"ATHLETE-MONITORING TO ASSESS PERFORMANCE, BIOMARKER RESPONSES, AND TRAINING LOAD IN FEMALE COLLEGIATE ATHLETES"

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ABSTRACT OF THE DISSERTATION

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National Collegiate Athletic Association (NCAA) sports face unique challenges to success. Unlike their professional counterparts, collegiate athletes experience the stress of the sport coupled with academic demands, frequent travel, and other additional stressors that coaches and training staff must account for when assessing the training and recovery needs of their athletes. Therefore, athlete-monitoring methods that can be used to evaluate an athlete's capabilities and limitations, determine adaptations to a training program, and assess fatigue status become crucial in order to optimize performance and decrease injury risk, especially in the collegiate setting. In addition female athletes are at an increased-risk for certain hormonal-related issues due to their sex that may have long-term impacts on performance and overall health. Therefore, the purpose of this dissertation is to provide insight into the use of athlete-monitoring methods for the female athlete in order to optimize performance and health. In addition, this dissertation seeks to evaluate and characterize training loads, performance, and biomarkers throughout the competitive season in NCAA women's soccer and beach volleyball teams.

AIMS AND OBJECTIVES

AIM 1: Soccer is a physiologically demanding sport, particularly at the collegiate level where teams face distinct challenges such as a congested-match schedule and frequent travel. In addition to this congested match fixture, in-conference (IC) games may be considered of greater importance than out-of-conference (OC) games due to implications for the post-season and league standings. Therefore, the purpose of this study was to compare physical workload, physiological responses, and performance variables between IC and OC games over the course of a NCAA Division I women's collegiate soccer season. Female players were evaluated during all in-game play using the Polar TeamPro system, which was individualized based on pre-season performance testing (height, weight, age, VO_{2max}, VT, HR_{max}). The Polar TeamPro system utilized GPS, HR, and accelerometry to determine training load (TL), calories expended (kcal), total distance covered (DIS), sprints, time spent in HR zones, and distance covered in speed zones. All data obtained from Polar TeamPro were analyzed as a rate per minute playing time (PT) for each athlete during all games. Additionally, percent passing accuracy (PA%), dribble success (DS%), tackling success (TS%), and challenges won (CW%) were generated for all games. These data will provide information on physiological responses and performance metrics between IC and OC games. This may provide coaches and sport scientists with the necessary information to enhance periodization strategies to manage the cumulative season demands in order to maintain player performance and output throughout the entire season.

AIM 2: High training demands throughout the competitive season in female collegiate soccer athletes have been shown to induce changes in biomarkers indicative of stress, inflammation, and reproduction. Additionally, oral contraceptive (OC) use has been observed to cause changes in these biomarkers, which may be exacerbated in athletes using OCs. Therefore, the purpose of this study was to compare biomarkers and body composition changes between OC-using and non-using (CON) soccer players throughout the competitive season. Female collegiate soccer players were stratified into two groups based on their reported OC use at the start of pre-season (OC vs. CON). Prior to the start of pre-season and immediately post-season, athletes underwent a battery of performance tests. Pre-season performance characteristics were used to individualize each athlete's integrative Global Positioning System (GPS) and heart rate monitor, which was used to monitor all practices and games for the determination of training load and exercise energy expenditure. Blood draws and body composition assessments were performed prior to pre-season, on weeks 2, 4, 8, & 12 of the season, and immediately following week 15 (post-season). This research sought to provide insight into the effects of OCs on biomarkers and body composition in female athletes over the course of the competitive season. Additionally, this data may provide important information regarding the physiological effects and implications of OC use on aspects of performance, health, and recovery in female athletes.

AIM 3: Over the past five years women's beach volleyball has become the fastest growing collegiate sport, but despite this the training demands of the sport and performance characteristics of collegiate beach volleyball athletes have yet to be

determined. The purpose of this study was to evaluate internal and external training loads throughout a competitive season and to quantify the performance characteristics of NCAA DI women's beach volleyball players. Female beach volleyball players underwent body composition and performance testing (aerobic capacity, vertical jump, jump velocity) during pre-season. Players were monitored throughout the six-week competitive season (T1-T6) using an integrative GPS and heart rate (HR) monitoring system, which was individualized based on pre-season performance testing, for the determination of workload metrics. In addition to team data, all variables were also analyzed between travel squad (regular match-participation) and non-travel squad athletes. This observational study sought to provide important information regarding performance characteristics and the training demands of beach volleyball as well as insight into useful athlete-monitoring and testing techniques for success in the sport. This information may be used for the design and implementation of training programs to optimize performance and decrease injury risk in the sport. Additionally this study sought to determine differences in performance and workload metrics between travel squad players, who participate in competition matches, and non-travel squad player, which may aid in optimizing future player management.

DEDICATION

This dissertation is dedicated my Grandpa George who passed away during my first year of graduate school. Thank you grandpa for being the best role model I could ever ask for and for teaching me to always believe in myself. You have always been my #1 fan and I hope this dissertation makes you proud.

I would also like to dedicate this dissertation to my parents, Sue and Tony, my sisters, Dani and Niccola, and my boyfriend Kevin for their continual support and guidance throughout my graduate career.

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I. **IMPORTANCE OF ATHLETE-MONITORING**

Over the course of a single season, athletes undergo various periods of training including pre-season, competition (in-season), and off-season. The duration of these training blocks vary from sport to sport, and within collegiate athletics the amount and frequency of team training sessions are heavily regulated by the National Collegiate Athletic Association (NCAA). These rules and regulations are one of the many unique challenges NCAA sports face on their road to success. Unlike their professional counterparts, collegiate athletes experience the stress of the sport coupled with academic demands, frequent travel, and additional stressors that coaches and training staff must account for when assessing the training and recovery needs of their athletes. Therefore, the use of athlete-monitoring methods, particularly in the collegiate setting, becomes crucial for team success. Monitoring techniques can be used to evaluate an athlete's capabilities and limitations, determine adaptations to a training program, and assess fatigue status in order to optimize performance and decrease injury risk.

One of the challenges for team sports is to provide the optimal training stimulus for each individual athlete. A single periodized training program may provide too little or too much training stimulus for some players, while just the right amount of training stimulus induce positive training adaptations for others. This is concept often referred to as the Goldilocks' Principle is based off of Hans Selye's general adaption syndrome. Selye's general adaptation theory, which is the basis for periodized training, involves three stages: alarm, resistance, and exhaustion (78). Alarm occurs after an initial training stimulus in which performance declines below baseline (88). Subsequently this alarm is followed by resistance, in which the body attempts to adapt to the training stimulus and with adequate recovery, performance above baseline, also known as supercompensation, occurs (88). However, if recovery is insufficient relative the training stimulus, then exhaustion occurs, and reduced performance remains, unable to return back to baseline. Ideally with a periodized training program, athletes achieve adequate recovery to induce supercompensation and avoid exhaustion.

When intensified training leads to long-term impairments in sport performance this is recognized in the literature as overtraining. Overtraining exists on a continuum depending on the duration of the decrement in performance (62). Overreaching (OR) is when the decrement in performance lasts short-term and can be divided into functional overreaching (FOR), when performance is impaired from days to weeks, and non-functional overreaching (NFOR), in which performance decrements last weeks to months (62). If insufficient recovery to meet training demands continues, overtraining syndrome (OTS) can develop (62). OTS is characterized as a syndrome due to its multifaceted etiology in which sport-specific performance impairments last months to years (62). Development of OTS could potentially end an athlete's career and their athletic performance may never recover. Diagnosis of OTS is often made retrospectively, therefore overtraining research has sought to examine potential causes of OTS and create criteria for diagnosis. Many different hypotheses regarding the cause of OTS have been proposed such as the cytokine hypothesis (81), central fatigue hypothesis (23), glutamine hypothesis (68), glycogen depletion (19) amongst others; however each one only accounts for a part of the symptoms and findings associated with overtraining. Currently, there is no set diagnostic criteria for OR nor OTS due to the many conflicting findings, differences of methodology, and definitions of overtraining within the literature (62). Additionally, the majority of overtraining research has been performed within controlled lab settings that induce overreaching, making conclusions in an applied sport setting difficult (31, 62).

Furthermore, recent advances in the literature surrounding adequate energy intake to meet training demands demonstrate additional potentially confounding factors to classical overtraining research (64). Formerly labelled the female athlete triad to describe the interrelated disorders commonly found in female athletes (67), this clinical syndrome has been expanded to include male athletes and is characterized by inadequate energy intake (EI) to meet exercise energy expenditure (EEE) (64). Termed relative energy deficiency in sport or RED-S, this syndrome is caused by low energy availability (LEA), which is defined as EI minus EEE per kilogram body weight (65). Symptoms of RED-S are multifaceted involving impairments of many physiological functions including metabolic rate, menstrual function, bone health, immunity, protein synthesis, cardiovascular health as a well as psychological functions, which can precede and/or be a result of LEA (65). RED-S may be considered a facet of overtraining, as the syndrome is caused be inadequate recovery through inappropriate EI relative to EEE. Further research is warranted examining OR and OTS when controlling for adequate EI to meet training demands in athletes. Overall, despite the lack of conclusive findings within the literature for specific markers of OR and OTS, various athlete-monitoring methods can be implemented to optimize and detect important changes in athlete readiness, health, and performance (10, 31, 44).

II. ATHLETE-MONITORING METHODS

i. Maximal Performance Tests

Maximal performance tests can be used to assess an athlete's strengths and limitations, assess the efficacy of a training program, and to track changes in performance capabilities in over time (42). Maximal testing can be used to assess a variety of performance metrics necessary for sport performance including speed, agility, power, endurance, and strength. A fundamental aspect of athletic performance testing is that the tests selected should emulate the biomechanics and energy systems/demands of the particular sport (42). Selecting tests that fit this criteria enhance the validity of the test (42). For example in soccer although endurance may be interpreted as the primary skill necessary, power, strength, speed, and agility are also all required for success in the sport (92). Therefore, carrying out a battery of performance tests to assess these variables will allow for quantification of an athlete's capabilities and readiness (92). Thus, it is important for researchers and strength & conditioning practitioners to understand the demands of the sport being investigated when implementing performance testing as testing methods can have good reliability for one sport, but moderate for others (42).

Endurance is a necessary skill component of many sports, particularly those of longer duration where the main metabolic pathways used are aerobic energy systems. The most common assessment of endurance fitness measures an athlete's aerobic capacity, denoted as maximal oxygen consumption during exercise (VO_{2max}), and is typically expressed in milliliters of oxygen consumption per kilogram body weight per minute ($mlO_2 \cdot kg^{-1}min^{-1}$) (48). The gold standard for measuring aerobic capacity (VO_{2max}) is a maximal graded exercise test (GXT) via a metabolic cart to measure gas exchange. The mode of exercise during the GXT should be as sport-specific as possible and thus, is typically performed on a treadmill. A cycle ergometer may also be appropriate mode for lower-body dominant sports such as hockey as well as for athlete's coming back from injury. During the VO_{2max} test, heart rate (HR) is continuously recorded and can be used for the determination of maximal heart rate (HR_{max}). This can be useful for programming certain athlete-monitoring technologies as HR_{max} calculated from age-prediction equations have been shown to vary ±11 bpm from direct measurements (48). Other methods for assessing aerobic capacity include on-field assessments such as the Yo-Yo Intermittent test and submaximal GXT protocols where VO_{2max} is estimated using normative metabolic equations (48). While these indirect measures can evaluate endurance performance, they do not allow for the assessment of other biological thresholds such at ventilatory and lactate thresholds which can be ascertained using direct measures. Ventilatory threshold (VT), defined as the point at which ventilation increases disproportionately with increases in oxygen consumption, can be assessed during the direct VO_{2max} GXT. Lactate threshold (LT) is performed separately using a discontinuous GXT protocol to allow for blood lactate sampling between stages. VT and LT represent aerobic "efficiency" as they are associated with a shift from aerobic to more anaerobic energy systems (92). Both are trainable thresholds to a greater extent than VO_{2max} especially in elite athletes as VO_{2max} is predominantly influenced by a preset genetic ceiling (58). Therefore, training at or around VT or LT over time may increase an athlete's ability to utilize aerobic energy systems at higher intensities (%VO_{2max}) or for longer duration before transitioning to anaerobic systems. This increased aerobic efficiency has been associated with increased endurance performance and delayed fatigue.

Strength, power, and anaerobic capacity are also important fitness characteristics necessary for success in sport. Muscular strength, defined as the force exerted by musculature during one maximal effort (1-RM) with proper form, is typically assessed using core lifts such as bench, deadlift, and squat (42). These dynamic strength assessments, as opposed to isometric strength assessments measured by transducers, are preferable as they more closely resemble the movements and abilities necessary in sport (42). As maximal 1-RM testing may not be possible or recommended during the season or in preparatory periods, 3- to 5-RM testing can be used to estimate an athlete's 1-RM and may be more comparable to loads lifted during training (92).

Power is a function of both force and velocity and thus, power tests assess an athlete's ability to exert maximal force while accelerating at the fastest possible rate (42). The primary test to assess maximal power output are vertical jump tests, with the most common being a countermovement vertical jump (CMJ). Due to the relatively little burden on the athlete and ease of the test, vertical jump testing can be employed throughout a competitive season where it has been shown to be sensitive enough to detect alterations in athlete fatigue (35). Anaerobic capacity measures the maximal work performed with anaerobic energy systems: ATP-PCr and glycolysis. Typically anaerobic capacity is evaluated via the Wingate anaerobic test (WAnT) on a cycle ergometer. The WAnT is a 30s maximal effort ride with relative resistance on the fly wheel proportional to an individual's body weight. The WAnT assesses an athlete's peak power, average power, and fatigue index (ratio of maximum and minimum power) which illustrates an individual's ability to sustain maximal power output.

The skills of speed and agility assess the time it takes an athlete to cover a set distance, with agility involving changes of direction, stops, and starts. Agility tests in addition to involving rapid change of directions can also include sport-specific perceptual cues (42). Standard protocol to evaluate agility include the T-test, 505 agility, and pro-agility tests (42). Performance tests for maximal speed involve distances of less than 100m, as longer duration tests represent anaerobic and aerobic capacities (42). Speed and agility tests can be administered using a stopwatch; however, this method has been shown to often be inaccurate due to the anticipatory response of the testers (42). Electronic timing gates are thus the preferred method to assess maximal speed and agility and that provide the ability to assess accelerations (split times) at multiple distances throughout a speed test.

Due to the variety of skills required for success in sports, using a battery of performance tests allows coaches and training staff to characterize and evaluate a complete fitness profile for each athlete. Interrelationships have been shown between speed, agility, power, endurance, and strength performance in athletes. For example, maximal strength (1-RM squat) has been strongly correlated to 10m and 30m sprint performance, agility, and vertical jump height in elite soccer players (96). Moreover, greater 1-RM strength did not necessarily correspond to a reduced aerobic capacity in these athletes, highlighting high levels of endurance and power are fundamental to the sport (96). Evidently, a variety of physical abilities are necessary for superior athletic performance (42). Additionally, a comprehensive performance profile allows for the identification of strength and weaknesses specific to each athlete, which can be used to set goals and evaluate progress.

An additional consideration when implementing a selection of maximal performance testing is testing order. Appropriate testing order is crucial so that the completion of one test does not adversely affect performance in later tests. Thus, selection of testing order relies heavily on the energy systems used during a performance test (42). For example, the complete recovery of a taxed ATP-PCr energy system takes three to five minutes, whereas anaerobic glycolytic energy system takes at least hour (42). The recommended efficient and reliable testing session order based on NSCA guidelines is: 1) non-fatiguing tests (body composition, height, weight, etc.), 2) agility, 3) maximum strength and power, 4) sprint tests, 5) local muscular endurance tests, 6) fatiguing anaerobic capacity tests, and 7) aerobic capacity tests (42). Additionally, standardizing the warm-up prior to testing as well as time-of-day of testing will increase a test's reliability (42). Particularly for team sports, test selection and order will depend on the time and days allotted for testing around normal training and competition as well as accessibility to equipment and laboratory-tests. These limitations are important to consider when designing a testing program. Overall, performance testing allows for the quantification of an athlete's strengths, limitations, and changes in performance in order to tailor training to address team and individual needs to reduce injury risks and optimize athletic performance and health.

ii. Body Composition

Factors that can impact athletic performance are an athlete's body composition and weight. Thus, monitoring changes in body composition and weight in conjunction with performance testing and other athlete-monitoring methods can provide context to performance changes and athlete readiness. Body composition determines the "leanness" of an athlete, the proportion of fat mass (FM) relative to body mass of an individual. Body composition measures divide the body into two- to four-compartment models depending on the equipment used. Traditionally considered the gold standard to evaluate body composition, dual energy x-ray absorptiometry (DXA) utilizes a three-compartment model to assess FM, lean mass, and bone mineral content (66). However, accessibility, cost, and radiation associated with DXA scan often are prohibitive and as a result, other more accessible and cost-effective two-compartment methods that evaluate body composition as FM and fat free mass (FFM) are often used in athletes. In the two-compartment model, the FFM measurement consists of water, muscle, bone, and bodily organs. Measuring body composition throughout a macrocycle allows for the assessment of changes in fat and muscle mass. These changes can reveal physiological adaptations to training and may also give an indication of dietary intake and energy balance in an athlete (92). In elite athletes, increased EEE without concomitant increase in EI over 4-weeks produced significant decreases in body mass and fat mass as well as significant reductions in resting metabolic rate (RMR) (97). These body composition changes also corresponded to decreased sport-performance, despite no significant changes in FFM (97). Higher body fat percentage (%BF) has been correlated to lower vertical jump, lower aerobic capacity, and slower speed (80). Therefore, integration of regular body composition measurements into an athlete-monitoring program delivers valuable information regarding an athlete's fitness profile and perspective to performance outcomes and training adaptations.

iii. Training Loads

With advances in technology, there has been an increase in the number of methods and devices used monitor and manage training loads in athletes. Athlete training loads can be monitored using both external and internal load measurements (10, 44), with dissociations between the two a potential indicator of fatigue (44). External training loads are objective measures of the work completed by the athlete and are typically assessed using global positioning systems (GPS) and accelerometers (10). A typical external load metric assessed is the total distance covered by the athlete during training, which can be expressed as a rate per minute of a session duration or time on the field/court for inter-session comparison (84). Total distance covered has been shown to vary between sports and within a sport depending on the level of play For instance during a match, elite female players typically cover about ~10km (21, 22), while collegiate female players cover ~8.3km (60). Limited research exists examining external loads in court-based sports; however current findings have shown similar distances covered per minute in court versus field sports despite the smaller playing area (84). Wearable technologies using GPS and accelerometry can also provide additional external load metrics including distances covered at certain speed thresholds as well as the number of accelerations and decelerations (eccentric work) performed by an athlete. However, standards for velocity and sprint thresholds vary throughout team-sport research, with no set criteria for determining appropriate threshold values (84). Additionally, external load thresholds determined in male sports have often been applied to female athletes even though differences exist between sexes in maximal aerobic capacity and power production (sprint) capabilities (84). Thus, further research is necessitated evaluating velocity and sprint capabilities and thresholds in females athletes.

Internal load refers to the physiological and/or psychological stress of training on the athlete (10). A simple, no cost way to monitor the internal training load of a session on an athlete is by measuring rate of perceived exertion (RPE) (44). RPE can be measured on a scale of 1-10 or 6-20 (Borg scale) with the highest number indicating maximal exertion; therefore, the higher the number reported, the harder the athlete perceived that training session to be. RPE has been shown to be a valid measure in athletes; however, it is a subjective measure and thus can be influenced by subject bias (44). RPE can also be multiplied by the duration of exercise session, which can provide a good indication of the total load a training session on the athlete. Previous work using this RPE method for quantifying game training load found that playing time, rather than match duration, was superior in reflecting the workload of a NCAA DI men's soccer game (76).

Unlike RPE, heart rate (HR) monitoring provides an objective assessment of internal load during training. The estimation of exercise intensity using HR monitoring is based on the linear relationship between HR and oxygen consumption during submaximal steady-state exercise (49). Although this relationship does not necessarily hold true at high intensities, HR monitoring can also provide a reliable estimation of the EEE due to the relationship between oxygen consumption (VO_2) and caloric expenditure (1L O_2 = ~5 kcals expended)(14). Knowledge of calories expended during exercise can be used to assess the energy demands of training and the sport as well as the fueling needs of the athlete. This information allows for individualized sport nutrition programming for all players on a team, which is important due to the implications of inadequate fueling to meet training demands (64). Additionally, HR technology that allows for the individualization of monitors to each athlete's physiological profile (height, weight, $HR_{max,}$ VO_{2max}), ideally from maximal performance tests, will allow for an enhanced assessment of exercise intensity relative to each athlete (8). While findings have been inconsistent using HR alone to detect OR/OTS, some studies have a reported an increased resting HR in OR athletes, while other have reported a decreased maximal HR during maximal exercise (62). Despite inconclusive findings, consistent HR monitoring can of an athlete could be useful in identifying alterations in HR response during training and recovery. In a systematic review of the literature, long-term intensified training may lead to decreases in submaximal and maximal HR responses (9). Therefore, examining the relationship between internal and external loads may favorable for identifying fatigue in an athlete (44). This integrated approach assessing both internal and external training loads allows for the management of training stress to reduce the risk of overtraining, injury, and illness as well as improve athletic performance (10, 26, 44).

iv. Biomarkers

Although athlete-monitoring methods such as HR and GPS allow for the determination of internal and external training loads and recovery during training sessions, tracking changes in blood biomarkers may offer a more comprehensive picture of the cumulative demands of a competitive season outside of just on-field training sessions (2). Assessing blood biomarkers could be particularly useful at the collegiate level, where the stress of the sport is coupled with academic demands, frequent travel, and additional stressors that coaches and training staff must account for when assessing the training and recovery needs of their athletes (29). Even though previous research has shown no one biomarker to be indicative of overtraining (62), assessing a panel of biomarkers may allow for a big picture snapshot on the overall health and training status of an athlete. An extensive panel of biomarkers assessing nutritional status, hydration, anabolism, catabolism, inflammation, reproduction, and immune function allows for comprehensive monitoring of physiologic changes throughout various periods of training (53). Regular blood draws throughout the course of a macrocycle can be useful in establishing 'baselines' and normal responses for each individual. This is important as significant interindividual variability has been shown in biomarker responses to exercise as well as only a few biomarkers have normative ranges for athletes (53, 94). Ideally, biomarker measurements should be made at critical timepoints over the course of a season such as before the start of training (pre-season) and at the termination of the competitive season (post-season), as well as incrementally through the season and during the off-season in order to assess the load of training each part of the macrocycle on the individual (53). For example, in an NCAA collegiate soccer, blood draws could be performed prior to the start of pre-season training, every month throughout the season, post-season, prior to the start of off-season training, and post-offseason to track changes in athlete health (93).

The hallmark biomarker evaluated when examining the stress response, to either physical and/or psychological stimuli, is cortisol. Cortisol, a glucocorticoid and the chief hormone secreted in response to stress, is the final hormone produced in hypothalamic-pituitary-adrenal (HPA)-axis cascade that is activated during the "flight or fight" response to a stressor. In the stress response, corticotropin-releasing hormone (CRH), the main regulator of the HPA-axis, is released from the paraventricular nucleus in the hypothalamus, triggering the secretion of adrenocorticotropin hormone (ACTH) from the anterior pituitary (15). During stress, vasopressin (AVP) acts synergistically to CRH in an additive effect by also stimulating ACTH production (59). The role of AVP on the HPA-axis occurs in intensity-dependent fashion during exercise and thus, is predominant during high intensity exercise (59). This enhanced ACTH secretion stimulated by both AVP and CRH during exercise leads to an abundant cortisol release from the adrenal cortex (59). This dual stimulation of ACTH allows for a feedforward amplification of stress hormones as exercise intensity increases. The circulating cortisol then acts to mobilize fuels throughout the body in order to meet the energy demands of exercise. Acutely, cortisol activates the gluconeogenic enzymes to increase glucose production and stimulates lipolysis by inhibiting glucose uptake and glycolytic production of α-glycerophosphate, and by increasing sensitivity of adipose to catecholamine-stimulated lipolysis (7). Cortisol also increases the availability of amino acids in circulation by stimulating muscle protein degradation by selectively degrading Type II and sparing Type I fibers (17).

Inverse to its acute effects, chronically elevated cortisol stimulates free fatty acid uptake and triglyceride synthesis and storage by stimulating the synthesis of adipose lipoprotein lipase (LPL) and potentiates insulin's activation of LPL (7). Cortisol also stimulates appetite, particularly of high sugar/high fat foods, and induces peripheral insulin resistance and over-secretion (7, 28). These actions in conjunction with cortisol's muscle protein degradation could have ramifications on an athlete's body composition and ability to maintain muscle mass during periods of intense training and competition. In addition to its acute metabolic effects, cortisol also exerts anti-inflammatory actions such as inhibiting the production of proinflammatory cytokines and eicosanoids, and reducing capillary permeability to leukocytes (7). Additionally, high levels of plasma cortisol released during high intensity exercise induce immunosuppressive effects by inhibiting phagocytosis by leukocytes and of the synthesis to thymus-derived lymphocytes (7). During periods of intense training without sufficient recovery, glucocorticoid-induced immunosuppression may persist instead of the immuno-stimulation/adaptation found with chronic exercise and proper recovery (7). In overtraining and depression, a dysregulation of the HPA-axis has been shown to occur with an impairment of the negative feedback by cortisol on CRH and ACTH (46, 62). Moreover, chronically high norepinephrine and cortisol levels have also been shown to damage neurons and cause memory loss (34). Thus, HPA-axis dysregulation may be one of the underlying mechanisms behind the physiological and psychological symptoms associated with OTS and RED-S (4, 62, 64). Overall, chronic abnormal elevations in cortisol in an athlete may indicate impaired recovery, capacity for protein synthesis, and immune function (53).

Conversely, an anabolic hormone that is often monitored in conjunction with cortisol is testosterone (53). Testosterone promotes muscular hypertrophy by stimulating muscular protein synthesis and amino acid uptake, and reducing protein degradation (91). In addition to its role in protein metabolism, testosterone also promotes glycogen replenishment (36) as well as red blood cell production (79). As such, testosterone levels may have implications on muscle growth, fuel availability and aerobic capacity in addition to reproductive function in males. In males, exercise elicits acute increases in androstenedione, a precursor to testosterone, as well as free and total testosterone in an intensity-dependent fashion (32, 87). Whereas in females, acute increases in testosterone have been shown to be either non-existent or delayed; however, long-term training may increase resting levels (27, 69, 91). Testosterone production by Leydig cells been shown to be primarily responsible for acute testosterone increases following exercise, thus explaining the disparity in findings between males and females (91). Alternatively, prolonged periods of intense training may produce decreases in resting testosterone levels (40). Decreased testosterone levels have been associated with decreases in performance, energy, and strength in athletes (52, 53, 98). Decreased testosterone levels in males have also been implicated in the development and diagnosis of RED-S, which can have long-term impacts on athlete health (64).

High cortisol levels can impact testosterone production (59) as well as its ability to bind to its androgen receptor, leading to the inhibition protein synthesis and other testosterone-mediated effects on target tissues (15). As a result, the testosterone:cortisol (T:C) ratio has been examined in overtraining research, mostly in male athletes, as an indication of the anabolic/catabolic balance (53). Previous research has indicated that a 30% decrease in an athlete's T:C ratio may represent inadequate recovery (3). In NCAA DI men's soccer it was shown that athletes, particularly starters, who began the season with lower testosterone in addition to elevated cortisol levels, had a decreased T:C ratio over the course of the season as well as decreased performance at the end of the season, indicating a potentially catabolic environment and increased recovery needs in these athletes (52). On the other hand, following a two-week taper in overreached elite male triathletes, a significant increase in a T:C ratio corresponded to increases in 3km time trial performance and VO_{2max} (20), providing support for T:C ratio as an indication of athlete readiness and fatigue status. However, the use of the T:C ratio in female athletes as a marker of fatigue has not been substantiated.

Similarly to testosterone for males, estrogen plays an important role in female athlete health and performance. In addition to its roles in growth and maturation in females estrogen, specifically estradiol, the most biologically active form of estrogen, influences substrate utilization during exercise (86). During exercise, females utilize greater fat and less carbohydrate and protein than males due to estradiol-mediated effects on metabolic pathways (86). Additionally in males and females, estradiol has been shown to exhibit a protective effect against skeletal muscle damage (32, 50). Estrogen is also the primary hormone responsible for regulating bone health in males and females (13). Estradiol concentration has been shown to be a predictor of fracture risk, with decreased estradiol levels correlating to decreased bone mineral densities and increased fracture risks in males and females (13). Similar to testosterone in males, in response to acute exercise estradiol increases in women in an intensity-dependent fashion (6); however, long-term intense training may decrease estradiol levels (7). The reduced production of sex hormones observed in athletes with training has been largely attributed to the overlap between the HPA- and hypothalamic-pituitary-gonadal (HPG)-axes (59).

In males and females, the HPA-axis acts as to inhibit the HPG-axis, through the influence of corticotropin-releasing hormone (CRH) on gonadotropin-releasing hormone (GnRH) either directly or indirectly through β-endorphin or cortisol (59). Cortisol, whose production can also be stimulated by vasopressin (AVP) during stress such as exercise, acts to inhibit all levels of the HPG-axis beyond just GnRH (59). Therefore, in times of chronic intense training without proper recovery, a downregulation of the HPG-axis can occur, leading to decreases in follicle-stimulating hormone (FSH) and luteinizing hormone (LH) and subsequent decreased production of sex-specific gonadotropins: estrogen and progesterone in females, and testosterone in males (38, 59). HPG-axis disruption in males and females has been shown to have a detrimental impact on sports performance and health and is one of the main components of RED-S (64). For instance, elite junior ovarian suppressed (OS) swimmers exhibited a 9.8% decrease in 400m performance after 12 weeks of training compared to an 8.2% increase in their cyclic (CYC) counterparts (90). In elite distance athletes, bone injuries were ~4.5-fold more prevalent in amenorrheic (ES=0.85) and low testosterone (ES=0.52) athletes (45). Therefore, monitoring changes in reproductive hormones (FSH, LH, testosterone, estradiol, and progesterone) in context with changes in cortisol may provide indications of overtraining and/or inadequate energy intake in athletes.

A potential confounding factor to the stress response of training specific to the female athletes is hormonal contraceptive (HC) use due to the overlap between HPA- and HPG-axes (59, 61). In females, HCs modify normal hormonal fluctuations, suppressing endogenous productions of estrogen and progesterone (77). A recent study investigating the effects oral contraceptive (OC) use on the HPA-axis demonstrated that OCs alter the activation of the HPA-axis, increasing circulating levels of cortisol, thereby inducing metabolic alterations as well such as increasing circulating levels of triglycerides (47). This finding demonstrates that OC use may have an analogous impact on the HPA-axis as the stress of training. Therefore, OC use in conjunction with training, especially during times of high training loads such as during the competitive season, may produce an augmented stress response in female athletes. As a result, further research is warranted examining OC use in athletes and its implications on performance and recovery.

An additional reproductive biomarker that may be useful to monitor the anabolic/catabolic balance in athletes is sex-hormone binding globulin (SHBG), the transporter for testosterone and estradiol (E_2) (53). In males, the testosterone:SHBG ratio was shown to be correlated to concentric power improvements over two years of strength training (43). Therefore, changes in testosterone:SHBG ratio may indicate the ability of an athlete to adapt to a training stimulus (43). In female endurance athletes a negative correlation between cortisol and SHBG has been observed, with higher cortisol and lower SHBG levels corresponding to the severity of menstrual dysfunction (54). Following 8-weeks of intense military training, overreached male servicemen displayed higher SHBG at baseline and higher SHBG and cortisol levels post-training than their non-overreached counterparts (85). Moreover, dietary intake and weight loss may influence SHBG levels (63). Therefore, chronic changes in SHBG could be indicative of suboptimal recovery and/or nutritional intake (53).

Other biomarkers used to evaluate anabolic status are growth hormone (GH) and insulin-like growth factor-1 (IGF-1). Exercise stimulates the release of growth hormone releasing hormone (GHRH) from the hypothalamus, which then binds to its receptor at the anterior pituitary initiating the synthesis and release of GH (51). GH release during exercise has been shown to be intensity-dependent, thought to be primarily driven by the catecholamine response to exercise (95). GH has pervasive effects on a multitude of target tissues throughout the body during exercise and plays important roles in growth, reproductive function, osmoregulation, and immune function amongst others (51). GH secretion occurs in a pulsatile manner with its largest pulses observed at night, and its anabolic effects observed during exercise are contingent on this intermittent interaction of GH with its receptor (7, 83). GH stimulates muscles protein synthesis indirectly through the actions of IGF-1, whose synthesis is modulated by GH, and other endocrine factors (51). Additionally, GH is postulated to potentially exert direct effects on muscle and connective tissue protein synthesis (25). As a result, GH and IGF-1 levels play critical roles in muscular adaptations to training in athletes.

GH also has potent lipolytic actions in adipose tissue including increasing the sensitivity of adipose to catecholamine-stimulated lipolysis by stimulating the synthesis of additional β-receptors (71), stimulating the activity of hormone-sensitive lipase, and inhibiting fat synthesis (7, 51). GH also stimulates hepatic gluconeogenesis and thus, can be considered a nutrient repartitioning agent, acting to spare carbohydrate and use fat for fuel (7). As a result, chronic changes in GH and IGF-1 concentrations in athletes could impact body composition, which may impact performance outcomes. In addition chronic GH and IGF-1 changes may also influence training adaptations, with decreases indicating an impaired ability to maintain/increase muscle mass (53). Chronic HPA-axis activation, such as during prolonged stress, suppresses GH secretion and yields glucocorticoid-induced inhibition of IGF-1-mediated effects on target tissues (15). During intense training combined with LEA, athletes experienced significant decreases in IGF-1, with declines becoming more pronounced over the 12-weeks of training indicating a potentially increased catabolic environment in these athletes (90). Thus, periods of high training loads accompanied with inadequate recovery may hinder the ability of athletes to adapt to a training program as well as maintain muscle mass. Therefore, monitoring changes in anabolic status in conjunction with body composition may offer enhanced insight into the anabolic/catabolic status in athletes over a season.

Assessing biomarkers including thyroid-stimulating hormone (TSH), thyroxine (T_4), and triiodothyronine (T_3) can provide insight into the metabolic function and provide context to nutritional intake by athletes. Various stimuli including changes in body temperature, dietary intake, stress (i.e. exercise), and hormones such as estrogen and catecholamines affect the release of thyroid releasing hormone (TRH) (7, 82). TRH, released from the hypothalamus, stimulates the release of TSH from the anterior pituitary, which in turn causes the production and release of T_4 and T_3 from the thyroid gland (7). The majority of circulating thyroid hormones are bound to protein carriers; however, free (unbound) T_3 is the biologically active form able to influence cellular metabolism. Thyroid hormones have an impact on a wide range physiological functions, often in a permissive manner, including metabolism, growth and development, and nerve function (7, 82). During exercise thyroid hormone release is dependent on exercise intensity and duration in addition to training-status, with training increasing their secretion and turnover (5, 16). Continual activation of the HPA-axis can cause abnormal thyroid function as glucocorticoids released during exercise (stress) suppress both TSH production and the conversion of T_4 into T_3, and increase the production of reverse T_3 (41, 59). Thus, hormonal alterations such as chronic elevations in cortisol as well as inflammatory markers observed during intense training (coupled with inadequate recovery) could have a negative impact on metabolic function (59). Low T_3 in athletes has been shown to be a consequence of inadequate energy intake to meet training demands and altered LH pulsatility in females (55). In US National Team swimmers, T_3 levels correlated to performance improvements, with low T_3 levels corresponding to low performance improvements over six months (89). Interestingly, all low performance athletes were oligomenorrheic or amenorrheic (89). This finding was corroborated in elite youth ovarian suppressed swimmers who displayed significantly lower T_3 (19% lower) and lower EA (90% lower) than their cyclic counterparts (90). Moreover, the ovarian suppressed swimmers displayed a 9.8% decrease in 400m performance compared to a 8.7% increase over 12-weeks of training, signifying a meaningful interplay between reproductive function, metabolism, and performance outcomes in athletes (90).

Another hormone that may provide insight into an athlete's nutritional intake is leptin. Leptin is an adipose-derived hormone whose plasma levels correlate to chronic changes energy stores and balance (1). Leptin also regulates bone set-points by acting on the bone hormone osteocalcin amongst other mechanisms and in females leptin levels have been shown to influence bone mineral density (38). In addition, leptin has been postulated to play a role in reproductive function through disruption GnRH pulsatility at the level of the hypothalamus leading to disruptions in LH pulsatility (38, 59). Leptin has been shown to be downregulated in female's with exercise-induced amenorrhea as known as functional hypothalamic amenorrhea (38, 59). Leptin has also been shown to correlate to resting energy expenditure per fat free mass (REE/FFM) in exercising women, explaining 12.8% of the variance in REE/FFM beyond the level of menstrual dysfunction (24). Moreover, increases in leptin have also been associated with elevations in inflammatory cytokines (1). Thus, decreased levels of leptin over time in athletes may indicate decreasing energy balance and availability as well as inflammation.

Inflammatory cytokines such as interleukins (IL-6, IL-1β) and tumor necrosis factor-α (TNF-α) are released during stress and are the central biomarkers of the cytokine hypothesis to overtraining (81). In response to muscular trauma during exercise, IL-6 is produced and released from skeletal muscle (73). Magnitude of IL-6 secretion during exercise has been correlated to catecholamine responses (72) and to creatine kinase (CK) levels, which is also released from the skeletal muscle from Z-line damage (30). One of the roles of IL-6 is to regulate local and systemic inflammation and immunity (81). Increased release of pro-inflammatory cytokines IL-1β and TNF-α are induced with exercise (70); however, their expression is directly inhibited by IL-6 (73). This inhibition could explain the magnitude of increase observed in these cytokines with exercise, as IL-1β and TNF-α increased 2-fold whereas IL-6 increased 128-fold post-marathon (70). Pro-inflammatory cytokines IL-1β and TNF-α have multiple diverse actions in the body including the activation of endothelial cells for further cytokine production, regulation of the synthesis of acute phase proteins and regulation of body temperature (81). Exercise induces an acute phase response (APR) in the body, with elevated IL-6 levels stimulating increased c-reactive protein (CRP) release (70). CRP has been shown to sensitive marker of systemic inflammation and tissue damage, with circulating levels related to cardiovascular disease risk (75). With exercise, the extent of the APR appears to related to the degree of muscle catabolism generated during exercise and the clearance of damaged tissue (12) and is thought to be a potential mechanism for muscular hypertrophy (7).

Another anti-inflammatory action is IL-6 amplifies the activation of the HPA-axis through stimulation of CRH release from the hypothalamus (7). Through negative feedback, especially after high-intensity exercise, circulating cortisol inhibits further IL-6 secretion (59) as well as potentially counteracts some of the effects of the APR (7). Long-term these elevated glucocorticoid levels can cause glucocorticoid immunosuppression and an increased susceptibility for infections, which may partially explain the increased number viral and upper respiratory infections observed in overreached and OTS athletes (62). Therefore monitoring changes in IL-6, IL-1β, TNF-α, CRP and CK may provide the insight into the recovery status of athletes.

Another hormone that has been implicated in overtraining research is prolactin. Prolactin release can be stimulated by IL-6 production (59) and occurs in an intensity-dependent fashion during exercise (56). In addition to its role in reproductive function and lactation in females, prolactin aids in maintaining body homeostasis through its regulation of immune function, osmotic balance, and angiogenesis (33). Blunted prolactin responses to exercise and reduced basal levels have been associated with OTS; however, findings are not consistent (11, 62).

Declines in athletic performance have also been associated with poor iron status in athletes. Iron plays an important role in oxygen transport throughout the body as well as during oxidative phosphorylation within the mitochondria (74). Markers of iron status such as iron, total iron binding capacity (TIBC), transferrin, and ferritin can be used to determine and track changes in iron status in athletes. Transferrin is a glycoprotein that binds to and transports iron throughout the body (37) and ferritin is the stored form of iron within a cell (74). TIBC represents the total amount of iron if all transferrin within the blood was saturated with iron while percent saturation (% saturation) refers to the actual amount of saturated transferrin. Classification of iron deficiency varies throughout the literature, but it is commonly evaluated using ferritin and % saturation levels (57). Female athletes in particular appear to be at increased risk for iron deficiency, with 31% to 82% of female athletes classified as iron deficient (18, 89). This increased risk for iron deficiency could be contributed to a multitude of factors including menstruation, sweating, dietary intake, gastrointestinal conditions, and elevated cytokine levels in addition to others (74). During an intense training period or the competitive season, these various mechanisms could produce iron losses in athletes, negatively effecting iron status and subsequent health and performance (74). Reductions in iron may have particular impact on endurance performance due to a diminished ability to deliver oxygen to working skeletal muscles, with declines in performance most pronounced in athletes with iron deficiency with anemia (39, 74). Thus, regular iron status assessments provide critical information regarding an athlete's capacity for optimal training adaptations and performance. Although biomarker monitoring may be expensive and time-consuming, it can provide useful information regarding the overall health and training status of individual athletes on a team. Additionally, tracking changes in biomarkers may provide context to changes observed with other monitoring-methods.

v. Conclusion

Although various athlete-monitoring methods exist, it is critical that the athlete-monitoring tools implemented into a sport science program are efficient, assessable, and are sensitive enough to detect meaningful changes on both an individual and group levels

(10, 44). Utilizing a battery of athlete-monitoring methods allows for a complete picture of an athlete's strengths and weaknesses, health, and performance (92). Strategic and systematic development of a plan for athlete testing and monitoring allows for the assessment of training needs in order to maximize player as well as team performance, reduce injury risk, and prioritize player health and well-being. The majority of previous research evaluating performance characteristics and demands of sport have been done in male athletes, with findings applied to female athletes despite well-known physiological differences between sexes. Additionally, of the limited research in female athletes, the bulk has been conducted in elite players. Therefore, further research examining training demands and performance specifically in collegiate female athletes is warranted.

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CHAPTER II:

VARYING DEMANDS AND QUALITY OF PLAY BETWEEN IN-CONFERENCE AND OUT-OF-CONFERENCE GAMES IN DIVISION I COLLEGIATE WOMEN'S SOCCER

ABSTRACT

The purpose of this study was to assess differences in physical workload, physiological responses, and performance variables between in-conference (IC) and out-of-conference (OC) games during a collegiate women's soccer season. **Methods:** Female field players (N=11), who played a minimum of 45 minutes for >50% of games, were evaluated using an integrative GPS and heart rate (HR) monitoring system to determine training load (TL), exercise energy expenditure (EEE), total distance covered (DIS), sprints, time spent in HR zones 4 and 5 (HR_{Z4}=80-89%max; HR_{Z5}=90-100%max), and distance covered in speed zones 4 and 5 (DIS_{Z4}=15.0-19.9km/h; DIS_{Z5}=≥20km/h). Additionally, percent passing accuracy (PA%), dribbling success (DS%), tackling success (TS%), and challenges won (CW%) were generated for all games. Workload data were analyzed as a rate per minute playing time (PT) per game to account for differences in game duration and PT between OC (n=7) and IC games (n=11). RM-MANOVAs with univariate follow-ups and effect sizes (Hedges' *g*) were conducted to compare conditions (OC vs. CON) ($p<0.05$). **Results:** There were significantly greater TL, DIS, EEE, and HR_{Z5} per minute PT in OC versus IC games (Hedges' *g*: TL=0.48; DIS=0.20, EEE=0.55; HR_{Z5}=0.83; $p<0.05$). PT (g=p=0.076) and TS% (p=0.073) favoring IC games approached significance, with no differences in any other variable ($p>0.05$). Further analysis found significant differences in first half play favoring OC games ($p<0.05$), but not second half play ($p>0.05$). **Conclusion:** Based on these findings, OC games appear to be more demanding compared to IC, particularly during first half play. **Practical Application:** Emphasis should be placed on tailoring TL to the accumulating in-season demands through athlete-monitoring technology to prevent declines in performance in the latter half of the season.

INTRODUCTION

Soccer is a physiologically demanding sport, particularly at the collegiate level where teams face unique challenges to success. Specifically, the rules and regulations the National Collegiate Athletic Association (NCAA) places on training and competition schedules for collegiate soccer results in a 16-week fall competitive season (16). The NCAA also specifies that soccer teams are permitted to a maximum of 20 matches within a 12-week period, which is preceded by a 21 unit (less than three-week) preseason (16). For collegiate soccer teams this results in a congested match schedule with an average of 1.66 matches played per week and, at best, a few days between games (9). In addition to this congested match fixture, typically in-conference (IC) games are more integral for team success than out-of-conference (OC) games due to implications for the post-season and league standings. This is particularly relevant in conferences that hold a final conference tournament where champions receive an automatic bid to the NCAA tournament. In highly competitive conferences with tournaments, such as the Big Ten which consistently has several nationally ranked teams every year, the IC schedule may prove particularly demanding and important. The NCAA post-season tournament (NCAA Women's College Cup) consists of 64 teams with 31 conference champion teams qualifying automatically and the remaining teams selected by the NCAA Soccer Committee (17). The selection process relies heavily on the Ratings Percentage Index (RPI), that takes into account both the strength of schedule (i.e. opponent difficulty) and a team's winning record (17). Therefore, the strength of a team's OC schedule is also factored into making the post-season through these at-large bids to the NCAA Women's College Cup. In women's collegiate soccer, OC games tend to be scheduled heavily in the beginning of the season whereas IC games are more commonly backloaded in the schedule. As a result, it is imperative for teams to be able to maintain player output in the transition from OC to IC games in order to have a successful season.

Previous research on the demands of soccer have demonstrated the intermittent, power-endurance nature of the sport. During a 90-minute soccer match, numerous explosive movements occur, with sprints occurring approximately every 90s (20). While the majority of the research involving game metrics has focused on male players, limited studies have examined the competition demands in females, with the majority being in elite professional female soccer players. During a match, elite female players typically cover about ~10km (4, 5), with about 1.7km (5) to 2.5km (4) performed at high-speed depending on the velocity threshold used (>18 versus 19.8–25.1 km/h respectively). In NCAA Division I women's soccer, distance covered is slightly reduced, with players covering ~8.3km and 0.40 km at speeds >19 km/h, in addition to performing on average 14 sprints (defined as any movement greater than 2.8 m/s^2) a game (14). Additionally, the high intensity nature of match play has been demonstrated in elite females with average and peak heart rates (HR) of 87% and 97% HR_{max} respectively (13) and in collegiate females with an average caloric expenditure of 15.4 kcal per kg body weight (14). During a game, player performance typically decreases in the second half, with less distance-covered and reduced exercise intensity (decreased high-speed running and number of sprints), compared to the first half (4, 20). Elite professional female players have been shown to perform greater high intensity running and a greater number of sprints in international (national team or UEFA Women's Cup) versus domestic club games (1). The increased demands of international game play present a challenge for coaches to not only prepare but manage their athletes for this intensified play. In addition to the limited research on female collegiate soccer, no research exists examining the demands of match play between IC and OC games in female collegiate players. Knowledge of these demands could enhance player development and management over the course of the season.

Therefore, the purpose of this study was to compare physical workload, physiological responses, and performance variables between IC and OC games over the course of a NCAA Division I women's collegiate soccer season. It was hypothesized that workloads, physiological responses and performance of the soccer players would differ between IC and OC games.

METHODS

i. *Experimental Approach to the Problem*

Female collegiate soccer players were monitored throughout the competitive season to determine differences in physical workload, physiological responses, and performance variables between IC and OC games. Players were evaluated during all in-game play using the Polar TeamPro system, which was individualized based on pre-season performance testing (height, weight, age, VO_{2max}, VT, HR_{max}). Polar TeamPro utilized global positioning system (GPS), HR, and accelerometry to determine training load (TL), exercise energy expenditure (EEE), total distance covered (DIS), sprints, time spent in HR zones, and distance covered in speed zones (3). These metrics were assessed during an athlete's playing time (PT) on the field for each game. All warm-up, half-time, and bench times were factored out of analysis so that only on-field game play were included in analysis. All data obtained from Polar TeamPro were analyzed as a rate per minute PT based on the Polar data to account for differences in game duration and PT between games. Additionally, game data were generated by InStat video and analytics system to determine percent passing accuracy (PA%), dribbling success (DS%), tackling success (TS%), and challenges won (CW%). These measures were included to compare technical performance between IC and OC games.

ii. Subjects

Female collegiate soccer players were monitored throughout the 2018 competitive season and field players who played a minimum of 45 minutes for >50% of games (N=11) were included in analysis. This playing time criteria was chosen in order to evaluate athletes who consistently played throughout the season in both OC and IC games, and saw significant amounts of on-field playing time in order to assess total accumulated effects of the season without artificially impacting metrics per minute playing time due to spurts of activities from substitutes. Descriptive and baseline performance characteristics are presented in *Table 1*. Informed consent was obtained from all subjects prior to the participation and all subjects received clearance by the

University sports medicine staff prior to testing. All players were on the same Division I NCAA women's soccer team in the Big Ten Conference. Research was approved by the Rutgers University Institutional Review Board for the Protection of Human Subjects and conducted in accordance with the Declaration of Helsinki

Table 1: Descriptive Characteristics

	Mean ± SD
Age *(years)*	19.00 ± 1.0
Weight *(kg)*	68.06 ± 5.4
BF%	19.81 ± 4.5
VO_{2max} *($ml \cdot kg^{-1} min^{-1}$)*	48.33 ± 5.0

BF%: body fat percentage; VO_{2max}: aerobic capacity

PROCEDURES

iii. Preseason Performance Testing

Athletes reported to the Rutgers University Center for Health and Human Performance (CHHP) prior to the start of preseason to complete a battery of tests. Subjects were instructed to arrive euhydrated, at least two-hours fasted, and having abstained from exercise 24-hours prior to testing. First, body composition was assessed using air displacement plethysmography via the BodPod (BODPOD, COSMED, Concord, CA) to determine percent body fat (%BF) with a predicted lung volume using the Brozek formula (2, 6). After a standardized warm-up, a maximal graded treadmill exercise test (GXT) was used to measure maximal aerobic capacity (VO_{2max}) and ventilatory threshold (VT) via direct gas exchange measured by a COSMED Quark CPET (COSMED, Concord, CA).

A speed-based protocol was used with stages that were equated using metabolic equivalents (MET) to the Bruce protocol. This protocol included two-minute stages at a constant 2% incline, with increasing speeds of 6.43, 7.88, 9.97, 11.74, 13.67, 15.61, 17.06, 18.18, 19.79, 21.08 km/h (14). Subjects continued the test with encouragement from researcher assistants until volitional fatigue. At least two of the following criteria were met for attainment of VO_{2max}: RER ≥1.1, observation of a plateau in O_2 consumption (increase ≤150 ml/min with increasing workload), and HR >85% age-predicted HR_{max} (208 – 0.7 x age). For three players (n=3) who did not meet the above criteria VO_{2peak} was used. Subject's VT was calculated after the completion of each test as the point where ventilation increased nonlinearly with VO_2, expressed as a percentage of VO_{2max} (10). HR was continuously monitored using a Polar S610 HR monitor to obtain maximal heart rate (HR_{max}) (Polar Electro Co., Woodbury, NY, USA). The Polar TeamPro system was then individualized using each subject's testing results of height, weight, age, VO_{2max}, VT, and HR_{max}.

iv. In-Game Monitoring

Players were evaluated during all in-game play using the Polar TeamPro system. The Polar TeamPro utilized GPS, accelerometry, and HR technology to determine TL, EEE, DIS, sprints, time spent in HR zones 4 and 5 (HR_{Z4}=80-89%HR_{max}; HR_{Z5}=90-100%HR_{max}), and distance covered in speed zones 4 and 5 (DIS_{Z4}=15.0-19.9 km/h; DIS_{Z5}= ≥20 km/h) for each game. The top two HR zones and speed zones were used to examine differences in high intensity work between IC and OC games. EEE was calculated by the Polar TeamPro system based on the established relationship between

HR, oxygen consumption, and caloric expenditure using each individual's information established from VO_{2max} testing (3, 8, 12). Similarly, TL was calculated via an algorithm developed by Polar™ based on the quantification of an individual player's caloric expenditure, time spent in different HR zones, speed, distance, and acceleration data. A sprint was considered to be any movement greater than 2.8 m/s^2 (21, 22). Additionally, game data were generated by InStat video and analytics system to determine percent passing accuracy (PA%), dribbling success (DS%), tackling success (TS%), and challenges won (CW%) to examine technical performance differences between OC and IC games. A challenge was an all-inclusive metric used to describe all types of struggles for the ball such as air challenges, neutral balls, and tackles. In this study there were seven OC games played from August 17th through September 9th followed by 11 IC games from September 14th through October 21st. Win-tie-loss records against opponents were 4-2-1 for OC games and 7-3-1 for IC games.

v. Statistical Analyses

All data obtained from Polar TeamPro were analyzed as a rate per minute PT to account for differences in PT and game length due to stoppage time and overtime periods. During each game, an individual's total scores for each metric were divided by the number of minutes played on the field. There were six instances out of 190 where the HR monitor either fell off (n=4) or had poor HR signal reception (n=2) during game play and were subsequently excluded from analysis. Additionally, Polar TeamPro data were further analyzed as rate per minute PT in first half and second half game play to provide greater insight between performance in OC and IC games and to account for differences in overtime included in the previous full-game analysis. Overtime added on average 13.0 minutes of PT to IC games (9 out of 11 games went to overtime) and 18.9 minutes of PT to OC games (2 out of 7 games). Due the varied duration of overtime played between games, separate overtime analysis between IC and OC games was not made. Separate repeated measures (RM)-MANOVAs were conducted to compare all in-game data between conditions (OC vs. IC) using each player's averages for internal workloads (TL, EEE, HR_{Z5}, HR_{Z4}), external workloads (DIS, DIS_{Z5}, DIS_{Z4}, sprints), and technical performance metrics (PA%, DS%, TS%, CW%). RM-MANOVAs for both internal and external workloads were also conducted for first-half and second-half comparisons. RM univariate follow-ups were then conducted to examine the source of the multivariate differences. Average PT for IC and OC for the full match, first half, and second half were analyzed using RM-ANOVAs. All analyses were conducted using SPSS (IBM SPSS v26), with significance set at $p<0.05$. Hedges' *g* was used to calculate effect sizes (ES), with 0.20, 0.50, and 0.80 considered indicative of small, medium, and large effects, respectively. ES and 95% confidence intervals (CIs) were calculated between conditions (OC vs. IC).

RESULTS

The final RPI ranking of teams played during the season averaged 78.5 for IC games and 135.9 for OC games. There was a significantly higher physical workload including TL and DIS ($Mean_{Diff}\pm SE$: TL= 0.14±0.05 TL/minPT; DIS= 1.76±0.5 m/minPT; $p<0.05$) and physiological response including calories expended and time spent in HR_{Z5} (EEE= 0.50±0.2 kcal/minPT; HR_{Z5}= 0.10±0.04 min/minPT; $p<0.05$) in OC compared to IC games *(Table 2)*. There were no significant differences between OC and IC games in distance covered in the fastest speed zones (DIS_{Z4} and DIS_{Z5}), number of sprints, or time spent in HR_{Z4} ($p>0.05$) *(Table 2)*. Differences in PT ($p=0.076$) and TS% ($p=0.073$) approached significance favoring IC games, with no differences in any other in-game technical performance variable ($p>0.05$) *(Table 3)*.

Table 2: Comparison of Workload Rates between OC and IC games

	OC games	**IC games**	**ES (95% CI)**
TL *(TL/minPT)*	3.14 ± 0.3*	3.00 ± 0.3	0.48 (-0.37 – 1.37)
EEE *(kcal/minPT)*	13.23 ± 0.9*	12.73 ± 0.9	0.55 (-0.30 – 1.41)
DIS *(m/minPT)*	104.99 ± 9.1*	103.23 ± 8.7	0.20 (-0.64 – 1.03)
DIS_{Z4} *(m/minPT)*	10.26 ± 2.8	10.04 ± 2.1	0.09 (-0.75 – 0.92)
DIS_{Z5} *(m/minPT)*	3.20 ± 2.1	3.06 ± 1.8	0.08 (-0.76 – 0.91)
sprints *(# of sprints/minPT)*	0.16 ± 0.05	0.15 ± 0.07	0.20 (-0.64 – 1.04)
HR_{Z4} *(min/minPT)*	0.42 ± 0.1	0.47 ± 0.08	-0.57 (-0.37 – 1.37)
HR_{Z5} *(min/minPT)*	0.48 ± 0.1*	0.38 ± 0.1	0.83 (-0.04 – 1.70)
PT *(min)*	79.68 ± 17.3†	89.9 ± 15.3	-0.62 (-1.48 – 0.23)

Values are expressed as Mean ± SD; TL= training load; EEE= exercise energy expenditure; DIS= total distance covered; PT= playing time
*denotes significant difference between OC and IC games ($p<0.05$)
†denotes that the difference between OC and IC games approached significance ($p<0.10$)

Table 3: Comparison of InStat Performance Variables between OC and IC games

	OC games	**IC games**	**ES (95% CI)**
PA% *(Accurate passes/total passes attempted)*	75.82 ± 5.1	73.52 ± 6.1	0.41 (-0.43 – 1.26)
DS% *(Successful dribbles/total dribbles attempted)*	53.12 ± 23.2	45.9 ± 14.4	0.38 (-0.47 – 1.04)

TS% *(Successful tackles/total tackles attempted)*	53. 31 ± 14.8	64.7 ± 11.1†	-0.87 (-1.74 – 0.01)
CW% *(Successful challenges/total challenges attempted)*	56. 21 ± 8.6	57.75 ± 10.9	-0.16 (-0.99 – 0.68)

Values are expressed as Mean ± SD; PA= passing accuracy, DS= dribbling success; TS= tackling success; CW= challenges won
†denotes that the difference between OC and IC games approached significance ($p<0.10$)

Upon further analysis investigating the differences in first half and second half gameplay, significant differences were observed in first, but not second halves between OC and IC games *(Table 4)*. Between first half play, OC games had significantly greater TL and number of sprints than IC (TL= 0.17±0.03 TL/minPT; sprints= 0.29±0.01 sprints/minPT; $p<0.05$). Furthermore, OC games had significantly greater caloric expenditure and time spent in HR_{Z5} (EEE= 0.53±0.09 kcal/minPT; HR_{Z5}=0.13±0.02 min/minPT; $p<0.05$), but less time spent in HR_{Z4} (HR_{Z4}=-0.08±0.03 min/minPT; $p<0.05$) than IC in the first half play. There were no significant differences between OC and IC games for all first half distance measures (DIS, DIS_{Z4}, DIS_{Z5}) ($p>0.05$). Greater DIS ($p=0.075$) and kcal ($p=0.087$) in OC second half play than IC approached significance; however no differences were observed between second half TL, sprints, DIS_{Z4}, DIS_{Z5}, and HR data (HR_{Z5} and HR_{Z4}) ($p>0.05$). Moreover, there was no significant differences in first or second half PT between OC versus IC games ($p>0.05$).

Table 4: Comparison of First Half and Second Half Workload Rates between OC and IC games

	OC First Half	**IC First Half**	**ES (95% CI)**	**OC Second Half**	**IC Second Half**	**ES (95% CI)**
TL *(TL/minPT)*	3.26 ± 0.2*	3.09 ± 0.3	0.67 (-0.19 –1.52)	3.01 ± 0.3	2.90 ± 0.4	0.31 (-0.53 – 1.15)

EEE *(kcal/minPT)*	13.59 ± 0.9*	13.06 ± 0.9	0.60 (-0.25 – 1.46)	12.87 ± 0.8†	12.50 ± 1.0	0.40 (-0.44 – 1.24)
DIS *(m/minPT)*	108.78 ± 9.5	107.93 ± 8.9	0.09 (-0.74 – 0.93)	100.81 ± 8.6†	99.47 ± 8.5	0.16 (-0.68 – 0.99)
DIS_{Z4} *(m/minPT)*	10.89 ± 3.1	11.12 ± 2.3	-0.09 (-0.92 – 0.75)	9.42 ± 2.3	9.10 ± 2.0	0.15 (-0.69 – 0.98)
DIS_{Z5} *(m/minPT)*	3.30 ± 2.8	3.21 ± 1.9	0.04 (-0.79 – 0.88)	2.90 ± 1.7	2.90 ± 1.8	0.00 (-0.83 – 0.84)
sprints *(# of sprints/minPT)*	0.17 ± 0.06*	0.14 ± 0.05	0.49 (-0.36 – 1.34)	0.16 ± 0.04	0.15 ± 0.07	0.05 (-0.79 – 0.88)
HR_{Z4} *(min/minPT)*	0.35 ± 0.1*	0.43 ± 0.09	-0.84 (-0.36 – 1.34)	0.48 ± 0.1	0.49 ± 0.08	-0.10 (-0.94 – 0.73)
HR_{Z5} *(min/minPT)*	0.58 ± 0.1*	0.46 ± 0.1	1.06 (0.17 – 1.96)	0.38 ± 0.1	0.32 ± 0.1	0.45 (-0.40 – 1.30)
PT *(min)*	37.39 ± 9.4	38.42 ± 6.4	-0.13 (-0.96 – 0.71)	37.00 ± 7.4	40.76 ± 7.3	-0.51 (-1.36 – 0.34)

TL= training load; EEE= exercise energy expenditure; DIS= total distance covered; PT= playing time
Values are expressed as Mean ± SD;
*denotes significant difference between OC and IC games ($p<0.05$)
†denotes that the difference between OC and IC games approached significance ($p<0.10$)

DISCUSSION

Based on these findings, OC games appear to be more physiologically and physically demanding compared to IC, despite no significant differences in technical performance. Additionally, it appears the increased demands of OC play is both a result of elevated workloads in the first half as well as the cumulative load of the game. One possible explanation for these observed differences could be the order in which OC and IC games are scheduled throughout the competitive fall season. Typically, OC games in collegiate women's soccer are scheduled in the beginning of the season whereas IC games are more commonly backloaded in the schedule. Having healthier, less fatigued players at the onset of season game play may result in greater physiological and physical outputs in OC games. These differences seem to be driven by first half play with OC games exhibiting a greater number of sprints performed as well as more time spent in the top heart zone (HR_{Z5}). This may indicate enhanced capacity to perform high intensity work at the start of games and points to importance of maintaining player readiness throughout a long season.

Previous research has shown decrements in technical performance during extra-time (overtime) in professional soccer game-play (11). An acknowledged limitation of this study was that technical performance differences were unable to be assessed between halves due to the analytical output generated by InStat. The full game analysis revealed no significant differences in technical performance, but the large effect for increased tackling success in IC games may indicate greater physicality in IC competition. In terms of overall match outputs, performance decrements have been observed in professional players who competed in the World Cup following their club season (7). Interestingly, players who underperformed in the World Cup played more matches before the World Cup than those who did not, with the majority of players who averaged more than one game a week underperforming (7). In NCAA DI women's soccer, typically there are two games played per week on a Thursday-Sunday or Friday-Sunday game schedule. In this study, all seven OC games were played in a three-week span before the start of IC games. The chronically congested match fixture of collegiate soccer and the subsequent fatigue incurred by the players as the season progressed could potentially explain some of differences observed between OC and IC games. We do not see this as a limitation in the study, but rather a reflection of the inherent nature of NCAA women's soccer.

In addition, player readiness may also be of increasing importance come IC games, as starters may have more PT and games tend to be more physical and competitive. The noted moderate effect did support slightly greater PT for these players in IC games. The level of competition in IC play may also impact PT and game-duration, as games may be more likely to go into overtime play. In the current study, nine out of the 11 IC games went into overtime whereas only two of the seven OC games did. The prevalence of overtime in IC games could also contribute to increased fatigue and therefore the decreased player output (decreased DIS) and physiological response (decreased EEE and time in HR_{Z5}) per minute PT observed. As previously mentioned, player output typically decreases throughout the game, with less distance-covered and reduced exercise intensity (decreased high-speed running and number of sprints) in the second half compared to the first half (4, 20). These decrements in performance may be then further exacerbated in overtime situations (11). An increase in PT in IC games could also be a result of the starting line-up being solidified by this point in the season, with player substitutions occurring more frequently in OC games at the beginning of season. This could also potentially contribute to the increased physical workload and physiological demand observed in OC games, as earlier in the season players are fighting for a starting spot on the field. Substitution strategies by coaches, in both OC and IC games, may aid in player management and recovery throughout the season. Additionally, given the RPI differences between IC and OC opponents coupled with the high-ranking of this team (RPI=35), OC opponents may have elevated their level as an underdog while IC opponents may have implemented a more cautious strategy. We speculate that aggressive play by OC opponents at game-onset could potentially contribute to the increased player outputs observed in the first-half of OC games. These data support that all games in women's collegiate soccer games are demanding and that coaches and training staff should treat all games as such regardless of opponent ranking or whether they are IC or OC games.

Another potential factor to consider that may play a role is the environment. As an outdoor sport, a hot and humid environment could negatively affect player performance through dehydration and increased cardiovascular strain (15). Thus, games played during typically hotter, more humid months (i.e. August/early September) may present differences in soccer performance than those performed in cooler weather (18). Although a limitation is that ambient temperature and humidity was not recorded during games, the increased time spent in HR_{Z5} and caloric expenditure displayed in OC games was matched with increased distance covered and TL. Therefore, it appears that the greater physiological response observed during OC could be a combined effect of both greater player output and the increased ambient temperature. Future research is required to determine the effects of temperature changes on cardiac responses as well as physiological loads throughout the season. It is also important to note the small sample size of eleven players used in this study. Although this is typical of an average team's rotational roster as only ten field players are permitted to play at a time, the small sample size may limit the interpretation of these results. Future research may consider utilizing multiple teams across a similar conference schedule. Finally, the analysis of both physiological and performance variables as a rate per minute PT appears to be a favorable method to allow player comparisons between games. Expressing these metrics as a rate accounts for differences in player PT and total game duration between games. Substitution strategies, opponent difficulty and formation, amongst other variables may impact a player's PT between games. Previous work using the RPE method for quantifying training load found that PT, rather than match duration, was superior in reflecting the workload of a NCAA DI men's soccer game (19). The use of rate to examine player performance between games may be advantageous to assess the production and workload of a player rather than using cumulative data that is easily influenced by total PT in a match. This method allows coaches and training staff to assess player production between games and may be more perceptive to changes in player workload and performance measures.

PRACTICAL APPLICATION

Success in collegiate soccer may be a result of a greater ability to sustain in-game performance as the season progresses. Emphasis should be placed on incorporating athlete-monitoring technology to track physical and physiological demands in conjunction with in-game performance metrics. This may enable coaches and sport scientists to tailor TL and recovery with the accumulating in-season demands in order to prevent declines in performance in the latter half of the season. Additionally, when implementing periodization strategies to manage the cumulative season demands, it appears game-load rather than simply the difficulty of the opponent may be an important factor to consider to increase player longevity and output throughout the season. Coaches and support staff may consider different strategies to mitigate accumulating season demands and stress placed on the athlete through the modification of practice load (intensity and/or duration), individualized rest and recovery strategies, nutritional support, and in-game player management through improved substitution strategies.

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CHAPTER III:

EVALUATING THE EFFECTS OF ORAL CONTRACEPTIVE USE ON CHANGES IN BIOMARKERS AND BODY COMPOSITION DURING A COMPETITIVE SEASON IN COLLEGIATE FEMALE SOCCER PLAYERS

ABSTRACT

High training demands throughout the competitive season in female collegiate soccer players have been shown to induce changes in biomarkers indicative of stress, inflammation, and reproduction. Additionally, oral contraceptive (OC) use has been observed to cause changes in these biomarkers, which may be exacerbated in athletes using OCs. Therefore, the purpose of this study was to compare biomarkers and body composition changes between OC-using and non-using (CON) female soccer players throughout the competitive season. **Methods**: Female collegiate soccer players were stratified into two groups based on their reported OC use at the start of pre-season (OC: n=6, M_{age}=19±1yr, $M_{BF\%}$=22.9±6.4kg, M_{VO2MAX}=48.5±2.9 ml·kg^{-1}min^{-1}; CON: n=17, M_{age}=19±1yr, $M_{BF\%}$=19.9±4.5kg, M_{VO2MAX} =49.2±4.6 ml·kg^{-1}min^{-1}). Prior to the start of pre-season and immediately post-season, athletes underwent a battery of performance tests. Pre-season performance characteristics were used to individualize each athlete's integrative Global Positioning System (GPS) and heart rate monitor. Athletes were monitored at all practices and games for the determination of training load and exercise energy expenditure. Blood draws and body composition assessments were performed prior to pre-season, on weeks 2, 4, 8, & 12 of the season, and immediately following week 15 (post-season). Markers of stress, inflammation, reproduction, metabolism, and nutritional status were analyzed using continuous models under a Bayesian framework.

Results: Across the competitive season both groups experienced perturbations in biomarkers. Area under the curve ratios (OC_{AUC} : CON_{AUC}) indicated the OC group were exposed to substantially higher levels of sex-hormone binding globulin (AUC_{ratio}: 1.4, probability OC > CON: p>0.999), total cortisol (1.7; p>0.999), c-reactive protein (5.2; p>0.999), leptin (1.4; p>0.990), growth hormone (1.5; p=0.97), but a substantively lower amount of estradiol (0.36; p<0.001), progesterone (0.48; p=0.008), free testosterone (0.58; p<0.001), follicle-stimulating hormone (0.67; p<0.001) and creatine kinase (0.33, p<0.001) compared with the CON across the season. Both groups increased fat free mass over the season, but CON experienced a greater magnitude of increase along with decreased %BF. **Conclusion:** Although similar biomarker response patterns were observed between groups over the season, the elevated exposure to stress, inflammatory, and metabolic biomarkers over the competitive in OC users season may have implications on body composition, training adaptations, and recovery in female athletes.

INTRODUCTION

Due to its power-endurance nature, soccer is a physically and physiologically demanding sport. Particularly at the collegiate level, the training demands of the sport is coupled with the stress of academics, frequent travel, and environmental stressors that coaches and support staff must account for when assessing the training and recovery needs of their athletes (19). Athlete-monitoring methods, such as heart rate (HR) and global positioning (GPS) systems, allow for the assessment of internal and external workloads and recovery during training and competition; however tracking changes in blood biomarkers may offer a more comprehensive picture of the cumulative demands of a collegiate season outside of just on-field training sessions (2). In National Collegiate Athletic Association (NCAA) Division I (DI) soccer, the high training demands throughout the competitive season have been shown to induce changes in biomarkers of stress and reproduction in male (23, 25) and female players (49). Chronic elevations in stress and inflammatory biomarkers such as cortisol and interleukin-6 (IL-6) and decreases in reproductive markers (testosterone, estrogen) amongst others changes can be indicative of inadequate recovery (28), and thus have implications on performance (25) and health (17).

Current research shows that the majority of elite female athletes have at some point in their career taken hormonal contraceptives (HC) with almost half (49.5%) of those athletes surveyed reporting current HC usage (30). Of the various HC methods reported, oral contraceptives (OC) were the most widely used (78.4%) amongst female athletes (30). As such, it is important to understand any implications HCs, especially OCs, have on responses to training, recovery, and performance. HC use is a potential confounding factor in the stress response from training in female athletes due to the overlap between hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes (31, 33). In females, HCs modify normal hormonal fluctuations, suppressing endogenous productions of estrogen and progesterone (41). HPA-axis activation inhibits the HPG-axis, through the influence of corticotropin-releasing hormone (CRH) on gonadotropin-releasing hormone (GnRH) either directly or indirectly through β-endorphin or cortisol (31). Cortisol, whose production can also be stimulated by vasopressin (AVP) during stress, acts to inhibit all levels of the HPG-axis beyond just GnRH (31). A recent study investigating the effects of OC use on the HPA-axis demonstrated that OCs alter the activation of the HPA-axis by increasing circulating levels of cortisol, thereby inducing metabolic alterations such as increasing circulating levels of triglycerides (22). This finding demonstrates that OC use may have an analogous impact on the HPA-axis as training, with both activating this stress response. Therefore, OC use in conjunction with training, particularly during times of high training demands, such as during the competitive soccer season (49), may produce an augmented stress response in female athletes.

OC use has also been linked to increased c-reactive protein (CRP) levels at rest in female athletes, but not other acute phase proteins (13). Moreover, this finding has been shown in active females who underwent 10-weeks of high intensity training, with HC users (7 out of 8 subjects on OCs) displaying increased CRP levels as well as reduced lean mass gains post-intervention than non-HC users (24). In elite female athletes, increased resting cortisol concentrations (6) and blunted cortisol responses to high intensity training sessions have been reported with OC use (15). In addition to the blunted cortisol responses, elite female hockey players on OCs also had decreased resting testosterone levels and a reduced testosterone response to training over 15 days compared to their non-user teammates (15). This mirrors previous findings in which OC use has been shown to decrease free testosterone and increase sex hormone-binding globulin (SHBG) levels in healthy women (50). As such, changes in biomarkers may be exacerbated or altered in athletes using OCs in response to prolonged periods of intense training. This possible enhanced activation of stress and inflammatory responses in female athletes using OCs may indicate a greater recovery need. Furthermore, side effects such as increases in body weight or fat mass have been reported in female endurance athletes and active females on OCs (12, 39), which may impact performance outcomes; however, these findings have not been consistent (38, 39). The purpose of this study was to compare biomarker and body composition responses in female soccer players with and without OC use during a NCAA DI competitive soccer season. It was hypothesized that the players using OCs would have altered physiological responses compared to their non-user counterparts over the competitive season.

METHODS

i. Experimental Design

Female collegiate soccer players were monitored throughout a competitive fall season to determine the effects of OC use on body composition and biomarkers indicative of stress, inflammation, reproduction, anabolism, metabolism, and hematological status. Prior to the start of pre-season, players underwent performance testing to determine maximal aerobic capacity, power, strength and speed, as well as to individualize each player's Polar TeamPro monitor. The Polar TeamPro system utilized GPS, accelerometry, and HR monitoring technology to determine training load (TL) and exercise energy expenditure (EEE) for all team training sessions, practices, and games. Additionally, body composition and biomarkers assessments were performed prior to pre-season as well as on weeks 2, 4, 8, 12, and immediately post-season.

ii. Subjects

Female collegiate soccer players (N=30) were monitored throughout the course of the competitive season. Players were stratified into two groups: oral contraceptive (OC:

n=6; M_{age}=19±1yr) and control (CON: n=17; M_{age}=19±1yr) based on their reported OC use. OC usage was determined by a Menstrual Status Questionnaire completed prior to the start of pre-season, which was also repeated post-season for confirmation of OC status. At baseline, all OC players reported at least one-year of OC use and all CON players reported menstrual cycles lengths of 25-35 days. Players were excluded from analysis if they were using intrauterine contraception (n=4), altered contraception method mid-season (n=1), did not participate in team training (n=1), or had a known metabolic disorder (n=1). Written, informed consent was obtained from all subjects prior to participation and all subjects received clearance by the university Sports Medicine staff prior to testing. All players competed in the same NCAA DI women's soccer team in the Big Ten Conference. Research was approved by the Rutgers University Institutional Review Board for the Protection of Human Subjects and conducted in accordance with the Declaration of Helsinki.

iii. Performance Testing

Prior to the start of pre-season and upon completion of the competitive season, players underwent a battery of performance tests and body composition assessments. All pre- and post-season testing sessions, as well as blood draws, occurred within a one-week period. Prior to the start of season, players reported to the IFNH Center for Health and Human Performance (CHHP) following a two-hour fast, having refrained from exercise in the preceding 12-hours. Body composition was assessed using air displacement plethysmography via the BodPod (BODPOD, COSMED, Concord, CA) to determine percent body fat (%BF) and fat free mass (FFM), and a predicted lung volume was determined using the Brozek formula (7, 16). After a ~10-minute standardized dynamic warm-up, players performed maximal countermovement vertical jumps with hands-on-hips (CMJ_{HOH}). Players were allowed two attempts with highest jump height recorded.

Afterwards, a maximal graded exercise test (GXT) on a treadmill was used to measure maximal aerobic capacity (VO_{2max}) and ventilatory threshold (VT) via direct gas exchange by a COSMED Quark CPET (COSMED, Concord, CA). HR was continuously monitored throughout the test using a Polar S610 HR monitor to obtain maximal heart rate (HR_{max}) (Polar Electro Co., Woodbury, NY, USA). A speed-based protocol was used with stages that were metabolic equivalents (MET) to the standard Bruce protocol. This protocol has previously been used in collegiate soccer players and consisted of two-minute stages at a constant 2% incline, with increasing speeds of 6.4, 7.9, 10.0, 11.7, 13.7, 15.6, 17.1, 18.2, 19.8, 21.1 km/h (32). Players continued the test with encouragement from research assistants until volitional fatigue. At least two of the following criteria were met for attainment of VO_{2max}: RER $\geq$1.1, observation of a plateau in O_2 consumption (increase $\leq$150 ml/min with increasing workload), and HR >85% age-predicted HR_{max} (208 – 0.7 x age). For athletes who did not meet the above criteria, VO_{2peak} was used (n=3). Player's VT was analyzed after the completion of each test as the inflection point where VCO_2 increased nonlinearly with VO_2, expressed as a percentage of VO_{2max} (5).

All performance tests were repeated post-season and body composition assessments were repeated during all blood draw timepoints in addition to post-season. One athlete at baseline (n=1) and four athletes at post-testing (n=4) were limited in participation for maximal testing by the team physician and did not participate in all testing sessions (see *Table 8*).

iv. Blood Draws

Blood draws were performed prior to pre-season, on weeks 2 (end of pre-season), 4, 8, & 12 of the season, and post-season. Athletes reported to the CHHP between 0700 and 0900h and were instructed to arrive in an euhydrated state following an overnight fast. All draws during the season were performed between 18-24 hours following a game (T2-T5), with the exception of pre-season (T1: 'baseline') and post-season draws (T6: ~58h post-game). For all draws, blood samples were drawn from participants while seated via the antecubital fossa (21G, BD Vacutainer, Safety-Lok) by three experienced phlebotomists into clot activator collection tubes (SST and gel-free tubes). Blood samples were centrifuged for 10-minutes at 4,750 rpm (Allegra x-15R; Beckman Coulter, Brea, CA, USA), serum/plasma were aliquoted from centrifuged tubes and immediately shipped, in containers designed to maintain 4º, 20º, or -20ºC depending on the analyte, to a Clinical Laboratory Improvements Amendment (CLIA)-certified processing facility for analysis (Quest Diagnostics, Secaucus, NJ, USA). Samples were run in duplicate and the coefficient of variation (CV) was between 0.5-10.0 % for all biomarkers. Results were provided to the researchers via the Quest Diagnostics Care360 online portal. Biomarkers analyzed included total cortisol (TCORT), free cortisol (FCORT), creatine kinase (CK), CRP, IL-6, tumor necrosis factor-α (TNF-α), estradiol (E_2), growth hormone (GH), insulin-like growth factor-1 (IGF-1), ferritin (Fer), iron (Fe), total iron binding capacity (TIBC), percent transferrin saturation (%SAT), transferrin, leptin, total triiodothyronine

(TT_3), free triiodothyronine (FT_3), total thyroxine (TT_4), free thyroxine (FT_4), thyroid-stimulating hormone (TSH), prolactin, sex-hormone binding globulin (SHBG), follicle-stimulating hormone (FSH), progesterone (P_4), total testosterone (TTEST), and free testosterone (FTEST).

v. In-season athlete-monitoring

Players were evaluated during all team training sessions using the Polar TeamPro system during the fall competitive season. The Polar TeamPro system utilized GPS, accelerometry, and HR technology to determine TL and EEE (14, 18) for all lifts, practices, and games. The Polar TeamPro system was individualized to each athlete using their pre-season testing results of height, weight, age, VO_{2max}, VT, and HR_{max}. TL, expressed as arbitrary units (au), was calculated via an algorithm developed by Polar™ based on the quantification of an individual player's EEE, time spent in different HR zones, speeds, distance, and acceleration data. EEE was normalized for body weight (EEE_{REL}, expressed as kcal/kg), which was obtained from body composition assessments, in order to account for relative size differences between players.

vi. Statistical Analysis

The purpose of the statistical analysis was to model the time series nature of biomarker and body composition data and assess the extent to which values changed across the season for both OC users and CON. To conduct the analyses, hierarchical generalized linear models (HGLMs) were fitted within a Bayesian framework. HGLMs accounted for structure in the data and were fitted to smooth the time series data, identifying the underlying shape of the physiological signal (36). With a Bayesian framework, dichotomous interpretations of results (*e.g.* with p values) can be avoided and greater emphasis placed on describing the most likely results and their practical consequences (26). Analogous to mixed-effect models with varying slopes, the HGLMs were fitted with a single common smoother plus group-level smoothers with the same "wiggliness" (36). The HGLMs also accounted for the repeated measures nature of the data by including random intercepts for each player. All models were fitted within the brms package (8) that interfaced with the Bayesian software Stan (10). Models were fitted with 5 chains each comprising 10,000 sets of posterior estimates. These model estimates with smoothers were then used to generate 50,000 new data sets to account for uncertainty in coefficients and variance parameters. Means were then calculated in each data set across time intervals for both OC users and CON. Visual inspection of the distribution of means revealed that most outcomes exhibited linear behavior (e.g. constant throughout the season or consistent increase/decrease). The proportion of gradients with for example a positive slope was interpreted as the probability of an increase in the outcome across the season. To quantify the magnitude of any increase, effect sizes (Cohen's *d*) were calculated for each data set by dividing the change in value across the season by the pre-season standard deviation. Effect sizes (*d*) of 0.20, 0.50, and 0.80 considered indicative of small, medium, and large effects, respectively. To quantify differences in biomarker levels across the season between OC users and CON, the ratio of the area under the curve (AUC) was calculated. The distribution of all calculations across the generated data sets were used to derive percentage credible intervals (%CrIs). Descriptive statistics (Mean ± SD) were used to quantify team, OC, and CON performance characteristics pre- and post-season. Frequency counts for OC and CON were used to present changes in performance from baseline values (increase, maintain, decrease) due to changes in sample size for each performance variable from pre- to post-season. Changes were considered an increase or decrease based on sensitively of equipment to detect significant changes (VO_{2max}: ± 2.3 ml·kg^{-1}min^{-1}; VT: ± 2.0%; CMJ_{HOH}: ± 1.7 cm) (21, 34), otherwise no change (maintenance) was indicated.

RESULTS

vii. Reproductive Markers: E_2, P_4, FSH, SHBG, TTEST, FTEST, Prolactin

Inspection of modelled time series indicated linear (constant or increasing/decreasing) responses for all reproductive biomarkers across the season. However, median point estimates describing linear changes were below a medium threshold ($|d|$<0.5) for all reproductive markers (*Table 1*) in both OC (-0.38: FSH; to 0.14: TTEST) and CON (-0.27: SHBG; to 0.43: TTEST) groups. The area under the curve ratios indicated the OC users were exposed to substantively higher levels of SHBG (AUC ratio: 1.4 [95%CrI: 1.3 – 1.5]; p>0.99), but a substantively lower levels of E_2 (AUC ratio: 0.36 [95%CrI: 0.11 – 0.61]; p<0.001), P_4 (AUC ratio: 0.48 [0.13 – 0.89]; p =0.008), FTEST (AUC ratio: 0.58 [95%CrI: 0.47 – 0.70]; p<0.001) and FSH (AUC ratio: 0.67 [95%CrI: 0.51 – 0.85]; p<0.001) compared with the CON group across the season.

Table 1: Changes in Reproductive Biomarkers Over Time and Differences in Exposure Between Groups

	Effect size [50% CrI] ***Probability (p) of increase ↑ or decrease ↓ across the season***	**Area Under Curve Ratio [95% CrI];** ***Probability (p) OC exposure > CON***

	OC	CON	OC_{AUC} : CON_{AUC}
E_2 *(pmol/L)*	-0.03 [-0.64 – 0.55] ↓p=0.53	0.14 [-0.28 – 0.56] ↑p=0.75	0.36 [0.11 – 0.61]; p <0.001
P_4 *(nmol/L)*	-0.00 [-0.19 - 0.19] ↓p=0.50	0.06 [-0.20 – 0.12] ↓p=0.62	0.48 [0.13 – 0.89]; p =0.008
FSH *(IU/L)*	-0.38 [-1.2 – 0.45] ↓p=0.83	0.14 [-0.68 – 0.40] ↓p=0.70	0.67 [0.51 – 0.85]; p <0.001
SHBG *(nmol/L)*	-0.08 [-0.53 – 0.39] ↓p=0.64	0.27 [-0.58 – 0.02] ↓p=0.97	1.4 [1.3 – 1.5]; p >0.99
FTEST *(nmol/L)*	-0.06 [-0.22 – 0.10] ↓p=0.61	-0.05 [-0.14 – 0.05] ↓p=0.62	0.58 [0.47 – 0.70]; p =0.150
TTEST *(nmol/L)*	0.14 [-0.10 – 0.37] ↑p=0.65	0.43 [0.28 – 0.58] ↑p=0.98	0.94 [0.85 – 1.0]; p <0.001
Prolactin *(nmol/L)*	0.02 [-0.22 – 0.28] ↑p=0.53	0.24 [0.08 – 0.40] ↑p=0.85	0.92 [0.76 – 1.09]; p =0.178

CrI: Credible Interval; E_2: estradiol, P_4: progesterone, FSH: follicle-stimulating hormone, SHBG: sex-hormone binding globulin, FTEST: free testosterone, TTEST: total testosterone; Effect sizes (*d*) indicated the magnitude of change from baseline across the season; OC_{AUC} : CON_{AUC} ratio of >1 indicative of greater exposure in OC group, of <1 indication of greater exposure in CON

viii. Stress & Inflammatory Markers: TCORT, FCORT, CRP, IL-6, TNF-α

Inspection of modelled time series indicated linear responses for the majority of stress and inflammatory biomarkers across the season. Results indicated that OC users experienced a large increase for CRP (*d*=0.85) and moderate increases for IL-6 (*d*=0.66) and TNF-α (d=0.67) (*Table 2*). In contrast, median point estimates describing linear changes were below a medium threshold ($|d|$<0.5) for all stress and inflammatory biomarkers in CON (0.07: TNF-α; to 0.46: IL-6) (*Table 2*). A similar non-linear response was identified for FCORT in both OC users and the CON group, with values increasing between T1-T4 (combined d =0.40; [50%CrI: 0.21 – 0.59]) followed by a return towards original values between T4-T6 (combined d=-0.23; [50%CrI: -0.42 – 0.05]). Both OC and CON groups also experienced a similar non-linear trend with decreasing TNF-α values between T1-T5 (combined d=-0.89; [50%CrI: -1.1 – 0.57]), followed by a subsequent large increase between T5-T6 (combined d=1.2; [50%CrI: 1.0 – 1.4]). The area under the curve ratios indicated the OC group were exposed to a substantially greater amount of TCORT (AUC ratio: 1.7 [95%CrI: 1.6 – 1.8]; p>0.99) and CRP (AUC ratio: 5.2 [95%CrI: 3.7 – 8.3]; p>0.99) compared with the CON across the season.

Table 2: Changes in Stress & Inflammatory Biomarkers Over Time and Differences In Exposure Between Groups

	Effect size [50% CrI] *Probability (p) of increase ↑ or decrease ↓ across the season*		**Area Under Curve Ratio [95% CrI];** *Probability (p) OC exposure > CON*
	OC	**CON**	**OC_{AUC} : CON_{AUC}**
FCORT *(nmol/L)*	0.15 [-0.08 – 0.37] ↑p=0.68	0.18 [0.01 – 0.34] ↑p=0.77	0.99 [0.89 – 1.1]; p =0.420
TCORT *(nmol/L)*	0.11 [-0.02 – 0.25] ↑p=0.72	0.12 [0.03 – 0.22] ↑p=0.81	1.7 [1.6 – 1.8]; p >0.99
CRP *(IU/L)*	0.85 [0.64 – 1.1] ↑p=0.99	0.10 [-0.03 – 0.05] ↑p=0.70	5.2 [3.7 – 8.3]; p >0.99

IL-6 *(pg/mL)*	0.66 [-0.73 – 2.1] ↑p=0.84	0.46 (-0.49 – 1.3) ↑p=0.84	1.0 [0.80 – 1.2]; p =0.491
TNF-α *(pg/mL)*	0.67 [0.42 – 0.89] ↑p=0.96	0.07 [-0.08 – 0.22] ↓p=0.63	1.02 [0.95 – 1.1]; p =0.724

CrI: Credible Interval; FCORT: free cortisol, TTCORT: total cortisol, CRP: c-reactive protein, IL-6: interleukin-6, TNF-α: tumor necrosis factor-alpha; Effect sizes (*d*) indicated the magnitude of change from baseline across the season; OC_{AUC} : CON_{AUC} ratio of >1 indicative of greater exposure in OC group, of <1 indication of greater exposure in CON

ix. Markers of Muscular Growth & Breakdown: GH, IGF-1, CK

Linear responses were identified for all biomarkers indicative of growth and muscular breakdown across the season. The OC group experienced a large increase in GH (d=1.5), but a moderate decrease in IGF-1 (d=-0.52) across the season (*Table 3*). In contrast, median point estimates were below a medium threshold ($|d|$<0.5) for all muscular anabolic and catabolic biomarkers in the CON group (-0.14: IGF-1; to -0.07: CK) (*Table 3*). The area under the curve ratios indicated OC users were exposed to substantively higher levels of GH (AUC ratio: 1.5 [95%CrI: 0.97– 2.2]; p=0.97), but substantively lower levels of CK (AUC ratio: 0.33 [95%CrI: 0.16 – 0.50]; p<0.001) compared with CON across the season.

Table 3: Changes in Biomarkers of Muscular Growth and Breakdown Over Time and Differences in Exposure Between Groups

	Effect size [50% CrI] *Probability (p) of increase ↑ or decrease ↓ across the season*		**Area Under Curve Ratio [95% CrI];** *Probability (p) OC exposure > CON*
	OC	**CON**	**OC_{AUC} : CON_{AUC}**

GH *(µg/L)*	1.5 [1.0 – 1.9] ↑p=0.99	-0.12 [-0.42 – 0.15] ↓p=0.62	1.5 [0.97 – 2.2); p =0.97
IGF-1 *(µg/L)*	-0.52 [-0.68 – -0.35] ↓p=0.99	-0.14 [-0.23 – -0.03] ↓p=0.81	0.88 [0.81 – 0.96]; p =0.002
CK *(U/L)*	-0.11 [-0.35 – 0.12] ↓p=0.63	-0.07 [-0.24 – 0.11] ↓p=0.60	0.33 [0.16 – 0.50]; p <0.001

CrI: Credible Interval; GH: growth hormone, IGF-1: insulin-like growth factor-1, CK: creatine kinase; Effect sizes (*d*) indicated the magnitude of change from baseline across the season; OC_{AUC} : CON_{AUC} ratio of >1 indicative of greater exposure in OC group, of <1 indication of greater exposure in CON

x. *Markers of Iron Status*: *Fe, Fer, %Sat, TIBC, Transferrin*

Linear responses were identified for the majority of biomarkers indicative of iron status in the athletes across the season. Both OC and CON groups were found to experience a moderate decrease in Fe (*d*=-0.51, *d*=-0.56), with CON also demonstrating a moderate increase in TIBC (*d*=0.63) (*Table 4*). Similar non-linear responses were identified for %SAT with OC and CON groups experiencing a decrease between T1-T5 (combined *d* = -0.42; [50%CrI: -0.60 – -0.23]), followed by a subsequent increase between T5-T6 (combined *d*= 0.34; [50%CrI: 0.17 – 0.51]).

Table 4: Changes in Iron Status Over Time and Differences in Exposure Between Groups

	Effect size [50% CrI] *Probability (p) of increase ↑ or decrease ↓ across the season*		**Area Under Curve Ratio [95% CrI];** *Probability (p) OC exposure > CON*
	OC	**CON**	**OC_{AUC} : CON_{AUC}**

Fe *(µmol/L)*	-0.51 [-0.75 – -0.28] ↓p=0.98	-0.56 [-0.73 – -0.39] ↓p=0.99	1.2 [1.0 – 1.4]; p =0.974
Fer *(pmol/L)*	-0.25 [-0.42 – -0.05] ↓p=0.80	-0.36 [-0.49 – -0.22] ↓p=0.96	1.1 [0.98 – 1.2]; p =0.95
%SAT *(%)*	0.07 [-0.13 – 0.29] ↑p=0.60	-0.20 [-0.33 – 0.06] ↓p=0.83	0.99 [0.82 – 1.2]; p=0.487
TIBC *(µmol/L)*	0.22 [0.04 – 0.38] ↑p=0.80	0.42 [0.30 – 0.54] ↑p=0.99	1.10 [1.07 – 1.13]; p>0.99
Transferrin *(g/L)*	0.25 [0.07 – 0.42] ↑p=0.83	0.63 [0.52 – 0.75] ↑p=0.99	1.09 [1.05 – 1.13]; p>0.99

CrI: Credible Interval; Fe: iron, Fer: ferritin, %Sat: percent transferrin saturation, TIBC: total iron binding capacity; Effect sizes (*d*) indicated the magnitude of change from baseline across the season; OC_{AUC} : CON_{AUC} ratio of >1 indicative of greater exposure in OC group, of <1 indication of greater exposure in CON

xi. Markers of Metabolism: TSH, TT_4, FT_4, TT_3, FT_3, Leptin

Linear responses were identified for all biomarkers indicative of metabolism and energy balance across the season. OC users were found to experience increases in the majority of biomarkers with large effects for TT_4 (*d*=0.91) and leptin (*d*=1.2), and moderate effects for TT_3 (*d*=0.71) and FT_3 (*d*=0.78), but a moderate effect for a decrease in FT_4 (*d*=-0.52) (*Table 5*). Similarly, CON experienced moderate effect for increases in TT_4 (*d*=0.53) and leptin (*d*=0.51), and moderate effect for decreases in both TSH (*d*=-0.61) and FT_4 (*d*=-0.70) (*Table 5*). The area under the curve ratios indicated the OC group were exposed to substantially greater amounts of TSH (AUC ratio: 1.4 [95%CrI: 1.3– 1.6]; p>0.99), TT_4 (AUC ratio: 1.3 [95%CrI: 1.2– 1.4]; p>0.99), TT_3 (AUC ratio:

1.3 [95%CrI: 1.2– 1.3]; p>0.99), and leptin (AUC ratio: 1.4 [95%CrI: 1.3– 1.6]; p>0.99) compared with the CON across the season.

Table 5: Changes in Metabolic Biomarkers Over Time and Differences in Exposure Between Groups

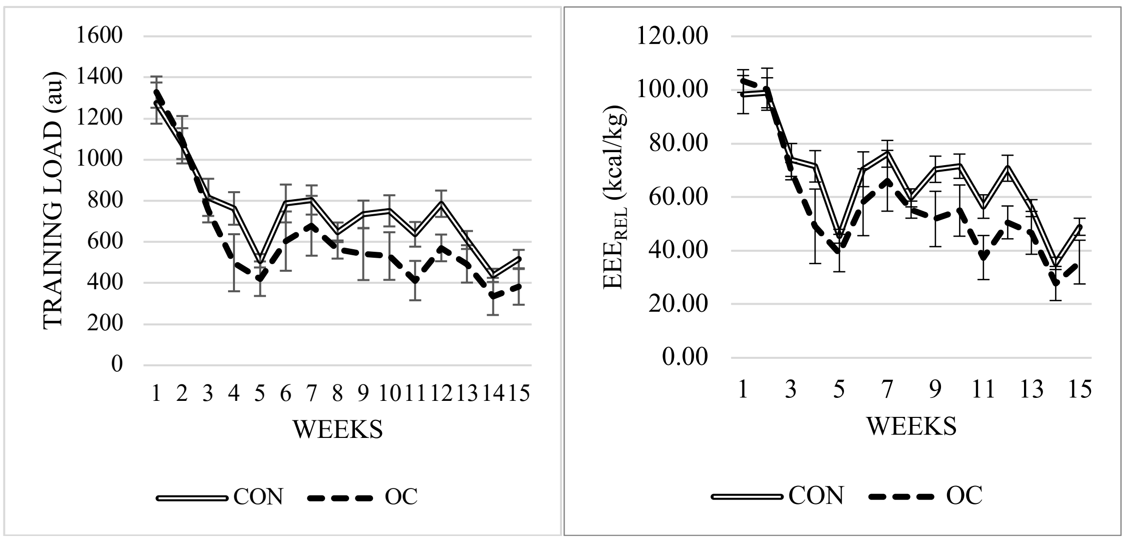
	Effect size [50% CrI] *Probability (p) of increase ↑ or decrease↓ across the season*		**Area Under Curve Ratio [95% CrI];** *Probability (p) OC exposure > CON*
	OC	**CON**	**OC_{AUC} : CON_{AUC}**
TSH *(mIU/L)*	-0.41 [-0.56 – 0.24] ↓p=0.95	-0.61 [-0.71 – 0.50] ↓p>0.99	1.4 [1.3 – 1.6]; p >0.99
TT_4 *(nmol/L)*	0.91 [0.72 – 1.1] ↑p>0.99	0.53 [0.41 – 0.64] ↑p>0.99	1.3 [1.2 – 1.4]; p >0.99
FT_4 *(pmol/L)*	-0.52 [-0.72 – 0.30] ↓p=0.95	-0.70 [-0.83 – 0.57] ↓p>0.99	0.97 (0.94 – 1.0); p =0.045
TT_3 *(nmol/L)*	0.71 [0.50 – 0.92] ↑p=0.99	-0.32 [-0.45 – 0.18] ↓p=0.94	1.3 [1.2 – 1.3]; p >0.99
FT_3 *(pmol/L)*	0.78 [0.55 – 1.0] ↑p=0.99	0.18 [0.03 – 0.33] ↑p=0.79	0.98 (0.95 – 1.0]; p =0.141
Leptin *(µg/L)*	1.2 [0.48 – 1.9]; ↑p>0.99	0.51 [0.08 – 0.95] ↑p>0.99	1.4 [1.3 – 1.6]; p >0.99

CrI: Credible Interval; TSH: thyroid stimulating hormone, TT_4: total thyroxine, FT_4: free thyroxine, TT_3: total triiodothyronine, FT_3: free triiodothyronine; Effect sizes (*d*) indicated the magnitude of change from baseline across the season; OC_{AUC} : CON_{AUC} ratio of >1 indicative of greater exposure in OC group, of <1 indication of greater exposure in CON

xii. Training Load / Exercise Energy Expenditure

Large linear decreases were found for TL and EEE_{REL} across the season in both OC and CON (*Table 6*); however, OC users were identified to exhibit a lower TL (AUC ratio: 0.83 [95%CrI: 0.76 – 0.89]) and EEE_{REL} (AUC ratio: 0.85 [95%CrI: 0.79 – 0.90]) across the season than the CON group *(Figure 1)*.

Figure 1: Changes in Training Load and Exercise Energy Expenditure Over Time



Values expressed as Mean ± SE; EEE_{REL}: relative exercise energy expenditure

Table 6: Changes in Training Load and Exercise Energy Expenditure Over Time and Differences Between Groups

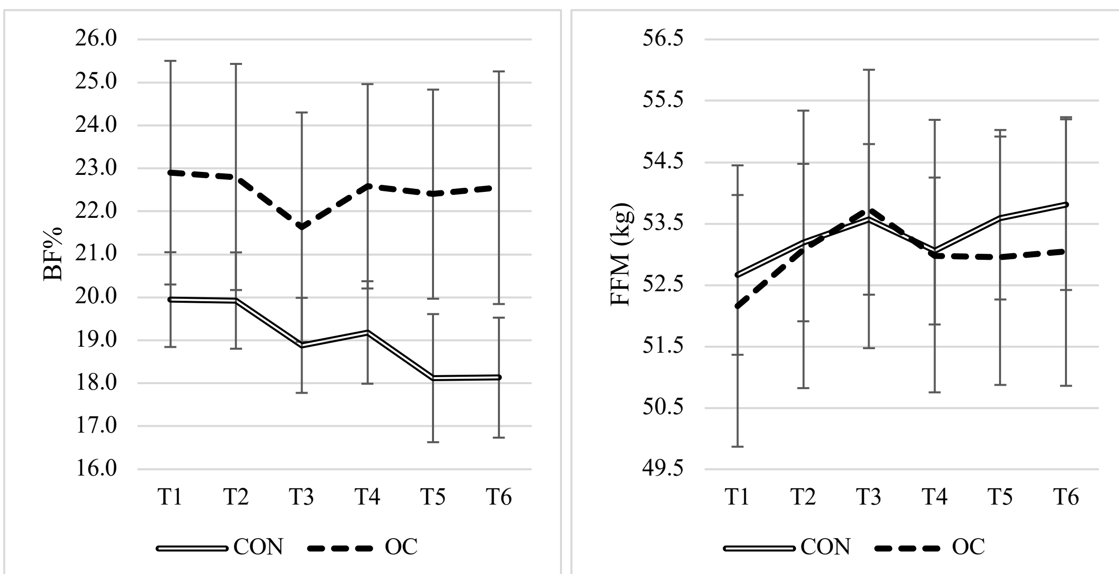
	Effect size [50% CrI] *Probability (p) of increase ↑ or decrease↓ across the season*		**Area Under Curve Ratio [95% CrI];** *Probability (p) OC exposure > CON*
	OC	**CON**	**OC_{AUC} : CON_{AUC}**
TL *(au)*	-2.5 [-2.6 – -2.3] ↓p>0.99	-2.2 [-2.3 – -2.1] ↓p>0.99	0.83 [0.76 – 0.89]; p <0.001
EEE_{REL} *(kcal/kg)*	-2.5 [-2.6 – -2.3] ↓p>0.99	-2.1 [-2.3 – -2.0] ↓p>0.99	0.85 [0.79 – 0.90]; p <0.001

CrI: Credible Interval; TL: Training Load, EEE_{REL}: relative exercise energy expenditure; Effect sizes (*d*) indicated the magnitude of change from baseline across the season; OC_{AUC} : CON_{AUC} ratio of >1 indicative of greater exposure in OC group, of <1 indication of greater exposure in CON

xiii. Body Composition

Investigation of body composition data indicated that both OC users and CON maintained body mass across the season (d_{OC} = 0.04 [50%CrI: -0.06 – 0.14]; d_{CON} = -0.03 [50%CrI: -0.09 – 0.04]; *Table 7*), with limited evidence that both groups increased FFM slightly (d_{OC} = 0.11 [50%CrI: 0.02 – 0.20]; d_{CON} = 0.20 [50%CrI: 0.14 – 0.26]). CON also experienced moderate decreases %BF (d_{CON} = -0.50 [50%CrI: -0.58 – -0.43]), with no such changes identified for OC users (d_{OC} = -0.08 [50%CrI: -0.19 – 0.04]; *Figure 2*).

Figure 2: Changes in Body Fat Percentage and Fat Free Mass Over the Season



Values expressed as Mean ± SE; BF%: Body Fat Percentage, FFM: Fat Free Mass

Table 7: Changes in Body Composition Over Time

	Effect size [50% CrI] ***Probability (p) of increase ↑ or decrease↓ across the season***

	OC	CON
Body Mass *(kg)*	0.04 [-0.06– 0.14] ↑p=0.61	-0.03 [-0.09 – 0.04] ↓p=0.61
BF% *(%)*	-0.08 [-0.19 – 0.04] ↓p=0.67	-0.50 [-0.58 – 0.43] ↓p>0.99
FFM *(kg)*	0.11 [0.02 – 0.20] ↑p=0.81	0.20 [0.14 – 0.26] ↑p=0.99

CrI: Credible Interval; BF%: body fat percentage, FFM: fat free mass; Effect sizes (*d*) indicated the magnitude of change from baseline across the season

xiv. Performance Variables

Table 8: Team and Group Performance Characteristics Pre- and Post-season

	TEAM *(baseline)*	**TEAM** *(post-season)*	**CON** *(baseline)*	**CON** *(post-season)*	**OC** *(baseline)*	**OC** *(post-season)*
VO_{2max} *($ml \cdot kg^{-1} min^{-1}$)*	49.03 ± 4.1	47.67 ± 4.3	49.21 ± 4.6	Increase(n=3) Maintain(n=5) Decrease(n=6)	48.53 ± 2.9	Increase(n=1) Maintain(n=1) Decrease(n=3)
VT *($\%VO_{2max}$)*	79.62 ± 4.6	80.74 ± 4.0	80.13 ± 4.2	Increase(n=5) Maintain(n=6) Decrease(n=2)	78.33 ± 5.9	Increase(n=2) Maintain(n=1) Decrease(n=2)
CMJ_{HOH} *(cm)*	46.82 ± 4.8	48.01 ± 5.0	46.59 ± 4.6	Increase(n=8) Maintain(n=5) Decrease(n=1)	47.41 ± 5.9	Increase(n=1) Maintain(n=4) Decrease(n=0)

Values are expressed as Mean ± SD; VO_{2max}: aerobic capacity, VT: ventilatory threshold, CMJ_{HOH}: hands on hip countermovement jump; Frequency counts for individual changes in performance variables (increase, maintenance, decrease) are presented for post-season testing values for OC and CON

DISCUSSION

The TLs and EEEs experienced by female collegiate soccer players throughout a competitive season corresponded with various perturbations in blood biomarkers and changes in body composition. TL and EEE_{REL} were highest for both groups during the first two weeks of pre-season, with players experiencing reductions in workload as the season progressed. Between OC and CON groups, however, there were substantially different exposures to biomarkers of reproduction, stress, inflammation, metabolism, and muscular anabolism/catabolism throughout the competitive season. These differences were observed despite similar training loads, although OC users exhibited an accumulative 15% lower training load across the season. Additionally, neither OC and CON groups exhibited any changes in body mass across the season; however, findings indicated that CON players experienced greater increases in FFM and substantially greater decreases in %BF compared with OC users. These findings indicate that although both groups displayed similar biomarker response patterns overall, the magnitude of these responses to training were exacerbated in OC users, particularly for CRP, GH, and leptin. This study highlights the influence of OC use on physiological changes that occur over a four-month intense competitive season and the differential systemic exposure to biomarkers, specifically those of inflammation, stress, anabolism, and energy balance. These differences observed as a result of OC use may have implications on body composition, training adaptations, and recovery during the competitive season in female athletes.

Over the season, effect sizes revealed concentrations of sex hormones E_2 and P_4 were relatively stable; however, the CON group experienced a ~3x greater exposure to E_2

and ~2x greater exposure to P_4 compared to OC users over the season. This is as expected as OCs act by suppressing endogenous production of E_2 and P_4 through inhibition of the HPG-axis (41). Oral contraceptive-mediated suppression of ovarian hormone production is coupled with a decreased production and secretion of FSH and luteinizing hormone (LH) (41). This is supported by the finding that the CON group exhibited larger concentrations of FSH (~2x greater exposure) over the season than the OC group. Although LH concentrations were not quantified in this study, the differences in female reproductive hormones between OC and CON groups illustrate the typical reproductive hormonal profiles associated with oral contraceptive use. Unlike the CON group, OC users experienced a small effect for a decreased FSH concentrations over the season. This increased suppression of FSH levels may in part be mediated by HPA-axis interactions and inhibition on the HPG-axis as TCORT was elevated in OC versus CON groups. Previous research has shown decreased FTEST and increased SHBG levels with OC use (50). This mirrored the findings in this study as the OC group had about ~2x less FTEST and ~1.5x greater SHBG exposure over the season compared with the CON group. This builds upon acute findings in elite athletes where salivary testosterone levels remained lower in OC users after exercise regardless of training session intensity (15). Finally, no differences in prolactin AUC were observed between groups. Prolactin levels can be influenced by IL-6 production (31), potentially explaining the similar prolactin levels across the season as both groups experienced similar increases in IL-6. Overall, these findings underscore the consistent differences over time in circulating sex hormones in female athletes with OC use.

Across the season, athletes exhibited an initial increase in FCORT followed by a small decrease during the second-half of season (T4-T6). This continued increase in FCORT in the first two-months of the season occurred despite dramatic decreases in weekly TL and EEE_{REL} following preseason. This increased catabolic environment observed in first-half of the season may be a result of high TL and EEE_{REL} that occurred during pre-season (T1-T2), where workloads were nearly double those observed from weeks 4 to 15 of the season. Previous research in collegiate fall-sport athletes has characterized the deleterious effects of a condensed pre-season (48, 49), with similar effects sizes observed for increased FCORT in female field-hockey players (48). The observed perturbations in FCORT described herein occurred earlier and to a smaller magnitude than those previously reported in female soccer players (49), which may point to differences in player management between studies. Interestingly, OC players were exposed to nearly ~2x greater TCORT throughout the season compared to CON players, with no differences in FCORT between groups. OC use been shown to enhance corticosteroid-binding globulin binding capacity, which may influence circulating FCORT levels (51). In female athletes on OCs, increased resting cortisol concentrations have been reported (6), with blunted acute cortisol responses to exercise (6, 15). This study adds further support to the notion that OCs alter the activation of the HPA-axis by increasing circulating levels of cortisol (22). Research regarding cortisol and OC use in athletes has, however, been equivocal. For example Larsen and colleagues showed no differences in cortisol concentrations between elite female athletes on OCs (27); however, exercise participation prior to blood draws and time of day varied between subjects, potentially washing out any between group differences as both factors have been shown to impact cortisol levels. The elevated TCORT levels across the season in the OC group may indicate an increased catabolic environment in these athletes and thus, a reduced capacity for protein synthesis (28), especially when taken in conjunction with the smaller FFM gains observed in OC users. The sustained elevated TCORT levels, along with the exacerbated inflammatory responses observed in OC athletes, may also have implications on recovery and immune function (28), through the inhibition of muscle protein synthesis (20) and immunosuppression (20, 42).

For inflammatory biomarkers, the athletes TNF-α levels decreased through week-12 of the season followed by an increase from weeks 12 to 15. Interestingly, this contrasting response in TNF-α is opposite of that by FCORT over the season, and may be due to an interaction and feedback between FCORT, IL-6, and TNF-α responses (35). Compared with pre-season baseline values, OC users experienced large increases in CRP and moderate increases in IL-6 and TNF-α concentrations, whereas the CON group had a small overall increase in IL-6. Thus, there appears to be greater inflammatory responses to training with OC use, despite the increased resting TCORT levels. This may lead to augmented systemic inflammation in these athletes as OC users exposure to CRP was over 5x greater than CON players over the season. This aligns with previous findings that have shown increased CRP at rest and in response to intense training with OC use (13, 24, 27). The heightened systemic inflammation seen with OCs may have long-term implications on athlete health as elevated CRP levels have been associated with an increased cardiovascular disease risk (37). Additionally, chronic inflammation may influence training adaptations, as reduced FFM gains and FM loss alongside elevated CRP levels have been shown over a 10-week training block (24) and similar changes in body composition measures were observed in the present study. It appears OCs may exacerbate inflammatory responses to training, with the enhanced systemic inflammation contributing to a hindered ability to adapt to a training stimulus.

While the CON group experienced no changes in biomarkers indicative of muscular anabolism, OC users displayed a large increase in GH accompanied by a concomitant moderate decrease in IGF-1 from pre- to post-season. Moreover, AUC comparisons revealed ~1.5x greater exposure to GH in the OC group than CON group throughout the season. This is in agreement with previous findings in female endurance athletes, in which increased GH levels without changes in IGF-1 were observed following OC treatment (40). Similar declines in IGF-1 have been observed in ovarian suppressed female athletes with intense training, with declines becoming more pronounced over the 12-weeks of training indicating a potentially increased catabolic environment in these athletes (46). The decreased IGF-1 levels observed over the season in OC users may indicate an impaired ability to induce muscular adaptations in these athletes (28).

Overall, CK levels in the CON group started and remained elevated above OC users, yielding about a ~3x greater exposure in the CON group throughout the season. Previous research has shown E_2 to potentially play a protective role against muscle damage through mechanisms such as increased membrane stabilization (44). Findings on acute elevations in CK post-exercise with OC use remain equivocal (45), however, greater reductions in CK values 72-hours post-exercise have been observed in OC users (11). The greater CK levels observed in the CON group may be indicative of greater skeletal muscle turnover in these athletes (3), especially when taken into context with the FFM gains over the competitive season.

Overall, linear trends for decreases in Fer and Fe and increases in TIBC and transferrin were shown in the players over the soccer season. Additionally, a small decrease occurred through week 12 for %SAT followed by a small increase during the remainder of the season. These changes may indicate a trend towards a training-induced Fe deficiency particularly over the first 12-weeks of the season before the final decline in TL/EEE_{REL} as observed in previous research (49). Fe deficiency, Fer concentrations <12 μg/L and percent saturation <16%, has been reported in endurance and team sport athletes, with females experiencing a greater risk for reduced Fe status (29). The similar responses between groups in iron status over the collegiate season reflect previous findings that Fer and Fe concentrations are not affected with OC use (45).

For all athletes, FT_3 levels increased from baseline through week 12 before declining through week 15, demonstrating a similar response to that previously described in female collegiate soccer players (49). Decreased or no change in FT_3 levels have often been shown over training periods in athletes, potentially as an effort to promote energy conservation during high EEE (4, 46). Perhaps the FT_3 decline observed indicates decreased muscular metabolism "needs" as FT_3 regulates skeletal muscle metabolism (43) and declines corresponded to further decreases in TL/EEE_{REL} in weeks 12-15. Future research examining the relationship between changes in TL, EEE, and energy intake along with thyroid hormones responses in female athletes is warranted due to the conflicted findings in these hormones over periods of intense training. Between groups, OC athletes had considerably greater TSH, TT_4, and TT_3 levels, yet no differences were observed for FT_3 exposure compared to CON players. It appears that OCs potentially influence thyroid hormone levels, however, this does not necessarily correspond to increased levels of the biologically active FT_3 above non-OC users. This lends support to previous findings that OCs may increase TSH as well as TT_4 and TT_3 levels due to increased binding capacity of thyroxine-binding globulin, without significant changes in FT_4 and FT_3 levels (51).

For both groups, moderate to large increases were observed in leptin, an adipose-derived hormone whose levels are reflective of changes in energy balance (1), over the season. Previously in collegiate rowers, changes in FT_3 levels were related to leptin changes, with rowers experiencing either a decrease in both FT_3 and leptin or no change in the hormones over 20-weeks of training (4). Conversely, in this study increases in FT_3 and leptin were observed. It appears a relationship exists between thyroid hormones and leptin production that may be reflective of energy balance in athletes. Throughout the season OC athletes exhibited an almost ~1.5x greater exposure to leptin compared CON. The elevated leptin levels in OC correspond to the increased BF% demonstrated in these athletes. Leptin expression has been shown to correlate with adipose stores (1), supporting the disparity in leptin levels observed at baseline and maintained throughout the season between groups. Previous research examining the effects of OC use on body composition is inconsistent in its findings, with some studies reporting no change (38, 39), while others reporting increases in body weight (9, 12, 39). It appears however, that changes in leptin across a training block may occur independent of body composition changes, as previously evidenced in collegiate rowers (4). The authors speculate that while leptin may indicate fat storage, changes may be primarily influenced by fluctuations in energy balance (1) with training.

Team performance characteristics demonstrated the power-endurance nature of the sport with similar average team aerobic capacity and greater CMJ_{HOH} ability as those previously reported in DI female soccer players (47). Additionally in female collegiate soccer athletes, body composition changes and biomarker perturbations across a competitive season have been shown to occur alongside performance changes pre- to post-season (49). Although statistical comparison of performance changes between groups was not possible in this study due to reduced sample size at post-season testing; visual inspection of the data appears to show no discernable differences in aerobic performance metrics between groups pre- to post-season. In terms of power, it seems players in the CON group tended to experience increases in CMJ_{HOH} across the season (n=8), while the OC group tended to maintain baseline values (n=4). Future research investigating the effects of OC use on long-term changes in athletic performance in a larger sample size is warranted in light of the increased catabolic and inflammatory environment that exists in OC athletes.

CONCLUSION

Overall, the TL and EEE_{REL} incurred during a NCAA DI soccer season corresponded to perturbations in biomarkers of stress, inflammation, hematologic status, metabolism, anabolism, and reproduction as well as changes in body composition. The majority of biomarker response patterns were similar between groups; however large differences in biomarker exposures existed over the season. Specifically, OC use was related to exacerbated stress, inflammatory, and metabolic disruptions that corresponded to a potentially reduced capacity for training adaptations and recovery. This study highlights the need for further research examining the impact of OCs on changes in performance with training as well as to investigate the effect of other hormonal contraceptive methods on biomarkers and body composition changes.

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CHAPTER IV:

EVALUATION OF PERFORMANCE CHARACTERISTICS AND INTERNAL AND EXTERNAL TRAINING LOADS IN FEMALE COLLEGIATE BEACH VOLLEYBALL PLAYERS

ABSTRACT

Although women's beach volleyball is the fastest growing collegiate sport, the training demands and performance characteristics have yet to be determined. The purpose of this study was to evaluate internal and external training loads throughout a competitive season and to quantify the performance characteristics of NCAA DI women's beach volleyball players. **Methods:** Female beach volleyball players (N=20, M_{height}=175.3±5.2cm, M_{weight}=68.3±6.3kg) were monitored throughout the six-week competitive season (T1-T6) using an integrative GPS and heart rate (HR) monitoring system, which was individualized based on pre-season testing, for the determination of workload metrics. In addition to team data, all variables were analyzed between travel (n=11, regular match-participation) and non-travel squad (n=7) athletes ($p<0.05$). **Results:** Team performance metrics demonstrated the explosive power emphasis of the sport, with travel squad players exhibiting significantly greater vertical jump and jump velocity abilities than their non-travel counterparts ($p<0.05$). Although few time main effects from T1-T6 were observed for team workload metrics, follow-ups revealed significant time*group interactions for training load (TL), exercise energy expenditure (EEE), total distance covered, and minutes spent in HR zones (HR_{Z1-Z5}) over the season ($p<0.05$). Finally, although average workloads were greater in practices than matches, when accounting for pre-match warm-ups, competition load was greater than practice ($p<0.05$). **Conclusion:** NCAA DI women's beach volleyball is a demanding, explosive power sport characterized by overall large TL and EEE, particularly in-season when athletes compete in four matches per weekend. **Practical Application:** The workloads observed point to the need to assess and manage training loads and fueling requirements to optimize performance and decrease injury risk.

INTRODUCTION

Appropriate monitoring can be used to evaluate an athlete's capabilities and limitations, determine adaptations to a training program, and assess fatigue status in order to optimize performance and decrease injury risk (10). With advancements in monitoring technology, coaches, sport-scientists, and training staffs have the ability to assess and design training programs to maximize exercise adaptations and minimize injury risk for athletes in a multitude of sports (2). Training loads can be monitored using both internal and external load measurements, with the dissociation between these metrics potentially indicating fatigue in an athlete (10). Internal load refers to the physiological impact of training on the athlete, which can be assessed through heart rate (HR) monitoring during exercise (2). HR monitoring can also provide a reliable estimation of caloric expenditure during exercise which can be useful to gauge recovery and fueling needs of the athlete (3). External training loads are objective measures of the work completed by the athlete and are typically assessed using global positioning systems (GPS) and accelerometers (2). Additionally, monitoring systems that allow for the individualization to an athlete's physiological profile allow for an improved assessment of training intensity specific to each athlete. An integrated approach to assessing both internal and external training loads allows for the management of training stress to reduce the risk of overtraining, injury, and illness (2, 10). Though much research exists examining the workloads of sports such as soccer and rugby, research examining the internal and external loads in other Olympic sports such as beach volleyball is extremely limited.

To date, few studies have investigated the demands of beach volleyball. Hank and colleagues analyzed three sets of professional female beach volleyball match play and found that average horizontal distance covered during a rally-play in one set was 287.5±19.7m, with no significant differences between blockers and defenders (11). In a case study of an Olympic male beach volleyball team (n=2), internal training load was assessed by multiplying the session duration by a rating of perceived exertion (RPE) for practices over 10 weeks (17). However, match load was not assessed during the monitoring period, thus failing to account for the complete training stress on the athletes (17). In a simulated 3-set match, male beach volleyball players spent the majority of the match between 71-80% HR_{max}, with an average intensity of 75% HR_{max} (13). In addition to the lack of research in beach volleyball, there is a need for research evaluating female athletes, as the majority of sport performance and nutrition guidelines are based on research conducted in men. Additionally, unlike professional athletes, collegiate athletes face unique challenges and stressors such as maintaining required academic loads and frequent travel which can have implications on training adaptations and recovery for their sport (6).

Since its inclusion as a National Collegiate Athletic Association (NCAA) championship sport in the 2015, women's collegiate beach volleyball has become the fastest growing collegiate sport (15). Previous research in athletes of other NCAA sports, such as soccer and lacrosse, has provided key insight into the demands of sport at the collegiate level as well as the efficacy and benefits of using various athlete monitoring methods to evaluate workload, reduce injury risk, and optimize performance (14, 22, 23). Despite the exponential increase in participation in NCAA beach volleyball over the years, to our knowledge no studies examining workloads or performance characteristics in collegiate beach volleyball players exist. In NCAA beach volleyball, a competition between two teams is defined as a dual, with each team comprised of 5 "starting" pairs and a sixth "alternate" pair. Starting pairs (1-5) match results are counted towards the result of the dual (win/loss), while the alternate pair typically plays in an "exhibition" match. Unlike other NCAA collegiate sports' game schedules, collegiate beach volleyball teams typically play four duals over a weekend, with two duals played per day and only a maximum of a few hours rest in between. This provides a unique challenge to the athletes as well as coaches to optimize player management.

The aim of this study was to evaluate internal and external training loads in NCAA DI beach volleyball players during all training sessions and matches over the course of a competitive season. Additionally, this study sought to quantify the performance characteristics of women's collegiate beach volleyball players. This observational study may provide important information regarding performance characteristics and the training demands of beach volleyball as well as insight into useful athlete-monitoring and testing techniques for success in the sport. Additionally, this study has the potential to determine differences in performance and workload metrics between travel squad players, who participate in competition matches, and non-travel squad players, which may aid in optimizing future player management.

METHODS

i. Experimental Design

This study was designed to assess performance characteristics and internal and external training loads in beach volleyball throughout a competitive season. Female collegiate beach volleyball underwent pre-season testing to assess performance characteristics and body composition as well as to individualize each athlete's wearable HR+GPS monitor. In this testing session, body composition, maximal counter-movement vertical jumps, aerobic capacity (VO_{2max}), ventilatory threshold (VT), and maximal HR (HR_{max}) were assessed. On a second testing session as part of their regular team program, athletes performed sport-specific maximal vertical jump height and jump velocity tests. Throughout the season, internal and external training load metrics were derived from heart rate (HR), GPS, and accelerometry data for all strength and conditioning sessions, practices, and matches. Due to the impact of COVID-19, the NCAA 2020 beach volleyball season was ended prematurely as was subsequent data collection.

ii. Subjects

Female collegiate beach volleyball players (N=20, Age=20±1yr, Height=175.3±5.2cm, Weight=68.3±6.3kg; Mean±SD) on a NCAA beach volleyball program ranked in the Top 20 in the nation and located in the southeast region of the United States were monitored throughout the course of the 2020 competitive season. Due to the impact of COVID-19 on the NCAA season, six weeks of data were available for analysis including three weeks of pre-season training (T1-T3) followed by three weeks of the competitive season (T4-T6) in which four matches occurred per week. Written informed consent was obtained from all subjects prior to participation and all subjects received clearance by the University sports medicine staff prior to testing. Research was approved by the University Institutional Review Board for the Protection of Human Subjects and conducted in accordance with the Declaration of Helsinki.

iii. Performance testing

After the determination of eligibility and upon enrollment into the study, athletes underwent team testing in the first week of preseason to determine performance characteristics of the athletes as well as to individualize each athlete's HR+GPS monitor. Athletes reported to the University of South Carolina Clinical Exercise Research Center for body composition and performance testing having abstained from exercise ≥24h prior and from caffeine intake on the day of testing. Upon arrival subjects' height and weight were measured using a stadiometer and calibrated scale, respectively. Next, body composition (FFM, BF%) was assessed via ultrasound (BodyMetrix, Intelametrix, Brentwood, CA, USA) using a 7-site model (24). Athletes then performed a standardized dynamic warm-up followed by maximal vertical jump testing using a Just Jump Mat (Probotics, Inc, Huntsville, AL, USA) (16). Participants performed maximal countermovement vertical jumps using both hands-on-hips (CMJ_{HOH}) and arm-swing methods (CMJ). Three efforts were performed for each jump with 30s rest between jumps. Depth of the eccentric phase of the CMJ and CMJ_{HOH} were self-selected by the athlete to maximize jump height. Peak Power (PP) was calculated for both CMJ_{HOH} and CMJ jumps using the Sayers equation: (60.7*jump height (cm)) + (45.3*weight (kg)) – 2055 (20).

Participants next performed a maximal graded exercise test on the treadmill using a metabolic cart (Parvo Medics, Sandy, UT, USA) and Polar H7 HR monitor (Polar, Lake Success, NY, USA) for the determination of VO_{2max}, VT, HR at VT (HR_{VT}), and HR_{max}. A speed-based protocol was used with stages that were MET equated to the Bruce protocol. The protocol consisted of two-minute stages at a constant 2% incline, with increasing speeds of 6.4, 7.9, 10.0, 11.7, 13.7, 15.6, 17.1, 18.2, 19.8, 21.1km/h (14). Subjects continued the test with encouragement from the lab staff until volitional fatigue. At least two of the following criteria were met for attainment of VO_{2max}: RER $\geq$1.1, observation of a plateau in O_2 consumption (increase $\leq$150 ml/min with increasing workload), Borg scale rating of perceived exertion (RPE) $\geq$17, and HR >85% age-predicted HR_{max} (208 – (0.7 x age). For one subject who did not meet the above criteria, VO_{2peak} was used. Each athlete's VT was calculated after the completion of each test as the point where ventilation increased nonlinearly with VO_2, expressed as a percentage of VO_{2max} (7). The Polar TeamPro system (Polar Electro Co., Woodbury, NY, USA) was individualized using each athlete's profile and pre-season testing results (age, height, weight, VO_{2max}, VT, HR_{VT}, and HR_{max}).

On a separate day during pre-season as part of their regular team testing with their strength and conditioning coach (and aided by the researchers), athletes participated in maximal vertical jump testing. Power and vertical jump ability were assessed using a two-legged counter-movement jump with single-arm reach and a volleyball approach (an offensive volleyball attack) vertical jump using a Vertec (Sports Imports, Hilliard, OH, USA). Additionally, jump velocity was assessed using a TENDO unit (TENDO Sports Machines UK LTD, London, UK). Athletes performed a barbell (20 kg) back squat and maximal jump with the TENDO unit attached to the barbell. Three maximal efforts were performed of each jump variation, with the highest values recorded. Three athletes were limited in participation for maximal testing by sports medicine and did not participate in all jump variations (see *Table 1*).

iv. In-season athlete-monitoring

Players were evaluated during all team training sessions as well as during all matches for those players on the travel squad using the Polar TeamPro system during the spring competitive season. This included 13 indoor strength and conditioning sessions, 34 practices, and 12 matches. Average durations were 57 mins (range: 41-68 mins) for strength and conditioning sessions, 121 mins (range: 58-201 mins) for practices, and 46 mins (range: 32-76 mins) for matches. The Polar TeamPro system utilized both GPS, HR and accelerometry technology to determine training load (TL), absolute exercise energy expenditure (EEE_{ABS}, expressed as kcals), total distance covered (DIS), sprints, time spent in HR zones, distance covered in speed zones, and acceleration/deceleration data. TL, expressed as arbitrary units (au), was calculated via an algorithm developed by Polar™ based on the quantification of an individual player's caloric expenditure, time spent in different HR zones, speed, distance, and acceleration data. EEE_{ABS} was adjusted for body weight (EEE_{REL}, expressed as kcal/kg), which was obtained during body composition assessments, in order to account for relative size differences between players. HR zones were classified as 1, 2, 3, 4, and 5 (HR_{Z1}=0-59%HR_{max}; HR_{Z2}=60-69%HR_{max}; HR_{z3}=70-79%HR_{max}; HR_{Z4}=80-89%HR_{max}; HR_{Z5}=90-100%HR_{max}). For all practices and matches in the sand, speed zones were based off available previous research in a sand sport (4) and were set as follows: DIS_{Z1}= 0-3.9 km/h, DIS_{Z2}= 4.0-6.9 km/h, DIS_{Z3}= 7.0- 12.9 km/h, DIS_{Z4}= 13.0-17.9 km/h, DIS_{Z5}= ≥18 km/h). For all strength and conditioning sessions (indoors), speed zones were set using established indoor zones that were faster than those observed in sand sport in order for the zones to be relative to the ground surface used during training sessions (DIS_{Z1}= 0-6.9 km/h, DIS_{Z2}= 7.0-10.9 km/h, DIS_{Z3}= 11.0- 14.9 km/h, DIS_{Z4}= 15.0-18.9 km/h, DIS_{Z5}= ≥19 km/h). A sprint was considered to be any movement greater than 2.8 m/s^2 (21). Accelerations and decelerations performed by players during practices and matches were categorized in four zones to quantify dynamic characteristics of movement ($ACEL_{Z4}$= 3.0-50.0 m/s^2, $ACEL_{Z3}$= 2.0-2.99 m/s^2, $ACEL_{Z2}$= 1.0-1.99 m/s^2, $ACEL_{Z1}$= 0.5-0.99 m/s^2; $DECEL_{Z4}$= -3.0--50.0 m/s^2, $DECEL_{Z3}$= -2.0--2.99 m/s^2, $DECEL_{Z2}$= -1.0--1.99 m/s^2, $DECEL_{Z1}$= -0.5--0.99 m/s^2).

v. Statistical Analysis

Descriptive statistics (Mean ± SD) were used to quantify physical and performance characteristics. RM-ANOVAS with polynomial contrasts were calculated for weekly internal and external load metrics for all strength and conditioning sessions, practices, and matches throughout the season. Due to impact of different ground surfaces on velocity, acceleration, and deceleration outputs, summed weekly external load velocity/acceleration threshold metrics ($DIS_{Z1\text{-}Z5}$, $ACCEL_{1\text{-}4}$, $DECEL_{1\text{-}4}$, sprints) were not included in this analysis (1). Sub-analysis of performance characteristics (ANOVAs) and internal and external load metrics (RM-ANOVAs) were performed between travel squad athletes who participated in matches on the weekend (n=11) and non-travel players (n=7). Two players who alternated between travel and non-travel squads were not included in the sub-analysis. Furthermore, for travel and non-travel squad, RM-ANOVAs with polynomial contrasts were calculated for weekly internal and external load metrics (T1-T6) to determine changes over time within each group. In order to compare match and practice loads in the sand, RM-ANOVAs with simple contrasts were performed between conditions, defined as the average loads for practices, matches, and total match sessions for each travel player. A match was defined as the period of time from the start of the match until the completion of match-point. For determination of match load, matches for pairs 1-5 and exhibition matches for pair 6 were included in analysis. A total match session (match+) was defined as the start of team warm-up until the completion of each player's respective wave of matches, in order to account for the full load of competition on the athlete. Hedges' *g* was used to calculate effect sizes (ES), with 0.20, 0.50, and 0.80 considered indicative of small, medium, and large effects, respectively. ES and 95% confidence intervals (CIs) were calculated between conditions. All analyses were performed using SPSS Statistical Software (SPSS version 26, IBM). For RM-ANOVA analysis, the Huynh–Feldt correction was applied in cases where the assumption of sphericity was not met. Significance was set at $p<0.05$.

RESULTS

vi. Body Composition and Performance

Body composition and performance characteristics of the team are presented in *Table 1.* Athletes on the travel squad displayed significantly greater single-arm reach ($p=0.028$), volleyball approach vertical jumps ($p=0.024$), and maximal jump velocity ($p=0.049$) than the non-travel players. Additionally, greater CMJ_{HOH} favoring the travel athletes approached significance ($p=0.068$, $ES=0.70$), while VT ($p=0.022$) was significantly greater for non-travel players (*Table 1*).

Table 1: Body Composition and Performance Characteristics

	Team	**Travel squad**	**Non-Travel squad**	**ES (95% CI)**
BF%	21.80 ± 3.5	21.39 ± 3.0	22.01 ± 4.1	-0.18 (-1.13 – 0.77)
FFM *(kg)*	53.37 ± 4.9	55.05 ± 5.4	51.96 ± 3.6	0.64 (-0.33 - 1.61)
VO_{2max} *($ml \cdot kg^{-1} min^{-1}$)*	43.59 ± 4.0	43.89 ± 3.1	42.14 ± 4.4	0.48 (-0.48 – 1.44)
VT *($\%VO_{2max}$)*	79.0 ± 0.04	77.82 ± 0.04*	82.29 ± 0.02	-1.23 (-2.26 – 0.20)
CMJ_{HOH} *(cm)*	49.11 ± 5.5	51.15 ± 3.9†	47.43 ± 7.1	0.70 (-0.27 – 1.68)
CMJ *(cm)*	54.06 ± 7.6	56.46 ± 5.1	51.87 ± 10.4	0.61 (-0.36 – 1.57)
PP CMJ_{HOH} *(W)*	4020.48 ± 440.1	4223.05 ± 344.3	3850.44 ± 465.9	0.94 (-0.05 – 1.94)
PP CMJ *(W)*	4321.12 ± 558.1	4545.43 ± 435.2	4119.11 ± 654.0	0.81 (-0.18 – 1.79)
Single-arm reach VJ *(cm)*	53.37 ± 4.9	63.63 ± 5.5*	56.24 ± 7.0	1.20 (0.15 – 2.25)
Volleyball approach VJ *(cm)*	60.11 ± 7.1	69.22 ± 4.8*	60.96 ± 8.3	1.31 (0.20 – 2.42)
Maximal Jump Velocity *(m/s^2)*	2.58 ± 0.2	2.67 ± 0.2*	2.48 ± 0.1	1.05 (0.03 – 2.08)

Values are expressed as Mean ± SD; n-values for sport-specific tests were as follows: Team single-arm reach VJ (n=18), volleyball approach VJ (n=17), maximal jump velocity (n=19), Travel (n=10), and Non-travel (n=6) for volleyball approach VJ; BF%= body fat percentage; FFM= fat free mass; VO_{2max}= aerobic capacity; VT= ventilatory threshold; CMJ_{HOH}= hands-on-hips countermovement jump; CMJ= countermovement jump; PP= peak power; VJ= vertical jump;
*denotes significant difference between travel and non-travel squads ($p<0.05$)

†denotes that the time main effect approached significance ($p<0.10$)

vii. Team Internal and External Load Metrics

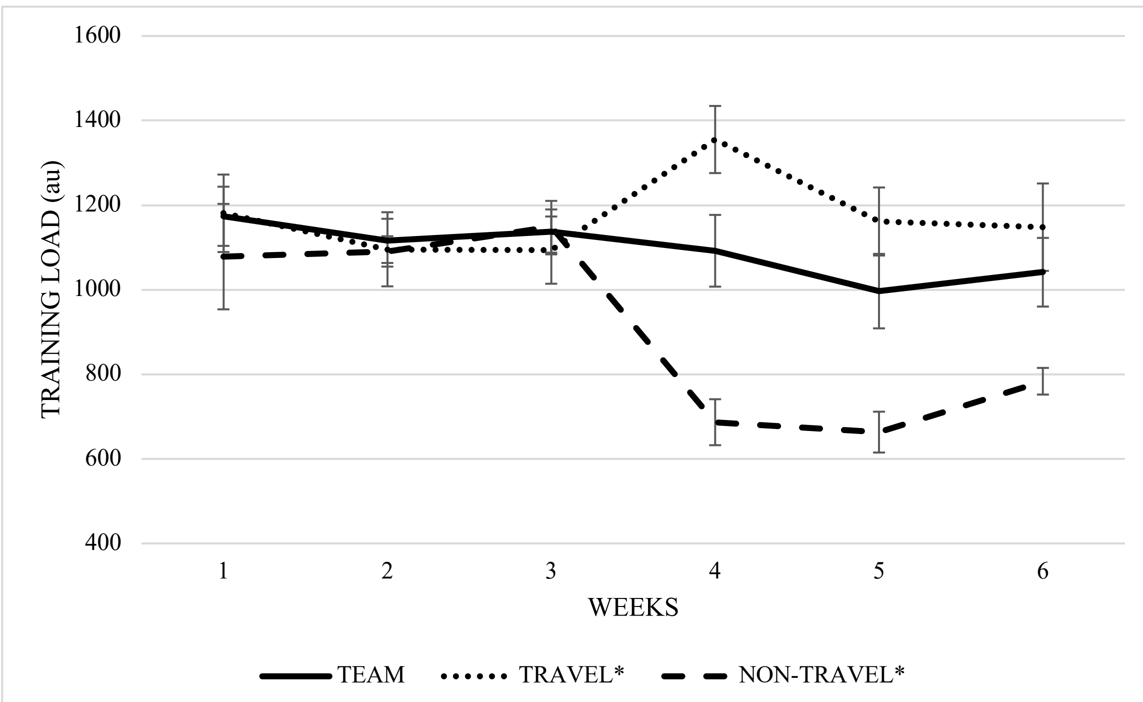
Significant time main effects were observed in team HR_{Z4} ($p<0.001$), HR_{Z3} ($p=0.001$) and HR_{Z1} ($p<0.001$), with DIS approaching significance ($p=0.056$) from T1-T6. Over the season, no significant time main effects were displayed for team TL (*Figure 1*), EEE_{REL} (*Figure 2*), EEE_{ABS}, HR_{Z5}, and HR_{Z2} ($p>0.05$). Polynomial contrasts revealed significant effects for decreases in HR_{Z4} ($p=0.001$) and HR_{Z3} ($p=0.004$) and for curvilinear responses in DIS and HR_{Z1} ($p<0.05$). Overall DIS trended to decrease pre- to in-season, with a decrease T1-T2, an increase T2-T3, a decrease T3-T4, and a slight increase from T4-T6 ($p=0.011$). HR_{Z1} decreased from T1-T2, followed by a return back to baseline values at T2-T6 ($p=0.012$). No effects were observed for HR_{Z2} ($p>0.05$). Weekly team EEE_{ABS}, DIS, and HR_{Z5-Z1} are presented in *Table 2.*

viii. Travel vs. Non-travel Internal and External Load Metrics

Significant time*group interactions were observed for TL, EEE_{REL}, EEE_{ABS}, DIS, HR_{Z5}, HR_{Z4}, HR_{Z3}, HR_{Z2}, and HR_{Z1} over the season ($p<0.001$). The travel squad demonstrated significant time main effects from T1-T6 for TL ($p<0.001$, *Figure* 1), EEE_{REL} ($p<0.001$, *Figure 2*), and EEE_{ABS} ($p<0.001$), DIS ($p<0.001$), HR_{Z5} ($p<0.001$), HR_{Z4} ($p=0.004$), HR_{Z2} ($p<0.001$) and HR_{Z1} ($p=0.001$). No significant time main effects were observed for HR_{Z3} for travel squad players ($p>0.05$). Polynomial contrasts displayed significant effects for increased EEE_{ABS} ($p=0.003$) and HR_{Z2} ($p<0.001$) pre- to in-season, and significant curvilinear responses in TL, EEE_{REL}, and DIS ($p<0.05$). Travel players' TL was maintained in pre-season from T1-T3, followed by an increase at the start of competition season (T3-T4), a decrease from T4-T5, and then a plateau from T5-T6 ($p=0.003$). EEE_{REL} experienced an initial decrease T1-T2, followed by an increase T3-T4 at the in-season transition for travel squad athletes ($p=0.002$). DIS decreased from T1-T2, followed by an increase T2-T5 for travel players ($p=0.002$). HR_{Z5} was maintained throughout pre-season (T1-T3), with a spike at T4 and subsequent decrease followed by a plateau T5-T6 ($p=0.036$). HR_{Z4} initially decreased from T1-T2 and then rebounded T2-T3 followed by a decrease T4-T5 ($p=0.020$). For travel players over the season, HR_{Z1} initially decreased from T1-T2, followed by an increase T2-T6 above baseline ($p=0.007$).

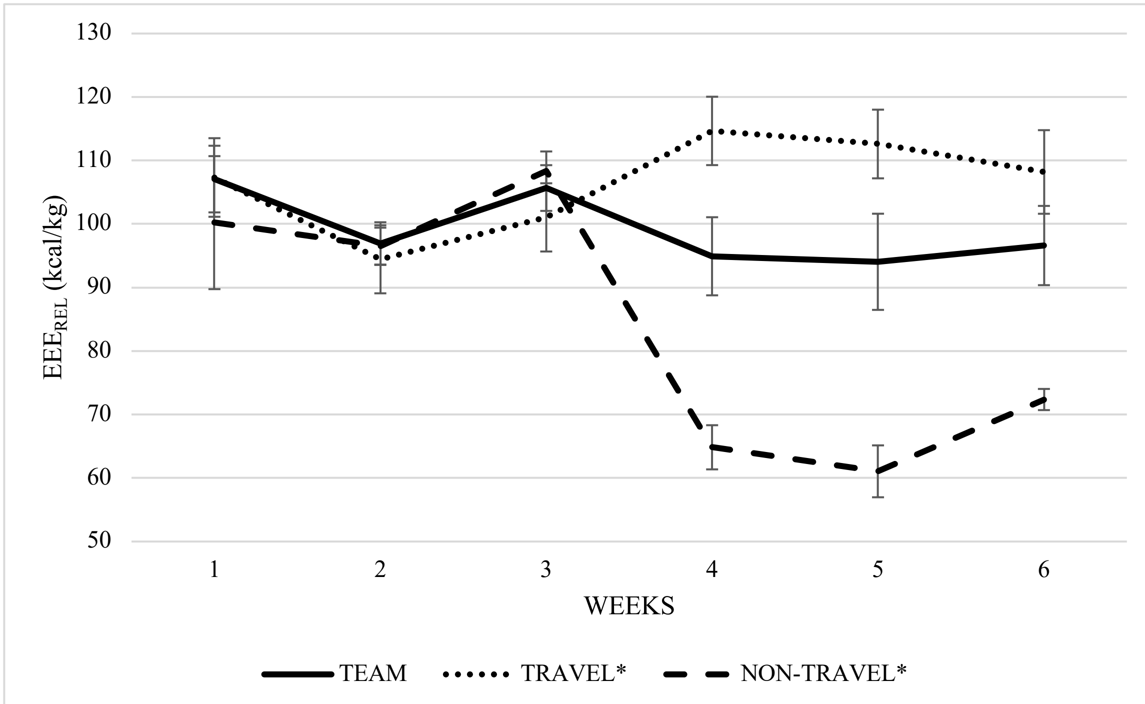
Over the season, significant time main effects were shown for TL ($p<0.001$, *Figure 1*), EEE_{REL} ($p=0.001$; *Figure 2*), EEE_{ABS} ($p=0.001$), DIS ($p<0.001$), HR_{Z4} ($p<0.001$), HR_{Z3} ($p<0.001$) HR_{Z2} ($p<0.001$), HR_{Z1} ($p<0.001$), with a time main effect for HR_{Z5} approaching significance ($p=0.090$) for non-travel players from T1-T6. Polynomial contrasts revealed significant curvilinear responses in TL, EEE_{REL}, EEE_{ABS}, DIS, and $HR_{Z5\text{-}Z1}$ ($p<0.05$). Non-travel squad athletes experienced a major decrease in TL at the transition from pre- to in-season (T3-T4) and then an increase from T5-T6 ($p=0.005$). Both EEE_{REL} and EEE_{ABS} increased from T2-T3, decreased drastically from T3-T4 at the transition from pre-season to in-season, followed by a plateau then minor increase from T5-T6 in non-travel players ($p=0.001$; $p=0.001$). Similarly, DIS increased from T2-T3, decreased drastically from T3-T4 and continued to decrease from T4-T5 until a slight increase from T5-T6 ($p<0.001$). HR_{Z5} increased the last week of pre-season (T2-T3), then decreased T3-T4, followed by a slight increase in-season (T4-T6) ($p=0.002$). In $HR_{Z4,}$ non-travel players experienced a maintenance over pre-season (T3-T4), followed by a decrease (T3-T4) and subsequent plateau throughout in-season (T4-T6) ($p=0.004$). Non-travel athletes displayed a decrease in HR_{Z3} from T3-T4, followed by an increase from T5-T6 (p=0.005). An increase was observed in HR_{Z2} from T2-T3, with decreases from T3-T5 and then an increase from T5-T6 (p=0.019). HR_{Z1} (p=0.057) initially decreased from T1-T2, then increased from T2-T3, then declined again from T3-T4 and was maintained over the rest of the season (p=0.005). Weekly EEE_{ABS}, DIS, and HR_{Z1-Z5} for travel and non-travel players are presented in *Table 2.*

Figure 1: Changes in Training Load Over the Season



Values are expressed as Mean ± SE
*denotes significant time main effect (p<0.05)
†denotes that the time main effect approached significance (p<0.10)

Figure 2: Changes in Relative Exercise Energy Expenditure Over the Season



Values are expressed as Mean ± SE; EEE_{REL}= relative exercise energy expenditure
*denotes significant time main effect ($p<0.05$)
†denotes that the time main effect approached significance ($p<0.10$)

Table 2: Weekly Total Exercise Energy Expenditure, Distance Covered, and Time Spent in Heart Rate Zones Over the Season

		WK 1	WK 2	WK 3	WK 4	WK 5	WK 6
EEE_{ABS} *(kcal)*	***Team***	7265.00 ±1510.2	6585.80 ±974.2	7159.40 ±873.4	6496.75 ±1959.7	6401.35 ±2241.7	6573.80 ±1864.6
	Travel squad*	7461.36 ±1275.3	6579.64 ±1181.4	7006.73 ±927.8	7982.09 ±1057.7	7823.55 ±924.9	7522.91 ±1396.0
	Non-travel squad*	6665.43 ±1882.9	6435.71 ±648.0	7226.57 ±718.6	4343.43 ±818.1	4047.86 ±601.1	4821.29 ±354.8
DIS *(m)*	***Team***†	25134.25 ±5019.8	22448.52 ±2796.2	24296.93 ±2629.1	21211.61 ±5591.8	21517.29 ±7219.4	21914.83 ±5976.6
	Travel squad*	25637.03 ±3559.2	22010.44 ±2868.0	23466.08 ±3109.8	25481.17 ±2709.2	26605.88 ±2037.1	25625.90 ±3476.0

	Non-travel squad*	23754.28 ±7387.2	22597.17 ±3077.4	25240.38 ±1672.1	15044.36 ±2465.3	13539.26 ±2122.9	15629.58 ±1386.8
HR_{Z5} *(mins)*	***Team***	39.91 ±34.8	38.56 ±35.8	42.19 ±32.2	57.57 ±41.3	41.72 ±34.8	46.46 ±42.1
	Travel squad*	43.05 ±39.5	41.39 ±37.3	36.95 ±27.9	86.85 ±29.9	48.45 ±30.7	51.96 ±45.3
	***Non-travel squad*†**	29.81 ±29.2	30.50 ±35.3	47.74 ±35.9	16.33 ±15.2	23.21 ±20.8	30.34 ±32.7
HR_{Z4} *(mins)*	***Team****	195.97 ±76.9	181.97 ±62.4	195.17 ±67.8	154.04 ±59.3	141.22 ±48.2	145.77 ±64.0
	Travel squad*	184.41 ±67.6	167.85 ±66.8	189.79 ±66.0	181.55 ±46.7	152.76 ±38.1	151.12 ±73.4
	Non-travel squad*	200.54 ±99.9	197.17 ±62.8	202.27 ±83.0	107.67 ±59.2	110.46 ±27.3	121.87 ±23.4
HR_{Z3} *(mins)*	***Team****	231.44 ±49.3	242.57 ±52.7	218.16 ±42.8	201.36 ±56.9	186.52 ±60.8	198.19 ±44.3
	Travel squad	228.35 ±44.9	229.90 ±47.2	204.17 ±46.1	228.61 ±56.9	217.05 ±59.6	209.86 ±49.5
	Non-travel squad*	232.03 ±62.8	260.04 ±60.2	232.29 ±31.7	160.80 ±30.5	140.94 ±24.2	173.27 ±28.5
HR_{Z2} *(mins)*	***Team***	214.87 ±51.8	189.07 ±44.4	214.26 ±55.0	201.12 ±52.4	209.91 ±75.6	219.44 ±55.1
	Travel squad*	219.49 ±39.7	187.09 ±34.8	195.09 ±48.3	230.22 ±44.3	262.67 ±42.0	250.95 ±48.3
	Non-travel squad*	200.33 ±71.7	189.49 ±62.7	237.29 ±63.6	161.07 ±31.9	131.29 ±19.5	170.59 ±30.1
HR_{Z1} *(mins)*	***Team****	163.26 ±51.4	94.97 ±39.3	167.15 ±49.3	124.06 ±52.1	174.84 ±92.9	160.74 ±71.2
	Travel squad*	169.97 ±32.0	101.90 ±37.5	170.59 ±50.7	144.92 ±56.3	237.76 ±79.2	209.86 ±53.3

	Non-travel squad *	155.6 ±80.3	88.16 ±48.1	164.91 ±57.2	99.50 ±39.0	95.50 ±25.2	99.46 ±32.9

Values are expressed as Mean ± SD; EEE_{ABS}= absolute exercise energy expenditure; DIS= total distance covered; HR_{Z1-Z5}= heart rate zones 1-5
*denotes significant time main effect ($p<0.05$)
†denotes that the time main effect approached significance ($p<0.10$)

ix. Practices vs. Matches & Total Match Sessions

Significant differences were observed between conditions for all workload variables ($p<0.05$). Simple contrasts revealed practices had significantly greater TL than matches (M_{Diff} ± SE: 42.40±8.1 TL-points, $p<0.001$), but significantly less TL than match+ (-22.96±5.7 TL-points, $p=0.002$). Similarly, EEE_{ABS} and EEE_{REL} were significantly greater in practices than matches (EEE_{ABS}=469.52 ± 31.3 kcal, $p<0.001$; EEE_{REL}=6.75 ± 0.5 kcal/kg, $p<0.001$), but significantly less than match+ (EEE_{ABS}= -59.5 ± 25.4 kcal, $p=0.041$; EEE_{REL}= -0.89 ± 0.4 kcal/kg, $p=0.040$). Time spent in HR_{Z5} was significantly greater for matches (8.62±1.6 mins, $p<0.001$), but significantly less for all other HR zones compared to practices ($p>0.05$). Match+ had significantly more time spent in HR_{Z5} (9.04±1.5 mins; $p<0.001$) and less time spend in HR_{Z3} (-6.36±1.2 mins, $p<0.001$) than practices. Match+ and practices displayed no differences in time spent in HR_{Z4}, HR_{Z2}, and HR_{Z1} ($p>0.05$).

Total DIS covered was significantly greater in practices than both matches (1876.74±55.9 m; $p<0.001$) and match+ (207.56±56.3 m; $p=0.004$). Athletes also performed significantly fewer sprints in matches versus practices ($p=0.046$), but they tended to perform more sprints in match+ compared to practices ($p=0.059$). Athletes covered significantly more distance in sand DIS_{Z1-Z3} and DIS_{Z5} ($p<0.001$), but not DIS_{Z4} ($p>0.05$) in practices versus matches. Between practices and match+, less DIS_{Z4}

(p=0.002), but more DIS_{Z1} (p<0.001) was covered in practices. Greater high velocity accelerations and decelerations were performed in match+ versus practices ($ACCEL_{Z4}$: p=0.042; $DECEL_{Z4}$: p=0.046). However, in practices athletes performed a significantly greater number of accelerations and decelerations in zones 1-3 than matches ($ACCEL_{Z3}$: p=0.003; $ACCEL_{Z2}$: p<0.001; $ACCEL_{Z1}$: p<0.001; $DECEL_{Z1}$: p<0.001; $DECEL_{Z2}$: p<0.001; $DECEL_{Z3}$: p=0.006) and in zones 1 and 2 than match+ ($ACCEL_{Z2}$: p<0.001; $ACCEL_{Z1}$: p=0.005; $DECEL_{Z1}$: p=0.001; $DECEL_{Z2}$: p<0.001). Differences and effect sizes between practices versus matches and match+ are presented in *Table 3*.

Table 3: Beach Volleyball Practices versus Matches and Total Match Sessions

	Practices	**Matches**	**ES (95% CI)**	**Total Match Sessions (Match+)**	**ES (95% CI)**
TL *(au)*	155.83 ± 32.5	113.42 ± 14.4*	-1.69 (-2.66 – -0.71)	178.78 ± 33.8*	0.69 (-0.17 – 1.55)
DIS *(m)*	3545.42 ± 213.7	1668.68 ± 94.3*	-11.36 (-14.82 – -7.90)	3337.86 ± 238.8*	-0.92 (-1.79 – -0.04)
EEE_{ABS} *(kcal)*	997.05 ± 108.7	527.53 ± 29.8*	-5.89 (-7.82 – -3.96)	1056.55 ± 80.2*	0.62 (-0.23 – 1.48)
EEE_{REL} *(kcal/kg)*	14.33 ± 1.9	7.58 ± 0.7*	-4.72 (-6.35 – -3.10)	15.21 ± 1.9*	0.46 (-0.39 –1.31)
SPRINTS *(#)*	3.00 ± 1.0	2.00 ± 1.0*	-0.58 (-1.43 – 0.27)	3.00 ± 1.0†	0.56 (-0.29 –1.41)
HR_{Z5} *(mins)*	5.13 ± 4.3	13.74 ± 5.7*	1.70 (0.73 – 2.68)	14.17 ± 5.8*	1.78 (-0.79 – 2.77)
HR_{Z4} *(mins)*	24.18 ± 8.0	18.66 ± 2.3*	-0.94 (-1.82 – -0.06)	24.67 ± 5.5	0.07 (-0.77 – 0.91)
HR_{Z3} *(mins)*	31.66 ± 4.1	8.74 ± 1.8*	-7.22 (-9.52 – -4.93)	25.30 ± 5.8*	-1.26 (-2.18 – -0.35)
HR_{Z2} *(mins)*	30.24 ± 3.9	3.97 ± 1.9*	-8.60 (-11.27 – -5.92)	28.87 ± 3.8	-0.35 (0.49 – -1.20)
HR_{Z1} *(mins)*	22.80 ± 5.9	1.10 ± 1.1*	-5.08 (-6.80 – -3.36)	21.05 ± 9.2	-0.23 (-1.06 – 0.61)
DIS_{Z5} *(m)*	2.06 ± 0.7	0.42 ± 0.5*	-2.62 (-3.76 – -1.48)	1.47 ± 1.7	-0.45 (-1.30 – 0.39)

DIS_{Z4} *(m)*	15.13 ± 3.9	15.16 ± 8.6	0.01 (-0.83 – 0.84)	27.39 ± 10.5*	1.55 (0.59 – 2.50)
DIS_{Z3} *(m)*	461.17 ± 82.9	264.81 ± 56.7*	-2.77 (-3.93 – -1.60)	480.68 ± 103.8	0.21 (-0.63 – 1.05)
DIS_{Z2} *(m)*	1387.64 ± 162.0	734.51 ± 74.6*	-5.18 (-6.92 – -3.44)	1355.85 ± 126.8	-0.22 (-1.06 – 0.62)
DIS_{Z1} *(m)*	1679.10 ± 44.8	652.50 ± 74.3*	-16.73 (-21.75 – -11.72)	1471.42 ± 123.9*	-2.23 (-3.29 – -1.17)
$DECEL_{Z4}$ *(#)*	2.00 ± 1.0	2.00 ± 1.0	-0.10 (-0.93 – 0.74)	3.00 ± 1.0*	0.61 (-0.24 – 1.47)
$DECEL_{Z3}$ *(#)*	21.00 ± 4.0	17.00 ± 4.0*	-0.95 (-1.83 – -0.07)	22.00 ± 5.0	0.37 (-0.47 – 1.21)
$DECEL_{Z2}$ *(#)*	217.00 ± 27.0	108.00 ± 13.0*	-5.15 (-6.89 – -3.41)	191.00 ± 26.0*	-0.99 (-1.88 – -0.11)
$DECEL_{Z1}$ *(#)*	707.00 ± 45.0	307.00 ± 18.0*	-11.69 (-15.25 – -8.14)	662.00 ± 48.0*	-0.97 (-1.86 – -0.09)
$ACCEL_{Z1}$ *(#)*	551.00 ± 46.0	240.00 ± 28.0*	-8.14 (-10.69 – -5.60)	515.00 ± 49.0*	-0.77 (-1.63 – 0.10)
$ACCEL_{Z2}$ *(#)*	212.00 ± 24.0	112.00 ± 10.0*	-5.52 (-7.35 – -3.69)	193.00 ± 23.0*	-0.85 (-1.72 – 0.02)
$ACCEL_{Z3}$ *(#)*	24.00 ± 5.0	19.00 ± 5.0*	-1.01 (-1.90 – -0.12)	25.00 ± 6.0	0.23 (-0.61 – 1.07)
$ACCEL_{Z4}$ *(#)*	1.00 ± 1.0	1.00 ± 1.0	-0.49 (-1.34 – 0.35)	2.00 ± 1.0*	0.66 (-0.20 – 1.52)

Values are expressed as Mean ± SD; TL= training load; DIS= total distance covered; EEE_{ABS}= absolute exercise energy expenditure; EEE_{REL}= relative exercise energy expenditure; HR_{Z1-Z5}= heart rate zones 1-5; DIS_{Z1-Z5}= distance covered in zones 1-5; $DECEL_{Z1-Z4}$= decelerations in zones 1-4; $ACCEL_{Z1-Z4}$= accelerations in zones 1-4
*denotes significant difference from practices ($p<0.05$)
†denotes that the difference from practices approached significance ($p<0.10$)

DISCUSSION

Based on these findings, NCAA DI women's beach volleyball is a demanding sport characterized by high training loads and caloric expenditures, particularly during the onset of competition match-play. Additionally, player performance metrics demonstrate the explosive power emphasis of the sport. Vertical jump ability is essential to beach volleyball as players perform repeated maximal vertical jumps during game-play while executing skills such as blocking, hitting, and serving. Previous research in indoor volleyball has demonstrated a relationship between vertical jump performance and player proficiency (19, 25). Typically, "better" players exhibited superior vertical jump abilities; however, strength of this relationship differed between vertical jump types (19, 25). Similarly, in this study, beach volleyball travel squad players demonstrated significantly greater vertical jump ability in the single arm-reach and volleyball approach jumps as well as greater maximal jump velocity over non-travel squad players. Moreover, although not statistically significant, large effects were also observed for greater peak power outputs and moderate effects for greater CMJ and CMJ_{HOH} in the travel players compared to non-travel. Interestingly, the enhanced explosive power capabilities in travel squad players corresponded to decreased VT with no differences in aerobic capacity compared with non-travel players. This appears to reflect a power-endurance trade-off in these athletes, underlining explosive power as the primary fitness trait critical for beach volleyball sport performance. Despite differences in performance variables, no differences in body composition were observed between travel and non-travel players. Future research examining the influence of the high training loads of the competitive season on body composition and performance metrics. This could provide insight on both the preferred 'baseline' performance attributes for beach volleyball athletes as well as whether or not exposure to these workloads corresponds to changes in performance and body composition variables.

As a team, few changes were observed in various internal and external training load metrics over the three weeks of pre-season and three weeks of the regular season. Specifically, overall decreases in time spent in HR_{Z4} and HR_{Z3} were observed over the six weeks. Similarly, the team experienced a decrease in total weekly DIS covered, mirroring the changes seen in internal loads. Subsequent follow-up analysis of internal and external loads revealed that overall lack of team changes were largely driven by opposing load changes between travel and non-travel squad players. Over the first three weeks of pre-season training, travel and non-travel squad players experienced similar internal and external training loads; however, at the onset of season drastic disparities in training loads were observed between groups. During week 4, travel players faced an increase in workload during the first week of competition match-play of an average of 262 TL points and 975 kcal (13.6 kcal/kg) whereas non-travel players experienced 462-point TL and 2883 kcal (43.5 kcal/kg) decrease. In reference to practice-load, that is essentially having travel athletes play an additional ~1.7 practices and non-travel athletes play in ~3 fewer practices than the previous week of pre-season training. This separation in workloads between travel and non-travel players reveals important considerations for coaches and training staff in regard to load periodization and player management.

Coaches and training staff must be cognizant of match-load when competition begins in order to adjust weekly workloads to accommodate for the increased TL experienced by travel players on a weekend, but decreased TL for non-travel players. Large acute increases in internal and external training loads have been associated with an increased risk for injury and illness in athletes (5). Moreover, large fluctuations in these training loads can impact sport performance and fitness (12). Large decreases in workload during a season can result in detraining while long-term exposure to high workloads may have implications on fatigue and player readiness (2). In-season, non-travel players sustained large decreases in internal and external training loads compared to pre-season workloads. This can have considerable repercussions on athlete readiness and injury risk, specifically for athletes who are "non-travel" one week and "travel" the next week as these athletes experience major acute workload fluctuations. Therefore, coaches and training staff must incorporate strategies to 'recapture' loads in-season for non-travel players to avoid substantial workload declines following pre-season. Although some strategies, such as non-travel practices and lifts, were loosely employed in-season in this study, the above findings indicate need for planned, deliberate weekly strategies to maintain workload in these players in-season. Following week 4, travel players' weekly TL returned towards baseline values along with reductions in EEE with the removal of one practice-day for these athletes. During weeks 5 and 6 of the regular season, travel players practiced one-less day per week compared to week 4, highlighting the importance of workload modifications during the transition from pre-season to in-season. Thus, managing in-season training loads in order to account for the greater load experienced by travel players on a competition weekend becomes essential at the immediate onset of weekly game-play to avoid large acute increases in TL and EEE.

The competition game-schedule for NCAA DI women's beach volleyball involves four matches played over a weekend, with two matches played per day with as little as ~1hr rest between same-day matches. This compact in-season game-schedule unique to beach volleyball poses a challenge in terms of player management. Examination of load metrics revealed the high intensity of match-play with players spending the majority of a match in the top two HR zones: HR_{Z4} and HR_{Z5} (80-100% HR_{max}). This is greater intensity than previously assessed in elite male beach volleyball players who spent the majority of a simulated 3-set match between 71-80% HR_{max} (13). This discrepancy in intensity could be attributed to the fact that these were simulated matches not a part of a tournament or competition play, and were automatically played to 3-sets regardless of score (13). When compared to practices, matches consisted of significantly less workload in both internal and external load metrics on an athlete; however, comparison of practices to match+ revealed that when team and on-court pre-game warm-ups were included with game-play analysis, loads were significantly greater than during an average practice. During a single match, players expended an average of over 500 calories (~8.0 kcal/kg), but over 1000 calories (~15.0 kcal/kg) within a match session (match+). The increased load observed for match session is likely a result of players performing both a standardized full team warm-up plus a pairs-only warm-up prior to the start of a match. In comparison to other collegiate athletes, NCAA DI women's soccer players typically have EEE_{REL} and TL of 15.36 kcal/kg and 243-points per 90-minute soccer match and 11.39 kcal/kg and 128-points per practice (14). In this study, matches lasted an average of 46.4 minutes corresponding to a 113-point TL and EEE_{REL} ~8.0 kcal/kg. Thus, when doubled to account for two matches played per day, beach volleyball players performed within-match workloads comparable to two 90-minute soccer games over a weekend.

These large total and relative energy expenditures and training loads during a collegiate beach volleyball match/match session emphasize the game demands and fueling needs in these athletes. Particularly over a competition weekend, where players compete in four matches over two days, this equates to over 4000 calories expended and an accumulated TL of over 700 within a 48-hour period. Thus, these athletes have considerable re-fueling needs within and between matches. Moreover, beach volleyball match duration is variable, as matches are played as best two out of three sets. Players could potentially experience even higher workloads if matches over a weekend were consistently played to three-sets. The rules and regulations of NCAA beach volleyball stipulate that substitutions are not allowed during match-play. Coaches and training staff must prioritize player nutrition and load management in order to optimize game performance. This could include strategies such as shortening pre-match warm-up duration in order to decrease overall load of competition, as match+ observed in this study were close to double the workload of a match itself.

Higher TL and EEE, and subsequent athlete fueling needs are also illustrated in practices. These findings echo previous research examining the impact of training surface on exercise intensity and energy cost (1). Higher energy cost of exercise as well as greater HR and blood lactate responses during exercise have been observed in training on sand compared to firm surfaces (1). Exercise on the sand also provides a greater recruitment of lower leg musculature (18), which may also contribute to the performance characteristics observed in the athletes in this study. Due to the impact of sand on exercise intensity and energy expenditure, manipulation of training duration may be particularly useful for coaches and training staff for the management of training loads, especially at the onset of in-season competition. Additionally, as a result of the increased energy cost of exercise in sand, beach volleyball players in particular may be at a greater risk for inadequate fueling to meet the energy demands of the sport. Proper nutritional intake surrounding (and during) both practices and matches becomes paramount to meet the EEE observed in this study. Further research evaluating energy availability in beach volleyball players, especially in female athletes, is warranted in order to understand optimal energy intakes and nutrient timing around matches in these athletes.

Additionally, examining the effects of the high EEEs in beach volleyball on body composition variables across the competitive season may provide further emphasis for and insight into the fueling needs of these athletes.

Evaluation of distances covered in various speed zones in practices and matches displayed the speed dynamics of beach volleyball in sand. Athletes covered very minimal distance (<2 m) in speed zone 5 in both practices and matches. This may indicate that the top speed zone used ≥18 km/h, although based off previous literature in beach soccer to account for sand surfaces, may not be optimal for quantification and thus, may be underestimating high velocity work in beach volleyball due to differences in sport dynamics. Although beach volleyball and beach soccer both are intermittent in nature, beach volleyball is characterized by repeated bursts of volleyball-skill movements combined with frequent explosive jumps and rapid decelerations (17). It is likely that beach volleyball play may not cover distances large enough to allow for athletes to reach their peak velocity capabilities. Additionally, these speed zones were based on male athletes. When comparing between sexes, known differences exist in physiological capacities, with males exhibiting superior speed and power output capabilities (21). DIS_{Z4} was similar between practices and matches, indicating that the upper end of this speed zone may be more indicative of high velocity work performed by these athletes. Between practices and match+ no large differences were observed between velocity threshold except DIS_{Z4} and DIS_{Z1}. Pre-match warm-ups were designed by the coaching staff to mimic the speed demands of a match, thus supporting the increased DIS_{Z4} observed. Further research is warranted to determine appropriate velocity thresholds for the purposes of monitoring beach volleyball athletes of various playing levels as well as between sexes.

Acceleration and deceleration data indicate the overall capacity of beach volleyball athletes to perform frequent velocity changes. Although a greater number of sprints were performed during practices compared to matches, overall a relatively low number of sprints were performed by athletes in practice and competition. In order to evaluate and monitor high-intensity external workloads, the sprint threshold of 2.8 m/s^2, which has been previously used in female collegiate athletes (14), may be inappropriate for sports on sand surfaces. Previous research in professional male soccer players displayed decreased maximal and average acceleration and speed values when shuttle runs and sprint tests were performed in sand (8). Instability of sand has been shown to reduce maximal force production in elite beach volleyball players (9) and thus may be a contributing factor in the acceleration and deceleration data observed in this study. Thus, further research investigating velocity and acceleration/deceleration thresholds and capabilities in male and female beach volleyball players is needed. Overall, this study highlights the physiological and physical demands of NCAA DI women's beach volleyball and underscores the high training loads and fueling needs of these athletes, particularly at the onset of competition. It also points to the need to assess workloads between athletes participating in competition and those in reserve, as evaluated together may mask major discrepancies in workloads, that unacknowledged may have impacts on athlete health and performance.

PRACTICAL APPLICATION

The high internal and external training loads observed in NCAA DI women's beach volleyball warrant the use of athlete-monitoring methods coupled with systematic performance testing in order to accurately assess and manage training loads and subsequent fueling needs of these athletes. Differences in TL and EEE observed between travel and non-travel squad players indicate the need for coaches and training staff to implement strategies designed to minimize large acute fluctuations in workloads between groups. Specifically, shortened pre-match warm-ups and periodized practice loads in travel players may be effective to manage training loads during competition weeks; whereas incorporation of greater high intensity drills and training for non-travel players may mitigate large reductions in workloads in-season. Additionally, the continued emphasis on developing explosive power by strength and conditioning coaches may be advantageous for sport success in female beach volleyball athletes.

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