment. You will only have 1 DEXA scan during this study.

6) Allergic reactions to the nutritional supplements is a risk. If you are allergic to any of the nutritional supplements, you may NOT participate in the study. If you participate and notice any of the following, bloating, flatulence (gas), diarrhea, stomach cramps, nausea, loss of coordination, headache, increased insulin resistance, extreme fatigue, you should discontinue using the supplement and contact the researchers immediately. If you experience tingling in the mouth, hives, itching, swelling in the face, lips, tongue, throat, have trouble breathing, are wheezing, or faint, consider this a medical emergency and call 911.

7) There is a risk of coercion, as some subjects may have known Mr. Oldham or Dr. Bernhardt from classes or TAMUC events. Again, participation is completely voluntary. If you feel you have been coerced to join, begin, or continue the study against your will, you should contact the IRB at either campus, or the Faculty Advisors listed at the top of the page.

_____

Initials

8) Another risk in this study is loss of confidentiality. Confidentiality will be protected to the extent that is allowed by law. No one but the researcher will know your real name. All records will be kept in a locked cabinet in Field House 110C at Texas A&M University – Commerce.

The signed consent form will be stored separately from all collected information and will be destroyed three years after the study is closed. The results of the study may be reported in scientific magazines or journals, but your name or any other identifying information will not be included. There is a potential risk of loss of confidentiality in all email, downloading, electronic meetings and internet transactions.

The researchers will remove all of your personal or identifiable information (e.g. your name, date of birth, contact information) from any saliva samples for the study. After all identifiable information is removed, any saliva sample collected for this study may be used for future research or be given to another researcher for future research without additional informed consent.

> If you would like to participate in the current study but not allow your de-identified data to be used for future research, please initial here _____.

This research study will not include whole genome sequencing (i.e., sequencing of a human germline or somatic specimen with the intent to generate the genome or exome sequence of that specimen) by using your saliva samples in any way, at any time.

In case of a medical emergency, the fire department's Emergency Medical Team will be alerted. Telephones are available in the testing laboratories. We will try to prevent any problem that could result from this research study. Please let us know at once if there is a problem and we will help you. You should understand, however, that Texas A&M University - Commerce does not provide medical services or financial assistance for injuries that might happen because you are taking part in this research. The investigators are prepared to advise you in case of adverse effects, which you should report to them promptly. Phone numbers where the investigators may be reached are provided in this form.

Texas Woman's University Disclaimer Statement:
The researchers will try to prevent any problem that could happen because of this research. You should let the researchers know at once if there is a problem and they will help you. However, TWU does not provide medical services or financial assistance for injuries that might happen because you are taking part in this research.

Participation and Benefits

Your involvement in this study is completely voluntary and you may withdraw from the study at any time. Following the completion of first trial week, you will be given a $25 gift card, and you will receive a $50 gift card after completing the entire study, for your participation. If you would like to know the results of this study we will email or mail them to you.*

Questions Regarding the Study

You will be given a copy of this signed and dated consent form to keep. If you have any questions about the research study you should ask the researchers; their contact information is at the top of this form. If you have questions about your rights as a participant in this research or the way this study has been conducted, you may contact the TWU Office of Research and Sponsored Programs at 940-898-3378 or via e-mail at IRB@twu.edu, or by contacting Dr. Lucy Pickering - IRB Chair at Texas A&M University, at IRB@tamuc.edu

Approved by the
Texas Woman's University
Institutional Review Board
Approved: May 1, 2019

_____
Initials

The signature below affirms that the undersigned is at least 18 years old, has received a copy of this consent form, has understood the above information, and agrees to voluntarily participate in this research.

Participant's name:_____Date:_____

_____
Signature of Participant

*If you would like to know the results of this study tell us where you want them to be sent:

Email: _____ or Address: _____
_____

Appendix E

Health History Form – PAR-Q + 2018

2018 PAR-Q+

FOLLOW-UP QUESTIONS ABOUT YOUR MEDICAL CONDITION(S)

1. **Do you have Arthritis, Osteoporosis, or Back Problems?**
   If the above condition(s) is/are present answer questions 1a-1c If **NO** ☐ go to question 2

1a	Do you have difficulty controlling your condition with medications or other physician prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	**YES** ☐	**NO** ☐
1b	Do you have joint problems causing pain, a recent fracture or fracture caused by osteoporosis or cancer, displaced vertebra (e.g., spondylolisthesis), and/or spondylolysis/pars defect (a crack in the bony ring on the back of the spinal column)?	**YES** ☐	**NO** ☐
1c	Have you had steroid injections or taken steroid tablets regularly for more than 3 months?	**YES** ☐	**NO** ☐

2. **Do you have Cancer of any kind?**
   If the above condition(s) is/are present, answer questions 2a-2b If **NO** ☐ go to question 3

2a	Does your cancer diagnosis include any of the following types; lung/bronchogenic, multiple myeloma (cancer of plasma cells), head, and neck?	**YES** ☐	**NO** ☐
2b	Are you currently receiving cancer therapy (such as chemotherapy or radiotherapy)?	**YES** ☐	**NO** ☐

3. **Do you have a Heart or Cardiovascular Condition?** *This includes Coronary Artery Disease, Heart Failure, Diagnosed Abnormality of Heart Rhythm*
   If the above condition(s) is/are present, answer questions 3a-3d If **NO** ☐ go to question 4

3a	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	**YES** ☐	**NO** ☐
3b	Do you have an irregular heart beat that required medical management? (e.g., atrial fibrillation, premature ventricular contraction)	**YES** ☐	**NO** ☐
3c	Do you have chronic heart failure?	**YES** ☐	**NO** ☐
3d	Do you have diagnosed coronary artery (cardiovascular) disease and have not participated in regular physical activity in the last 2 months?	**YES** ☐	**NO** ☐

4. **Do you have High Blood Pressure?**
   If the above condition(s) is/are present, answer questions 4a-4b If **NO** ☐ go to question 5

4a	Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments)	**YES** ☐	**NO** ☐
4b	Do you have a resting blood pressure equal to or greater than 160/90 mmHg with or without medication? (Answer YES if you do not know your resting blood pressure)	**YES** ☐	**NO** ☐

5. **Do you have any Metabolic Conditions?** *This includes Type 1 Diabetes, Type 2 Diabetes, Pre-Diabetes*
   If the above condition(s) is/are present, answer questions 5a-5e If **NO** ☐ go to question 6

5a	Do you often have difficulty controlling your blood sugar levels with foods, medications, or other physician-prescribed therapies?	**YES** ☐	**NO** ☐
5b	Do you often suffer from signs and symptoms of low blood sugar (hypoglycemia) following exercise and/or during activities of daily living? Signs of hypoglycemia may include shakiness, nervousness, unusual irritability, abnormal sweating, dizziness or light-headedness, mental confusion, difficulty speaking, weakness, or sleepiness.	**YES** ☐	**NO** ☐
5c	Do you have any signs or symptoms of diabetes complications such as heart or vascular disease and/or complications affecting your eyes, kidneys, **OR** the sensation in your toes and feet?	**YES** ☐	**NO** ☐
5d.	Do you have other metabolic conditions (such as current pregnancy-related diabetes, chronic kidney disease or liver problems)?	**YES** ☐	**NO** ☐
5e	Are you planning to engage in what for you is unusually high (or vigorous) intensity exercise in the near future?	**YES** ☐	**NO** ☐

2018 PAR-Q+

The Physical Activity Readiness Questionnaire for Everyone

The health benefits of regular physical activity are clear; more people should engage in physical activity every day of the week. Participating in physical activity is very safe for MOST people. This questionnaire will tell you whether it is necessary for you to seek further advice from your doctor OR a qualified exercise professional before becoming more physically active.

GENERAL HEALTH QUESTIONS

Please read the 7 questions below carefully and answer each one honestly: check YES or No	**YES**	**NO**
1) Has your doctor ever said that you have a heart condition ☐ **OR** high blood pressure ☐ ?	☐	☐
2) Do you feel pain in your chest at rest, during your daily activities of living, **OR** when you do physical activity	☐	☐
3) Do you lose balance because of dizziness **OR** have you lost consciousness in the last 12 months? (Please answer **NO** if your dizziness was associated with over-breathing (including during vigorous exercise).	☐	☐
4) Have you ever been diagnosed with another chronic medical condition (other than heart disease or high blood pressure)? **PLEASE LIST CONDITION(S) HERE**:_____	☐	☐
5) Are you currently taking prescribed medications for a chronic medical condition: **PLEASE LIST CONDITION(S) AND MEDICATIONS HERE**:_____	☐	☐
6) Do you currently have (or have had within the past 12 months) a bone, joint, or soft tissue (muscle, ligament, or tendon) problem that could be made worse by becoming more physically active? (Please answer **NO** if you had a problem in the past, but it does not limit your current ability to be physically active). **PLEASE LIST CONDITION(S) HERE:**_____	☐	☐
7) Has your doctor ever said that you should only do medically supervised physical activity?	☐	☐

☑ **If you answered NO to all of the questions above, you are cleared for physical activity. Go to page 4 to sign the PARTICIPANT DECLARATION. <u>You do not need to complete Pages 2 and 3.</u>**

- Start becoming much more physically active – start slowly and build up gradually.
- Follow International Physical Activity Guidelines for your age (www.who.int/dietphysicalactivity/en/).
- You may take part in a health and fitness appraisal.
- If you are over the age of 45 and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.
- If you have any further questions, contact a qualified exercise professional.

If you answered YES to one or more of the questions above, COMPLETE PAGES 2 AND 3.

⚠ **Delay becoming more active if:**

- ✓ You have a temporary illness such as a cold or fever, it is best to wait until you feel better.
- ✓ You are pregnant – talk to your health care practitioner, your physician, a qualified exercise professional, and/or complete the ePARmed-X+ at www.eparmedx.com before becoming more physically active.
- ✓ Your health changes – answer the questions of Pages 2and 3 of this document and/or talk to your doctor or a qualified exercise professional before continuing with any physical activity program.

2018 PAR-Q+

6. **Do you have any Mental Health Problems or Learning Difficulties?** *This includes Alzheimer's Dementia, Depression, Anxiety Disorder, Eating Disorder, Psychotic Disorder, Intellectual Disability, Down Syndrome*
If the above condition(s) is/are present, answer questions 6a-6b If **NO**☐go to question 7

6a Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) **YES** ☐ **NO** ☐

6b Do you ALSO have back problems affecting nerves or muscles? **YES** ☐ **NO** ☐

7. **Do you have a Respiratory Disease?** *This includes Chronic Obstructive Pulmonary Disease, Asthma, Pulmonary High Blood Pressure*
If the above condition(s) is/are present, answer questions 7a-7d If **NO**☐go to question 8

7a Do you have difficulty controlling your condition with medications or other physician-prescribed therapies? (Answer **NO** if you are not currently taking medications or other treatments) **YES** ☐ **NO** ☐

7b Has your doctor ever said your blood oxygen level is low at rest or during exercise and/or that you require supplemental oxygen therapy? **YES** ☐ **NO** ☐

7c If asthmatic, do you currently have symptoms of chest tightness, wheezing, labored breathing, consistent cough (more than 2 days/week), or have you used your rescue medication more than twice in the last week? **YES** ☐ **NO** ☐

7d Has your doctor ever said you have high blood pressure in the blood vessels of your lungs? **YES** ☐ **NO** ☐

8. Do you have a Spinal Cord Injury? *This includes Tetraplegia and Paraplegia*
If the above condition(s) is/are present, answer questions 8a-8c If **NO**☐go to question 9

8a Do you have difficulty controlling your condition with medications or other physician-prescribed therapies?
(Answer **NO** if you are not currently taking medications or other treatments) **YES** ☐ **NO** ☐

8b Do you commonly exhibit low resting blood pressure significant enough to cause dizziness, light-headedness, and/or fainting? **YES** ☐ **NO** ☐

8c Has your physician indicated that you exhibit sudden bouts of high blood pressure (known as Autonomic Dysreflexia) **YES** ☐ **NO** ☐

9. **Have you had a Stroke?** *This includes Transient Ischemic Attack (TIA) or Cerebrovascular Event*
If the above condition(s) is/are present, answer questions 9a-9c If **NO**☐go to question 10

9a Do you have difficulty controlling you condition with medications or other physician-prescribed therapies?
(Answer **NO** if you are not currently taking medications or other treatments) **YES** ☐ **NO** ☐

9b Do you have any impairment in walking or mobility? **YES** ☐ **NO** ☐

9c Have you experienced a stroke or impairment in nerves or muscles in the past 6 months? **YES** ☐ **NO** ☐

10. Do you have any other medical condition not listed above or do you have two or more medical conditions?
If you have other medical conditions, answer questions 10a-10c If **NO**☐read the Page 4 recommendations

10a Have you experienced a blackout, fainted, or lost consciousness as a result of a head injury within the last 12 months **OR** have you had a diagnosed concussion within the last 12 months? **YES** ☐ **NO** ☐

10b Do you have a medical condition that is not listed (such as epilepsy, neurological conditions, kidney problems)? **YES** ☐ **NO** ☐

10c Do you currently live with two or more medical conditions? **YES** ☐ **NO** ☐
PLEASE LIST YOUR MEDICAL CONDITIONS(S)_____
AND ANY RELATED MEDICATIONS HERE:_____

GO to Page 4 for recommendations about your current medical condition(s) and sign the PARTICIPANT DECLARATION.

2018 PAR-Q+

☑ **If you answered NO to all of the follow-up questions about your medical condition, you are ready to become more physically active – sign the PARTICIPANT DECLARATION below:**

- It is advised that you consult a qualified exercise professional to help you develop a safe and effective physical activity plan to meet your health needs.
- You are encouraged to start slowly and build up gradually – 20 to 60 minutes of low to moderate intensity exercise, 3-5 days per week including aerobic and muscle strengthening exercises.
- As you progress, you should aim to accumulate 150 minutes or more of moderate intensity physical activity per week.
- If you are over the age of 45 yr and **NOT** accustomed to regular vigorous to maximal effort exercise, consult a qualified exercise professional before engaging in this intensity of exercise.

If you answered **YES** to **one or more of the follow-up questions** about your medical condition:
You should seek further information before becoming more physically active or engaging in a fitness appraisal. You should complete the specially designed online screening and exercise recommendations program – the ePARmed-X+at www.eparmedx.com and/or visit a qualified exercise professional to work through the ePARmed-X+ and for further information.

⚠ Delay becoming more active if:

- ✓ You have a temporary illness such as a cold or fever; it is best to wait until you feel better.
- ✓ You are pregnant – talk to your health care practioner, you physician, and qualified exercise professional, and/or complete the ePARmed-X+ at www.eparmedx.com before becoming more physically active.
- ✓ Your health changes – talk to your doctor or qualified exercise professional before continuing with any physical activity program.

- You are encouraged to photocopy the PAR-Q+. You must use the entire questionnaire and NO changes are permitted.
- The authors, the PAR-Q+ Collaboration, partner organizations, and their agents assume no liability for persons who undertake physical activity and/or make use of the PAR-Q+ or ePARmed-X+. If in doubt after completing the questionnaire, consult your doctor prior to physical activity.

NAME _____ DATE _____

Submit

Appendix F

Dietary Recall Sheet

Readiness – Dissertation Study Subject #:_____

Dietary Recall Form

Date	Time	Food / Drink Item	Servings Consumed	Serving Size

Appendix G

Data Collection Sheet

Repetitive Performance Effects and Placebo / Whey Protein

Subject Data Form:

Subject Number:_____

Age:_____

Height(cm):_____ Weight(kg):_____

___

Last meal time:_____ Approximate calories in last meal:_____

START TIME:_____ pm

Date: _____

Data Collection Sheet

Subject #:_____

Resting HR:_____

Age:_____

APMHR = 220 - _____ =

VO2max Test - Speed / Grade and Heart Rate Timepoints

Time(min:sec)	Speed(mph)	Grade(%)	HR(bpm)
0:00 @ _____	1.7	10	
3:00	2.5	12	
6:00	3.4	14	
9:00	4.2	16	
12:00	5	18	
15:00	5.5	20	
18:00	6	22	

*** = Start stopwatch when treadmill starts

VO2max = _____

Treadmill Protocol - Speed / Grade and Heart Rate Timepoints

Time(min:sec)	Speed(mph)	Grade(%)	VO2 - 1 min Avg
0:00 @ _____			
5:00 – 10:00			
15:00 – 20:00			
25:00 – 30:00			

*** = Start stopwatch when treadmill starts

VO2max = _____
Time Off Treadmill:_____

WAIT 5 MINUTES!!!!!!!

Wingate Start Time:_____

Wingate Data Collection:
Body Weight (BW): _____ / 2.2 = _____

7.5% of BW: 0.075 x _____ (BW (lb)) = _____ (BW (kg))

Peak Power:_____ (BW (kg))
Relative Peak Power:_____
Anaerobic Fatigue Index:_____
Anaerobic Capacity:_____ (LOAD (kp))

Appendix H

Biomarker Analysis Technique

Cortisol

Samples and Dissociation Reagent will be warmed to room temperature. Five microliters of warmed Dissociation Reagent will be added into each microcentrifuge tube. Five microliters of sample will be added to the microcentrifuge tube. Microcentrifuge tubes will be vortexed gently and incubated at room temperature for 5 min. Four-hundred and ninety microliters of 1X Assay Buffer will be added to prepare a 1:100 dilution of the sample. Standards will be produced by adding 50 μL Cortisol Standard to one tube containing 450 μL distilled or deionized water and label as 10 pg/mL cortisol. The Standard will be pipetted up and down several times to wet the pipette tip before transferring, to ensure that volumes are dispensed accurately. Two-hundred microliters of distilled or deionized water will be added to each of 7 tubes labeled as follows: 3200, 1600, 800, 400, 200, 100, and 0 pg/mL cortisol. A serial dilution of the standards will be prepared by adding 250 μL from the preceding tube into the tube containing 200 μL of distilled or deionized water. Each tube will be mixed thoroughly between steps by a tube vortexing machine for 5 s. Standards will be used within 2 hr of preparation. To bind antigen 50 μL of standards or samples will be added to the appropriate wells on the 96-well ELISA plate (Salimetrics, LLC., Carlsbad, CA, USA). Seventy-five microliters of 1X Assay Buffer will be added into each well for detecting non-specific binding (NSB). Twenty-five microliters of Cortisol Conjugate will be added to each well. Twenty-five microliters of Cortisol Antibody will be added to each well, except NSB wells. The side of the plate will be tapped 10 times to mix. The plate will be covered with plate sealer and incubated for 1 hr at room temperature with

shaking, at 200 rpm. The solution in each well will be thoroughly aspirated and washed 4 times with 300 μL of 1X Wash Buffer. One-hundred microliters of TMB Substrate will be added to each well. The substrate solution will begin to turn blue within a few seconds. The plate will be incubated for 30 min at room temperature. Interaction with TMB and aluminum foil or other metals will be avoided per manufacturer instructions. After 1 hr, 50 μL of Stop Solution will be added to each well. The plate will be gently tapped 5 times to mix. The solution in the wells will change from blue to yellow. Plates will be read with a BioTek (BioTek Instruments, Inc., Winooski, VT, USA) at 450 nm, within 10 min after adding the Stop Solution. Curve-fitting software will be used to generate the standard curve, on a four-parameter algorithm for best standard curve fit. The analytical sensitivity of the assay is 17.3 pg/mL cortisol. This will be determined by adding two standard deviations to the mean O.D. obtained when the zero standard is assayed 19 times and calculating the corresponding concentration. After analysis, plates will be discarded in the appropriate biohazard disposal unit.

Appendix I

Passive Drool Technique

(Salimetrics, LLC, Carlsbad, CA, USA)

1. Open foil pouch and remove the Saliva Collection Aid (SCA)
2. Place ribbed-end of the SCA securely into a prelabeled collection vial.
3. Allow saliva to pool in mouth. Then, with head tilted forward, gently guide saliva through the SCA into the vial. Fill to the required volume (2 ml).
4. Remove and discard SCA. Attach cap to collection vial and tighten.

Appendix J

Saliva Collection Guidelines

1. You MUST avoid the following AFTER you wake up:

a. Smoking
b. Caffeinated drinks
c. Food/breakfast
d. Physical exercise
e. Gum with Red Dye #40
2. There is NO PROBLEM with doing the following:
a. Getting up and moving around
b. Going to the restroom
c. Brushing teeth, gently - make sure you stop AT LEAST 5 min before sampling, and rinse mouth thoroughly

What does it mean to be awake?

When you are awake, you are conscious, know who you are, and where you are. You are in a state that is clearly different from when you were sleeping, even though you may still feel tired.

What if I wake up in the middle of the night?

If you wake up in the middle of the night and plan to go back to sleep, DO NOT begin sampling; please only begin sampling when you awake for the final time when you plan to get up for the day.

Can I fall back asleep or doze off?

During this study, please DO NOT fall back asleep, doze, or hit the snooze button on your alarm. You can stay in bed or get out of bed and move around, but please stay awake (even if you are not fully alert) during and after the saliva sampling time period.

Appendix K

POMS Short Form Questionnaire and Scoring Key

Item	Not At All	Somewhat	Moderately So	Very Much So	Very Very Much So
Tense	0	1	2	3	4
Angry	0	1	2	3	4
Worn Out	0	1	2	3	4
Unhappy	0	1	2	3	4
Proud	0	1	2	3	4
Lively	0	1	2	3	4
Confused	0	1	2	3	4
Sad	0	1	2	3	4
Active	0	1	2	3	4
On-edge	0	1	2	3	4
Grouchy	0	1	2	3	4
Energetic	0	1	2	3	4
Hopeless	0	1	2	3	4
Uneasy	0	1	2	3	4
Restless	0	1	2	3	4
Can’t	0	1	2	3	4
Fatigued	0	1	2	3	4
Annoyed	0	1	2	3	4
Discouraged	0	1	2	3	4
Resentful	0	1	2	3	4
Nervous	0	1	2	3	4
Miserable	0	1	2	3	4
Confident	0	1	2	3	4
Bitter	0	1	2	3	4
Exhausted	0	1	2	3	4
Anxious	0	1	2	3	4
Helpless	0	1	2	3	4
Weary	0	1	2	3	4
Bewildered	0	1	2	3	4
Furious	0	1	2	3	4
Full of Pep	0	1	2	3	4
Forgetful	0	1	2	3	4
Vigorous	0	1	2	3	4
Uncertain	0	1	2	3	4
Bushed	0	1	2	3	4

POMS Short Form Scoring Key

Item	Scoring Category
Tense	Tension
Angry	Anger
Worn Out	Fatigue
Unhappy	Depression
Proud	Vigor
Lively	Vigor
Confused	Confusion
Sad	Depression
Active	Vigor
On-edge	Tension
Grouchy	Anger
Energetic	Vigor
Hopeless	Depression
Uneasy	Tension
Restless	Tension
Can’t concentrate	Confusion
Fatigued	Fatigue
Annoyed	Anger
Discouraged	Depression

Resentful	Anger
Nervous	Tension
Miserable	Depression
Bitter	Anger
Confident	Vigor
Exhausted	Fatigue
Anxious	Tension
Helpless	Depression
Weary	Fatigue
Bewildered	Confusion
Furious	Anger
Full of Pep	Vigor
Forgetful	Confusion
Vigorous	Vigor
Uncertain	Confusion
Bushed	Fatigue

Total Mood Disturbance (TMD) = (((depression-dejection) + (tension-anxiety) + (anger-hostility) + (fatigue-inertia) + (confusion-bewilderment)) – (vigor-activity))

Appendix L

Individual Participant Data

Subject 001

Anthropometric Data

Age	Height (cm)	Weight (Kg)	VO2 ABS	VO2 REL	BMI
20	168	64.9	2.42	37.7	23

Dosage Order

Week 1 Week 2

Whey Placebo

Cortisol Data (pg/ml)

Placebo

D1/S1	D1/15	D1/30	D1/45	D1/60		D2/S1	D2/15	D2/30	D2/45	D2/60
0.5165	0.549	0.731	0.1069	0.7135		1.007	1.001	0.9455	0.792	0.546

D3/S1	D3/15	D3/30	D3/45	D3/60		D4/S1	D4/15	D4/30	D4/45	D4/60
0.665	0.8235	1.1895	0.5145	0.4835		0.1015	0.137	0.144	0.0955	0.108

Whey

D1/S1	D1/15	D1/30	D1/45	D1/60		D2/S1	D2/15	D2/30	D2/45	D2/60
0.5555	0.72	0.5805	0.622	0.474		0.351	0.3335	0.506	0.624	0.4265

D3/S1	D3/15	D3/30	D3/45	D3/60		D4/S1	D4/15	D4/30	D4/45	D4/60
0.395	0.571	0.5955	0.554	0.708		0.439	0.541	0.9145	0.8215	0.73

POMS

Placebo

D1 - Depr	D1 - Tens	D1 - Angr	D1 - Fatig	D1 - Conf	D1 - Vigor	D2 - Depr	D2 - Tens	D2 - Angr	D2 - Fatig	D2 - Conf	D2 - Vigor
0	0	4	1	4	0	0	0	4	0	4	0

D3 - Depr	D3 - Tens	D3 - Angr	D3 - Fatig	D3 - Conf	D3 - Vigor	D4 - Depr	D4 - Tens	D4 - Angr	D4 - Fatig	D4 - Conf	D4 - Vigor
0	6	0	1	4	0	0	5	0	1	4	0

D1TMD	D2TMD	D3TMD	D4TMD

9	8	11	10

Whey

D1 - Depr	D1 - Tens	D1 - Angr	D1 - Fatig	D1 - Conf	D1 – Vigor	D2 - Depr	D2 - Tens	D2 - Angr	D2 - Fatig	D2 - Conf	D2 - Vigor
0	6	0	3	4	2	0	5	0	3	4	0

D3 - Depr	D3 - Tens	D3 - Angr	D3 - Fatig	D3 - Conf	D3 - Vigor	D4 - Depr	D4 - Tens	D4 - Angr	D4 - Fatig	D4 - Conf	D4 - Vigor
0	4	0	2	4	0	0	4	0	0	4	0

D1TMD	D2TMD	D3TMD	D4TMD
11	12	10	8

HRV rMSSD

Placebo

Day 1	Day 2	Day 3	Day 4
36.1	24.6	60.4	51.6

Whey

Day 1	Day 2	Day 3	Day 4
35.7	102.8	16.9	17.7

Diet

Placebo

Day 1	Day 2	Day 3	Day 4
1177	3240	2429	1211

Macros Day 1 (g)			Macros Day 2 (g)			Macros Day 3 (g)		
CHO	PRO	FAT	CHO	PRO	FAT	CHO	PRO	FAT
126	60	50	309	163	155	336	116	92

Average Macros

CHO	PRO	FAT
257	113	99

Average kcal

CHO	PRO	FAT
1028	452	891

Average kcal with Supplement

CHO	PRO	FAT
1228	452	891

Average Total kcal

PL	WH
2282	2264.33

Average Total kcal with Supplement

PL	WH
2482	2664.33

Whey

Day 1	Day 2	Day 3	Day 4
1246	2314	3233	2264

Macros Day 1 (g)			Macros Day 2 (g)			Macros Day 3 (g)		
CHO	PRO	FAT	CHO	PRO	FAT	CHO	PRO	FAT
103	55	51	201	90	127	345	98	140

Average Macros

CHO	PRO	FAT
216.3	81	106

Average kcal

CHO	PRO	FAT
865.33	324	954

Average kcal with Supplement

CHO	PRO	FAT
1065.33	524	954

Average Total kcal

PL	WH
2371	2143.33

Average Total kcal with Supplement

PL	WH
2571	2543.33

VO2

Placebo

VO2peak	Day 1 - Warm	Day 1 - 5min	Day 1 - 15 min	Day 1 - 25 min	Day 1 Mean
35.1	25.5	24.4	26.1	25	25.25

Day 2 - Warm	Day 2 - 5 min	Day 2 - 15 min	Day 2 - 25 min	Day 2 Mean
26.2	25.6	24.5	26.1	25.6

Whey

VO2peak	Day 1 - Warm	Day 1 - 5min	Day 1 - 15 min	Day 1 - 25 min	Day 1 Mean
35.1	23.6	27.1	26.2	25.8	25.68

Day 2 - Warm	Day 2 - 5 min	Day 2 - 15 min	Day 2 - 25 min	Day 2 Mean
25.1	25.8	26.6	25.4	25.73

WAnT

Placebo

Peak Power

Day 1	Day 2
699	751

Relative Peak Power

Day 1	Day 2
10.9	11.6

Fatigue Index

Day 1	Day 2
10.9	13.1

Anaerobic Capacity

Day 1	Day 2
7.6	7.7

Whey

Peak Power

Day 1	Day 2
752	807

Relative Peak Power

Day 1	Day 2
11.6	12.4

Fatigue Index

Day 1	Day 2
14.8	16.5

Anaerobic Capacity

Day 1	Day 2
7.3	7.4

OC Dosage

Oral Contraceptives Dosing (mg E2)

0.2

RER

Wk 1 Ex 1	WK 1 EX2	Wk 2 EX 1	Wk 2 Ex 2
0.91	0.84	0.9	0.93
0.92	0.85	0.96	1
0.9	0.93	0.98	0.99

0.98	0.93	0.96	1
0.97	0.98	0.96	0.97
0.99	1.01	0.93	0.99
1.01	1.01	0.97	1.01
1.04	1.01	0.96	0.96
0.98	0.99	0.96	0.99
0.96	1		0.96
0.97	1.04		1
0.98	0.99		0.99
0.93	1.01		
0.89	0.97		
0.92	0.95		
0.93			
0.93			
0.95			
0.93			
0.91			
0.92			
0.94			

0.98			
1			
Mean			
0.952	0.967	0.953	0.983
WH	WH	PL	PL

Subject 002

Anthropometric Data

Age	Height (cm)	Weight (Kg)	VO2 ABS	VO2 REL	BMI
20	161	55.6	1.92	35.1	21.4

Dosage Order

Week 1 Week 2

Placebo Whey

Cortisol Data (pg/ml)

Placebo

D1/S1	D1/15	D1/30	D1/45	D1/60		D2/S1	D2/15	D2/30	D2/45	D2/60
0.174	0.361	0.658	0.8345	0.59		0.441	0.9625	1.1235	1.136	1.109

D3/S1	D3/15	D3/30	D3/45	D3/60		D4/S1	D4/15	D4/30	D4/45	D4/60
0.305	0.976	0.801	1.018	0.908		0.304	0.4655	0.625	0.884	0.562

Whey

D1/S1	D1/15	D1/30	D1/45	D1/60		D2/S1	D2/15	D2/30	D2/45	D2/60
0.332	0.4755	0.7155	0.722	1.116		0.4405	0.57	0.834	0.7535	0.74

D3/S1	D3/15	D3/30	D3/45	D3/60		D4/S1	D4/15	D4/30	D4/45	D4/60
0.302	0.3615	0.828	0.903	0.956		0.26	0.375	0.6945	0.6315	0.507

POMS

Placebo

D1 - Depr	D1 - Tens	D1 - Angr	D1 - Fatig	D1 - Conf	D1 - Vigor	D2 - Depr	D2 - Tens	D1 - Angr	D2 - Fatig	D2 - Conf	D2 - Vigor
0	5	0	6	5	1	0	5	1	5	4	0

D3 - Depr	D3 - Tens	D3 - Angr	D3 - Fatig	D3 - Conf	D3 - Vigor	D4 - Depr	D4 - Tens	D4 - Angr	D4 - Fatig	D4 - Conf	D4 - Vigor
0	5	1	3	4	1	0	7	1	4	5	0

D1TMD	D2TMD	D3TMD	D4TMD

15	15	12	17

Whey

D1 - Depr	D1 - Tens	D1 - Angr	D1 - Fatig	D1 - Conf	D1 – Vigor	D2 - Depr	D2 - Tens	D2 - Angr	D2 - Fatig	D2 - Conf	D2 - Vigor
0	6	2	3	5	0	0	6	3	3	5	2

D3 - Depr	D3 - Tens	D3 - Angr	D3 - Fatig	D3 - Conf	D3 – Vigor	D4 - Depr	D4 - Tens	D4 - Angr	D4 - Fatig	D4 - Conf	D4 - Vigor
0	8	3	4	5	0	0	6	0	2	5	1

D1TMD	D2TMD	D3TMD	D4TMD
16	15	20	12

HRV rMSSD

Placebo

Day 1	Day 2	Day 3	Day 4
48.9	31.1	64.8	52.3

Whey

Day 1	Day 2	Day 3	Day 4
48.9	31.1	64.8	52.3

Diet

Placebo

Day 1	Day 2	Day 3	Day 4
1835	1712	1686.5	1745

Macros Day 1 (g)			Macros Day 2 (g)			Macros Day 3 (g)		
CHO	PRO	FAT	CHO	PRO	FAT	CHO	PRO	FAT
228	52	80	217.5	43.5	62	165.5	30.5	36

Average Macros

CHO	PRO	FAT
203.7	42	59.3

Average kcal

CHO	PRO	FAT
814.67	168	534

Average kcal with Supplement

CHO	PRO	FAT
1014.67	168	534

Average Total kcal

PL	WH
1744.5	1115.67

Average Total kcal with Supplement

PL	WH
1944.5	1515.67

Whey

Day 1	Day 2	Day 3	Day 4
1569	690	1088	2424

Macros Day 1 (g)			Macros Day 2 (g)			Macros Day 3 (g)		
CHO	PRO	FAT	CHO	PRO	FAT	CHO	PRO	FAT
127	58	64	123	14	10	178	20	31

Average Macros

CHO	PRO	FAT
142.7	30.7	35

Average kcal

CHO	PRO	FAT
570.67	122.67	315

Average kcal with Supplement

CHO	PRO	FAT
770.67	322.67	315

Average Total kcal

PL	WH
1516.67	1008.33

Average Total kcal with Supplement

PL	WH
1716.67	1408.33

VO_2

Placebo

VO2peak	Day 1 - Warm	Day 1 - 5min	Day 1 - 15 min	Day 1 -25 min	Day 1 Mean
35.2	21.7	24.4	25.1	25.5	24.18

Day 2 - Warm	Day 2 - 5 min	Day 2 - 15 min	Day 2 - 25 min	Day 2 Mean
24.2	25.1	26.3	24.8	25.1

Whey

VO2peak	Day 1 - Warm	Day 1 - 5 min	Day 1 -15 min	Day 1 -25 min	Day 1 Mean
35.2	23.5	25.6	25.8	25.9	25.2

Day 2 - Warm	Day 2 - 5 min	Day 2 - 15 min	Day 2 - 25 min	Day 2 Mean
23.4	25.9	24.2	23.1	24.15

WAnT

Placebo

Peak Power

Day 1	Day 2
678	753

Relative Peak Power

Day 1	Day 2
12.3	13.6

Fatigue Index

Day 1	Day 2
17.4	22.1

Anaerobic Capacity

Day 1	Day 2
7.1	6.8

Whey

Peak Power

Day 1	Day 2
760	727

Relative Peak Power

Day 1	Day 2
13.7	13.2

Fatigue Index

Day 1	Day 2
21.8	20.7

Anaerobic Capacity

Day 1	Day 2
7.1	7.2

OC Dosage

Oral Contraceptives Dosing (mg E2)

0.02

RER

Subject 002			
Wk 1 Ex 1	WK 1 EX2	Wk 2 EX 1	Wk 2 Ex 2

0.99	1.03	1.03	1
1	1.03	1.07	1
1	1.02	1.08	1.03
1.02	1.05	1.07	1.04
1.06	1.04	1.06	1.01
1.02	1.02	1.03	1
1	1	1.06	1.02
1.02	0.97	1.05	1.04
1.01	1.01	1.01	1.02
1	0.98	0.99	1.06
0.93	1	1.02	1.02
0.95	1.02	0.97	1.01
0.94	0.94	1.01	0.95
0.96	0.96		0.92
0.95			0.96
0.95			0.97
0.96			0.94
0.95			0.94
0.89			
0.979	1.005	1.035	0.996
PL	Pl	WH	WH

Subject 003

Anthropometric Data

Age	Height (cm)	Weight (Kg)	VO2 ABS	VO2 REL	BMI
19	161	68.5	2.33	34.7	26.4

Dosage Order

Week 1 Week 2

Placebo Whey

Cortisol Data (pg/ml)

Placebo

D1/S1	D1/15	D1/30	D1/45	D1/60		D2/S1	D2/15	D2/30	D2/45	D2/60
0.1395	0.1475	0.2435	0.472	0.68		0.13	0.1465	0.1685	0.1415	0.1135

D3/S1	D3/15	D3/30	D3/45	D3/60		D4/S1	D4/15	D4/30	D4/45	D4/60
0.066	0.116	0.104	0.1225	0.255		0.1045	0.141	0.192	0.4825	0.305

Whey

D1/S1	D1/15	D1/30	D1/45	D1/60		D2/S1	D2/15	D2/30	D2/45	D2/60
0.1305	0.1915	0.1805	0.1645	0.171		0.6	0.4815	0.373	0.4385	0.5165

D3/S1	D3/15	D3/30	D3/45	D3/60		D4/S1	D4/15	D4/30	D4/45	D4/60
0.1995	0.094	0.078	0.0805	0.109		0.1135	0.1685	0.225	0.131	0.0935

POMS

Placebo

D1 - Depr	D1 - Tens	D1 - Angr	D1 - Fatig	D1 - Conf	D1 – Vigor	D2 - Depr	D2 - Tens	D2 - Angr	D2 - Fatig	D2 - Conf	D2 - Vigor
0	4	0	0	0	0	0	4	0	0	4	0

D3 - Depr	D3 - Tens	D3 - Angr	D3 - Fatig	D3 - Conf	D3 - Vigor	D4 - Depr	D4 - Tens	D4 - Angr	D4 - Fatig	D4 - Conf	D4 - Vigor
0	4	0	0	4	0	2	4	2	0	4	0

D1TMD	D2TMD	D3TMD	D4TMD
4	8	8	12

Whey

D1 - Depr	D1 - Tens	D1 - Angr	D1 - Fatig	D1 - Conf	D1 – Vigor	D2 - Depr	D2 - Tens	D2 - Angr	D2 - Fatig	D2 - Conf	D2 - Vigor
0	4	0	0	4	0	0	4	0	0	4	0

D3 - Depr	D3 - Tens	D3 - Angr	D3 - Fatig	D3 - Conf	D3 – Vigor	D4 - Depr	D4 - Tens	D4 - Angr	D4 - Fatig	D4 - Conf	D4 - Vigor
0	4	0	0	4	0	0	4	0	0	4	0

D1TMD	D2TMD	D3TMD	D4TMD
8	8	8	8

HRV rMSSD

Placebo

Day 1	Day 2	Day 3	Day 4
170.3	91.8	253.2	59.9

Whey

Day 1	Day 2	Day 3	Day 4
112.9	109.9	253.9	176.7

Diet

Placebo

Day 1	Day 2	Day 3	Day 4
2416.5	1151.55	3024	1598

Macros Day 1 (g)			Macros Day 2 (g)			Macros Day 3 (g)		
CHO	PRO	FAT	CHO	PRO	FAT	CHO	PRO	FAT
278	71	94	130.8	44.7	40.2	337.0	115.4	109.7

Average Macros

CHO	PRO	FAT
248.7	77	81.3

Average kcal

CHO	PRO	FAT
994.67	308.13	731.7

Average kcal with Supplement

CHO	PRO	FAT
1194.67	308.13	731.7

Average Total kcal

PL	WH
2197.35	1426.5

Average Total kcal with Supplement

PL	WH
2397.35	1826.5

Whey

Day 1	Day 2	Day 3	Day 4
1555.2	1162.35	1561.95	1451.7

Macros Day 1 (g)		Macros Day 2 (g)		Macros Day 3 (g)	

CHO	PRO	FAT	CHO	PRO	FAT	CHO	PRO	FAT
191.4	45.2	54.5	121.4	41.5	47.7	146.3	70.8	65

Average Macros

CHO	PRO	FAT
153	52.5	55.7

Average kcal

CHO	PRO	FAT
612.13	210	501.6

Average kcal with Supplement

CHO	PRO	FAT
812.13	410	501.6

Average Total kcal

PL	WH
2034.5	1323.73

Average Total kcal with Supplement

PL	WH
2234.5	1723.73

VO2

Placebo

VO2peak	Day 1 - Warm	Day 1 - 5 min	Day 1 – 15 min	Day 1 – 25 min	Day 1 Mean
34.7	19.6	22	22.5	22.8	21.73

Day 2 - Warm	Day 2 - 5 min	Day 2 - 15 min	Day 2 - 25 min	Day 2 Mean
23.2	24	24.2	24.4	23.95

Whey

VO2peak	Day 1 - Warm	Day 1 - 5min	Day 1 - 15 min	Day 1 – 25 min	Day 1 Mean
34.7	22.4	23.9	23.8	24.9	23.75

Day 2 - Warm	Day 2 - 5 min	Day 2 - 15 min	Day 2 - 25 min	Day 2 Mean
24.4	23.8	23.6	24	23.95

WAnT

Placebo

Peak Power

Day 1	Day 2
833	831

Relative Peak Power

Day 1	Day 2
12.3	12.4

Fatigue Index

Day 1	Day 2
18.9	17.9

Anaerobic Capacity

Day 1	Day 2
6.9	7.1

Whey

Peak Power

Day 1	Day 2
851	844

Relative Peak Power

Day 1	Day 2
12.7	12.2

Fatigue Index

Day 1	Day 2
19.2	17.8

Anaerobic Capacity

Day 1	Day 2
6.8	7.1

OC Dosage

Oral Contraceptives Dosing (mg E2)

0.1

RER

Subject 003			

Wk 1 Ex 1	WK 1 EX2	Wk 2 EX 1	Wk 2 Ex 2
0.76	0.9	0.9	0.94
0.85	0.93	0.96	0.95
0.94	0.96	0.93	0.95
0.92	0.95	0.94	0.95
0.88	0.98	0.93	0.94
0.94	0.95	0.96	0.94
0.96	0.94	0.95	0.94
0.97	0.98	0.99	0.89
0.95	0.94	0.94	0.91
0.96	0.95	0.95	0.9
0.95	0.89	0.93	0.89
0.94	0.88		0.92
0.93	0.89		0.94
0.95	0.89		0.92
0.91	0.88		0.93
0.91			
0.88			
0.918	0.927	0.944	0.927
PL	PL	WH	WH

Subject 004

Anthropometric Data

Age	Height (cm)	Weight (Kg)	VO2 ABS	VO2 REL	BMI
21	169	88.6	1.91	21.8	31

Dosage Order

Week 1 Week 2

Whey Placebo

Cortisol Data (pg/ml)

Placebo

D1/S1	D1/15	D1/30	D1/45	D1/60		D2/S1	D2/15	D2/30	D2/45	D2/60
0.118	0.2295	0.3405	0.337	0.6145		0.1935	0.384	0.2625	0.257	0.3325

D3/S1	D3/15	D3/30	D3/45	D3/60		D4/S1	D4/15	D4/30	D4/45	D4/60
0.0765	0.1195	0.143	0.223	0.2765		0.13	0.176	0.3155	0.424	0.3745

Whey

D1/S1	D1/15	D1/30	D1/45	D1/60		D2/S1	D2/15	D2/30	D2/45	D2/60
0.2115	0.4045	0.6285	0.364	0.3135		0.2355	0.318	0.422	0.4225	0.393

D3/S1	D3/15	D3/30	D3/45	D3/60		D4/S1	D4/15	D4/30	D4/45	D4/60
0.2145	0.245	0.38	0.473	0.319		0.1165	0.298	0.6865	0.5855	0.3755

POMS

Placebo

D1 - Depr	D1 - Tens	D1 - Angr	D1 - Fatig	D1 - Conf	D1 – Vigor	D2 - Depr	D2 - Tens	D2 - Angr	D2 - Fatig	D2 - Conf	D2 - Vigor
0	4	0	0	4	9	0	4	0	0	4	7

D3 - Depr	D3 - Tens	D3 - Angr	D3 - Fatig	D3 - Conf	D3 – Vigor	D4 - Depr	D4 - Tens	D4 - Angr	D4 - Fatig	D4 - Conf	D4 - Vigor
0	6	1	1	4	5	0	4	0	0	4	9

D1TMD	D2TMD	D3TMD	D4TMD

-1	1	7	-1

Whey

D1 - Depr	D1 - Tens	D1 - Angr	D1 - Fatig	D1 - Conf	D1 – Vigor	D2 - Depr	D2 - Tens	D2 - Angr	D2 - Fatig	D2 - Conf	D2 - Vigor
0	4	0	0	4	10	0	4	0	0	4	11

D3 - Depr	D3 - Tens	D3 - Angr	D3 - Fatig	D3 - Conf	D3 – Vigor	D4 - Depr	D4 - Tens	D4 - Angr	D4 - Fatig	D4 - Conf	D4 - Vigor
0	4	0	0	4	11	0	4	0	0	4	10

D1TMD	D2TMD	D3TMD	D4TMD
-2	-3	-3	-2

HRV rMSSD

Placebo

Day 1	Day 2	Day 3	Day 4
70.9	76.8	71.5	72.5

Whey

Day 1	Day 2	Day 3	Day 4
46.5	17.4	30.4	24.9

Diet

Placebo

Day 1	Day 2	Day 3	Day 4
1683	1877	1402	1654

Macros Day 1 (g)			Macros Day 2 (g)			Macros Day 3 (g)		
CHO	PRO	FAT	CHO	PRO	FAT	CHO	PRO	FAT
177	62	78	209	96	76	137	35	37

Average Macros

CHO	PRO	FAT
174.3	64.3	63.7

Average kcal

CHO	PRO	FAT

697.33	257.33	573

Average kcal with Supplement

CHO	PRO	FAT
897.33	257.33	573

Average Total kcal

PL	WH
1654	1466.67

Average Total kcal with Supplement

PL	WH
1854	1866.67

Whey

Day 1	Day 2	Day 3	Day 4
1612	1332	1456	1467

Macros Day 1 (g)			Macros Day 2 (g)			Macros Day 3 (g)		
CHO	PRO	FAT	CHO	PRO	FAT	CHO	PRO	FAT

204	61	64	140	75	54	147	81	73

Average Macros

CHO	PRO	FAT
163.7	72.3	63.7

Average kcal

CHO	PRO	FAT
654.67	289.33	573

Average kcal with Supplement

CHO	PRO	FAT
854.67	489.33	573

Average Total kcal

PL	WH
1527.67	1517

Average Total kcal with Supplement

PL	WH

1727.67	1917

VO2

Placebo

VO2peak Day	Day 1 – Warm	Day 1 – 5 min	Day 1 – 15 min	Day 1 – 25 min	Day 1 Mean
21.8	16.2	15.7	16	16.1	16

Day 2 - Warm	Day 2 - 5 min	Day 2 - 15 min	Day 2 - 25 min	Day 2 Mean
16.4	15.7	16.6	16.2	16.23

Whey

VO2peak	Day 1 – Warm	Day 1 – 5min	Day 1 – 15 min	Day 1 – 25 min	Day 1 Mean
21.8	15.1	16.2	15.4	15.2	15.48

Day 2 - Warm	Day 2 - 5 min	Day 2 - 15 min	Day 2 - 25 min	Day 2 Mean
16.4	16.22	15.9	15.9	16.11

WAnT

Placebo

Peak Power

Day 1	Day 2
1098	1143

Relative Peak Power

Day 1	Day 2
12.4	12.9

Fatigue Index

Day 1	Day 2
29.1	30.2

Anaerobic Capacity

Day 1	Day 2
4.8	4.8

Whey

Peak Power

Day 1	Day 2
1070	1091

Relative Peak Power

Day 1	Day 2
12.3	12.4

Fatigue Index

Day 1	Day 2
27.6	28.5

Anaerobic Capacity

Day 1	Day 2
4.9	4.7

OC Dosage

Oral Contraceptives Dosing (mg E2)

0.35

RER

Subject 004			

Wk 1 Ex 1	WK 1 EX2	Wk 2 EX 1	Wk 2 Ex 2
0.85	0.93	0.99	0.97
0.84	0.91	0.98	0.99
0.84	0.86	1.01	0.98
0.86	0.9	0.99	0.95
0.86	0.88	0.95	0.94
0.86	0.9	0.96	1
0.88	0.94	0.95	1
0.91	0.89	0.97	0.98
0.91	0.91	1.01	1.06
0.9	0.89	1.02	1.04
0.92	0.89	0.91	1.02
0.94	0.92	0.96	1.03
0.96	0.87	0.94	1
0.97			
0.893	0.899	0.972	0.997
WH	WH	PL	PL

Subject 006

Anthropometric Data

Age	Height (cm)	Weight (Kg)	VO2 ABS	VO2 REL	BMI
20	160	73.3	2.25	33.9	28.6

Dosage Order

Week 1 Week 2

Whey Placebo

Cortisol Data (pg/ml)

Placebo

D1/S1	D1/15	D1/30	D1/45	D1/60		D2/S1	D2/15	D2/30	D2/45	D2/60
0.234	0.361	0.5165	0.179	0.176		0.297	0.2675	0.39	0.3255	0.572

D3/S1	D3/15	D3/30	D3/45	D3/60		D4/S1	D4/15	D4/30	D4/45	D4/60
0.427	0.2865	0.2185	0.2625	0.27		0.27	0.3885	0.4255	0.4795	0.228

Whey

D1/S1	D1/15	D1/30	D1/45	D1/60		D2/S1	D2/15	D2/30	D2/45	D2/60
0.111	0.148	0.227	0.291	0.224		0.273	0.316	0.179	0.244	0.328

D3/S1	D3/15	D3/30	D3/45	D3/60		D4/S1	D4/15	D4/30	D4/45	D4/60
0.141	0.1715	0.417	0.5535	0.7005		0.149	0.1465	0.129	0.178	0.185

POMS

Placebo

D1 - Depr	D1 - Tens	D1 - Angr	D1 - Fatig	D1 - Conf	D1 – Vigor	D2 - Depr	D2 - Tens	D2 - Angr	D2 - Fatig	D2 - Conf	D2 - Vigor
0	4	0	0	4	8	0	6	0	1	4	9

D3 - Depr	D3 - Tens	D3 - Angr	D3 - Fatig	D3 - Conf	D3 – Vigor	D4 - Depr	D4 - Tens	D4 - Angr	D4 - Fatig	D4 - Conf	D4 - Vigor
0	4	0	1	4	5	0	4	0	0	4	6

D1TMD	D2TMD	D3TMD	D4TMD
0	2	4	2

Whey

D1 - Depr	D1 - Tens	D1 - Angr	D1 - Fatig	D1 - Conf	D1 – Vigor	D2 - Depr	D2 - Tens	D2 – Angr	D2 - Fatig	D2 - Conf	D2 - Vigor
0	10	0	5	5	1	0	4	1	1	4	2

D3 - Depr	D3 - Tens	D3 - Angr	D3 - Fatig	D3 - Conf	D3 – Vigor	D4 - Depr	D4 - Tens	D4 - Angr	D4 - Fatig	D4 - Conf	D4 - Vigor
0	4	0	0	4	2	0	4	0	0	4	6

D1TMD	D2TMD	D3TMD	D4TMD
19	8	6	2

HRV rMSSD

Placebo

Day 1	Day 2	Day 3	Day 4
211.1	269.7	190.2	191.1

Whey

Day 1	Day 2	Day 3	Day 4
269.5	180	179.4	178.2

Diet

Placebo

Day 1	Day 2	Day 3	Day 4
957.6	1341	1026.9	1282.5

Macros Day 1 (g)			Macros Day 2 (g)			Macros Day 3 (g)		
CHO	PRO	FAT	CHO	PRO	FAT	CHO	PRO	FAT
99	63	25.4	98.1	74.5	62.7	139.9	28.1	30.1

Average Macros

CHO	PRO	FAT
112.3	55.2	39.4

Average kcal

CHO	PRO	FAT
449.33	220.8	354.6

Average kcal with Supplement

CHO	PRO	FAT
649.33	220.8	354.6

Average Total kcal

PL	WH
1108.5	1240.07

Average Total kcal with Supplement

PL	WH
1308.5	1640.07

Whey

Day 1	Day 2	Day 3	Day 4
1179	621	243	681

Macros Day 1 (g)			Macros Day 2 (g)			Macros Day 3 (g)		
CHO	PRO	FAT	CHO	PRO	FAT	CHO	PRO	FAT
90	50	61	91	11	18	26	2	13

Average Macros

CHO	PRO	FAT
126.1	49.2	50.1

Average kcal

CHO	PRO	FAT
504.53	196.93	450.9

Average kcal with Supplement

CHO	PRO	FAT
704.53	396.93	450.9

Average Total kcal

PL	WH
1024.73	1152.37

Average Total kcal with Supplement

PL	WH
1224.73	1552.37

VO2

Placebo

VO2peak	Day 1 - Warm	Day 1 - 5min	Day 1 - 15 min	Day 1 - 25 min	Day 1 Mean
33.9	20.9	21.5	22.9	23.1	22.1

Day 2 - Warm	Day 2 - 5 min	Day 2 - 15 min	Day 2 - 25 min	Day 2 Mean
22	21.1	21.2	22.1	21.6

Whey

VO2peak	Day 1 - Warm	Day 1 - 5min	Day 1 - 15 min	Day 1 - 25 min	Day 1 Mean
33.9	20.2	21.6	24	22.8	22.15

Day 2 - Warm	Day 2 - 5 min	Day 2 - 15 min	Day 2 - 25 min	Day 2 Mean
22.6	23.5	23	22.6	22.93

WAnT

Placebo

Peak Power

Day 1	Day 2
961	1051

Relative Peak Power

Day 1	Day 2
13	14.5

Fatigue Index

Day 1	Day 2
23	27.8

Anaerobic Capacity

Day 1	Day 2
6.9	7.1

Whey

Peak Power

Day 1	Day 2
1016	1083

Relative Peak Power

Day 1	Day 2
13.9	14.9

Fatigue Index

Day 1	Day 2
26.8	29.6

Anaerobic Capacity

Day 1	Day 2
6.3	6.3

OC Dosage

Oral Contraceptives Dosing (mg E2)

0.03

RER

Subject 006

Wk 1 Ex 1	WK 1 EX2	Wk 2 EX 1	Wk 2 Ex 2
0.86	1.22	1.21	0.95
0.88	1.27	1.03	1.01
0.85	0.98	1.01	1.05
0.88	0.98	1.02	1.04
0.92	0.99	1	1.06
0.92	0.97	1.03	0.99
0.93	0.92	1.02	0.97

0.93	0.94	1	0.97
0.95	0.93	1.02	0.94
0.95	0.97	1.01	0.97
0.9	0.97	1	0.93
0.93	0.91	1.01	0.96
0.9	0.9	0.95	
0.85	0.9	0.94	
0.89		0.95	
0.89		0.97	
0.92		0.98	
		0.94	
		0.95	
		0.94	
		0.98	
0.903	0.989	0.998	0.987
WH	WH	PL	PL

Subject 007

Anthropometric Data

Age	Height (cm)	Weight (Kg)	VO2 ABS	VO2 REL	BMI
20	166	78.58	2.41	30.9	28.5

Dosage Order

Week 1 Week 2

Placebo Whey

Cortisol Data (pg/ml)

Placebo

D1/S1	D1/15	D1/30	D1/45	D1/60		D2/S1	D2/15	D2/30	D2/45	D2/60
0.222	0.479	0.6595	1.098	1.181		0.475	0.963	0.9505	1.46	1.333

D3/S1	D3/15	D3/30	D3/45	D3/60		D4/S1	D4/15	D4/30	D4/45	D4/60
0.8125	1.335	1.4085	1.41	1.1505		0.5775	0.9595	1.302	1.268	1.1405

Whey

D1/S1	D1/15	D1/30	D1/45	D1/60		D2/S1	D2/15	D2/30	D2/45	D2/60

0.2325	0.3095	0.7925	0.9595	0.8845		0.511	0.517	0.916	1.04	1.089

D3/S1	D3/15	D3/30	D3/45	D3/60		D4/S1	D4/15	D4/30	D4/45	D4/60
0.1735	0.362	0.282	0.5975	0.7505		0.199	0.257	0.416	0.623	0.496

POMS

Placebo

D1 - Depr	D1 - Tens	D1 - Angr	D1 - Fatig	D1 - Conf	D1 – Vigor	D2 - Depr	D2 - Tens	D2 - Angr	D2 - Fatig	D2 - Conf	D2 - Vigor
0	8	0	3	12	4	1	5	1	6	6	0

D3 - Depr	D3 - Tens	D3 - Angr	D3 - Fatig	D3 - Conf	D3 - Vigor	D4 - Depr	D4 - Tens	D4 - Angr	D4 - Fatig	D4 - Conf	D4 - Vigor
0	5	0	6	5	5	0	6	0	0	4	8

D1TMD	D2TMD	D3TMD	D4TMD
19	19	11	2

Whey

D1 - Depr	D1 - Tens	D1 - Angr	D1 - Fatig	D1 - Conf	D1 – Vigor	D2 - Depr	D2 - Tens	D2 - Angr	D2 - Fatig	D2 - Conf	D2 - Vigor

1	4	0	5	6	0	0	4	0	4	6	0

D3 - Depr	D3 - Tens	D3 - Angr	D3 - Fatig	D3 - Conf	D3 - Vigor	D4 - Depr	D4 - Tens	D4 – Angr	D4 - Fatig	D4 - Conf	D4 - Vigor
0	6	0	2	4	1	0	4	1	1	7	0

D1TMD	D2TMD	D3TMD	D4TMD
16	14	11	13

HRV rMSSD

Placebo

Day 1	Day 2	Day 3	Day 4
80	93.6	95.4	109

Whey

Day 1	Day 2	Day 3	Day 4
98.5	91.9	96.5	95.6

Diet

Placebo

Day 1	Day 2	Day 3	Day 4

2074.5	1903.5	1440	1806

Macros Day 1 (g)			Macros Day 2 (g)			Macros Day 3 (g)		
CHO	PRO	FAT	CHO	PRO	FAT	CHO	PRO	FAT
238	65	79	196	56	85.5	110	63	73.5

Average Macros

CHO	PRO	FAT
181.3	61.3	79.3

Average kcal

CHO	PRO	FAT
725.33	245.33	714

Average kcal with Supplement

CHO	PRO	FAT
925.33	245.33	714

Average Total kcal

PL	WH
1806	681

Average Total kcal with Supplement

PL	WH
2006	1081

Whey

Day 1	Day 2	Day 3	Day 4
750.15	1242.45	1137.6	1432.8

Macros Day 1 (g)			Macros Day 2 (g)			Macros Day 3 (g)		
CHO	PRO	FAT	CHO	PRO	FAT	CHO	PRO	FAT
93.9	14.8	29	165.4	24.7	43	142.2	20.8	44.4

Average Macros

CHO	PRO	FAT
133.8	20.1	38.8

Average kcal

CHO	PRO	FAT
535.33	80.4	349.2

Average kcal with Supplement

CHO	PRO	FAT
735.33	280.4	349.2

Average Total kcal

PL	WH
1029.6	964.93

Average Total kcal with Supplement

PL	WH
1229.6	1364.93

VO2

Placebo

VO2peak	Day 1 - Warm	Day 1 - 5min	Day 1 - 15 min	Day 1 - 25 min	Day 1 Mean
30.9	22.4	21.9	20.9	22.1	21.83
Day 2 - Warm	Day 2 - 5 min	Day 2 - 15 min	Day 2 - 25 min	Day 2 Mean	
21.6	22	21.6	21.6	21.7	

Whey

VO2peak	Day 1 - Warm	Day 1 - 5min	Day 1 - 15 min	Day 1 - 25 min	Day 1 Mean
30.9	23.6	24.1	25.2	20.2	23.28

Day 2 - Warm	Day 2 - 5 min	Day 2 - 15 min	Day 2 - 25 min	Day 2 Mean
20.9	21.8	22.4	21.1	21.55

WAnT

Placebo

Peak Power

Day 1	Day 2
1077	1122

Relative Peak Power

Day 1	Day 2
13.9	14.5

Fatigue Index

Day 1	Day 2
27	28

Anaerobic Capacity

Day 1	Day 2
7.3	7.1

Whey

Peak Power

Day 1	Day 2
1196	1249

Relative Peak Power

Day 1	Day 2
15.2	15.6

Fatigue Index

Day 1	Day 2
32.1	33.9

Anaerobic Capacity

Day 1	Day 2
6.6	6.7

OC Dosage

Oral Contraceptives Dosing (mg E2)

0.03

RER

Subject 007			
Wk 1 Ex 1	WK 1 EX2	Wk 2 EX 1	Wk 2 Ex 2
1.11	1.01	0.94	1.08
1.01	1.03	1.32	1
1.03	1.01	1.01	0.94
0.95	1	1.05	0.96
1.02	0.94	1.05	0.96
1	0.95	1.01	0.92
0.99	0.92	0.99	0.95
0.99	0.91	0.94	0.94
0.96	0.87	0.99	0.95
0.94	0.87	1.01	0.92
0.9	0.88	0.93	0.92
0.95		0.9	0.89
			0.92
			0.95
			0.93
			0.91

			0.92
0.988	0.945	1.012	0.945
PL	PL	WH	WH

Subject 008

Anthropometric Data

Age	Height (cm)	Weight (Kg)	VO2 ABS	VO2 REL	BMI
19	166	61.5	1.96	32.6	22.3

Dosage Order

Week 1 Week 2

Placebo Whey

Cortisol Data (pg/ml)

Placebo

D1/S1	D1/15	D1/30	D1/45	D1/60		D2/S1	D2/15	D2/30	D2/45	D2/60
0.386	0.498	0.648	0.613	0.557		0.4755	0.5	0.3865	0.492	0.464

D3/S1	D3/15	D3/30	D3/45	D3/60		D4/S1	D4/15	D4/30	D4/45	D4/60
0.354	0.4915	0.528	0.528	0.4925		0.543	0.4655	0.4915	0.374	0.445

Whey

D1/S1	D1/15	D1/30	D1/45	D1/60		D2/S1	D2/15	D2/30	D2/45	D2/60
0.371	0.436	0.5745	0.684	0.7305		0.32	0.3875	0.722	0.787	0.698

D3/S1	D3/15	D3/30	D3/45	D3/60		D4/S1	D4/15	D4/30	D4/45	D4/60
0.437	0.5775	0.49	0.5165	0.4		0.147	0.302	0.36	0.394	0.5525

POMS

Placebo

D1 - Depr	D1 - Tens	D1 - Angr	D1 - Fatig	D1 - Conf	D1 – Vigor	D2 - Depr	D2 - Tens	D2 - Angr	D2 - Fatig	D2 - Conf	D2 - Vigor
0	5	1	7	5	0	0	4	0	1	4	6

D3 - Depr	D3 - Tens	D3 - Angr	D3 - Fatig	D3 - Conf	D3 – Vigor	D4 - Depr	D4 - Tens	D4 - Angr	D4 - Fatig	D4 - Conf	D4 - Vigor
0	6	0	3	4	2	0	4	0	1	5	3

D1TMD	D2TMD	D3TMD	D4TMD
18	3	11	7

Whey

D1 - Depr	D1 - Tens	D1 - Angr	D1 - Fatig	D1 - Conf	D1 – Vigor	D2 - Depr	D2 - Tens	D2 - Angr	D2 - Fatig	D2 - Conf	D2 - Vigor
0	7	1	2	5	4	0	9	1	2	5	2

D3 - Depr	D3 - Tens	D3 - Angr	D3 - Fatig	D3 - Conf	D3 – Vigor	D4 - Depr	D4 - Tens	D4 - Angr	D4 - Fatig	D4 - Conf	D4 - Vigor

0	8	1	3	5	0	0	5	0	3	4	4

D1TMD	D2TMD	D3TMD	D4TMD
11	15	17	8

HRV rMSSD

Placebo

Day 1	Day 2	Day 3	Day 4
97.6	80.2	79.6	78.7

Whey

Day 1	Day 2	Day 3	Day 4
61.7	101.7	31.3	90.7

Diet

Placebo

Day 1	Day 2	Day 3	Day 4
1735.65	1270.8	299.25	465.7

Macros Day 1 (g)		Macros Day 2 (g)		Macros Day 3 (g)	

CHO	PRO	FAT	CHO	PRO	FAT	CHO	PRO	FAT
98.6	82.7	102.2	166.5	41.5	37.2	33.4	11.1	11

Average Macros

CHO	PRO	FAT
99.5	45.1	50.1

Average kcal

CHO	PRO	FAT
398	180.4	451.2

Average kcal with Supplement

CHO	PRO	FAT
598	180.4	451.2

Average Total kcal

PL	WH
1101.9	1043.4

Average Total kcal with Supplement

PL	WH
1301.9	1443.4

Whey

Day 1	Day 2	Day 3	Day 4
1317.6	1179	814.5	2061

Macros Day 1 (g)			Macros Day 2 (g)			Macros Day 3 (g)		
CHO	PRO	FAT	CHO	PRO	FAT	CHO	PRO	FAT
85	87	60.4	147	43	36	108	29	22

Average Macros

CHO	PRO	FAT
113.3	53	39.5

Average kcal

CHO	PRO	FAT
453.33	212	355.2

Average kcal with Supplement

CHO	PRO	FAT
653.33	412	355.2

Average Total kcal

PL	WH
1507.73	1020.53

Average Total kcal with Supplement

PL	WH
1707.73	1420.53

VO2

Placebo

VO2peak	Day 1 -Warm	Day 1 - 5min	Day 1 - 15 min	Day 1 - 25 min	Day 1 Mean
32.6	21.5	22.1	21.5	24	22.28
Day 2 - Warm	Day 2 - 5 min	Day 2 - 15 min	Day 2 - 25 min	Day 2 Mean	
21.3	21.7	22.6	22.5	22.03	

Whey

VO2peak	Day 1 - Warm	Day 1 - 5min	Day 1 - 15 min	Day 1 - 25 min	Day 1 Mean
32.6	21.2	21.1	22.6	22.2	21.78

Day 2 - Warm	Day 2 - 5 min	Day 2 - 15 min	Day 2 - 25 min	Day 2 Mean
21.1	22.2	21.3	22.6	21.8

WAnT

Placebo

Peak Power

Day 1	Day 2
720	676

Relative Peak Power

Day 1	Day 2
11.6	10.8

Fatigue Index

Day 1	Day 2
18.4	18.2

Anaerobic Capacity

Day 1	Day 2
5.3	4.8

Whey

Peak Power

Day 1	Day 2
698	715

Relative Peak Power

Day 1	Day 2
11.5	11.9

Fatigue Index

Day 1	Day 2
18.4	18.5

Anaerobic Capacity

Day 1	Day 2
5.2	5.5

OC Dosage

Oral Contraceptives Dosing (mg E2)

0.03

RER

Subject 008			
Wk 1 Ex 1	WK 1 EX2	Wk 2 EX 1	Wk 2 Ex 2
0.87	0.94	0.93	0.9
0.87	0.95	0.96	0.94
0.99	0.95	0.93	0.95
1.01	0.94	0.92	0.94
1.03	0.94	0.94	1.01
1.03	0.93	1.01	0.98
1.01	0.91	0.98	0.98
0.99	0.91	0.97	0.99
1	0.94	1.01	0.98
1.01	0.91	0.98	0.96
0.99	0.91	1.02	0.95
0.94	0.95	0.99	0.95
0.94		1	0.9
0.96		1	0.93
0.94		0.96	0.97
0.94		0.94	
0.96			

0.95			
0.94			
0.92			
0.9			
0.961	0.932	0.971	0.955
PL	PL	WH	WH

Subject 009

Anthropometric Data

Age	Height (cm)	Weight (Kg)	VO2 ABS	VO2 REL	BMI
22	157	72.6	2.43	34.3	29.5

Dosage Order

Week 1 Week 2

Placebo Whey

Cortisol Data (pg/ml)

Placebo

D1/S1	D1/15	D1/30	D1/45	D1/60		D2/S1	D2/15	D2/30	D2/45	D2/60
0.126	0.3505	0.5265	0.6605	0.531		0.271	0.2835	0.4135	0.588	0.6405

D3/S1	D3/15	D3/30	D3/45	D3/60		D4/S1	D4/15	D4/30	D4/45	D4/60
0.086	0.2125	0.3265	0.7755	0.625		0.298	0.4035	0.3145	0.3095	0.4235

Whey

D1/S1	D1/15	D1/30	D1/45	D1/60		D2/S1	D2/15	D2/30	D2/45	D2/60
0.2785	0.5145	0.3955	0.4695	0.448		0.245	0.2995	0.447	0.6075	0.6755

D3/S1	D3/15	D3/30	D3/45	D3/60		D4/S1	D4/15	D4/30	D4/45	D4/60

0.181	0.2905	0.4885	0.469	0.4215		0.3325	0.4	0.4325	0.26	0.3855

POMS

Placebo

D1 - Depr	D1 - Tens	D1 - Angr	D1 - Fatig	D1 - Conf	D1 – Vigor	D2 - Depr	D2 - Tens	D2 - Angr	D2 - Fatig	D2 - Conf	D2 - Vigor
0	16	4	4	10	1	0	4	0	0	4	2

D3 - Depr	D3 - Tens	D3 - Angr	D3 - Fatig	D3 - Conf	D3 - Vigor	D4 - Depr	D4 - Tens	D4 - Angr	D4 - Fatig	D4 - Conf	D4 - Vigor
0	4	2	6	4	0	0	4	0	0	4	2

D1TMD	D2TMD	D3TMD	D4TMD
33	6	16	6

Whey

D1 - Depr	D1 - Tens	D1 - Angr	D1 - Fatig	D1 - Conf	D1 – Vigor	D2 - Depr	D2 - Tens	D2 - Angr	D2 - Fatig	D2 - Conf	D2 - Vigor
0	4	0	0	4	3	0	4	3	0	4	0

D3 - Depr	D3 - Tens	D3 - Angr	D3 - Fatig	D3 - Conf	D3 - Vigor	D4 - Depr	D4 - Tens	D4 - Angr	D4 - Fatig	D4 - Conf	D4 - Vigor
0	4	0	8	4	0	0	4	0	0	4	0

D1TMD	D2TMD	D3TMD	D4TMD
5	11	16	8

HRV rMSSD

Placebo

Day 1	Day 2	Day 3	Day 4
78.8	81.1	34	38.7

Whey

Day 1	Day 2	Day 3	Day 4
74.1	35.8	60.1	54.8

Diet

Placebo

Day 1	Day 2	Day 3	Day 4
1625.85	1868.85	1293.75	1596.15

Macros Day 1 (g)			Macros Day 2 (g)			Macros Day 3 (g)		
CHO	PRO	FAT	CHO	PRO	FAT	CHO	PRO	FAT
112.8	58.7	94.7	106.8	58.7	124.9	148.8	41.1	48.8

Average Macros

CHO	PRO	FAT
122.8	52.8	89.5

Average kcal

CHO	PRO	FAT
491.2	211.33	805.2

Average kcal with Supplement

CHO	PRO	FAT
691.2	211.33	805.2

Average Total kcal

PL	WH
1596.15	1103.7

Average Total kcal with Supplement

PL	WH
1796.15	1503.7

Whey

Day 1	Day 2	Day 3	Day 4
984.15	913.5	447.3	781.65

Macros Day 1 (g)			Macros Day 2 (g)			Macros Day 3 (g)		
CHO	PRO	FAT	CHO	PRO	FAT	CHO	PRO	FAT
121.4	31.3	33	110	27	33	52	15	16.2

Average Macros

CHO	PRO	FAT
94.5	24.4	27.4

Average kcal

CHO	PRO	FAT
377.87	97.73	246.6

Average kcal with Supplement

CHO	PRO	FAT
577.87	297.73	246.6

Average Total kcal

PL	WH
845.63	722.2

Average Total kcal with Supplement

PL	WH
1045.63	1122.2

VO2

Placebo

VO2peak	Day 1 - Warm	Day 1 - 5min	Day 1 - 15 min	Day 1 - 25 min	Day 1 Mean
34.3	20.7	22.4	23.6	20.5	21.8

Day 2 - Warm	Day 2 - 5 min	Day 2 - 15 min	Day 2 - 25 min	Day 2 Mean
21.7	21.6	21.4	23.1	21.95

Whey

VO2peak	Day 1 - Warm	Day 1 - 5min	Day 1 - 15 min	Day 1 - 25 min	Day 1 Mean
34.3	21.9	22.3	22.4	21.5	22.03

Day 2 - Warm	Day 2 - 5 min	Day 2 - 15 min	Day 2 - 25 min	Day 2 Mean
22.8	20	21	20.4	21.05

WAnT

Placebo

Peak Power

Day 1	Day 2
900	960

Relative Peak Power

Day 1	Day 2
12.3	13.3

Fatigue Index

Day 1	Day 2
23.3	24.6

Anaerobic Capacity

Day 1	Day 2
5.4	5.8

Whey

Peak Power

Day 1	Day 2
959	974

Relative Peak Power

Day 1	Day 2
13.3	13.5

Fatigue Index

Day 1	Day 2
23.8	24.6

Anaerobic Capacity

Day 1	Day 2

5.7	6.3

OC Dosage

Oral Contraceptives Dosing (mg E2)

0.035

RER

Subject 009			
Wk 1 Ex 1	WK 1 EX2	Wk 2 EX 1	Wk 2 Ex 2
0.87	0.94	0.93	0.9
0.87	0.95	0.96	0.94
0.99	0.95	0.93	0.95
1.01	0.94	0.92	0.94
1.03	0.94	0.94	1.01
1.03	0.93	1.01	0.98
1.01	0.91	0.98	0.98
0.99	0.91	0.97	0.99
1	0.94	1.01	0.98
1.01	0.91	0.98	0.96
0.99	0.91	1.02	0.95
0.94	0.95	0.99	0.95

0.94		1	0.9
0.96		1	0.93
0.94		0.96	0.97
0.94		0.94	
0.96			
0.95			
0.94			
0.92			
0.9			
0.961	0.932	0.971	0.955
PL	PL	WH	WH

Subject 010

Anthropometric Data

Age	Height (cm)	Weight (Kg)	VO2 ABS	VO2 REL	BMI
21	168	80.68	2.11	26.5	28.6

Dosage Order

Week 1 Week 2

Whey Placebo

Cortisol Data (pg/ml)

Placebo

D1/S1	D1/15	D1/30	D1/45	D1/60		D2/S1	D2/15	D2/30	D2/45	D2/60

0.2195	0.3755	0.564	0.5735	0.6265		0.4375	0.599	0.51	0.493	0.4655

D3/S1	D3/15	D3/30	D3/45	D3/60		D4/S1	D4/15	D4/30	D4/45	D4/60
0.343	0.4725	0.589	0.5295	0.5085		0.2765	0.307	0.4815	0.439	0.404

Whey

D1/S1	D1/15	D1/30	D1/45	D1/60		D2/S1	D2/15	D2/30	D2/45	D2/60
0.3115	0.456	0.404	0.2505	0.2035		0.248	0.779	0.813	0.78	0.7045

D3/S1	D3/15	D3/30	D3/45	D3/60		D4/S1	D4/15	D4/30	D4/45	D4/60
0.401	0.4855	0.691	0.6915	0.6305		0.533	0.619	0.738	0.5585	0.284

POMS

Placebo

D1 - Depr	D1 - Tens	D1 - Angr	D1 - Fatig	D1 - Conf	D1 – Vigor	D2 - Depr	D2 - Tens	D2 - Angr	D2 - Fatig	D2 - Conf	D2 - Vigor
0	5	0	0	4	14	0	5	0	3	4	6

D3 - Depr	D3 - Tens	D3 - Angr	D3 - Fatig	D3 - Conf	D3 – Vigor	D4 - Depr	D4 - Tens	D4 - Angr	D4 - Fatig	D4 - Conf	D4 - Vigor
0	4	0	0	4	9	0	4	0	5	5	4

D1TMD	D2TMD	D3TMD	D4TMD
-5	6	-1	10

Whey

D1 - Depr	D1 - Tens	D1 - Angr	D1 - Fatig	D1 - Conf	D1 – Vigor	D2 - Depr	D2 - Tens	D2 - Angr	D2 - Fatig	D2 - Conf	D2 - Vigor
0	9	0	4	6	8	0	6	0	9	7	6

D3 - Depr	D3 - Tens	D3 - Angr	D3 - Fatig	D3 - Conf	D3 – Vigor	D4 - Depr	D4 - Tens	D4 - Angr	D4 - Fatig	D4 - Conf	D4 - Vigor
0	4	0	0	4	13	0	4	0	2	4	9

D1TMD	D2TMD	D3TMD	D4TMD
11	16	-5	1

HRV rMSSD

Placebo

Day 1	Day 2	Day 3	Day 4
33.2	33.1	42	27.3

Whey

Day 1	Day 2	Day 3	Day 4
25	36.7	31	29.9

Diet

Placebo

Day 1	Day 2	Day 3	Day 4
1075.05	666	1048.5	1102.5

Macros Day 1 (g)			Macros Day 2 (g)			Macros Day 3 (g)		
CHO	PRO	FAT	CHO	PRO	FAT	CHO	PRO	FAT
159.5	30.8	24.3	71	27	22	117	46	35

Average Macros

CHO	PRO	FAT
115.8	34.6	27.1

Average kcal

CHO	PRO	FAT
463.33	138.4	243.9

Average kcal with Supplement

CHO	PRO	FAT
663.33	138.4	243.9

Average Total kcal

PL	WH
929.85	781.65

Average Total kcal with Supplement

PL	WH
1129.85	1181.65

Whey

Day 1	Day 2	Day 3	Day 4
1296	1665	1937.25	1161.9

Macros Day 1 (g)			Macros Day 2 (g)			Macros Day 3 (g)		
CHO	PRO	FAT	CHO	PRO	FAT	CHO	PRO	FAT
155	47	43	178	63	64.5	227.5	69	67

Average Macros

CHO	PRO	FAT
186.8	59.7	58.2

Average kcal

CHO	PRO	FAT
747.33	238.67	523.5

Average kcal with Supplement

CHO	PRO	FAT
947.33	438.67	523.5

Average Total kcal

PL	WH
1748.27	1509.5

Average Total kcal with Supplement

PL	WH
1948.27	1909.5

VO2

Placebo

VO2peak	Day 1 - Warm	Day 1 - 5min	Day 1 - 15 min	Day 1 - 25 min	Day 1 Mean
26.5	18.6	18.6	20.2	18.5	18.98

Day 2 - Warm	Day 2 - 5 min	Day 2 - 15 min	Day 2 - 25 min	Day 2 Mean
19.6	19.2	18.5	18.6	18.98

Whey

VO2peak	Day 1 - Warm	Day 1 - 5min	Day 1 - 15 min	Day 1 - 25 min	Day 1 Mean

26.5	18.8	19.1	19.6	19.2	19.18

Day 2 - Warm	Day 2 - 5 min	Day 2 - 15 min	Day 2 - 25 min	Day 2 Mean
19.5	19.5	19	19.6	19.4

WAnT

Placebo

Peak Power

Day 1	Day 2
1080	1069

Relative Peak Power

Day 1	Day 2
13.4	13.3

Fatigue Index

Day 1	Day 2
29.6	29.7

Anaerobic Capacity

Day 1	Day 2
5.4	5.2

Whey

Peak Power

Day 1	Day 2
1070	1052

Relative Peak Power

Day 1	Day 2
13.3	13

Fatigue Index

Day 1	Day 2
30.8	28

Anaerobic Capacity

Day 1	Day 2
5.3	5.4

OC Dosage

Oral Contraceptives Dosing (mg E2)

0.02

RER

Subject 010			
Wk 1 Ex 1	WK 1 EX2	Wk 2 EX 1	Wk 2 Ex 2
0.82	0.8	0.87	0.81
0.87	0.83	0.86	0.79
0.88	0.81	0.85	0.81
0.85	0.75	0.84	0.84
0.86	0.77	0.82	0.83
0.89	0.77	0.84	0.86
0.89	0.77	0.85	0.84
0.82	0.77	0.81	0.8
0.85		0.83	0.83
0.89		0.86	0.83
0.84		0.87	0.79
0.82		0.8	0.81
		0.82	

		0.85	
0.857	0.784	0.841	0.82
WH	WH	PL	PL

Subject 011

Anthropometric Data

Age	Height (cm)	Weight (Kg)	VO2 ABS	VO2 REL	BMI
22	162	50.9	1.82	36.7	19.4

Dosage Order

Week 1 Week 2

Placebo Whey

Cortisol Data (pg/ml)

Placebo

D1/S1	D1/15	D1/30	D1/45	D1/60		D2/S 1	D2/1 5	D2/30	D2/4 5	D2/60

0.5915	0.9825	1.2835	1.2925	0.9585		0.89	2.075	1.6115	1.146	0.5135

D3/S1	D3/15	D3/30	D3/45	D3/60		D4/S1	D4/15	D4/30	D4/45	D4/60
0.6095	0.6385	1.548	1.121	0.6625		0.3755	1.161	1.251	0.987	0.756

Whey

D1/S1	D1/15	D1/30	D1/45	D1/60		D2/S1	D2/15	D2/30	D2/45	D2/60
0.597	1.032	1.2325	1.7055	1.207		0.89	2.075	1.6115	1.146	0.5135

D3/S1	D3/15	D3/30	D3/45	D3/60		D4/S1	D4/15	D4/30	D4/45	D4/60
0.1955	0.4015	0.5745	0.4975	0.5605		1.026	1.999	1.241	0.48	0.1455

POMS

Placebo

D1 - Depr	D1 - Tens	D1 - Angr	D1 - Fatig	D1 - Conf	D1 – Vigor	D2 - Depr	D2 - Tens	D2 - Angr	D2 – Fatig	D2 - Conf	D2 - Vigor

0	4	1	1	4	0	0	4	2	1	5	0

D3 - Depr	D3 - Tens	D3 - Angr	D3 - Fatig	D3 - Conf	D3 – Vigor	D4 - Depr	D4 - Tens	D4 - Angr	D4 - Fatig	D4 - Conf	D4 - Vigor
0	4	1	5	4	0	0	4	2	1	4	1

D1TMD	D2TMD	D3TMD	D4TMD
10	12	14	10

Whey

D1 - Depr	D1 - Tens	D1 - Angr	D1 - Fatig	D1 - Conf	D1 – Vigor	D2 - Depr	D2 - Tens	D2 - Angr	D2 - Fatig	D2 - Conf	D2 - Vigor
0	5	0	1	4	0	0	4	0	0	4	4

D3 - Depr	D3 - Tens	D3 - Angr	D3 - Fatig	D3 - Conf	D3 – Vigor	D4 - Depr	D4 - Tens	D4 - Angr	D4 - Fatig	D4 - Conf	D4 - Vigor
0	5	0	1	4	3	0	4	0	2	4	1

D1TMD	D2TMD	D3TMD	D4TMD
10	4	7	9

HRV rMSSD

Placebo

Day 1	Day 2	Day 3	Day 4
47.7	41.7	43.5	38.5

Whey

Day 1	Day 2	Day 3	Day 4
38.1	33.3	38.7	40.8

Diet

Placebo

Day 1	Day 2	Day 3	Day 4
1342.35	1899	2398.5	1648.35

Macros Day 1 (g)			Macros Day 2 (g)			Macros Day 3 (g)		
CHO	PRO	FAT	CHO	PRO	FAT	CHO	PRO	FAT
165.8	49.3	41.6	192	60	85	257	66	105

Average Macros

CHO	PRO	FAT
204.9	58.4	77.2

Average kcal

CHO	PRO	FAT
819.73	233.73	694.8

Average kcal with Supplement

CHO	PRO	FAT
1019.73	233.73	694.8

Average Total kcal

PL	WH
1879.95	1632.75

Average Total kcal with Supplement

PL	WH
2079.95	2032.75

Whey

Day 1	Day 2	Day 3	Day 4
1296	1665	1937.25	1161.9

Macros Day 1 (g)			Macros Day 2 (g)			Macros Day 3 (g)		
CHO	PRO	FAT	CHO	PRO	FAT	CHO	PRO	FAT
155	47	43	178	63	64.5	227.5	69	67

Average Macros

CHO	PRO	FAT
186.8	59.7	58.2

Average kcal

CHO	PRO	FAT
747.33	238.67	523.5

Average kcal with Supplement

CHO	PRO	FAT
947.33	438.67	523.5

Average Total kcal

PL	WH
1047.3	1509.5

Average Total kcal with Supplement

PL	WH
1247.3	1909.5

VO2

Placebo

VO2peak	Day 1 - Warm	Day 1 - 5min	Day 1 - 15 min	Day 1 - 25 min	Day 1 Mean
36.7	23.4	24.5	26.2	24	24.53

Day 2 - Warm	Day 2 - 5 min	Day 2 - 15 min	Day 2 - 25 min	Day 2 Mean
23.8	23.9	22.8	23.6	23.53

Whey

VO2peak	Day 1 - Warm	Day 1 - 5min	Day 1 - 15 min	Day 1 - 25 min	Day 1 Mean
36.7	21.5	25	25	24.1	23.9

Day 2 - Warm	Day 2 - 5 min	Day 2 - 15 min	Day 2 - 25 min	Day 2 Mean

22.8	23.4	24.3	24.5	23.75

WAnT

Placebo

Peak Power

Day 1	Day 2
645	623

Relative Peak Power

Day 1	Day 2
12.7	12.2

Fatigue Index

Day 1	Day 2
14	13.4

Anaerobic Capacity

Day 1	Day 2
7.4	7.6

Whey

Peak Power

Day 1	Day 2
653	687

Relative Peak Power

Day 1	Day 2
13.1	13.4

Fatigue Index

Day 1	Day 2
14.5	15.4

Anaerobic Capacity

Day 1	Day 2
8.3	8.3

OC Dosage

Oral Contraceptives Dosing (mg E2)

0.3

RER

Subject 011			
Wk 1 Ex 1	WK 1 EX2	Wk 2 EX 1	Wk 2 Ex 2
0.95	1.03	0.86	0.81
1	1.04	0.89	0.81
1.04	0.96	0.97	0.93
1.03	0.95	0.99	0.96
0.96	0.95	0.96	0.96
1	0.96	0.95	0.92
0.98	0.9	0.94	0.9
0.96	0.91	0.94	0.91
0.94	0.91	0.89	0.86
0.91	0.91	0.9	0.87
0.9	0.91	0.92	0.88
0.91	0.91	0.91	0.88
0.94	0.92	0.88	0.86
0.91	0.91	0.86	0.86
	0.92	0.86	
0.959	0.939	0.915	0.886

PL	PL	WH	WH

Subject 012

Anthropometric Data

Age	Height (cm)	Weight (Kg)	VO2 ABS	VO2 REL	BMI
21	160	57.73	1.8	31.3	22.6

Dosage Order

Week 1 Week 2

Whey Placebo

Cortisol Data (pg/ml)

Placebo

D1/S1	D1/15	D1/30	D1/45	D1/60		D2/S1	D2/15	D2/30	D2/45	D2/60
0	0.06	0.259	0.6045	0.2775		0.047	0.027	0.2045	0.2645	0.136

D3/S1	D3/15	D3/30	D3/45	D3/60		D4/S1	D4/15	D4/30	D4/45	D4/60
0	0.0055	0.0615	0.071	0.1145		0.1275	0.1385	0.233	0.3285	0.248

Whey

D1/S1	D1/15	D1/30	D1/45	D1/60	D2/S1	D2/15	D2/30	D2/45	D2/60
0	0.0165	0.1405	0.3585	0.331	0	0.0265	0	0.0135	0.0945

D3/S1	D3/15	D3/30	D3/45	D3/60		D4/S1	D4/15	D4/30	D4/45	D4/60
0	0.0305	0.1685	0.2045	0.062		0.0515	0.1785	0.291	0.463	0.8325

POMS

Placebo

D1 - Depr	D1 - Tens	D1 - Angr	D1 - Fatig	D1 - Conf	D1 – Vigor	D2 - Depr	D2 - Tens	D2 - Angr	D2 - Fatig	D2 - Conf	D2 - Vigor
0	4	0	2	4	0	0	4	0	2	4	0

D3 - Depr	D3 - Tens	D3 - Angr	D3 - Fatig	D3 - Conf	D3 – Vigor	D4 - Depr	D4 - Tens	D4 - Angr	D4 - Fatig	D4 - Conf	D4 - Vigor
0	4	0	9	4	1	0	4	0	5	4	1

D1TMD	D2TMD	D3TMD	D4TMD
10	10	16	12

Whey

D1 - Depr	D1 - Tens	D1 - Angr	D1 - Fatig	D1 - Conf	D1 – Vigor	D2 - Depr	D2 - Tens	D2 - Angr	D2 - Fatig	D2 - Conf	D2 - Vigor
0	5	0	5	4	1	0	4	1	7	4	0

D3 - Depr	D3 - Tens	D3 - Angr	D3 - Fatig	D3 - Conf	D3 – Vigor	D4 - Depr	D4 - Tens	D4 - Angr	D4 - Fatig	D4 - Conf	D4 - Vigor
2	4	2	12	4	0	0	4	0	8	4	0

D1TMD	D2TMD	D3TMD	D4TMD
13	16	24	16

HRV rMSSD

Placebo

Day 1	Day 2	Day 3	Day 4
51.1	50	46.3	54.6

Whey

Day 1	Day 2	Day 3	Day 4
64.3	40.1	45.6	54

Diet

Placebo

Day 1	Day 2	Day 3	Day 4
1120.5	903.6	1404	1923.75

Macros Day 1 (g)			Macros Day 2 (g)			Macros Day 3 (g)		
CHO	PRO	FAT	CHO	PRO	FAT	CHO	PRO	FAT
159	28	31	84.8	39.6	38.2	222	39	25.5

Average Macros

CHO	PRO	FAT
155.3	35.5	31.6

Average kcal

CHO	PRO	FAT
621.07	142.13	284.1

Average kcal with Supplement

CHO	PRO	FAT
821.07	142.13	284.1

Average Total kcal

PL	WH
1142.7	1062.92

Average Total kcal with Supplement

PL	WH
1342.7	1462.92

Whey

Day 1	Day 2	Day 3	Day 4
662.4	1494.95	1031.4	1165.5

Macros Day 1 (g)			Macros Day 2 (g)			Macros Day 3 (g)		
CHO	PRO	FAT	CHO	PRO	FAT	CHO	PRO	FAT
83.7	29.7	16.9	234.4	55.9	40.5	149.7	35.7	21.9

Average Macros

CHO	PRO	FAT
155.9	40.4	26.4

Average kcal

CHO	PRO	FAT
623.73	161.73	237.9

Average kcal with Supplement

CHO	PRO	FAT
823.73	361.73	237.9

Average Total kcal

PL	WH
1023.37	1023.37

Average Total kcal with Supplement

PL	WH
1223.37	1423.37

VO2

Placebo

VO2peak	Day 1 - Warm	Day 1 - 5min	Day 1 - 15 min	Day 1 - 25 min	Day 1 Mean
31.3	21.5	20.9	20.9	20.5	20.95

Day 2 - Warm	Day 2 - 5 min	Day 2 - 15 min	Day 2 - 25 min	Day 2 Mean
20.2	21.5	20.6	22.7	21.25

Whey

VO2peak	Day 1 - Warm	Day 1 - 5min	Day 1 - 15 min	Day 1 - 25 min	Day 1 Mean
31.3	20.5	20.5	21.1	22.1	21.05

Day 2 - Warm	Day 2 - 5 min	Day 2 - 15 min	Day 2 - 25 min	Day 2 Mean

20.8	20.5	21.1	21.8	21.05

WAnT

Placebo

Peak Power

Day 1	Day 2
700	689

Relative Peak Power

Day 1	Day 2
12.1	12.1

Fatigue Index

Day 1	Day 2
17.1	16.7

Anaerobic Capacity

Day 1	Day 2
6.8	6.8

Whey

Peak Power

Day 1	Day 2
699	700

Relative Peak Power

Day 1	Day 2
12	12.1

Fatigue Index

Day 1	Day 2
17.9	17.1

Anaerobic Capacity

Day 1	Day 2
6.1	6.7

OC Dosage

Oral Contraceptives Dosing (mg E2)

0.3

RER

Subject 012			
Wk 1 Ex 1	WK 1 EX2	Wk 2 EX 1	Wk 2 Ex 2

0.91	0.92	0.94	0.81
0.94	0.94	0.95	0.8
0.96	0.98	0.96	0.82
0.95	0.95	0.95	0.86
0.89	0.91	0.93	0.88
0.91	0.91	0.94	0.87
0.94	0.91	0.94	0.88
0.94	0.93	0.89	0.91
0.94	0.91	0.87	0.87
0.92	0.88	0.9	0.83
0.88	0.9	0.91	0.84
0.88	0.91	0.89	0.84
0.89	0.92	0.89	0.84
0.91	0.92	0.91	
0.9	0.92	0.93	
0.88	0.87		
0.85			
0.911	0.918	0.92	0.85
WH	WH	PL	PL