“EVALUATING THE EFFECTS OF TRAINING LOAD, BIOMARKERS, SLEEP, AND MOOD STATUS ON PERFORMANCE OUTCOMES IN FEMALE COLLEGIATE SOCCER ATHLETES”

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ABSTRACT OF THE DISSERTATION

“EVALUATING THE EFFECTS OF TRAINING LOAD, BIOMARKERS, SLEEP, AND MOOD STATUS ON PERFORMANCE OUTCOMES IN FEMALE COLLEGIATE SOCCER ATHLETES”

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Soccer is a physically demanding sport requiring frequent bouts of high intensity activity coupled with high caloric expenditures and an average distance covered of 10 km. Collegiate soccer is further characterized by a short intense preseason, combined with a season of congested match fixtures, frequent travel, and academic requirements often with minimal recovery allotted. Therefore, methods designed to track recovery needs of the athletes and promote optimal performance are essential to a successful sports science program. Further, the effects of high training loads without adequate recovery has been shown to have negative implications on hormonal changes throughout the season. Female athletes in particular, are at an increased risk for health-related issues unique to their sex that may greatly impact sport performance and general well-being. The purpose of this dissertation is to provide a profile of the typical season demands placed on female collegiate athletes. This dissertation sought to evaluate the effect of training load, biomarkers, mood states, and sleep on performance outcomes and body composition in a Division I female collegiate soccer team.

AIMS AND OBJECTIVES

Aim 1: The physiological demands of a competitive soccer season are often thought to vary between males and females, yet these workload outputs as a function of sex have rarely been assessed. Research comparing the total competitive season demands on male and female soccer players is warranted to determine potential differences in recovery needs and practice strategies. The purpose of this study is to compare the internal and external training loads in males and females throughout an entire Division I soccer season during both practices and games. Players were evaluated during all practices and regulation game play using the Polar TeamPro system that utilizes heart rate (HR), global positioning satellites (GPS), and accelerometry technology. Differences in training load, HR, distance, calories expended per kilogram body weight, and speed were evaluated in males and females. This data may provide information regarding sex differences in internal and external loads during games and practices to determine differences in overall workload. Further, data may provide sex specific recommendations for recovery and training of collegiate soccer players.

Aim 2: Adequate recovery from stressors is an essential aspect of an athletes training program. When the balance between appropriate training stress and adequate recovery is disrupted, an abnormal training response may arise resulting in decrements in performance. The purpose of this observational study was to monitor the physiological and psychological profile of Division I female collegiate soccer players throughout the course of an entire season to determine the effects of training load as well as detect various markers of fatigue. Cumulative workload was assessed at all practices and games using the Polar TeamPro system that evaluates total distance covered and calories expended for each player. Biomarkers, mood profiles, and sleep quality and duration were evaluated every 28 days in conjunction with vertical jump performance in high level female collegiate athletes. This research can provide insight into the total demands of a competitive season on the female athlete. Further, this data can provide information on monitoring techniques that can detect fatigue and inadequate recovery.

Aim 3: In-season, increased soccer-specific demands and insufficient recovery may result in a loss of strength and fat free mass. The use of biomarker monitoring is an emerging method to track an individual players physiological response to overall stress as well as identify the balance between training and recovery. The purpose of this study was to identify the relationship between biomarker changes and changes in performance and body composition variables in female Division I college soccer players participating in a periodized strength and conditioning program over the course of a competitive season. Prior to the start of preseason and within one week of the final match, players participated in maximal performance testing for strength, power, and aerobic capacity. Additionally, body composition testing and biomarker monitoring occurred periodically throughout the season to determine the correlation between biomarkers, body composition, and performance outcomes. This research may provide information regarding the association of biomarkers with performance measures. Biomarker monitoring may be a useful tool to detect the impact of workload related stressors, particularly if related to performance outcomes.

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CHAPTER IV

1. Introduction: The Demands of Soccer

Soccer is a physically demanding power-endurance sport which involves frequent bouts of high intensity activity, including sprinting and high-speed running, coupled with low-intensity periods of walking and jogging (3). Soccer involves explosive bouts of activity, such as jumping, kicking, tackling, turning, sprinting, changing pace, and sustaining forceful contractions all while maintaining balance and control of the ball against an opponent (67). A 90-minute soccer match is further characterized by a large caloric expenditure estimated to be around 20.3 - 23.6 kcal/kg body weight and an average distance covered of 10 km at an intensity close to anaerobic threshold (80-90% HR_{max}) (67). Sprinting occurs approximately every 90 seconds and constitutes 1-11% of the total distance covered (48, 67), with players performing around 10-20 sprints during a game (67). In addition, high intensity running occurs approximately every 70 seconds in a game (67).

The physiological demands of soccer therefore require athletes to possess high levels of aerobic capacity, as well as muscular strength, power, speed, and speed-endurance (34, 67). An athlete’s success may depend on the ability to sprint to the ball, evade opponents and physically dominate on the ground as well as in the air through tackles, headers, passing, and change of direction. Therefore, determining an athlete’s physical capacity as well as maintaining and improving these metrics are imperative to success. Additionally, body composition, biomarkers, and psychological health are important measures for determining athlete’s training status and readiness to perform (73).

Throughout the season, high training volumes combined with minimal recovery, present a challenge to the maintenance of these performance metrics. Athlete monitoring techniques that track training stressors, including physiological and psychological metrics, in conjunction with performance status are necessary to detect maladaptive responses to training.

2. Collegiate Level Soccer Players

Soccer players experience high training volumes compounded by an intense season consisting of multiple games per week and often times with minimal recovery allotted. Collegiate soccer players, in particular, are further burdened with stressors that include a short preseason combined with a season of congested match fixtures, frequent travel, and academic requirements that can exacerbate their recovery requirements. One distinguishing characteristic that differentiates collegiate level soccer programs in the United States is they are governed by the National Collegiate Athletic Association (NCAA) which places restrictions on when coaches can train with their athletes. As a result, training programs designed for NCAA sports are presented with unique challenges in their efforts to maintain performance metrics throughout the year. *Figure 1* depicts time frames of a typical collegiate preseason, regular season, post season and off-season training cycle as compared to schedules consistent with the European Professional League (EPL) model. The restrictions on coaches-led training schedules are particularly relevant for athletes participating in fall sports, such as soccer, whereby athletes returning from summer break are only allotted two weeks to train with the coaching staff prior to the start of the season. These restrictions shape the nature of the preseason as it is a very short, intense, two-week period which utilizes multiple practices per day in conjunction with the pressure of competition amongst teammates for a starting role, academic requirements, and administrative meetings. These additional stressors all combine to compromise optimal recovery.

Appropriate monitoring techniques during this time period becomes imperative to maintaining the well-being of the collegiate athletes.

Figure 1: Collegiate Soccer Model vs European Professional League Model

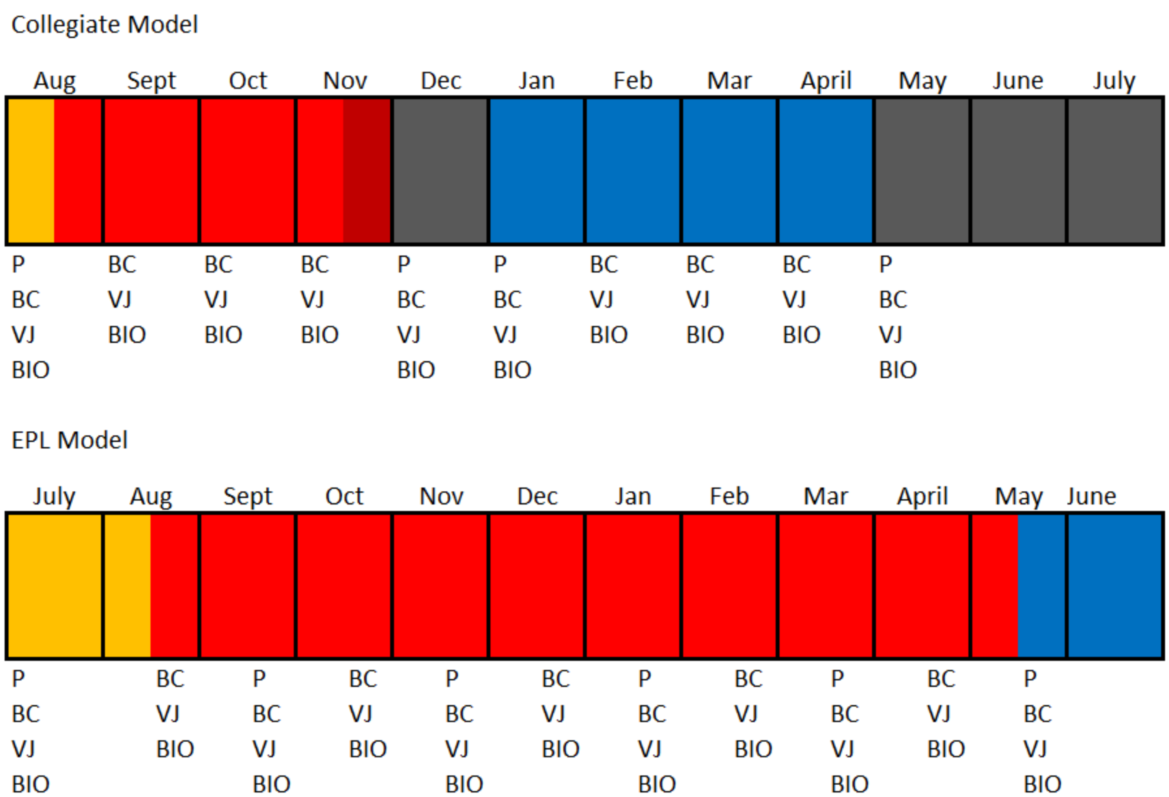

Figure 1: Shows example testing time frame for a collegiate and professional model of the course of a full year. =Preseason, =Regular Season, =Post Season, =Off-Season Training, =Unsupervised Training. P= PerformanceTesting (Aerobic, Strength, Speed, Agility), BC= Body Compostion, VJ= Vertical Jump, BIO= Biomarkers.

Walker et al., IN PRESS (73)

3. Determining Athlete Capabilities: Performance Testing

A. *Aerobic Capacity:*

A 90-minute soccer match is largely dependent on aerobic metabolism. Aerobic capacity is often represented by maximal oxygen uptake (VO_{2max}), which is defined as the maximal rate of oxygen consumption during exercise often expressed relative to body weight

($ml \cdot kg \cdot min^{-1}$) (73). The gold standard of measuring aerobic performance and VO_{2max} is through laboratory-based indirect calorimetry via direct gas exchange using a graded exercise test (GXT). This test uses a treadmill or cycle ergometer to increase speed, incline/resistance, or both as each stage progresses. Choosing a mode of exercise that is sport specific is preferential for testing. For soccer athletes, testing on a treadmill using a speed-based protocol rather than incline based is beneficial due to similarities in how the athletes train. There are also various field tests available and commonly implemented to estimate VO_{2max} in soccer athletes. Field tests do not directly measure VO_{2max} however they are commonly used tools to estimate aerobic capacity of the players. Field tests are less time consuming due to the ability to run groups of players simultaneously. Additionally, they require minimal equipment and tester skill, which greatly reduces the cost burden of performing a laboratory based GXT. However, an inability to objectively determine maximal capacity as well as motivation and tester judgement may limit the accuracy of determining VO_{2max} (73). Typical VO_{2max} values for national and professional male soccer players range between 55-65 $mL \cdot kg \cdot min^{-1}$, while a VO_{2max} for females range from 45-55 $mL \cdot kg \cdot min^{-1}$ (67).

VO_{2max} testing also allows for the determination of ventilatory threshold. Ventilatory threshold (VT) is the point at which ventilation no longer tightly couples to oxygen consumption and reflects a transition from an exercise intensity predominately fueled by aerobic energy systems to an intensity that requires greater anaerobic contributions (67, 73). Increases in VT can increase an athlete’s ability to sustain work at a greater percentage of maximum before transitioning into a more anaerobic state and this represents a greater ability to delay fatigue (73). Average VT for elite male soccer players has been found to occur at around 77% of VO_{2max} (67). Research has indicated VT to be more trainable than VO_{2max}, as VT has been shown to increase without the improvement of VO_{2max} (2, 10, 73)

In addition to VT, accurate HR_{max} values can be obtained through maximal aerobic capacity tests which can be used to quantify training loads as well as exercise energy expenditure. Measuring VO_2 during a match is difficult due to the necessary laboratory equipment required. Establishing the relationship between HR and VO_2 allows accurate indirect measurements of VO_2 during soccer matches (67). Mean and peak HRs during a soccer match have been reported to reach around 85-90% of maximal values (35). Typical heart rate measurements during a game suggest average oxygen uptake is around 70% of $VO_{2max.}$ (3). Further, establishing the HR-VO_2 relationship may be used to determine caloric expenditure (as 1 liter of O_2 consumed per min corresponds to 5 kcal expended) (11, 67). During a typical match for a player weighing 75kg, estimates for exercise energy expenditure range from 1519-1772 kcal (67).

B. *Strength and Power:*

In addition to aerobic capacity, anaerobic energy systems play a decisive role in the physiological demands of soccer. Anaerobic capacity is defined as the amount of work that can be performed from energy stored in the absence of oxygen (12) and involves the ATP-PCR system as well as anaerobic glycolysis. In a soccer match, anaerobic capacity translates to the ability to repeat power efforts (73). Soccer athletes are required to perform power efforts such as sprints, jumps, tackles and one-on-one play against an opponent. Anaerobic capacity may be a deciding factor in determining the players who can sprint and jump the fastest (67). Therefore, strength and power, which reflect anaerobic capacity, are important components of soccer success and athlete readiness. Strength is the force that a muscle group can maximally exert while maintaining proper form (44). Maximal strength is defined as the highest force that can be produced by the neuromuscular system during one maximum voluntary contraction (one repetition maximum (1RM) (67). Power is the result of a combination of strength and speed and refers to the ability of the neuromuscular system to produce the greatest force in the shortest amount of time (67). Maximal strength directly relates to power production, and therefore strength training should be applied to maximize power production and sprint speed essential for soccer success (73). In addition, increasing strength may serve to increase force production and aid in joint stability (73). Although no standardized protocols for testing strength of soccer players exists (67), testing the strength of soccer athletes are necessary to determine player readiness as well as identify areas that need improvement (73). Typically, repetition maximum (RM) testing in the 3-5 repetition range is advantageous to determining strength and may be more relevant to the loads applied during training. RM testing on the core lifts: bench press, squat, and deadlift are appropriate for comprehensive evaluations of maximal strength in this population (73). Data collected from strength testing, can be used to design periodized strength and conditioning programs which have the ability to enhance strength and improve soccer performance. In addition to testing for strength, periodic power testing is necessary to determine maximal power production. One simple method to determine power is through vertical jump (VJ). VJ is a useful performance measure because it is indicative of lower limb muscular power and has been shown to relate to maximal running speed which is an important metric of soccer performance (77). Further, VJ can be performed throughout the season as it is simple to administer and results in minimal amount of fatigue incurred by the athlete (24, 71). Periodic testing of VJ may be a useful monitoring tool to detect fatigue (19). One common method to test VJ is through counter movement vertical jump (CMJ) performed on a contact mat. CMJ requires the athlete to quickly move down into a semi squat position before takeoff to utilize the stretch shortening cycle (30). For elite male soccer players, average CMJ range from 47-71 cm while females range from 40-59 cm (21).

C. *Body Composition:*

Body composition is an important component of an athlete's fitness profile and is often described using the two-component model which divides the body into fat mass (FM) and fat free mass (FFM). Body fat percentage (BF%) is commonly expressed as general means to communicate testing results and is calculated as FM divided by total body mass. Systematic and periodic assessments of body composition provide valuable information related to whether an athlete is gaining or losing muscle or fat, which can help clarify physiological responses to training as well as determine energy needs and energy balance (58, 64). This becomes important during the competitive season when increased soccer-specific demands coupled with insufficient recovery may result in a loss of FFM. Further, the relationship between body composition and physical performance tests have been established in various studies. Silvestre et al found significant correlations between body composition variables and physical performance between total body tissue and vertical jump, speed, power, and cardiorespiratory capacity. Total fat positively correlated with increased speed in the 40-yard sprint (r= 0.60) and negatively correlated with cardiorespiratory capacity (r= -0.65). Percent fat negatively correlated with vertical jump (r = -0.55) and cardiorespiratory capacity (r = -0.65) and positively correlated with values for speed in male collegiate soccer players. Soccer is also a sport where very large values for FFM are not particularly conducive for performance either, as shown by a moderate negative correlation between VJ and FFM. (64). Currently, the laboratory-based gold standard methods of measuring body composition include dual x-ray absorptiometry (DEXA), air-displacement plethysmography (i.e., BOD POD), and hydrostatic (or underwater) weighing (29). Additional field-based methods of measuring body composition include skinfold thickness (SKF), bioelectrical impedance analysis (BIA), and other anthropometric measures, such as girth measurements. Equipment for these tests are readily accessible, portable, and relatively inexpensive, but these methods typically have a greater degree of error when compared to the laboratory-based methods (29). Although "normative" values have not been established for the soccer, research has suggested that an average BF% for elite male soccer players is around 10-11%, while that of collegiate female soccer players is approximately 22% (47, 58).

Periodic testing of the athletes' capabilities is necessary to gauge performance relative to normative data, track change and progress over time, and to develop appropriate training protocols to address needs of individual athletes. The decision of what type of performance test to use and how often to test these performance metrics may depend on the time commitment of the test, the physical burden placed on the athlete, the monetary cost of the equipment necessary to run the test, tester expertise, and accuracy of the test (73). A timeframe of a typical collegiate testing schedule is presented in *Figure 1.*

4. Principles of Training

There are four basic principles to consider when training for the competitive soccer season. These "principles of training" include reversibility, specificity, individualization, and overload (23). Reversibility applies to a state of detraining that occurs when a stimulus is removed. Specificity refers to applying specific protocols that resemble the sport in which an athlete trains to compete. This dissertation will mainly focus on the latter two principles of overload and individualization. Individualization states that every athlete is unique. To improve performance, training load should be individualized to each athlete. The intensity and duration of training that is suitable for one athlete may not apply to another athlete even within the same sport. This approach should be further undertaken when monitoring an athlete’s response to training particularly when competing in a team sport setting. In team sports, such as soccer, where training is done in a group setting, this presents a challenge to determining appropriate levels of training which can often lead to an inadequate training stimulus or more often than not, excessive training leading to fatigue or injury (1).

The principle of overload states that a stimulus should be challenging above a certain threshold in order for adaptations to occur (23). In order to improve performance, athletes must utilize the principle of overload to modify their training load. This can occur by making adjustments to the frequency, duration, and intensity of training at various times throughout a training cycle to either increase or decrease fatigue (24). This is the basis behind periodization models. Supercompensation refers to a state in which overload can initially lead to decrements in performance due to fatigue, but with adequate rest and recovery, adaptation to the training load can occur leading to improved performance. Overload training with adequate recovery periods presents an environment in which the athlete can achieve a higher level of performance. However, if overload training is not met with sufficient time to recover, a state of overtraining may occur (45).

5. Non-Functional Over-Reaching and Overtraining

When the balance between appropriate training stress and adequate recovery is disrupted, an abnormal training response may arise (28). Overtraining syndrome (OTS) is the term given to a state of excessive training stress and inadequate recovery leading to performance decrements. OTS lies on a spectrum of underperformance conditions which includes functional overreaching (FOR), non-functional overreaching (NFOR) and OTS (36, 45). FOR is an essential component of training and reflects the overload principle (57). FOR is often applied during a well periodized training cycle with the idea to continuously and progressively overload the athlete causing a short-term decrement in performance which can result in a supercompensation effect (i.e. improved performance) if sufficient recovery time is allowed (9). However, if recovery is not allotted, NFOR can occur leading to worsened performance lasting weeks or months (9). If not fully corrected, NFOR can develop into OTS resulting in long-term decrements in performance capacity accompanied by adverse psychological symptoms (45). The amount of time needed for restoring performance differentiates OTS from NFOR as it may take weeks or months to recover from NFOR while OTS may take months or years to fully recover (57).

When discussing OTS, researchers often refer back to the teachings of Hans Selye and the theory on General Adaptation Syndrome (GAS) which refers to the stress response and subsequent adaptations that occur in the body (63). Selye discusses the concept of eustress and distress and the impact these stressors can have on the body's maintenance of homeostasis. GAS has three distinct phases: Alarm, Resistance, and Exhaustion. Stress is considered a threat to homeostasis and causes disharmony. When presented with an exercise stressor, the body initially goes through an alarm phase resulting in an initial decrease in performance. If the body successfully responds and adapts (resistance phase), improvements in performance may occur. The stage of exhaustion may occur if the body is under too high of chronic training stress without recovery. In the exhaustion stage, resistance to the stressor may decline leading to maladaptation and performance decrements.

Signs and symptoms of NFOR are often varied but may include pronounced fatigue, insomnia, change in appetite, irritability, restlessness, and downturns in performance (45). Often, an athlete experiencing OR will report negative mood states and physiological markers that may precede the athlete's decrements in performance (45). Reliable markers of detecting OTS should be objective and sensitive to training load, unaffected by diet, change prior to the diagnosis of OTS, change in response to acute exercise, be distinguishable from chronic changes, and be easy to measure and determined on the basis of sound theoretical framework (45). However, the hallmark sign when diagnosing NFOR and OTS is chronic performance decrements (45).

6. Athlete Monitoring

Athlete monitoring throughout the season has emerged as a tool to prevent declines in performance stemming from inappropriate training load and inadequate recovery. Further, monitoring athlete training load is important to determine whether an athlete is adapting to their training program, to assess fatigue and recovery needs, and to avoid NFOR (6, 24). In order to prevent declines in performance stemming from inappropriate training load and insufficient recovery, sport programs and support staff have taken an increasingly scientific approach to monitoring training load.

Training load can be divided into two main categories: internal load and external load. Internal workload is considered the physiological or psychological response of the athlete to training while external workload is seen as the work completed by the athlete (24, 75). Training load is often determined by the duration and intensity of a training session. Yet the way an athlete responds to this training load varies due to individual differences (8). Therefore, quantifying an individual's response to training load is necessary for coaches and sports scientists to monitor their players and adapt their training protocols to fit individual player's needs (1).

Physiological, biochemical and subjective measures as well as performance outcomes are viable methods for athlete monitoring (62). When choosing a monitoring technique, it is important to consider the efficacy of that method to distinguish between sufficient and insufficient recovery. Useful methods to determine athlete readiness must first have a valid and reliable ability to detect maladaptive changes that occur in response to overall stress. Secondly, based on the results obtained from athlete monitoring, sports scientists must be able to provide accurate and objective feedback to the coaches and athletes. It is the role of the sports scientist to provide information to the coaches as to when training becomes maladaptive in order to better facilitate and enhance performance outcomes (8). Finally, in order for a monitoring technique to be feasible at preventing declines in performance, when maladaptation to training become apparent, sports scientists and coaches must have the ability to implement change to correct that marker. This may occur in the form of adjustments to training load or increased time for recovery. A combination of monitoring techniques may be useful in combating excess fatigue and insufficient recovery from the accumulated stress of the season.

7. Monitoring Techniques

A. *Performance Testing:*

Determining the physical capabilities of the athletes allow coaches and sports scientists to select training strategies to identify strengths and improve areas of weakness. Further, it provides a basis to measure if the athletes are progressing with their training programing or to identify when performance declines. Additionally, appropriate testing will provide the ability to input individualized values into monitoring systems to allow more accurate programing. Obtaining accurate HR_{max}, VT, VO_{2max} and body composition values from performance tests can be used to program monitoring systems to track player training load on an individualized basis. This allows quantification of training load to be more specific to the individual rather than based on general values (74).

Periodic maximal performance tests such as VO_{2max} tests for aerobic capacity, vertical jump for power, and repetition maximum tests for strength, are important to determine the individual athletes' capabilities as well as to monitor the effectiveness of a training program. While maximal performance tests are optimal to determine the player's development, the added fatigue incurred by the athlete makes these tests difficult to implement during the season. Further some performance tests lack sensitivity to detect physiological deterioration as an athlete may find a way to "grit through" a maximal test (73). Conversely, athletes may lack the motivation to achieve a maximal effort during tests not associated with competition (24). Therefore, it is important to incorporate other monitoring techniques rather than simply relying on decrements in performance to be seen during these maximal tests.

B. Heart Rate Monitoring:

One method of determining the internal load of the athlete is through technologies that measure HR. The utility of HR monitoring during exercise is based on the linear relationship between HR and the rate of oxygen consumption during steady state exercise (24, 31). HR monitoring can be used to measure exercise intensity (32). Reduced HR during intensified training has been shown in overreached athletes (36) . However, a meta-analytic review assessed the capability of HR monitoring to assess overreaching and overtraining and concluded accurate interpretation of HR data should be used in combination with other measures to provide meaningful information (5).

C. *GPS and Accelerometry:*

The physical demands of soccer, also known as external load, can be tracked using global positioning satellite (GPS) systems that determine distance covered as well as the speed at which the athletes travel (13). The incorporation of accelerometry allows these systems to quantify accelerations and decelerations as well as number of sprints performed, adding to the information that can be gathered to assess external load. Monitoring systems that combine GPS, accelerometry, HR technology provide the ability to track both an athletes on-field internal and external workload throughout a competitive season (24). Dissociation between external and internal load units may reveal a state of excessive fatigue (24).

D. *Biomarkers:*

A biomarker is defined as a "measurable product or a substance of an organism that is used as an indicator of biological state to objectively measure physiological or pathogenic processes in the body" (17, 55). While workload tracking systems that measure HR and GPS metrics can detect how an athlete responds to on-field training, the use of biomarker monitoring is an emerging method to track an individual player's physiological response to *overall* stress (including both on-field stressors and cumulative stress of everyday life) as well as to identify the balance between training and recovery (38). Periodic biomarker assessments during the competitive soccer season offers an accurate method for evaluating training related stress and recovery needs (4). Biomarker monitoring can provide insight into the physiological changes that athletes experience during the season (74). Currently, no gold standard panel for biomarker testing exists to determine fatigue status, however monitoring markers indicative of anabolic and catabolic response may prove beneficial to evaluating athletes' readiness to perform. In addition, measuring fluctuations in general health and nutritional markers may provide an opportunity to intervene before decrements in performance are apparent. Biomarkers can further serve as a guide to strength and conditioning coaches regarding periodization of strength training programs to optimize performance.

i. *Cortisol:* One important biomarker commonly seen with regards to training status is cortisol. Cortisol is an important hormone released in response to stress. Fluctuations in resting cortisol can be seen due to a disruption in hypothalamic pituitary adrenal (HPA) axis (45) leading to an increased catabolic environment making it difficult to build or maintain muscle or recover from training (38). Increases in training load and inadequate recovery may result in a rise in resting cortisol (74). Elevated resting cortisol can indicate an impaired capacity for recovery as well as an impaired capacity for protein synthesis (38, 72). If this progresses to OTS, the stress response can become desensitized which may result in a decreased cortisol response (45).

ii. *Sex hormones:* The sex hormones are important makers that provide information on HPA and hypothalamic pituitary gonadal (HPG) axis dysregulation. Testosterone is a hormone important for reducing protein breakdown and promoting protein synthesis (38). Testosterone is used as a biomarker often in conjunction with cortisol to provide a relative indication of an anabolic to catabolic state, particularly in male athletes (34, 38). Fluctuations in testosterone may imply greater HPA/HPG axis dysregulation (20, 38). Declines in testosterone may cause an increased catabolic state resulting in decreased protein synthesis (38). Estrogen, and the derivative estradiol, is an important reproductive hormone in the females with varying effects on performance as well as female athlete health. Estrogen enhances glycogen uptake in the liver and muscle (22, 37, 54) and may improve endurance performance through enhancing lipid oxidation and utilization as well as gluconeogenesis (37). Increased training loads without adequate recovery can cause the suppression of estrogen leading to the disruption of the menstrual cycle or the development oligomenorrhea and amenorrhea

(52). Estrogen is also affected by increased exercise energy expenditure and inadequate energy intake. Low energy availability is the term used to categorize insufficient energy intake and associates energy intake with the caloric expenditure that is needed for all body functions to produce optimal health (51). If caloric intake is inadequate, low energy availability may result in a suppression of estrogen leading to a host of maladaptive functions such as declines in bone health and dysregulation of menstrual function (51, 52). Special consideration is warranted for testing in females as estrogen becomes difficult to measure when an athlete uses contraceptives, as these may mask hormonal levels depending on the composition of the contraceptive. Sex hormone binding globulin (SHBG) is another sex hormone which may be particularly relevant in the female athlete. SHBG modulates the transport of various sex hormones and has been shown to increase in both males and females with regards to exercise (38). SHBG may provide a protective effect against the breakdown of both estradiol and testosterone (38). Fluctuations in SHBG has been shown in overreaching research and may indicate insufficient recovery or an inability to adapt to training (38, 69, 72). Additionally, the pituitary hormone, prolactin, has been shown in prior research to increase in response to stress, hypoglycemia and exercise (68). Prolactin serves many roles and is particularly relevant in the development of mammary glands and initiation of lactation (14). High levels of prolactin secreted by the pituitary, can result in decreased secretion of luteinizing hormone and follicle stimulating hormone which may results in subsequent suppression of estrogen leading to amenorrhea (76).

iii. *Growth Hormone (GH) and Insulin-like Growth Factor (IGF-1):* Additional biomarkers indicative of anabolism include GH and IGF-1, both of which are involved in muscle protein synthesis and the regulation of muscle mass (38). GH increases levels of IGF-1, an important mediator of anabolic response. GH has been shown to correlate to exercise volume and intensity (38). Previous research evaluating acute GH responses to exercise have found blunted effects in NFOR and OR compared to healthy subjects (9). In addition, IGF-1 has been associated with bone quality as reduced concentrations of IGF-1 has been found to relate to fracture risk in women (33, 38).

iv. *Creatine Kinase (CK)* is an important biomarker indicative of muscle damage (38). Increases in CK are caused by tears in the muscle membrane of a damaged muscle fiber that result in CK leaks into plasma (50). With regards to OTS, there are mixed conclusions on the utility of CK as an adequate marker to assess NFOR or OTS. Despite the mixed literature, CK is often used as a supporting marker in OTS evaluation (45). CK is unique as a biomarker as it is one of the few markers that has athlete specific values to represent the expected amount of muscle damage experienced with the sport (male athletes: 82-1083 U/L, female athletes: 47-513 U/L) (57). Chronically elevated CK in response to fluctuating training volumes can indicate insufficient recovery. CK may be a particularly relevant marker in soccer players as game movements and the stop and go characteristics inherent to the sport may impose high eccentric, biomechanical strain on skeletal muscle leading to microtraumas and the resulting release of CK into plasma (46). Markers such as CK can also provide insight into more strenuous and damaging times of the year (74) *v.* *Interleukin-6 (IL-6)* is a biomarker often associated with catabolism and fatigue (16). IL-6 belongs to group of cytokines that regulates the body's inflammatory response (17). It has been shown to increase in response to decreased muscle glycogen, muscle contraction, and muscle damage in order to activate an immune response (56). Cytokines have also been implicated in the production and enhancement of negative mood including fatigue (39, 42). Increases in circulating IL-6 have been found to be associated with depressed moods, sleep disturbances and fatigue. IL-6- is affected by chronic glycogen depletion and immune system dysfunction (78) often seen in fatigued soccer athletes.

vi. *Nutritional Markers:*

a. *Iron* is an essential mineral required for aerobic metabolism and endurance performance and may serve as an important biomarker in female athletes as suboptimal iron stores are associated with declines in exercise capacity (38). Iron deficiency can impair muscle function, and limit work capacity while iron deficiency can result from periods of rapid growth, training at high altitudes, menstrual blood loss, foot strike hemolysis, and injury (70). The RDA recommends > 18 mg for females >8 mg for males, making females more susceptible to deficiencies (70). Monitoring relative changes in iron stores throughout the season may provide an opportunity to intervene before deficiencies can occur. Reversing iron deficiency can require 3-6 months and therefore it is essential to begin nutrition interventions before iron deficiency develops (70). Monitoring the iron status of the athletes throughout the season is important to determine an early course of action if downturns in iron become apparent.

b. *Amino Acids:* Monitoring deficiencies in amino acids and other nutritional markers may be warranted to prevent declines in performance stemming from inadequate nutrition. One important amino acid in athletes is glutamine. Glutamine has been extensively studied in association with overreaching for its role in immune system function (53). During high intensity and prolonged exercise, glutamine values increase followed by significant decreases during the post-exercise recovery period (59). Decreases in glutamine may lead to an impaired immune system response and may be responsible for increased rates of infection, specifically upper respiratory tract infections, associated with OTS (53). Decreased concentrations of glutamine have also been reported during prolonged exercise and heavy training (27, 57).

Another important nutritional marker is the amino acid tryptophan, which is a precursor of serotonin. Tryptophan may provide a mechanism for understanding changes in mood typically reported with overreaching (65). Increased brain levels of serotonin are believed to result in mood and behavioral changes such as inducing sleep and reducing appetite, both behaviors evident in OTS (65). Increased tryptophan during increased training may be indicative of overreaching and associated with mood disturbances.

Further, deficiencies in other amino acids and micronutrients may play a role in evaluating the overall health and nutrition status of the athletes. For example, phenylalanine and taurine are important micronutrients involved in muscle quality (38). Deficiencies in these micronutrients may indicate a need for supplementation or improved nutrition strategies.

Although no single biomarker has been shown to effectively diagnose inadequate recovery, the use of multiple biomarkers may provide an objective snapshot of an athlete's general health, nutrition, performance and recovery status (74). However, it is important to consider that increases or declines in single biomarkers often do not occur independently from one another as seen with various convergent hormonal pathways (66). For example, disruptions in the HPA/HPG axis will result in a cascade of hormonal responses that may result in a disruption to homeostasis. Therefore, rather than looking at one biomarker to analyze the effects of training load on an athlete, it may be more advantageous to analyze multiple anabolic and catabolic biomarkers together when assessing adaptations to a training program.

Additionally, biomarker monitoring may be a useful tool in team sports to determine an individuals' differential response to the same team training stimulus. Biomarkers may be helpful in determining why some athletes do not adapt to their training in similar ways in order to take on a more individual approach to training and recovery. The use of biomarkers provides an objective means to evaluate the physiological response of the athlete to training as they may better indicate exercise related stress that is independent of other factors, such as motivation. When using biomarkers in team sports such as soccer, it is important to choose a standardized time course of assessments to allow accurate and precise measurements so that physiological alterations can be identified and addressed (38). Testing should be frequent enough so that results can be seen without being too invasive to the athlete. The associated costs and time-consuming nature of biomarker analysis need to be considered when determining a testing schedule.

E. *Perceptual Information:*

Determining how an athlete responds to the stress of the season using subjective measure of well-being is an important means to monitor internal training load. Changes in mood profiles have been established as an indicator of training distress and may be linked to decrements in performance (18). Increases in training load are associated with increases in mood disturbances (43). The classic study by Morgan et al monitoring mood states in competitive swimmers showed mood disturbances increase during times of high training volumes in a dose-response manner (49). When evaluating ideal psychological profiles of athletes, Morgan et al proposed what he calls the “iceberg principle” in which successful athletes exhibit below average scores for depression, fatigue, and tension in contrast to above average scores for vigor. An athlete experiencing negative adaptations to training may have an “inverse iceberg profile” (40, 49) in which vigor drops below average with a concomitant increase in fatigue. Monitoring the athletes mental and physical well-being through self-report measures may help to better understand an athletes readiness to perform and prevent changes in performance stemming from fatigue (41)

Questionnaires and surveys designed to assess mood disturbances have the ability to provide subjective information as to how the athlete is adapting to the imposed training load (24). Further these methods are efficient, inexpensive, non-invasive and easy to administer to monitor athletes progress throughout training (41). There are various valid and reliable questionnaires available to sports scientists and coaches to examine training distress through self-report. These include measuring mood fluctuations connected to training volume, utilizing a behavioral symptoms checklist that rates wellbeing, and measuring changes in perceived stress or state of recovery (41). Some of the more popular self-report measures include the use of a rating of perceived exertion scale (RPE), the Profile of Mood States (POMS), Recovery-Stress Questionnaire for athletes (REST-Q-Sports), Daily Analysis of Life Demands for Athletes

(DALDA), Total Recovery Scale (TQR), and the Multi-Component Training Distress Scale (MTDS) (41, 61).

Monitoring athlete's performance in conjunction with mood states at different periods during the season is important to understand relative changes in mood in connection to performance outcomes (7, 15). When choosing a self-report measure, it may be advantageous to consider the length of the questionnaire and the frequency of administration to improve compliance (24). Additionally, to improve reliability, clear instructions should be given to ensure understanding and to enhance motivation to respond accurately in order to reduce bias. Although subjective measures are a useful monitoring technique to track psychological well-being and athlete readiness to perform, these methods need to be used in conjunction with physiological data as athletes may over or underestimate training load (26).

F. *Sleep Assessments:*

Adequate sleep can help to buffer the effects of increased training and aid in athlete recovery (60). Sleep loss has effects on performance, motivation, perception of effort and cognition, along with a host of physiological functions (25). Impaired sleep patterns, increased wakefulness, decreased sleep quality, decreased perception of strength and recovery has been shown in overreached athletes during heavy training (28). Assessing sleep quality and quantity may provide context to decrements in performance that can occur with increased training demands. Monitoring sleep quality and duration can occur through subjective measures such as sleep diaries and questionnaires including the Pittsburgh Sleep Quality Index (PSQI) as well as wearable technologies such as actigraphy monitors (24),

8. Conclusion:

Collegiate soccer athletes experience fluctuations in training loads throughout the competitive season. When the balance between training and recovery becomes disrupted, perturbations in mood states and sleep may occur along with biochemical/hormonal changes that may result in decrements in performance. Monitoring techniques to track relative changes in physiological and psychological workload along with indicators of athlete readiness are important to detect maladaptation to training so that workloads can be appropriately programmed to fit individual needs (24).

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Chapter I: A Comparison of Internal and External Training Loads in Male and Female Collegiate Soccer Players During Practices vs. Games

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ABSTRACT

The purpose of this study was to compare the internal and external training loads in males and females throughout a Division I soccer season during practices versus games. **METHODS:** Players were evaluated during all practices and regulation game play using the Polar TeamPro system, utilizing Global Positioning Satellite (GPS) technology and heart rate (HR) monitoring to determine training load (TL), time spent in HR zones expressed as a percent of HR_{max} (HR_{Z1-Z5}), calories expended per kilogram body weight (Kcal/kg), distance covered (DIS), sprints, average speed (SPD_{AVG}), and distance covered in speed zones (DIS_{Z1-Z5}). **RESULTS:** During games, no significant differences were seen between men and women for TL, Kcal/kg, HR_{Z1}-$_{Z5}$, SPD_{AVG}, DIS, DIS_{Z1}, DIS_{Z3}, and DIS_{Z4}. However, males accumulated a significantly greater number of sprints ($P < 0.05$), and DIS_{Z5} ($P <0.05$) during games while females exhibited a greater DIS $_{Z2}$ ($P<0.05$). During practice, no differences were observed for TL, DIS, Sprints, Kcal/kg, DIS_{Z2}, DIS_{Z3}, HR_{Z1}-$_{Z5}$, but males exhibited higher SPD_{AVG}, ($P<0.05$), DIS_{Z1} ($P<0.05$), DIS_{Z4} ($P<0.05$), and DIS_{Z5} ($P<0.05$). **CONCLUSIONS:** The parallels in Kcal/kg, total DIS, HR, and TL indicate a similar relative workload between males and females. However, distance covered in higher speed zones was found to be greater in males than females across practice and games likely reflecting inherent sex differences in the ability to achieve those speeds. **PRACTICAL APPLICATION:** Monitoring techniques that track relative player workloads throughout practices and games may enhance player health and performance during the season. An individualized approach to tracking high intensity running may improve workload prescriptions on a per player basis.

Key Words: Athlete Monitoring, High Speed Running, Heart Rate, GPS

INTRODUCTION:

Soccer is a physically demanding sport which involves frequent bouts of high intensity activity, including sprinting and high-speed running, coupled with low-intensity periods of walking and jogging (2). A 90-minute soccer match is further characterized by a large caloric expenditure estimated to be around 20.3 - 23.6 kcal/kg BW and an average distance covered of 10 km at an intensity close to anaerobic threshold (80-90% HR_{max}) (14). Additionally, sprints constitute 1-11% of the total distance covered (12, 14), with players performing around 10-20 sprints during a game (14). In addition, high intensity running occurs approximately every 70 seconds in a game (14). While the physiological demands of a competitive soccer season are often thought to vary between males and females, these workload outputs as a function of sex have rarely been assessed.

Technological advances in athlete tracking have afforded the ability to monitor training workloads both in-game and during practice. Sports science programs have the capabilities to track the physiological response of the athlete, often termed internal load, using techniques such as heart rate (HR) monitoring. The physical demands, often described as external load, can be tracked using global positioning satellite (GPS) systems that determine distance covered as well as the speed at which the athletes travel. While research that quantifies these workload demands has mainly been limited to in-game performance (5, 11), equally important are the workload demands required at practices. More research comparing the total competitive season demands, specifically both internal and external loads, on male and female soccer players is warranted to determine potential differences in recovery needs and practice strategies.

Research designed to assess the current stressors placed on the players in competition as well as the performance characteristics of men’s soccer has grown rapidly (4), yet less progress has been made in women's soccer. Conclusions regarding sex differences are based on a relatively small number of in-game studies in high-level to elite female players (5). The few studies that have addressed sex differences in workload have found the relative physiological demands, represented by percent of HR_{max} and percent of maximum oxygen consumption (VO_{2max}), for male and female elite soccer players during a game to be similar (14). While previous research suggests that both female and male players tax the aerobic and anaerobic energy systems to a comparable level (9, 14), female soccer players often experience less of an external load (e.g. female players run a shorter distance during a typical game compared with male players) (5, 14). Further, high intensity running in an elite female game is reported to be ~30% lower than male athletes of a similar competitive standard (5, 10, 11). For example, elite female players cover less distance at high intensity (speed>15 km/h) than male players matched for age and competitive standards (11, 13). More research is needed to determine *total* competitive season demands including practice and games on combined internal and external load metrics in high-level male and female collegiate soccer players. With multiple games per week, frequent travel, and further burdens of academic requirements, quantifying workload becomes increasingly important in a collegiate population in order to enhance recovery throughout the season.

The purpose of this study was to compare the internal and external training loads in males and females throughout an entire Division 1 soccer season during both practices and games. It was hypothesized that male and female high-level soccer players would have a similar relative internal load in regards to both practices and games; however, males would accumulate more high-speed running on an absolute basis, which may be a function of greater power capabilities.

METHODS

Experimental Approach to the Problem: This observational study sought to determine differences in workload and performance characteristics between men's and women's Division I collegiate soccer teams. Differences in internal load and external load were monitored during both practice and games utilizing GPS and HR technology throughout the course of a competitive collegiate soccer season. Additionally, maximal performance testing and body composition characteristics were evaluated prior to the start of the season to determine differences in aerobic capacity, power output, and body composition.

Subjects: Female (N=16, M_{age} = 19.3±0.3 yrs, M_{weight} = 63.9±1.4 kg; M_{height}=166.61±5.3 cm) and Male (N=12, M_{age} = 20±0.4 yrs, M_{weight} = 77.3±2.8 kg; M_{height}=180.23±5.9 cm) Division I collegiate soccer players were monitored throughout the competitive season. Descriptive characteristics are presented in Table 1. All participants performed testing as part of regular team activity and in association with their sports science program. All subjects received clearance by the Rutgers University Sports Medicine staff prior to testing. Research was approved, and written consent waived, 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

	Subjects (N)	Age (yrs)	Height (cm)	Weight (kg)
Male	12	20.1 ± 1.3	180.2 ± 5.9	77.3 ± 9.6
Female	16	19.3 ± 1.4	166.6 ± 5.3	63.9 ± 5.7

Values are expressed as means ± standard deviation

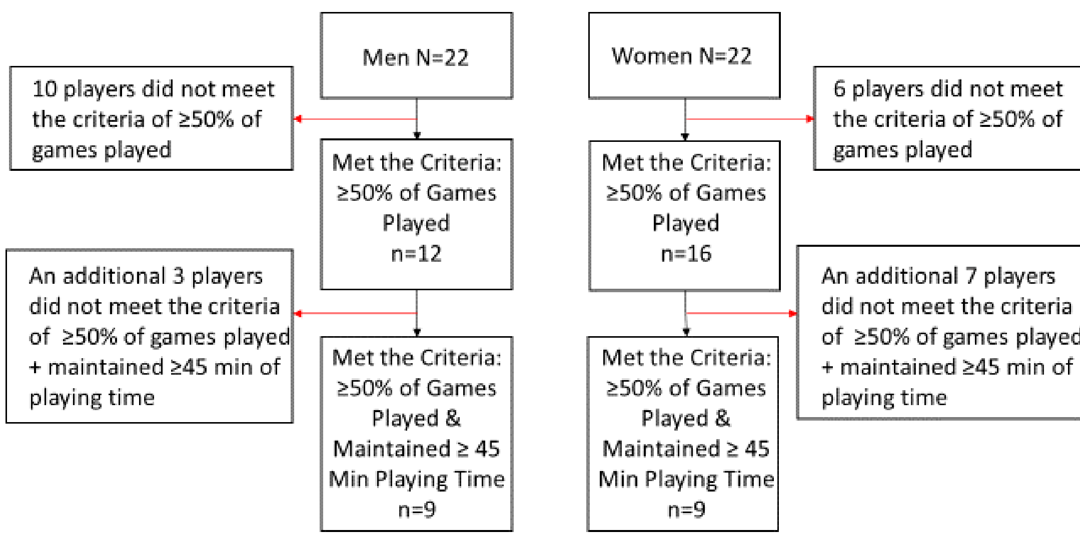
Procedures

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. Body composition was assessed by air displacement plethysmography (7) via the BodPod (BOD POD, COSMED, Concord, CA) to determine percent body fat (%BF), fat free mass (FFM), and fat mass (FM), using the Brozek formula (6) . Following a self-selected warm up, subjects were given three attempts for maximal countermovement vertical jump (CMJ) with arm swing methods assessed using the Just Jump system (Probotics, Huntsville, AL, USA). Performance was scored as the highest jump recorded. Following this, a maximal graded treadmill exercise test (GXT) was used to measure maximal aerobic capacity (VO_{2max}) and ventilatory threshold (VT) via direct gas exchange via a COSMED Quark CPET (COSMED, Concord, CA). A speed-based protocol was used with stages that were MET equated to the Bruce protocol. This protocol included two-minute stages at a constant 2% incline. The speeds were as follows: 6.4, 7.9, 10.0, 11.7, 13.7, 15.6, 17.1, 18.2, 19.8, 21.1 (km/h). Subjects continued the test with encouragement from the lab staff until volitional fatigue. At least three of the following criteria were met for attainment of VO_{2max}: a leveling off or plateauing of VO_2 with an increase in exercise intensity, attainment of age predicted HR_{max}, a RER>1.10, and/or an RPE ≥18. HR was continuously monitored using a Polar S610 HR monitor to accurately obtain HR_{max} (Polar Electro Co., Woodbury, NY, USA). Subject's VT was calculated after the completion of each test as the point where ventilation increased nonlinearly with VO_2, which is expressed as a percentage of VO_{2max}.

In-game and practice monitoring: Players were evaluated during all practices and regulation game play using the Polar TeamPro system that utilizes HR, GPS, and accelerometry technology. Physiological attributes of the player obtained from laboratory testing (age, height, weight, sex, VO_{2max}, HR_{max}, and VT) were used to program each individual player's monitor. The quantification of each individual player's workload was calculated by training load (TL), energy expenditure expressed as a function of body weight (Kcal/kg), time spent in HR zones expressed as a percent of HR_{max} (HR_{Z1}= 50-59%; HR_{Z2}=60-69%; HR_{Z3}=70-79% HR_{Z4}=80-89%; HR_{Z5}=90-100% of HR_{max}), total distance (DIS), number of sprints, average speed (SPD_{AVG}), and distance covered in each speed zone (DIS_{Z1}=3.0-6.99 km/h; DIS_{Z2}= 7.0-10.99 km/h; DIS_{Z3}=11.0-14.99 km/h; DIS_{Z4}=15.0-18.99 km/h; DIS_{Z5}= ≥ 19 km/h). Training load was calculated via an algorithm developed by Polar™ based on the quantification of an individual player's output. Speed zone thresholds were designated by the Polar TeamPro system. A sprint was considered to be any movement greater than 2.8 m/s^2 (15, 16).

Statistical Analysis: For the purposes of this study, only field players were included in analysis. Further, only players who participated in at least 50% of the matches and maintained a minimum playing time of 45 minutes per match were included in the analysis (male: N = 9; female: N = 9). A secondary analysis was run to account for players who were substituted into at least 50% of the matches but did not meet the 45-minute playing time criteria (male: N= 12; female: N =16). All overtime and half-time minutes were factored out of the match data so that only 90 minutes of regulation play was included in analysis. *Figure 1* depicts the flow of subjects included in primary and secondary analysis. Averages for both game and practice data were taken separately for all Polar TeamPro metrics. RM MANOVAS were conducted for the physiological attributes, internal load, and external load variables. Univariate follow-ups were used to compare differences between sexes. All analyses were conducted using SPSS Statistical Software (SPSS version 23; IBM) with significance set at P<0.05. Values are expressed as means ± standard deviation. Difference adjustments were examined and effect sizes (ES) were calculated using Hedge's *d*.

Figure 1: Flow Diagram of Subjects



RESULTS

When comparing performance and body composition characteristics between females and males, differences were seen for FFM (FFM_F=50.69±4.6kg; FFM_M=67.78±8.6kg; ES=2.59; P<0.05), %BF ($\%BF_F$=20.331±3.4; $\%BF_M$=12.27±3.5; ES= -2.34; P<0.05), CMJ (CMJ_F=53.64±7.3cm; CMJ_M=61.52±7.6cm; ES=1.06; P<0.05), and VO_{2max} (VO_{2maxF}=51.13±2.8 ml/kg/min; VO_{2maxM}=57.53±5.1 ml/kg/min; ES=1.62; P<0.05). Males exhibited higher aerobic capacity, power production, and FFM compared with female, while the females showed greater %BF. Differences in body composition and performance characteristics between men and women are presented in Table 2.

Table 2: Performance and Body Composition Comparison

	Subjects (N)	FFM (kg)	%BF	VO_{2max} ($ml \cdot kg \cdot min^{-1}$)	CMJ (cm)
Male	12	67.8 ± 8.6*	12.27 ± 3.5*	57.5 ± 5.1*	61.52 ± 7.6*
Female	16	50.7 ± 4.6*	20.33 ± 3.6*	51.1 ± 2.8*	53.64 ± 7.3*

Values are expressed as means ± standard deviation
*Denotes significant differences between males and females

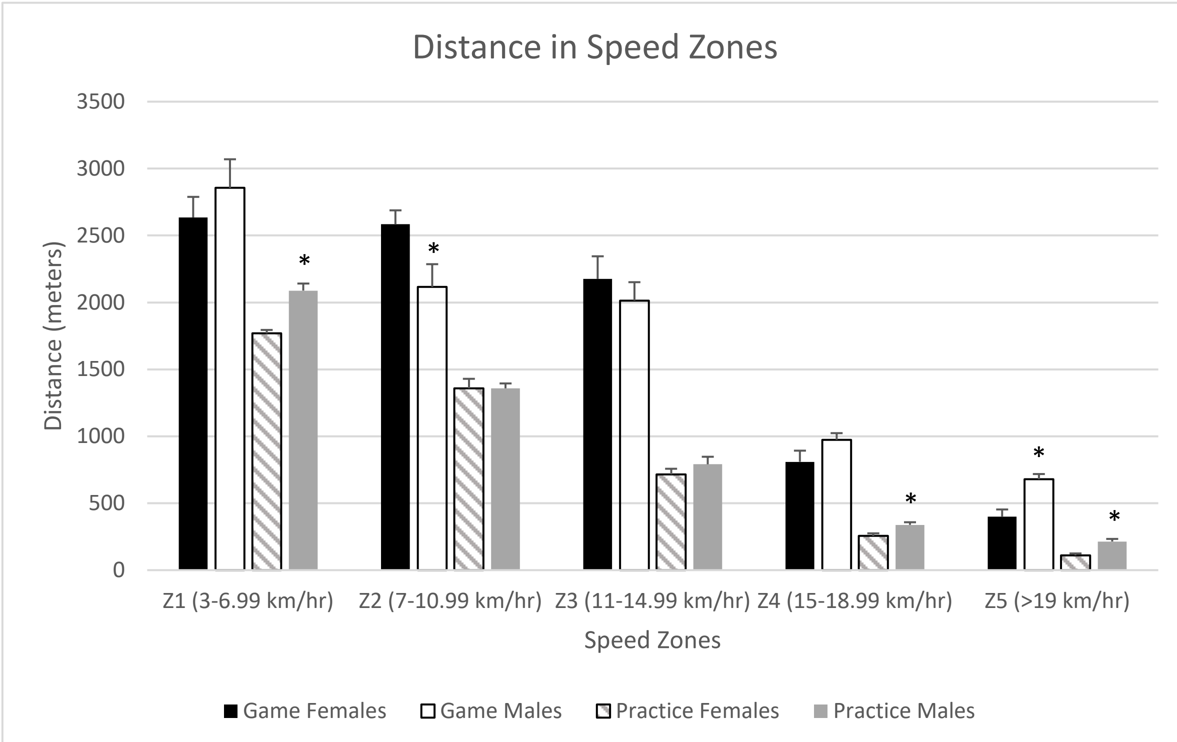
Internal and external load comparisons for males and females can be found in Table 3. No significant differences in game analytics between men and women were seen for TL, Kcal/kg, HR_{Z1} - HR_{Z5}, SPD_{AVG}, DIS, DIS_{Z1}, DIS_{Z3}, and DIS_{Z4} (P>0.05). All comparisons between distance covered in speed zones and time spent in heart rate zones are presented in Figures 2 and 3 (respectively). However, males accumulated a significantly greater number of sprints ($Sprints_F$ =13.8±5.0; $Sprints_M$ =21.9±3.2; ES=1.95; P < 0.05), and DIS_{Z5} (DIS_{Z5F} =400.9±157.6m; DIS_{Z5M} =680.0±114.2m; ES=2.02; P <0.05) during games. Females covered more ground in DIS_{Z2} (DIS_{Z2F}=2584.5±311.1m; DIS_{Z2M} =2116.4±508.3m; ES=-1.11; P < 0.05).

Table 3. Male v. Female Practice and Game Comparison

	Subjects (N)	**Average Speed (km/h)**	
		Game	*Practice*
Male	9	6.90 ± 0.4	3.58 ± 0.2*
Female	9	6.71 ± 0.7	3.06 ± 0.2*
Male†	12	7.03 ± 0.5	3.63 ± 0.3
Female†	16	6.92 ± 0.62	3.07 ± 0.2
		Training Load	
		Game	*Practice*
Male	9	257.52 ± 61.5	139.05 ± 20.7
Female	9	243.15 ± 38.9	128.04 ± 25.7
Male†	12	231.48 ± 73.1#	143.81 ± 19.7
Female†	16	179.06 ± 81.1#	143.95 ± 28.5
		Distance (km)	
		Game	*Practice*
Male	9	8.20 ± 1.4	4.81 ± 0.4
Female	9	8.31 ± 0.9	4.51 ± 0.3
Male†	12	7.41 ± 0.2	4.90 ± 0.4*
Female†	16	6.06 ± 2.7	4.54 ± 0.3*
		Sprints	
		Game	*Practice*
Male	9	21.93 ± 3.2*	11.11 ± 2.9
Female	9	13.78 ± 5.0*	9.64 ± 1.9
Male†	12	19.12 ± 5.9*	11.02 ± 2.8
Female†	16	10.75 ± 5.1*	10.51 ± 2.7
		Kcal/kg	
		Game	*Practice*
Male	9	15.41 ± 3.6	11.76 ± 1.5
Female	9	15.36 ± 2.0	11.39 ± 1.0
Male†	12	14.07 ± 4.15	12.19 ± 1.5
Female†	16	11.21 ± 5.11	12.28 ± 1.4

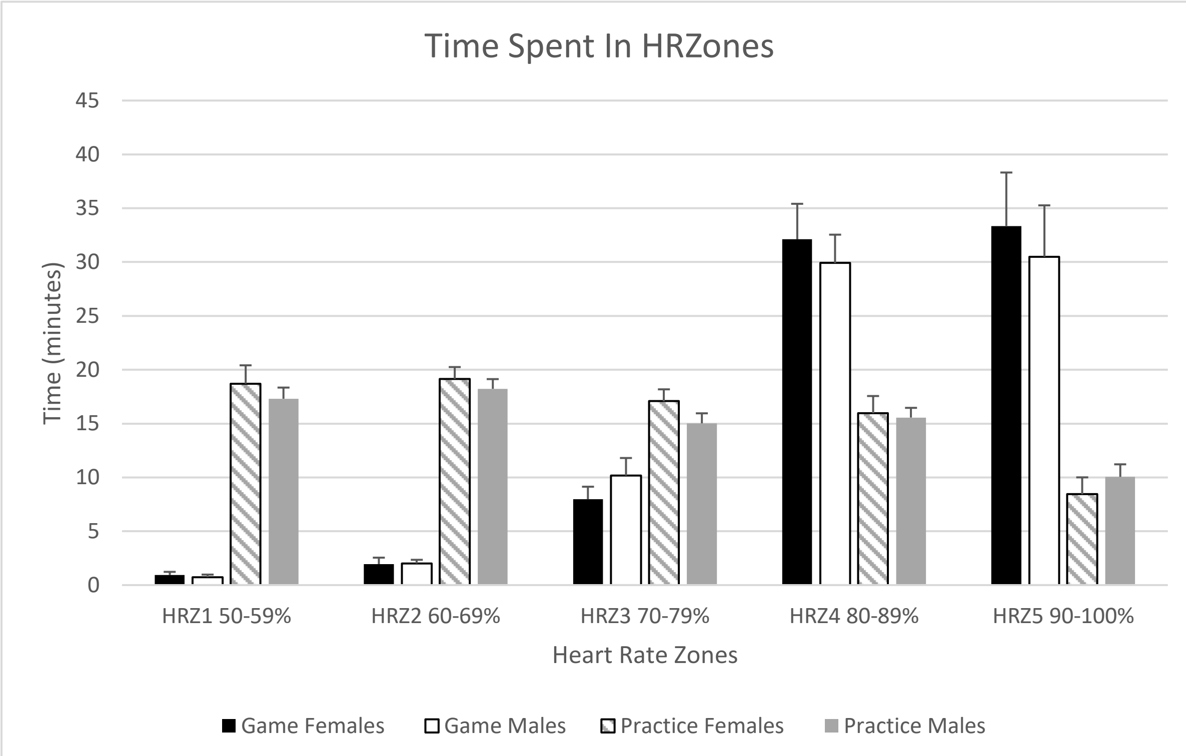
Values are expressed as Means ± Standard Deviation
*Denotes significant differences between males and females
#Denotes a trend towards significant differences between males and females
†Denotes analysis including substituted players

Figure 2: Distance Covered in Speed Zones (n_M=9; n_F=9).



*Denotes significant differences between males and females

Figure 3: Time Spent in HR Zones (n_M=9; n_F=9).



*Denotes significant differences between males and females

When substituted players were included in the game analysis, differences in DIS_{Z4} (ES=0.90; P<0.05) as well as time spent in HR_{Z3} (ES=0.79; P<0.05) were observed, with values found to be higher in males. Further, TL (ES=0.67; P=0.09) and DIS_{Z1} (ES=0.70; P=0.079) trended towards being higher in male than female players.

During practices, no differences were seen for TL, DIS, Sprints, Kcal/kg, DIS_{Z2}, DIS_{Z3}, HR_{Z1-Z5} (P>0.05). However, males exhibited higher SPD_{AVG} (ES=2.60; P<0.05), DIS_{Z1} (ES=2.57; P<0.05), DIS_{Z4} (ES=1.41; P<0.05), and DIS_{Z5} (ES=2.03; P<0.05). A higher average DIS (ES=1.04; P<0.05) was seen in males when substituted players were included in practice analysis, while a greater time spent in HR_{Z3} (HR_{Z3F}=18.7±3.6min; HR_{Z3M}=15.4±2.5 min; ES=-1.04; P<0.05) was seen in females.

DISCUSSION

Despite differences in performance characteristics, relative game and training demands were remarkably similar between male and female DI college soccer players over the competitive season. The parallels in TL, HR, Kcal/kg, DIS, and SPD_{AVG}, amongst the players indicate a similar relative workload between sexes. However, the distance covered in the higher speeds zones across practices and games were found to be greater in males than females, with the differences most pronounced at the highest speed zones. Additionally, male players accumulated a greater number of sprints during games than female players, although no differences were seen in the practice analysis.

Similar to the current study, Bradley et al., assessed sex differences in game performance characteristics of elite soccer players using a multi-camera system (5). Researchers found male players covered more distance than female players at higher speed thresholds (>15, 18-21, 21-23, 23-25, and >27 km/h), but minimal differences were depicted at speeds <12 km/h. Additionally, a study by Krustrup et al., evaluating the physical demands during an elite women's soccer game found the average distance covered by high intensity running (speeds >15 km/h) was 1.3 km, which was about 66% less than that of elite male players (1.9 -2.4 km) (10, 12). It appears that male players have an increased ability to reach (and sustain) higher speed thresholds than their female counterparts, which is not surprising. Although relative physiological loads in males and females have been shown to be similar, female players have a lower absolute aerobic and anaerobic physical fitness capacity (4, 13).Therefore, the increased ability to cover distance in higher speed zones is an expected finding in males due to a greater proportion of muscle mass and capacity for power production as seen with greater FFM values and higher CMJ performance amongst the males in the current study. This becomes further evident in a study by Mujika, et al., comparing sex differences in physical performance outcomes. Males produced 31.7-33.9% greater power via CMJ than females and 13.6%-16.2% faster 15-min sprint performance (13). Despite the greater ability to reach top speed thresholds, the current study shows both males and females covered the greatest proportion of their distance at speeds less than 15 km/h.

Additionally, the current study concluded both sexes covered a similar distance in games and practices, yet research comparing the total distance covered between males and females in a typical soccer game have varied (5, 10, 11, 14). Bradley, et al. (2014) found elite male players covered more distance in total than females (11.14 km and 10.75 km, respectively). However, sex differences were more pronounced at the higher speed thresholds (ES=0.7-1.4) than for the total distance covered in a match (ES=0.5) as the female players covered more of the distance at speeds <12 km/h (5). Krustrup, et al., found total distances covered in elite female soccer players ranged from 9.7-11.3 km (average 10.3 km), similar to the values reported for moderate and top-class male players (10.33 and 10.86 km, respectively) (12). Technical ability of the teams as well as their opponents may affect relative game workloads and differences seen in certain metrics (5). Further, it is important to consider that similarities in distance covered may not be reflective of the overall speed of play. Future studies may consider ball movement and change of field differences that may enhance or reduce the total distance traveled during a game.

As with the existing research on this topic, one limitation to the present study is the relatively small sample size used. However, the limited sample of athletes who met the playing time criteria is indicative of a typical collegiate soccer match. This nine-player rotation is a function of game substitution strategies typically seen at this level and important to consider in order to make reasonable male and female comparisons. Future studies evaluating sex differences in collegiate soccer players may consider utilizing multiple teams within a collegiate conference to reduce the variability inherent with the nature of the sport.

Another limitation that seems to be inherent across the existing literature is the differential ranges used to classify high intensity running. Speed zones are often defined according to distinct thresholds or determined by the proprietary software of the tracking system (15). A high intensity running speed threshold is typically set at >15 km/h for elite male soccer players (1, 3). The rationale for these thresholds are generally chosen based on speeds seen in soccer that equate to those obtained during maximal oxygen uptake (1, 5, 8); however these speed thresholds vary from study to study. Typically, elite male players reach VO_{2max} at ~19 km/h during treadmill running (1), which is ~3 km/h higher than seen with females (1, 5, 10). Though treadmill running does not equate to the intensities seen during match performance due to changes in acceleration and direction as well as movements on the ball, relative intensities may be comparable for males and females. Bradley et al., found males ran a greater distance at speeds >15 km/h, but found minimal differences at <12 km/h (5). The speed zones used in this study found males ran a greater distance in games at speed zones above >19 km/h, but no difference between 11 km/h–14.99 km/h. Although differential speed zones thresholds for males and females would have been desirable, the capabilities to individualize speed zones were not available in the Polar software at the time this study was conducted. Alternatively, individualized speed zones based on physical capacity tests, including maximal speed testing, may prove more beneficial than generalized male/female speed thresholds. This may aid in understanding differences between individual athletes as well as to more fully recognize relative sex differences in external load during practices and games and may be necessary if one hopes to optimize the utility of GPS monitoring.

Further, there is currently no consensus on the definition of a “sprint”, including the necessary acceleration/speed and the minimum duration required to be considered a sprint (15). While some studies categorize sprints as distances in certain speed thresholds, such as distance covered >25 km/h (11, 17), others (including the current study) define them using velocity thresholds, such as efforts exceeding greater than 2.8 m/s^2 (15, 16). It is important to note that linear movement does not take into consideration change of direction or acceleration. Future research is warranted to determine sprint classifications specific to the sport of soccer.

When all players were considered in analysis, the trends for higher TL seen in males versus females could indicate a possible difference in coaching strategies. Females may tend to share the workload amongst substituted players to a greater extent than males. Sex differences may be a function of substitution strategies rather than just physiological demands of the sport. For example, coaches of the female players tended to share the team’s TL by substituting different players more frequently, as evident with 16 players that were included in the greater than 50% of games criteria. This is in direct comparison to the males’ substitution strategies that only included a total of 12 players that met the minimum of 50% of games.

A strength of the current study is the duration of the season studied. Male and female collegiate players were analyzed during the whole competitive season to more fully understand the workload demands throughout practices and games. Further, HR_{max} and VO_{2max} were directly measured during a GXT test prior to the start of the season. This allowed for more accurate HR measurements and energy expenditure throughout the season. Establishing the relationship between HR and VO_2 during a game allows an accurate indirect measurement of VO_2 during soccer matches. Establishing each player’s relationship between HR and VO_2 may accurately reflect the energy expenditure in a soccer match (14) , thus giving a good representation of the internal physiological load of the player.

In this study, the internal physiological load was found to be similar for men and women despite differences seen in performance testing capabilities. Internal load metrics, HR and Kcal/kg, were determined on an individual basis based on data from VO_{2max} testing. However, differences in power production and muscle mass, as seen with CMJ testing and body composition testing, were not quantified in any load metrics. There may be a benefit to incorporating individual power and speed metrics into athlete tracking systems in the future. Additionally, due to the large variations seen in distances covered at different intensities, it has been suggested that game intensity be expressed as a percentage of HR_{max} and to include the number and duration of sprints (14). Future studies should consider both internal and external load derived from maximal capacity testing data in order to understand the relative differences in workload amongst individual players. This study further lends support for the notion that there are few apparent differences in workload between males and females during a typical soccer game if you are able to account for relative differences between sexes.

PRACTICAL APPLICATION

Coaches and sports scientists have the ability to implement monitoring techniques that track relative player workloads throughout practices and games in order to enhance player health and performance during the season. The similarities in workload throughout practices and games indicates similar relative demands for males and females. Further, the application of male-oriented speed zones to a female team tracking system may result in the underestimation of external load. For comparative purposes, relative speed zones based on individual speed testing data may be more useful then team speed zone thresholds. Further the capability to customize speed zones based on tested speed values would be a desirable characteristic in order to quantify an athlete's workload. An individualized approach to tracking high intensity running may improve workload prescriptions on a per player basis.

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Chapter II: Workload-Related Psychological and Physiological Changes in Women's College Soccer Players during a Competitive Season

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ABSTRACT:
Adequate recovery from stressors is an essential aspect of an athletes training program. Insufficient recovery can present itself in the form of psychological and physiological changes that manifest as performance decrements. The purpose of this study was to assess the influence of training demands on mood, sleep, biomarkers, and performance in D1 collegiate female athletes during a competitive season. **METHODS**: Female D1 college soccer players participated in blood draws prior to preseason (T1), and every 4 weeks thereafter (T2, T3 & T4). Athletes arrived for blood draws fasted and euhydrated. T2, T3, and T4 draws occurred ~18-24 hours after a game. Creatine kinase (CK), free cortisol (FCORT), total cortisol (TCORT), total testosterone (TTEST), free testosterone (FTEST), estradiol (E2), growth hormone (GH), interleukin-6 (IL-6), insulin-like growth factor (IGF-1), sex-hormone binding globulin (SHBG), prolactin (PRL), iron (FE), taurine (TAU), glutamine (GLN), phenylalanine (PHE) and tryptophan (TRP) were analyzed. The Multi-Component Training Distress Scale (MTDS; Main & Grove, 2009), the Pittsburgh Sleep Questionnaire Index (PSQI; Buysse et al., 1989), and countermovement vertical jump (CMJ) were also assessed at each time-point. Workload per session (km and kcal) was monitored using the Polar TeamPro system. **RESULTS:** Workload was at its highest from T1-T2, decreased from T2-T3 ($P<0.05$), and remained stable through T4. CMJ remained consistent from T1-T3, before beginning to modestly decline at T4. CK increased from T1-T2 ($P<0.05$), then returned to baseline. FCORT increased from T1-T2 ($P<0.05$) and returned to baseline by T4. TTEST increased from T1 to T2 ($P<0.05$) and returned to baseline by T4. No differences in E or PRL were seen ($P>0.05$). GH decreased from T1-T2 ($P<0.05$) and remained depressed. IL-6 significantly increased from T2-T4 ($P<0.05$) and remained elevated above baseline at T4 ($P<0.05$). No significant differences were seen in SHBG however there

were trends for a decline from T1 to T4 ($P=0.08$). IGF-1 significantly declined from T1-T3 ($P<0.05$) before returning to baseline at T4. FE declined from T1-T2 ($P<0.05$) before returning to baseline values at T3 to T4. GLN increased from T1-T2 ($P<0.05$) then returned to baseline. TRP decreased from T2-T3 ($P<0.05$) and remained depressed. There were no significant changes in PHE or TAU from baseline. Total training distress (TTD) increased from T2-T3 ($P<0.05$) and remained elevated. Sleep quality (SQ) did not change significantly from baseline ($P>0.05$), but sleep duration (SD) increased at T4 ($P<0.05$). **CONCLUSION:** Biomarkers showed the greatest change following the period of highest workload. Changes in biomarkers preceded decrements in mood, suggesting that they may be the earlier indicators of performance status.

Keywords: Female Athlete, Vertical Jump, Performance, Biomarkers

INTRODUCTION:

Adequate recovery from stressors is an essential aspect of an athletes training program. Insufficient recovery can present itself in the form of psychological and physiological changes that ultimately manifest as performance decrements. Soccer is one sport in which players experience high training volumes compounded by multiple games per week and often with minimal time allotted for recovery. Collegiate soccer players in particular, are further burdened with stressors that include a short preseason combined with a season of congested match fixtures, frequent travel, and academic requirements that can exacerbate their recovery requirements. There is a growing need for ways to detect insufficient recovery in order to optimize training and prevent declines in performance from occurring.

When the balance between appropriate training stress and sufficient recovery is disrupted, an abnormal training response may arise (18). Overtraining syndrome (OTS) is the term given to a state of excessive training stress and inadequate recovery leading to performance decrements. OTS lies on a spectrum of underperformance conditions which includes functional overreaching (FOR), non-functional overreaching (NFOR) and OTS (22, 29). FOR may often be applied during a well periodized training cycle with the idea to continuously and progressively overload the athlete causing a short-term decrement in performance which can result in a supercompensation effect (e.g. improvements in performance) if sufficient recovery time is allowed (6). However, if recovery is not allotted, NFOR can occur leading to worsened performance lasting weeks or months. If not fully corrected, NFOR can develop into OTS resulting in long-term decrements in performance capacity accompanied by adverse psychological symptoms (29).

Athletes participating in sports with high training volumes and limited recovery time are at risk of overreaching. Athlete monitoring throughout the season has emerged as a tool to prevent declines in performance stemming from inappropriate training loads and inadequate recovery. Further, monitoring athlete training loads is important to determine whether an athlete is adapting to their training program, to assess fatigue and recovery needs, and to avoid NFOR (1, 14). Ultimately, early detection and prevention (29) of excessive fatigue in athletes should be the goal of monitoring techniques in order to avoid OTS from developing. While no single reliable monitoring technique has been established (17), various methods of tracking an athlete's internal and external workload used in conjunction with psychological assessments may prove beneficial. Internal workload is considered the physiological response of the athlete to training while external workload is seen as the work completed by the athlete (14, 43). A dissociation between external and internal load is often used to determine a state of fatigue in athletes (14).

Physiological assessments of workload are used as a means to monitor an athlete's training and recovery. Technologies such as global positioning satellites (GPS) and heart rate (HR) monitoring provide the ability to track both an athletes on-field internal and external workload throughout a competitive season (14). While tracking on-field training load is an important step to gauge the athlete's recovery needs, other factors may also play a role in determining an athlete's readiness to perform. The use of blood-based biomarkers is an emerging technique with the capacity to provide an objective evaluation of the athletes' *overall* health, recovery, and performance status. Biomarkers such as creatine kinase (CK), total and free cortisol (TCORT & FCORT), and interleukin-6 (IL-6) may provide a general idea of breakdown in athletes and may indicate impaired recovery (23). Perturbations or relative declines in anabolic markers such as insulin-like growth factor (IGF-1), growth hormone (GH), total and free testosterone (TTEST and FTEST) and reproductive hormones such as estradiol (E2), prolactin (PRL) and sex-hormone binding globulin (SHBG) may provide an indication of hypothalamic pituitary adrenal (HPA) and gonadal (HPG) axis dysregulation (23). Iron (FE) is an important mineral involved in oxygen transport and oxidative phosphorylation and plays a role in aerobic metabolism (19, 23). Relative declines in FE throughout the season, particularly relevant in the female athletes (42), may lead to declines in performance (23). Further, several nutrient-based markers, such as the amino acids phenylalanine (PHE), taurine (TAU), tryptophan (TRP) and glutamine (GLN), may be indicative of muscle quality while deficiencies in these markers may indicate impaired muscular development (23) or inadequate nutrition. Monitoring the relative changes in amino acids may prove beneficial in evaluating training status and fatigue (10, 20, 23). Specifically, TRP and GLN have been explored in association with OTS (23, 34). More research is warranted to assess the utility of these markers as monitoring tools to discover how athletes are responding to the stress of training.

Changes in mood profiles have been established as an important indicator of training distress and may be linked to decrements in performance (13). The classic study by Morgan et al monitoring mood states in competitive swimmers showed mood disturbances increase during times of high training volumes in a dose-response manner (30). Questionnaires and surveys designed to assess mood disturbances have the ability to provide subjective information as to how the athlete is adapting to the imposed training load (14). Monitoring athlete’s performance and mood states at different periods during the season is important to understand relative changes in mood in connection to performance outcomes (2, 11).

Further, perturbations in sleep can have significant effects on performance, perceived effort, and cognition as well as other biological functions (15). Adequate sleep may act to buffer the negative effects of increased training demands, while conversely inadequate sleep may lead to a worsened state of fatigue. For example, one of the most commonly reported methods for managing fatigue and enhancing recovery is obtaining adequate sleep (18, 36). Therefore, evaluating the sleep quality of the individual athlete may help to better understand the athlete's overall readiness to perform. Ultimately, an imbalance between training and recovery can be compounded by inadequate nutrition, psychological stressors and sleep disorders (29) leading to the dysfunction of various metabolic pathways. Tracking changes in these metrics, may help to elucidate the impact of training stress on markers of fatigue.

Previous research has shown increased training stress can result in physiological, biochemical, and mood/behavioral alterations (38) that may occur before deteriorations in performance are apparent. Further research is warranted to evaluate the efficacy of multiple methods of tracking athlete well-being associated with changes in training volume throughout the course of a collegiate soccer season in order to determine an athlete's readiness to perform. The purpose of this observational study was to monitor the physiological and psychological profile of Division I female collegiate soccer players throughout the course of a season to determine the influence of training demands as well as detect various markers of fatigue. It was hypothesized that an increase in training load would be associated with greater perturbations in mood, sleep, biomarkers and declines in performance.

METHODS:

Experimental Approach to the Problem: This observational study sought to determine the effects of a competitive collegiate soccer season on biomarkers, mood profiles, sleep disturbances, and performance outcomes in high level female collegiate athletes. Biomarkers were analyzed before the start of preseason and every 4-weeks following to evaluate the effects of the accumulated stress of the season. Monthly performance testing occurred alongside subjective measures of mood states and sleep disturbance assessments on the corresponding 4-week schedule as biomarker testing. Training load variables were monitored at both practices and games throughout the duration of the season including preseason, regular season play, and tournament play in NCAA Division I female soccer players.

Subjects: Twenty- five collegiate female soccer players (M_{age} = 19.4 ± 1.4 yrs; M_{weight}= 66.1 ± 1.3 kg) participated in monitoring and assessments during an entire Division 1 season. All participants performed testing as part of the regular team activity and associated with their sports science program. All subjects received clearance by the Rutgers University sports medicine staff prior to testing. This research was approved, and written consent waived, by the Rutgers University Institutional Review Board for the Protection of Human Subjects. All procedures performed were in accordance with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standard.

Performance Testing: Athletes reported to the Rutgers University Center for Health and Human Performance (CHHP) prior to the start of preseason (P1) to complete a battery of fitness tests. Subjects were instructed to arrive euhydrated, 2-3 hours fasted, and having abstained from exercise 24 hours prior to testing. Body composition was assessed by air displacement plethysmography (9) via the BodPod (BOD POD, COSMED, Concord, CA) to determine percent body fat (%BF), fat free mass (FFM), and fat mass (FM), using the Brozek formula (4). Following a self-selected warm up, subjects were given three attempts for maximal countermovement vertical jump (CMJ) with hands on hips method and assessed using the Just Jump system (Probotics, Huntsville, AL, USA) with the highest vertical jump recorded. Following this, 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 MET equated to the Bruce protocol. This protocol included two min stages at a constant 2% incline. The speeds are as follows: 6.4, 7.9, 10.0, 11.7, 13.7, 15.6, 17.1, 18.2, 19.8, 21.1 (km/h). Subjects continued the test with encouragement from the lab staff until volitional fatigue. At least three of the following criteria were met verifying the attainment of VO_2max: a leveling off or plateauing of VO_2 with an increase in exercise intensity, attainment of age predicted heart rate max, a respiratory exchange ratio greater than 1.10, and/or an RPE ≥18. Heart rate was continuously monitored using a Polar S610 heart rate monitor to accurately obtain maximal heart rate (HR_{max}) (Polar Electro Co., Woodbury, NY, USA). Subject's VT was calculated after the completion of each test as the point where ventilation increased nonlinearly with VO_2, which is expressed as a percentage of VO_{2max}. Descriptive and baseline performance data is presented in Table 1.

Biomarker Collection and Analysis: Athletes reported to the CHHP in a euhydrated condition prior to the start of pre-season for blood draws (T1), and every four weeks following, (T2) (T3) & (T4). All blood draws were taken at the CHHP between 0700-0900 hrs following an overnight fast. Blood draws at T2, T3, and T4 occurred on the last day of the practice week, ~18-36 hours following a game. Blood samples were centrifuged for 10 minutes at 4,750 rpm (Allegra x-15R Centrifuge, Beckman Coulter, Brea, CA, USA) and were shipped to Quest Diagnostics for analysis via LC-MS/MS-based assays. Samples were run in duplicate and the coefficient of variation (CV) for all biomarkers were between 0.5 – 7.5 %. Biomarkers in the analysis included FCORT, TCORT, TTEST, FTEST, E2, GH, IGF-1, CK, IL-6, PRL, FE, TRP, GLN, PHE, and TAU (23).

Season Training and Monitoring: All practices and games were monitored using the Polar TeamPro system (Polar Electro Co., Woodbury, NY, USA) which utilizes heart rate (HR), global positioning satellite (GPS), and accelerometry technology. Physiological attributes of the player obtained from laboratory testing (age, height, weight, sex, VO_{2max}, HR_{max}, and VT) were used to program each individual players' monitor. Workload (i.e. distance covered (DIS), and exercise energy expenditure (EEE)) were assessed during all practices and games (7).

Mood disturbances including total training distress (TTD) and the six subscales: depression, vigor, physical signs and symptoms, sleep disturbance, perceived stress, and fatigue were measured utilizing the Multi-Component Training Distress Scale (MTDS) (26). Additionally, sleep quality (SQ) and sleep disturbances (SD) were measured using the Pittsburgh Sleep Quality Index (PSQI) (5). Prior to the start of the first practice of the week, paper-based surveys were administered and completed by the athletes. Survey completion was followed by a generalized dynamic warmup and subsequent CMJ assessments using the Just Jump system (Probotics, Huntsville, AL, USA) with the highest vertical jump recorded. This protocol was continued every 28 days during the first practice of the week, consistent with the same schedule as blood draws (T1, T2, T3 & T4). See *Figure 1* for a timeline of all assessments and *Figure 2* for a typical weekly testing schedule. The first practice of the week was chosen for assessments of sleep, mood states, and vertical jump while biomarker testing occurred on the last day of the practice week. As such with a typical NCAA female collegiate program, soccer matches occurred in the latter half of the week (typically a Thursday/Friday and Sunday schedule) and therefore we did not want match results (e.g. wins or losses) to become the main driver of mood states.

Statistical Analysis: RM MANOVAs were performed with univariate follow-ups. Simple contrasts were used to examine significant univariate effects. All analyses were conducted using

SPSS Statistical Software (SPSS version 23; IBM) with significance set at P<0.05. Cohen's *d* was used to calculate effect sizes (ES). Values are expressed as Means ± Standard deviation.

Table 1. Descriptive and Performance Data

Age (yr)	19 ± 0.3
Height (cm)	167.49 ± 1.2
Weight (kg)	66.12 ± 1.3
BF%	21.23 ± 0.6
VO_{2max} (ml·kg·min^{-1})	50.49 ± 0.8

Values represent Means ± Standard Deviations

Figure 1: Season-Long Timeline of Assessments

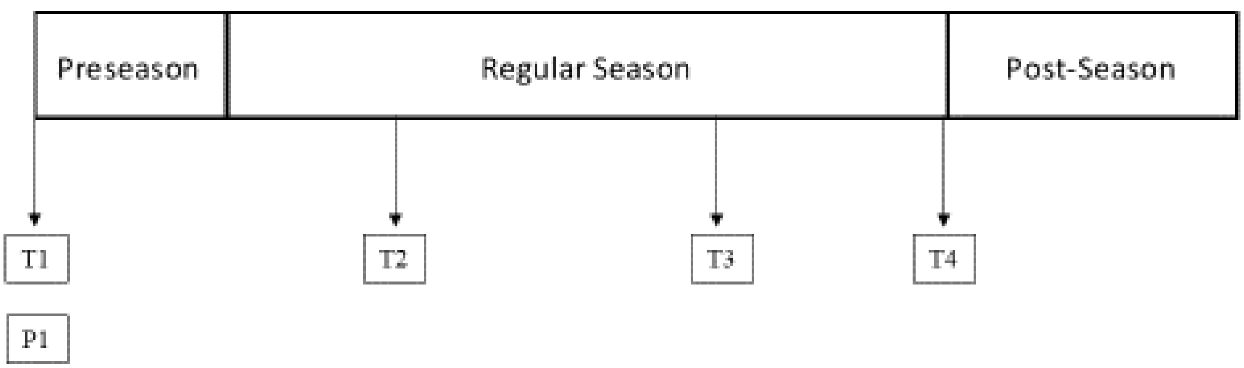
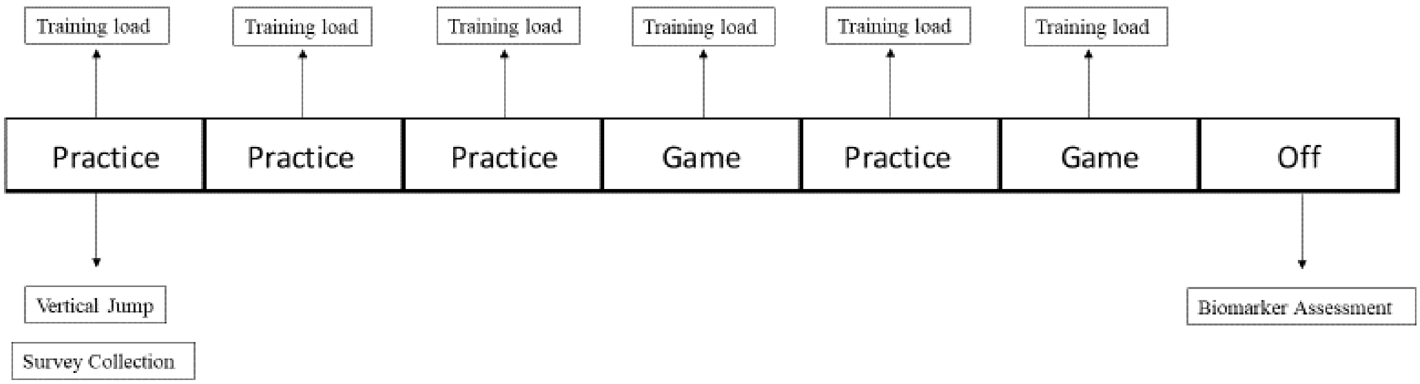


Figure 2: Typical Testing Week Occurring at Monthly Timepoints

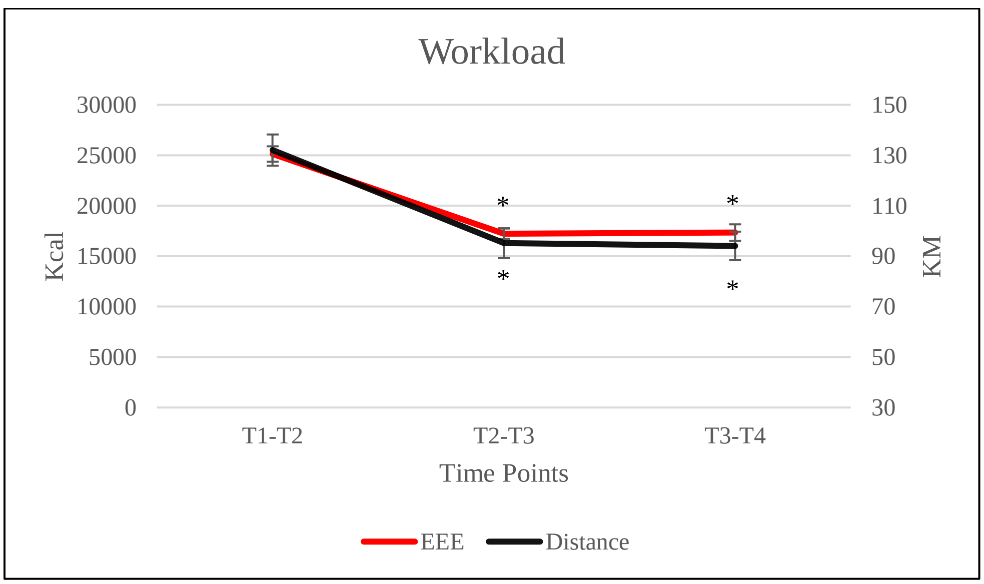


RESULTS

Workload

DIS was evaluated as the total sum of DIS covered during the 4-week training block between time points. All subsequent training blocks were significantly lower (P<0.05) than the initial preseason training block (T1-T2) (see *Figure 3*). Following T1-T2, there was a substantial decrease in DIS in the second training block (T2-T3; ES= -1.19) before remaining stable at (T3-T4). EEE was also evaluated as the total sum of calories expended during the 4-week training block between time points and followed the same pattern as DIS (see *Figure 3*). All subsequent training blocks were significantly lower (P<0.05) than the initial training block (T1-T2). Following the preseason block, there was a substantial decrease in EEE in the second training block (T2-T3; ES= -2.08) before normalizing through the last training block (T3-T4).

Figure 3: Changes in Workload Throughout the Season



Values represent Means ± Standard Error
(*) Denotes significant differences from baseline (T1)

Biomarker Response

All biomarker data can be found in Table 2. FCORT increased from T1-T2 (ES= 0.87; P<0.05) and returned to baseline by T4. Compared to T1, there was a trend for a decline in TCORT at T4 (ES= -0.35; P=0.056). CK increased from T1-T2 (ES= 1.71; P<0.05), then returned to baseline by T3. IL-6 significantly increased from T2-T4 (ES= 0.80; P<0.05) and remained elevated above baseline at T4 (ES= 0.71; P<0.05). FTEST increased from T1-T2 (ES=1.20; P<0.05) before returning to baseline by T4. TTEST followed a similar pattern with an increase from T1 to T2 (ES=3.64; P<0.05) and a returned to baseline by T4. No significant differences were seen in SHBG however there were trends for a decline from T1 to T4 (ES= -0.13; P=0.08). There were no differences seen in E2 or PRL (P>0.05). Compared to baseline, GH significantly decreased at T2 (ES= -0.50; P<0.05) and remained depressed (P<0.05). IGF-1 significantly declined from T1-T3 (ES= -0.57; P<0.05) before returning to baseline at T4. Iron declined from T1-T2 (ES= -0.70; P<0.05) before returning to baseline values at T3 to T4. Compared to baseline, GLN increased at T2 (ES= 1.07; P<0.05) and remained elevated at T3 (P<0.05) before returning to baseline at T4. There was a trend for an increase in TRP from T1-T2 (ES=0.62; P=0.08), decreased from T2-T3 (ES = -0.50; P<0.05) and remained depressed compared to baseline (P=0.09). Compared to baseline, no differences were seen with TAU however TAU showed a significant decline from T2-T4 (ES= -0.50; P<0.05) There were no significant changes in PHE from baseline.

Biomarkers	Units	T1	T2	T3	T4
FCORT	nmol/L	29.52 ± 11.0	38.07± 13.8*	34.76± 11.0*	30.90± 08.3†
TCORT	nmol/L	727.55± 339.4	675.40± 206.9	708.51± 286.9	608.36± 262.1$^{\#\dagger}$
CK	U/L	160.38 ± 120.5	366.54 ± 471.2*	204.33 ± 135.2	218.95 ± 143.5
IL-6	pg/ml	1.77 ± 1.3	1.84 ± 1.07	2.66 ± 2.5	2.69 ± 1.35*
FTEST	nmol/L	0.09±0.06	0.17±0.08*	0.12±0.07$^{\#\dagger}$	0.12±0.08
TTEST	nmol/L	1.12±0.4	2.50±2.9*	1.45±1.0	1.11±0.5
E2	pmol/L	734.27± 324.1	739.89± 422.2	634.24± 293.3	728.58± 391.7
SHBG	nmol/L	83.17 ± 61.9	80.13 ± 62.3	77.88 ± 59.7	74.83 ± 56.2$^{\#}$
GH	ng/mL	4.40 ± 4.2	2.30 ± 2.2*	2.00 ± 3.5*	1.85 ± 2.5*
IGF-1	ng/mL	302.21 ± 95.8	281.79 ± 97.6	247.74 ± 67.5*†	278.33 ± 77.82†
FE	Umol/L	16.22 ±7.4	11.02 ±5.8*	13.35 ±6.1	18.70 ±11.8†
PRL	ng/mL	17.32 ± 8.7	20.72 ± 12.8	19.01 ± 7.7	19.62 ± 8.3
GLN	Umol/L	530.32 ± 76.9	612.44 ± 105.6*	579.0 ± 86.6*	540.24 ± 93.1†
TAU	Umol/L	40.44 ± 9.6	44.44 ± 11.8	38.44 ± 12.6	38.40 ± 12.9
TRP	Umol/L	64.80 ± 13.4	73.12 ± 22.3$^{\#}$	62.24 ± 12.9†	59.32 ± 13.59$^{\#}$
PHE	Umol/L	70.56 ± 9.3	66.40 ± 10.9	72.08 ± 11.03	70.68 ± 9.2

Values represent Means ± Standard Deviations
(*) Denotes significant differences from baseline (T1)
(†) Denotes significant difference from previous time point
(#) Denotes trends for significant differences from baseline (T1)

Psychological Response: A trend for an increase in TTD was seen from T1-T2 (P=0.07), significantly increased from T2-T3 (ES= 0.70; P<0.05) and remained elevated above baseline at T4 (ES= 1.18; P<0.05). Compared to baseline values, scores on the depression scale trended for an increase at T3 (P=0.06) with a significant increase from baseline at T4 (ES= 0.73; P<0.05). Scores for vigor increased from T1-T2 (ES= 1.07; P<0.05) remained above baseline values. Scores for physical signs and symptoms decreased T1 to T2 (ES= -0.51; P<0.05) before returning to baseline at T4. Sleep disturbance increased from T1 to T3 (ES= 0.45; P<0.05) before returning to baseline at T4. Perceived stress decreased from T1-T2 (ES= -0.95; P<0.05) and remained depressed at T3 (P<0.05) before returning to baseline at T4. Compared to baseline, reported fatigue remained consistent from T1 to T3 before increasing above baseline at T4 (ES= 0.64; P<0.05). See *Table 3* for the full change in MTDS subscales.

Table 3: Changes in the Multi-Component Training Distress Scale

MTDS Subscale	T1	T2	T3	T4
TTD	24.4 ± 2.1	20.04 ± 1.9	26.50 ± 2.3	31.0 ± 2.1
Depression	1.08 ± 0.5	0.44 ± 0.2	1.72 ± 0.6	2.92 ± 0.6
Vigor	5.16 ± 0.6	8.20 ± 0.9	8.72 ± 0.8	9.56 ± 0.7
Phys Sign and Symptoms	6.12 ± 0.6	4.60 ± 0.7	4.52 ± 0.6	5.12 ± 0.7
Sleep Disturbance	1.84 ± 0.6	2.16 ± 0.6	3.12 ± 0.6	2.12 ± 0.5
Perceived Stress	5.00 ± 0.7	1.52 ± 0.4	3.44 ± 0.6	4.76 ± 0.6
Fatigue	4.16 ± 0.6	3.08 ± 0.5	4.88 ± 0.7	6.12 ± 0.7

MTDS Subscale	T1	T2	T3	T4
TTD	23.4 ± 10.5	20.04 ± 9.3#	26.50 ± 11.3†	31.0 ± 10.3*
Depression	1.08 ± 2.5	0.44 ± 0.8	1.72 ± 2.3#†	2.92 ± 3.2*
Vigor	5.16 ± 2.3	8.20 ± 4.3*	8.72 ± 4.0*	9.56 ± 3.6*
Phys Sign & Symptom	6.12 ± 3.0	4.60 ± 3.3*	4.52 ± 2.8*	5.12 ± 3.7
Sleep Disturbance	1.84 ± 2.8	2.16 ± 2.9	3.12 ± 3.1*	2.12 ± 2.4#
Perceived Stress	5.00 ± 3.7	1.52 ± 2.1*	3.44 ± 3.0*†	4.76 ± 3.0†
Fatigue	4.16 ± 3.1	3.08 ± 2.7	4.88 ± 3.3†	6.12 ± 3.5*

Values represent Means ± Standard Deviations
(*) Denotes significant differences from baseline (T1)
(†) Denotes significant difference from previous time point
(#) Denotes trends for significant differences from baseline (T1)

Sleep Response: Compared to baseline, trends for an increase in SQ were seen at T2 and T3 (P=0.09) but returned to baseline by T4. SD remained consistent from T1-T3 before significantly increasing from baseline at T4 (ES= 0.76; P<0.05). See *Table 4* for changes in sleep over the course of the season.

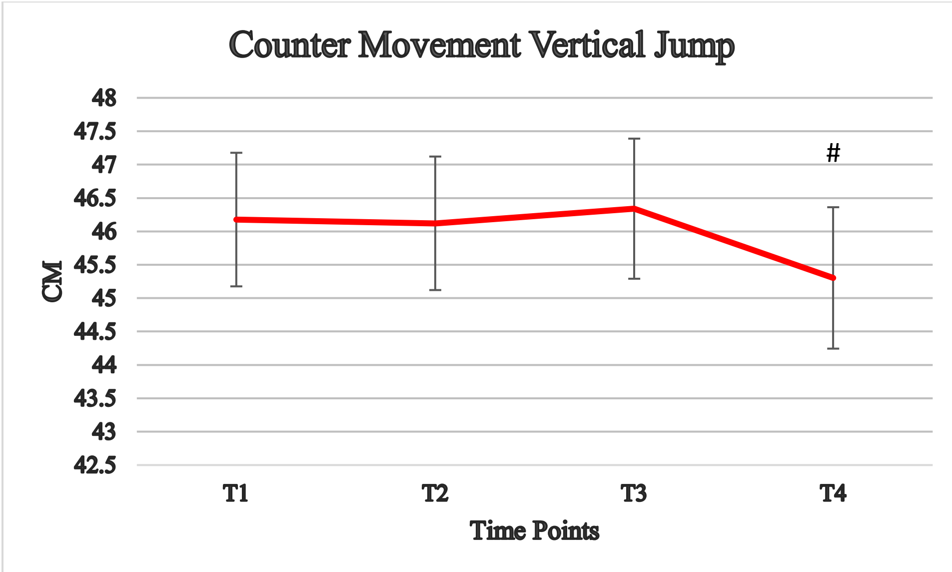
Table 4: Sleep Assessments

PSQI Subscales	T1	T2	T3	T4
Sleep Quality	0.96 ± 0.6	1.16 ± 0.6$^{\#}$	1.24 ± 0.6$^{\#}$	1.08 ± 0.5
Sleep Duration	0.32 ± 0.5	0.32 ± 0.6	0.28 ± 0.5	0.68 ± 0.6*†

Values represent Means ± Standard Deviation
(*) Denotes significant difference from baseline (T1)
(†) Denotes significant difference from previous time point
(#) Denotes trends for significant difference from baseline (T1)

Performance Response: CMJ remained consistent from T1-T3, before beginning to modestly decline (ES= -0.20; P=0.09) at T4. (See Figure 3)

Figure 3: Counter Movement Vertical Jump



(#) = Denotes a trends for significant differences from previous timepoint
Values represent Means ± Standard Error

CONCLUSION:

Monitoring the demands of training load through various assessment methods may provide a more complete picture of the stress incurred by the athlete. In the current study, workload was monitored to assess the influence of training on mood, sleep, biomarkers, and performance. The athletes experienced the highest training volume, including DIS and EEE, during the initial preseason training block (T1-T2). For collegiate soccer athletes, preseason is a short, intense, two-week period which comprises multiple practices per day in conjunction with the stress of competition and academic requirements (42). Biomarkers showed the greatest change following the period of highest workload. Several biomarker perturbations, including declines in GH and increases in FCORT, persisted well into the season which may indicate the athletes were never fully recovered from the stress incurred during preseason. Further, mood disturbances occurred in the latter half of the season (T3-T4) following biomarker changes. The time course of changes in various markers of fatigue throughout the season suggest biomarkers may provide an early indication of physiological disruption. Although there were trends for improvements in SQ and increases in SD by T4, trends were still seen for declines in CMJ. It appears sleep did not improve sufficiently to buffer the effects from accumulated workload.

Biomarker monitoring can provide insight into the physiological changes that athletes experience during the season (42). One important biomarker commonly seen with regards to training status is cortisol. Cortisol is an important hormone released in response to stress. An elevation in resting cortisol can be seen due to a disruption in HPA axis (29) leading to an increased catabolic environment making it difficult to build or maintain muscle or recover from training (23). In the current study, significant elevations in FCORT were apparent at T2 and T3, immediately following the preseason time block. Further, it is important to note that TCORT values were above the normal clinical reference ranges at all time points (4.6-20.6 mcg/dL when assessed 0800-1000h) indicating a high catabolic state in these athletes which persisted throughout the season.

Testosterone is a biomarker often used in conjunction with cortisol to provide a relative indication of the an anabolic state, particularly in male athletes (21, 23). Testosterone is important for reducing protein breakdown and promoting protein synthesis (23). Both FTEST and TTEST significantly increased following the preseason training block. This upregulation in the HPA-axis may have been a physiological response to combat the stress the first training block. Further the hormones, E2, SHBG and PRL, were analyzed as markers more pertinent to the female athlete. Increased training loads without adequate recovery can cause the suppression of E2 leading to the disruption of the menstrual cycle or the development oligomenorrhea and amenorrhea, one of the components of the female athlete triad (31). SHBG modulates the transport of various sex hormones and has been shown to increase in both males and females with exercise (23). Prior research has shown PRL to increase in response to stress, hypoglycemia and exercise (39). High levels of PRL secreted by the pituitary, can result in decreased secretion of LH and FSH and subsequent suppression of estrogen leading to amenorrhea (44). However, no significant changes in either E2, SHBG or PRL were seen throughout the season indicating no other major alterations in HPG axis were seen in these athletes.

Other HPA-axis markers that changed in response to the stress of the season included the anabolic hormones, IGF-1 and GH, both of which are involved in muscle protein synthesis and the regulation of muscle mass (23). GH has been shown to correlate to exercise volume and intensity (23). Previous research evaluating acute GH responses to exercise have found blunted effects in NFOR and OR compared to healthy subjects (6). GH increases levels of IGF-1, an important mediator of anabolic response. In this study, GH declined from T1 to T2 and remained suppressed for the remainder of the regular season while IGF-1 declined from baseline at T3. Relative declines in IGF-1 and chronic reductions in GH may indicate the athletes were experiencing impaired muscular adaptations to training.

CK, an important indicator of muscle damage (23), more than doubled (56% increase) from T1 values after the initial training block. Although within athlete specific reference ranges, the increased CK seen at T2 may be associated with the high training loads that occurred during the preseason and indicates the need for increased recovery strategies. Along with CK other catabolic markers include, IL-6, which belongs to group of cytokines that regulates the body’s inflammatory response (12). IL-6 has been shown to increase in response to decreased muscle glycogen, muscle contraction, and muscle damage in order to activate an immune response (33).

Cytokines have also been implicated in the production and enhancement of negative mood including fatigue (24, 27). Increases in circulating IL-6 have been found to be associated with depressed moods, sleep disturbances and fatigue. Interestingly, elevations in IL-6 occurred at T4 prior to the start of tournament play and in conjunction with increases in TTD, fatigue, and declines in CMJ.

Other nutritional markers may play a role in evaluating athlete recovery strategies. In this study, total FE was used to determine FE status. Total FE declined significantly after the first training block before returning to baseline values. Although not clinically classified as iron deficient, the decrease represents a 32% decline in FE levels. FE is an essential mineral required for aerobic metabolism and endurance performance with suboptimal FE stores leading to declines in exercise capacity (23). Monitoring relative changes in FE stores throughout the season may provide an opportunity to intervene before deficiencies can occur. Additionally, the amino acid GLN has been studied in association with overreaching for its role in immune system function (32). During high intensity and prolonged exercise, GLN values increase followed by significant decreases during the post-exercise recovery period (35). Decreases in GLN may lead to an impaired immune system response and may be responsible for increased rates of infection associated with OTS (32). Decreased concentrations of GLN have been reported during prolonged exercise and heavy training (17, 34). Interestingly, during the course of the season, GLN increased at T2 and T3 compared to initial values. Future research may consider tracking GLN in conjunction with rates of infection and illness throughout the season to better understand this marker and its role in recovery. Of additional importance is the amino acid TRP which is a precursor of serotonin. TRP may provide a mechanism for understanding changes in mood typically reported with overreaching (38). Increased brain levels of serotonin are believed to result in mood and behavioral changes such as inducing sleep and reducing appetite, both behaviors evident in OTS (38). In this study, TRP showed trends for a decline at T4 as mood disturbance increased. More research is needed to determine the mechanism by which changes in TRP occur. No changes in PHE or TAU were seen throughout the season, yet individual deficiencies in these micronutrients may indicate a need for supplementation or improved nutrition strategies. Monitoring deficiencies in amino acids and other nutritional markers may be warranted to prevent declines in performance stemming from inadequate nutrition or to provide justification for the implementation of nutrition education programs, particularly for athletes that experience high EEE.

Tracking athletes' psychological state is often used as a tool to identify athletes who are responding negatively to training. Throughout the season, the athletes experienced various changes in psychological states. The greatest perturbation in mood occurred at T4 which included significant increases in TTD, depression, and fatigue. While at T3, increases in sleep disturbances were noted. Lovell et al found similar results using the Profile of Mood States to measure psychological changes throughout the season in professional, university and recreational soccer players. Researchers found the greatest negative change in mood states occurred for the professional players as the season progressed suggesting psychological changes may be associated with the demands of the soccer season (25).

Interestingly, compared to baseline values, vigor was improved at all subsequent timepoints. Athletes who present with high measures of vigor and low rates of fatigue, depression, etc are said to have ideal psychological states (30). In the current study, declines in physical signs and symptoms occurred at T3 and rates of perceived stress declined from baseline throughout the season. The seemingly random perturbations throughout the season make it difficult to interpret all psychological subscale changes in combination. It may be that the greatest perceived stress was in anticipation of the upcoming season and therefore declines were noted as the season progressed. Alternatively, it may be that certain subscales were unresponsive to training loads in this team setting.

Psychological measures have been suggested to be a more sensitive and consistent indicator of overreaching than physiological indicators (1, 29). In a systematic review by Saw et al, researchers compared objective and subjective measures of athlete well-being and found that subjective measures were more responsive to variations in training loads (37). Further, an athlete's psychological profile can be reported more quickly than physiological or blood markers (1). Although psychological questionnaires are simple and inexpensive to administer, they rely on self-report data that can be manipulated either intentionally or unintentionally. Further, the frequency of administration and length of the survey can often lead to testing fatigue and inaccurate reporting. In the current study, increases in vigor and declines in perceived stress were seen in conjunction with trends for increased TTD after the period of highest training load indicating that other factors rather than training load alone may be driving psychological responses. Therefore, it may be beneficial to use subjective measures of athlete readiness in conjunction with objective measures of an athlete's physiological response to training.

Obtaining adequate sleep is often used as a method for enhancing recovery (36). Sleep is necessary for its restorative properties and the ability to improve cognitive processes and metabolic functions imperative to exercise performance all of which are affected by SQ and SD (36). A study by Matos et al found perceived sleep problems was one of the most reported physical symptoms by athletes who experienced fatigue which also coincided with significant decrements in performance (28). Hausswirth et al demonstrated a decrease in SD and SQ seen with overreaching (18). In the current study, sleep disturbances were reported midway through the season (T3). Yet an increase in SD occurred at T4, with trends for increases in SQ at T2 and T3. Despite these beneficial changes in sleep, CMJ performance declined at T4. Greater SQ may be more important for full recovery, as the improvements in SD did not prevent declines in CMJ. Therefore, implementing strategies to improve sleep quality and reduce sleep disturbances during times of high workload may better enhance recovery from exercise (36). Further, alternative methods to tracking sleep may be advantageous to determining sleep quality in response to fluctuating training demands. The current subjective monitoring technique may not be sensitive enough to see changes in sleep quality in a real world setting in actively training athletes.

In the current study, maximal CMJ was used as a measure of performance. It is important to note that the best test of fatigue is a maximal performance test replicating the athlete's competition. However, the limitations of maximal performance testing, including the added fatigue placed on the athletes during season, a lack of motivation to complete at maximal effort, and the difficulty in replicating maximal soccer performance (14), makes maximal performance tests difficult to implement during the season. Therefore, CMJ was chosen as it is simple to administer, with minimal amount of fatigue incurred by the athlete (14, 41). Additionally, CMJ is a useful performance measure because it is indicative of lower limb muscular power and has been shown to relate to maximal running speed which are important metrics of soccer performance (45). Changes in vertical jump throughout the season can be used as an objective means to evaluate an athlete's performance. Interestingly, CMJ began to modestly decline at T4 which is when the athletes were entering the most critical time of the competitive season, tournament play. Evaluating changes in performance outcomes may help coaches to better prepare training sessions and recovery strategies upon entering tournament play.

Increased training stress can result in physiological responses often in conjunction with psychological changes that result in imbalances to homeostasis (13, 16). Previous research on overload training has suggested mood disturbances accompany or precede negative physiological effects of overtraining (25). In the current study, changes in biomarkers preceded decrements in mood, suggesting that they may be the earlier indicators of performance status, in contrast to previous reports (2, 8, 16). It is important to note that a majority of overreaching research is performed in male athletes, particularly male endurance athletes, and occurs within a laboratory-based setting. These studies may not accurately translate to female athletes participating in a power-endurance sport. It is noted that this study was not designed to evaluate overtraining, nor do we believe the team as a whole was overreached. Nonetheless, there are some parallels that can be drawn between previous overload literature and the current study with regard to similarities in training protocols. During the initial preseason training block, the athletes experienced nearly double their regular workload which is consistent with overload training protocols. In addition, perturbations in biomarkers occurred after this period of overload training which was followed by a relatively modest decline in CMJ performance suggesting insufficient recovery and a fatigued state characteristic of overreaching.

Further, although no single biomarker has been shown to effectively diagnose inadequate recovery (23), it is important to consider that increases and decreases in hormones do not occur in isolation. For example, disruptions in the HPA/HPG axis will result in a cascade of hormonal responses that may result in a disruption to homeostasis. Therefore, it may be advantageous to evaluate a multitude of biomarkers in conjunction with training load to determine if an athlete is adapting to training stressors so that adequate recovery can be achieved.

This applied observational study sought to evaluate the effects of training load on various markers of athlete readiness in an applied setting within a team environment. However, we acknowledge a few limitations to the study. First dietary intake was not assessed during the season which may influence biomarker responses as well as overall recovery status. Adequate nutrition has been shown to improve recovery and enhance performance outcomes (40). However, the limitations to self-reported dietary analysis make accurate assessments of dietary intake difficult to achieve. Further, this study did not control for menstrual status or oral contraceptive use. Although biomarker monitoring did occur every 28 days to account for a typical menstrual cycle, the individual menstrual status of the athletes was not "controlled". Given the nature of this applied study, researchers sought to determine a typical hormonal environment that exists for a collegiate team during the season. Future research may consider ways to account for the differential hormonal environments that occur in the female athlete so that practical and comprehensive assessments may occur.

PRACTICAL APPLICATION:

Tracking athlete workload is an important first step to determine the on-field training stress imposed on the athlete. However, the use of multiple monitoring techniques may provide better clarity regarding the player's physiological response to the given training load in order to evaluate athlete well-being and readiness to perform. When choosing a monitoring technique, it is important to consider the efficacy of that method to detect fatigue and insufficient recovery in an effort to optimize performance. It is the role of the sports scientist to utilize the data collected to provide information to the coaches as to when training becomes maladaptive in order to better facilitate training strategies and enhance performance outcomes (3). A combination of subjective and objective monitoring techniques may be useful in combating insufficient recovery from the accumulated stress of the season that may ultimately lead to declines in performance. The time course of changes in athlete readiness markers indicated changes in biomarkers preceded changes in mood disturbances. Tracking changes in biomarkers may provide an earlier indication of maladaptive training and may provide an opportunity for intervention before decrements in performance can occur.

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Chapter III: Biomarkers Correlate with Strength, Endurance, and Body Composition Changes Throughout the Competitive Season in Women's Division I Collegiate Soccer Players

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ABSTRACT

Increased soccer specific demands and insufficient recovery during a season may result in loss of strength and fat free mass (FFM). Biomarker monitoring may be a useful tool to detect the impact of these stressors, particularly if related to performance outcomes. The purpose of this study was to identify the relationship between biomarker changes and changes in performance and body composition variables in female athletes over the course of a competitive season. **Methods:** Twenty-one women's DI college soccer players were monitored throughout their competitive season. Athletes performed a battery of tests pre- and post-season, including vertical jump (VJ), VO_{2max}, and 3 repetition maximum (RM) testing for bench press (BP), squat (SQ) and deadlift (DL). Body composition was also assessed via Bodpod prior to the start of the season and at weeks 6, 10, 14, and 17 (post-season). Blood draws were performed prior to the start of preseason and every 4-weeks thereafter. The athletes arrived fasted and euhydrated in the morning 18-36 hours post-game. Total cortisol (TCORT), free cortisol (FCORT), estradiol (E2), growth hormone (GH), insulin-like growth factor-1 (IGF1), total testosterone (TTEST), free testosterone (FTEST), c-reactive protein (CRP) and interleukin-6 (IL6) were analyzed. **Results:** TCORT was negatively correlated with change in fat free mass (FFM) ($r=-0.48$, $P<0.05$). TCORT had a positive correlation with changes in percent body fat (%BF) that approached significance ($r=0.39$; $P=0.08$). TCORT was positively correlated with change in VO_{2max} ($r=0.47$; $P<0.05$). The negative correlation between TCORT and SQ change approached significance ($r= -0.45$; $P=0.08$). IGF1 and GH were positively correlated to changes in DL ($r=0.56$; $P<0.05$) ($r=0.56$; $P<0.05$). IL6 was negatively correlated with change in BP ($r=-0.52$; $P<0.05$). **Conclusion:** These findings support a relationship between changes in anabolic and catabolic hormones and changes in performance and body composition. Greater elevations in GH and IGF1 from baseline were related to improvements in measures of strength, whereas greater elevations in TCORT and IL6 were related to a decline or mitigated improvement in FFM, BP and SQ. Interestingly, increases in TCORT were correlated with improvements in $VO_{2max.}$, which may be a result of aerobic training overload.

KEY WORDS: catabolic, anabolic, strength and conditioning, female athlete

INTRODUCTION:

The physiological demands of soccer require athletes to possess high levels of aerobic capacity, as well as muscular strength, power, speed, and speed-endurance (11, 20). However, collegiate soccer players face unique challenges to maintaining these metrics during the competitive season. Multiple games per week, sport practice, frequent travel, and the burdens of academic requirements can increase the athlete's stress and recovery needs and further hinder the ability to enhance aspects of sport performance that are required to succeed. In-season, increased soccer-specific demands coupled with insufficient recovery may result in a loss of strength and fat free mass (FFM). Monitoring techniques that track changes in an athlete's physiological state may prove beneficial in determining an athlete's readiness to perform. Identifying monitoring methods that are associated with performance outcomes may allow for prompt player evaluation and provide an opportunity to intervene before decrements in performance can occur.

Systematic assessments, specifically maximal performance tests such as VO_{2max} for aerobic capacity, vertical jump (VJ) for power, and repetition maximum (RM) tests for strength, are important to determine the individual athlete's capabilities as well as to monitor the effectiveness of training. While maximal performance tests are optimal to determine player development, the added fatigue incurred by the athletes make these tests difficult to implement during the season. Therefore, it is essential to incorporate other monitoring techniques during the competitive season and not rely solely on post-season maximal testing to reveal decrements in performance. The use of athlete tracking systems throughout the season plays an important role in assessing recovery needs, detecting non-functional overreaching, reducing risk of injury, and ultimately maximize athletic performance (9). While workload tracking systems that measure heart rate (HR) combined with global positioning satellite (GPS) metrics can detect an athletes' response to on-field training stressors, the use of biomarker monitoring is an emerging method to track an individual player's physiological response to *overall* stress as well as to identify the balance between training and recovery (13). Periodic biomarker asessments during the competitive soccer season offer an accurate method for evaluating training related stress and recovery needs (1).

Although there is no single definitive biomarker that can be used to monitor training progress (13), measuring markers that are indicative of an athlete's physiological state may prove to be a useful tool in determining athlete readiness. Kraemer et al. (2004), proposed that if the physical demands of training are too high, a catabolic state will persist throughout the season resulting in impaired performance. However, if athletes successfully recover from training demands, an anabolic environment will result as will maintenance or improvements in performance (11). Previous research investigating the relationship between exercise performance and hormonal concentrations over the course of a season in male soccer athletes has shown that decrements in sprint speed, vertical jump height, and knee extensor strength coincided with elevated concentrations of circulating cortisol and reduced testosterone indicating a predominance of catabolic processes. Markers associated with catabolism, such as total cortisol (TCORT), creatine kinase (CK), c-reactive protein (CRP), and interleukin-6 (IL-6), often released in response to excessive strain or muscle damage, have been shown to promote inflammation and muscle breakdown and may indicate impaired recovery (13). The reproductive hormone estradiol (E2) is pertinent to the female athlete as alterations in E2 have been shown to be reflective of an energy deficiency and hypothalamic pituitary gonadal (HPG) axis dysfunction

(5, 21). (11). In addition to testosterone, other anabolic hormones such as insulin-like growth factor-1 (IGF-1), and growth hormone (GH), have been shown to promote growth and development of muscle tissue (7, 13) which may lead to improvements in body composition and performance. While research has shown that an increase in maximal strength is correlated with an improvement in power, more specifically that lower body strength has been found to correlate with sprint and jump performance (18, 22) it is unclear if changes in markers of anabolic hormones correlate to changes in these performance outcomes.

For these reasons research is warranted to determine if anabolic and catabolic biomarkers can be associated with performance and body composition variables as well as to determine the nature of change in these variables over a competitive season, particularly while utilizing a periodized strength and conditioning program.

The purpose of this study was to identify the relationship between biomarkers, performance, and body composition changes in Division I (DI) female collegiate soccer athletes over the course of a competitive season, which included a periodized strength and conditioning program. It was hypothesized that a greater increase in concentrations of catabolic markers will correlate with decreased performance outcomes and loss of FFM while increased concentrations of anabolic hormones will be associated with improvements in performance and FFM. Additionally, this study sought to determine the impact of a women's collegiate soccer season on various measures of fitness and performance.

METHODS:

Experimental Approach to the Problem: This study sought to determine the correlation between biomarkers, body composition, and performance outcomes in DI female soccer players throughout the course of a competitive collegiate soccer season. Biomarkers were analyzed prior to the start of preseason and every 28 days following the initial analysis. Maximal performance testing occurred prior to the start of the season and within one week following the final match. Body composition testing occurred prior to the start of preseason, (week 0), and at weeks 6, 10, 14, and within one week following the final match, (week 17). A full timeline of assessments is presented in *Figure 1*.

Subjects: Women's DI college soccer players (N=21; M_{age}= 19.7± 1.5 yrs; M_{weight}= 66.3± 6.2kg) participated in monitoring and assessments during an entire competitive season. All participants performed testing as part of the regular team activity associated with their sports science program. Athletes participated in a periodized strength and conditioning program a minimum of one time per week throughout the course of the competitive season. The program was appropriately individualized by the strength and conditioning coach based on the capabilities of the player and taking into account injury and playing status. All subjects received clearance by the Rutgers University sports medicine staff prior to testing and at the start of the season. This research was approved, and written consent waived, by the Rutgers University Institutional Review Board for the Protection of Human Subjects. All procedures performed were in accordance with the 1964 Declaration of Helsinki and its later amendments or comparable ethical standard.

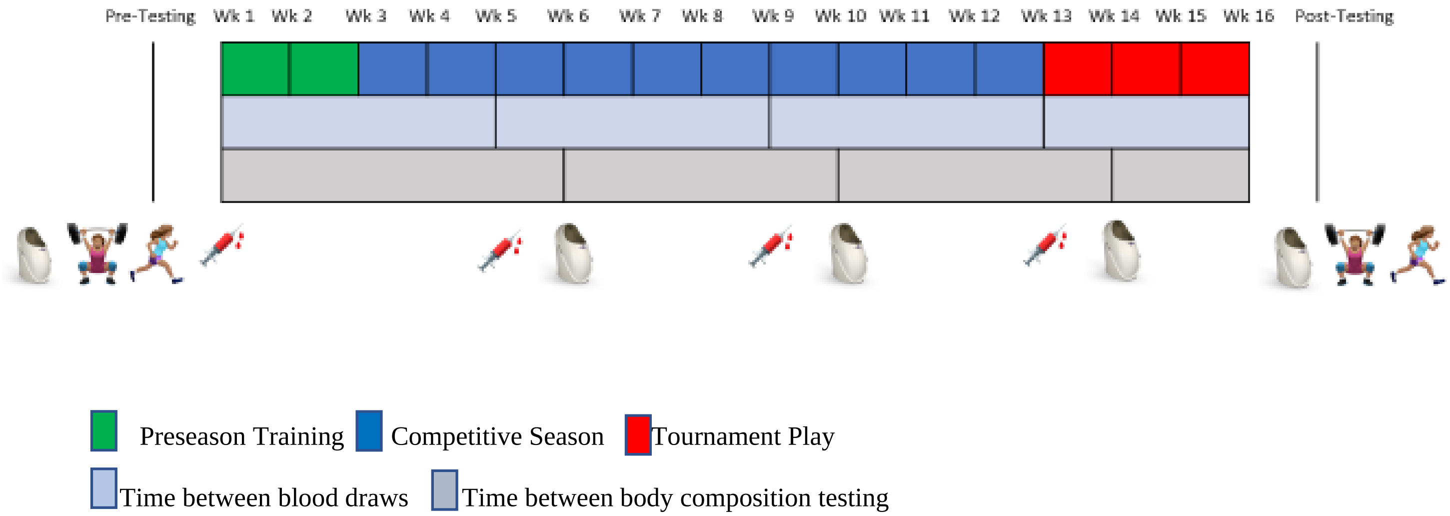
Performance Testing: Athletes reported to the Rutgers University Center for Health and Human Performance (CHHP) prior to the start of preseason (P1) and within one week following the final competitive match (P2) to complete a battery of fitness tests during a one-week period. Subjects were instructed to arrive euhydrated, 2-3 hours fasted, and having abstained from exercise 24 hours prior to testing. Body composition was assessed by air displacement plethysmography (4) via the BodPod (BOD POD, COSMED, Concord, CA) to determine percent body fat (%BF), fat free mass (FFM), and fat mass (FM), using the Brozeck formula (3). In addition to P1 and P2, body composition was also assessed at weeks 6, 10, and 14. Following a dynamic warm up, subjects were given three attempts for maximal countermovement vertical jump with both hands-on-hips method (VJHOH) as well as with arm swing method (VJ) assessed using the Just Jump system (Probotics, Huntsville, AL, USA) with the highest jump recorded. After completing power testing, a maximal graded treadmill exercise test (GXT) was used to measure maximal aerobic capacity (VO_{2max}) and ventilatory threshold (VT) via direct gas exchange using an indirect calorimeter (Quark CPET, COSMED, Concord, CA, USA). A speed-based protocol was used with stages that were MET-equated to the Bruce protocol. This protocol included two-minute stages at a constant 2% incline. The speeds were as follows: 6.4, 7.9, 10.0, 11.7, 13.7, 15.6, 17.1, 18.2, 19.8, 21.1 (km/h). Subjects continued the test with encouragement from lab staff until volitional fatigue. At least three of the following criteria were met verifying attainment of VO_{2max}: a leveling off or plateauing of VO_2 with an increase in exercise intensity, attainment of age predicted heart rate max, a respiratory exchange ratio greater than 1.10, and/or an RPE ≥18. Heart rate was continuously monitored using a Polar S610 heart rate monitor to accurately obtain maximal heart rate (HR_{max}) (Polar Electro Co., Woodbury, NY, USA).

Maximal strength testing was performed within a group setting under the instruction of the team's strength coach. Following a generalized dynamic warmup, 3RM tests were completed for squat (SQ), bench press (BP), and deadlift (DL) in that respective order and in accordance with NSCA guidelines (8).

Biomarker Collection and Analysis: Athletes reported to the CHHP in a euhydrated condition immediately prior to the start of pre-season for blood draws (T1), and every four weeks following (T2, T3, and T4). All blood draws were taken at the CHHP between 0700-0830 hours following an overnight fast; T2, T3, and T4 draws occurred ~18-36 hours following a game. Blood samples were centrifuged for 10 minutes at 4,750 rpm (Allegra x-15R Centrifuge, Beckman Coulter, Brea, CA, USA) and were shipped to Quest Diagnostics for analysis via LC-MS/MS-based assays. Samples were run in duplicate and the coefficient of variation (CV) for all biomarkers were between 0.5 – 7.5 %. Biomarkers in the analysis included CK, CRP, TCORT, E2, GH, IGF-1 and IL-6, free testosterone (FTEST), and total testosterone (TTEST).

Statistical Analysis: Delta area under the curve (DAUC) was calculated for the biomarkers and body composition variables using the trapezoidal method to account for seasonal changes adjusted for baseline. Pearson-product moment correlations were used to assess the relationships between biomarker changes and changes in performance and body composition with significance set at $P<0.05$. All analyses were conducted using SPSS Statistical Software (SPSS version 23; IBM) with significance set at $P<0.05$. Values are expressed as means ± standard deviation.

Figure 1: Timeline of Assessments



RESULTS:

As a team, no changes in VO_{2max}, VJ, and DL performance were seen from P1 to P2 ($P>0.05$). However, significant improvements in 3RM for SQ and BP performance were seen ($\Delta SQ=4.49\pm1.4$ kg; $P<0.05$; $\Delta BP=1.82\pm0.81$ kg; $P<0.05$). See *Table 1* for performance changes pre to post. No changes in FFM or weight were seen from P1 to P2 ($P>0.05$). A significant decline in %BF was seen from P1 to P2 ($\Delta\%BF=-1.44\pm0.62\%$; $P<0.05$). See *Table 2* for body composition changes pre to post.

Table 1: Preseason to Postseason Performance Changes

Performance Test	Preseason	Postseason
VO_{2Max} (mL·kg·min^{-1})	47.49 ± 4.0	47.80 ± 3.9
Vertical Jump (cm)	45.35 ± 5.4	46.34 ± 5.8
3RM Bench Press (kg)	43.33 ± 7.4	45.15 ± 5.1 *
3RM Squat (kg)	82.12 ± 11.6	86.61 ± 11.8 *
3RM Deadlift (kg)	84.55 ± 14.7	81.82 ± 16.5

Values are expressed as means and standard deviations
* Indicates a significant difference from pre to post

Table 2: Preseason to Postseason Body Composition Changes

Body Composition Test	Preseason	Postseason
FFM (kg)	53.762±1.4	54.841± 1.3
% BF	18.88±1.04	17.438±1.1*
Weight (kg)	66.26±1.4	65.84±1.3

Values are expressed as means and standard deviations
* Indicates a significant difference from pre to post

Biomarkers that correlate to performance and body composition variables are presented in *Table 3.* TCORT was negatively correlated with change in FFM (r=-0.48, P<0.05). TCORT also showed a positive correlation with changes in %BF that approached significance (r=0.39; P=0.08). Additionally, a negative correlation with TCORT and SQ change approached significance (r= -0.45; P=0.08). However, TCORT was positively correlated with change in VO_{2max} (r=0.47; P<0.05). IL-6 was negatively correlated with change in BP (r=-0.52; P<0.05) while IGF-1 was positively correlated with changes in DL (r=0.56; P<0.05). The correlations between TTEST and DL approached significance (r=0.46; P=0.10). No significant correlations were seen for biomarkers and changes in VJ/VJHOH (P>0.05). No significant correlations were seen for CRP, FTEST, CK, and measures of body composition or performance (P>0.05).

Within the biomarker analysis, a negative correlation between TCORT and E2 trended towards significance (r=-0.36; P=0.10). GH was positively correlated with changes in IGF-1 (r=0.48; p<0.05) and changes in DL (r=0.56; P<0.05). IL-6 positively correlated with change in CRP (r=0.76; P<0.05). IGF-1 positively correlated with changes in FTEST (r=0.60; P<0.05) and E2 (r=0.49; P<0.05). The correlation between E2 and FTEST approached significance (r=0.45, P=0.06). Biomarker correlations are presented in *Table 4.*

Within performance and body composition measures, a trend was seen for a positive correlation with VJHOH and SQ performance (r=0.43; P=0.097). Additionally, a trend was seen for a negative correlation between VO_{2max} and FFM (r=-3.73; P= 0.106) and a positive correlation with %BF (r=0.420; P=0.065). Performance correlations are presented in *Table 5.*

Table 3: Biomarkers and Performance/Body Composition Correlations

		FFM	%BF	BW	SQ	BP	DL	VO_{2max}	VJ	VJ_HOH
TCORT	r	-0.477*	0.390#	0.089	-0.449#	-0.049	0.116	0.467*	0.260	0.032
CK	r	0.021	-0.187	-0.198	-0.194	-0.429	0.429	0.011	0.025	0.19
E2	r	0.198	-0.276	-0.205	0.015	-0.180	0.387	-0.165	-0.304	-0.277
GH	r	-0.002	-0.085	-0.092	0.160	0.346	0.560*	0.211	-0.131	-0.256
CRP	r	0.135	-0.076	0.027	0.369	-0.287	-0.223	0.148	0.287	0.347
IGF-1	r	-0.027	-0.050	-0.114	0.117	-0.028	0.558*	0.119	-0.182	-0.278
IL-6	r	0.112	-0.088	-0.014	0.083	-0.521*	-0.198	0.043	0.077	0.303
FTEST	r	0.309	0.014	0.251	-0.028	0.119	0.316	-0.086	-0.179	-0.291
TTEST	r	-0.005	0.088	0.068	-0.196	-0.107	0.456#	0.272	-0.078	-0.230

*** =**Indicates a significant correlation between biomarkers and performance

#=Indicates a trend towards significant correlation between biomarkers and performance

Table 4: Biomarker Correlations

		TCORT	CK	E2	GH	CRP	IGF-1	IL-6	FTEST	TTEST
TCORT	r	1								
CK	r	0.148	1							
E2	r	-0.362#	0.133	1						
GH	r	0.301	-0.317	0.02	1					
CRP	r	0.05	0.082	-0.044	-0.127	1				
IGF-1	r	0.045	-0.002	.491*	.480*	-0.122	1			
IL-6	r	0.108	0.099	0.041	-0.177	.756*	-0.03	1		
FTEST	r	-0.13	-0.197	0.426#	0.287	-0.055	.600*	0.113	1	
TTEST	r	0.22	0.097	0.253	0.325	0.003	.718*	0.082	.747*	1

*** =**Indicates a significant correlation between biomarkers

#=Indicates a trend towards significant correlation between biomarkers

Table 5: Performance and Body Composition Correlations

		FFM	%BF	BW	SQ	BP	DL	VO_{2max}	VJ	VJ HOH
FFM	r	1								
%BF	r	-0.533*	1							
BW	r	0.205	0.711*	1						
SQ	r	-0.007	-0.067	-0.077	1					
BP	r	0.263	0.253	0.569*	0.133	1				
DL	r	0.161	-0.332	-0.238	-0.184	-0.115	1			
VO2max	r	-0.373#	0.420#	0.062	0.005	-0.155	0.052	1		
VJ	r	-0.329	0.227	0.057	0.231	0.123	-0.221	0.095	1	
VJ HOH	r	-0.265	0.035	-0.157	0.429#	-0.230	-0.305	0.203	0.560*	1

*** =**Indicates a significant correlation between performance markers
#=Indicates a trend towards significant correlation between performance markers

DISCUSSION:

After adjusting for individual baseline values, changes in the anabolic biomarkers, GH and IGF-1, were positively associated with DL strength measures. Additionally, greater changes in markers of stress and inflammation, such as IL-6 and TCORT, were negatively associated with strength and FFM measures. Interestingly, TCORT was also positively correlated with improvements in VO_{2max}. These findings support a relationship between changes in anabolic and catabolic markers and changes in performance and body composition in female collegiate athletes throughout a competitive season.

These results suggest biomarker changes during a season may be related to performance and body composition changes and, as such, may be useful in the evaluation of athlete adaptation and readiness. A lack of adequate recovery from soccer-related training stressors may result in a proinflammatory state exhibited by high concentrations of cytokines. One marker in particular, IL-6, which is often associated with muscle damage (1) and decreased muscular strength and physical function (6, 19), was negatively correlated to BP in the current study. Likewise, chronic elevations in TCORT promote a catabolic environment making it difficult for an athlete to build or maintain muscle or to recover from training (13), which was reflected in the negative correlations with FFM and SQ. Further, low levels of the key anabolic hormones, GH and IGF-1, have been associated with low muscle mass and strength (Stenholm et al 2010; Araujo et al 2008) as these hormones play a role in regulating muscle growth (7). Monitoring changes in anabolic and catabolic hormones may provide an opportunity to intervene before decrements in performance can occur.

A power/endurance tradeoff (12) is typically noted in times of increased aerobic activity and might be seen during times of high volumes of soccer-specific training, particularly if coupled with an insufficient resistance training stimulus. TCORT showed a negative correlation with both FFM and 3RM SQ performance which may be indicative of insufficient recovery in certain individuals; however, interestingly, increases in TCORT were also correlated with improvements in VO_{2max}. Intensive aerobic training can improve endurance performance reflected by improvements in VO_{2max} (2). However, if intensive aerobic training in not met with adequate recovery, altered hormonal responses including increases in TCORT may persist. The positive correlation between VO_{2max} and TCORT may be a result of an overload of aerobic training, although more research is needed to determine the underlying mechanism.

While soccer training alone has no effect on maximal strength (16-18) and large volumes of endurance training and concurrent resistance training may inhibit strength adaptations (12, 18) negatively impacting force production and rate of force development, resistance training has been shown to be an effective way to improve strength (Latham et al 2003) or, at the very least, maintain initial preseason strength levels. During the season, limited time is available to devote to resistance training, yet incorporating resistance training into the competitive season training program when increased soccer-specific aerobic work dominates is imperative to the maintenance of FFM and strength. Ronnestad et al demonstrated that one strength training session per week in professional soccer players during the first 12 weeks of the season maintained the initial gain in strength achieved during the preparatory period (18). In the current study, as a team, no decrements in strength or FFM were observed with the implementation of just a one-day per week periodized strength and conditioning program from preseason to postseason. The results of this study support the ability of this type of training to offset decrements in strength and FFM often seen during the competitive season. Given the in-season time demands on the players, this is an important finding due to the feasibility of implementation and underscores the utility of this type of “supplemental” training. Additional studies in female collegiate athletes are warranted to show the impact of a collegiate soccer season on various strength and fitness variables in athletes participating in a periodized strength and conditioning program.

Considering the female athlete specifically, one area of particular interest is E2 and its relationship to performance outcomes. Although no correlations between E2 and performance or body composition outcomes were seen, changes in TCORT were negatively correlated with changes in E2. Lowered E2 in conjunction with increased TCORT has also been associated with relative energy deficiencies caused by an inadequate caloric intake coupled with high exercise energy expenditures (14). Low energy availability is shown to alter levels of various hormones leading to disruptions in optimal health and performance (14, 21). Further, IGF-1 was positively correlated with change in E2 while correlations between E2 and FTEST approached significance.

IGF-1 has been shown to reflect energy status, with a combination of low energy intake and high volumes of training shown to reduce IGF-1 (23). Ultimately, the positive correlation with IGF-1 and E2 may be the result of a more favorable anabolic environment while declines in IGF-1 in conjunction with E2 may be a response to an energy deficit (10, 15, 21) associated with the high caloric expenditures characteristic of soccer athletes. More research is warranted to determine the impact of energy availability on biomarker changes as well as performance outcomes. It is worth noting that, although biomarker monitoring did occur every 28 days to account for a typical menstrual cycle, the individual menstrual status of the athletes was not "controlled". Given the nature of this applied study, researchers sought to determine a typical hormonal environment that exists for a collegiate team during the season. Further, since menstrual cycle does not control playing time, we believe that this is an accurate representative profile of a female collegiate soccer team.

It is important to consider that increases or decreases in single biomarkers often do not occur independently as illustrated by various convergent hormonal pathways (19). This is further evident in the correlations amongst biomarkers in this study, such as the associations of CRP and IL-6 along with IGF-1 and GH. Therefore, rather than looking at one biomarker to determine the effects of training load on an athlete, it may be more advantageous to analyze multiple anabolic and catabolic biomarkers together when assessing adaptations to a training program.

It would appear that biomarker monitoring may be a useful tool in team sports to determine an individual athlete's recovery needs and player readiness status. The use of biomarkers presents an objective means to evaluate the physiological response of the athlete to training as this method may better indicate exercise related stress independent of other factors, such as motivation, which may confound the results of performance tests or other subjective measures (1).

Practical Application:

The results of this study indicate a greater change in anabolic hormones from baseline were related to improvements in measures of strength, specifically DL performance, whereas greater changes in catabolic or inflammatory hormones were related to declines in FFM, BP, and SQ. Further, VO_{2max} was positively correlated with TCORT indicating that an overload of aerobic soccer specific training may predominate during the season. Biomarker monitoring may be useful to detect individual player's physiological response to a given training load and serve as a guide to strength and conditioning coaches regarding periodization of strength training programs to optimize performance. Additionally, the use of multiple biomarkers may better detect training stressors related to recovery status and performance outcomes rather than relying on performance tests alone. Through properly structured strength and conditioning programs and in conjunction with appropriate assessment methods, it may be possible to avoid the power/endurance tradeoff and loss of FFM often seen over the course of the season in these athletes. While the results are likely to maximize on-field performance.

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