"EFFECTS OF TRAINING LOAD AND PHYSICAL STRESS ON PERFORMANCE

AND BIOMARKERS INDICATIVE OF HEALTH, NUTRITION, RECOVERY, AND

PERFORMANCE IN COLLEGIATE MALE AND FEMALE ATHLETES"

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ABSTRACT OF THE DISSERTATION

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AND BIOMARKERS INDICATIVE OF HEALTH, NUTRITION, RECOVERY, AND

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Athlete tracking and monitoring have made recent technological advancements to encompass internal physiological markers (heart rate, heart rate variability, biomarkers) and external workload markers (GPS, accelerometry). Heart rate monitoring is a commonly used technique to monitor on-field training load (TL) which represents internal "effort" to complete a physical task. This effort is quantified as TL via algorithms based on heart rate response specific to each athlete or as exercise energy expenditure (EEE). Unfortunately, many TL monitoring techniques (including heart rate monitoring) only account for what is happening on the field and are unable to capture off-field stressors. Implementation of additional monitoring tools, such as blood biomarkers, can give insight as to athlete's health, performance, and recovery status by encompassing both on and off the field stressors. Blood biomarkers can provide a comprehensive analysis of the physiological and biochemical response to TL that would otherwise be undetected through the more traditional monitoring techniques. Therefore, the purpose of

this dissertation is to evaluate the cumulative effects of season long TL in male and female collegiate soccer players. Our primary hypothesis is the training load and blood biomarkers will change over the course of the season, the secondary hypothesis is there will be a difference in these parameters between males and females.

AIMS AND OBJECTIVES

Aim 1:

The primary aim of this study was to evaluate the effects of a 28-day training period in Division I female field hockey players on a panel of hormonal and biochemical markers as a result of the accumulated stress. This period encompasses the most demanding portion of the competitive season for fall athletes including two weeks of preseason and the first two weeks of the competitive season prior to the start of the academic year. All training sessions were monitored via the PolarTeam2 system which provided heart rate-based training load. The secondary aim was to identify physical and performance characteristics that best predict changes in hormonal and biochemical markers related to stress and recovery. This study provided real world data on female athletes, highlighting possible biomarkers and physical characteristics to use as indicators of excessive stress in this population.

Aim 2:

The primary aim of this study was to evaluate the cumulative effects of season long training load in conjunction with changes in performance and blood biomarkers associated with health, performance, and recovery in Division I female soccer players. This study encompassed the entire competitive season including preseason, regular season, conference tournament, and NCAA tournament. All training sessions were monitored via the PolarTeam2 system to provide heart rate-based training load. The primary outcome of this study was to evaluate biomarker changes in high-level female athletes throughout the season. These data provided additional insight into the effects of season long stress and training load on physiological changes female athletes experience.

These data are unique in evaluating a top tier collegiate program that made it to the final four, making this the longest possible time frame a college soccer athlete could have played in a season. Furthermore, this data provided one of the most extensive biomarker evaluations in female athletes over an extended time period providing critical data on free living athletes.

Aim 3:

The primary aim of this study was to evaluate the cumulative effects of season long training load in conjunction with changes in performance and blood biomarkers associated with health, performance, and recovery in Division I male and female soccer players. Training load incorporated both an internal and external load component via heart rate and GPS/accelerometry from the PolarPro system. This study encompassed the entire competitive season including preseason, regular season, conference tournament, and NCAA tournament play. The primary aim of this study was to evaluate the effects of season-long stress in various biomarkers in both male and female athletes. The secondary aim was to evaluate the differences in male vs. female soccer players to investigate potential differences in both workload factors as well as any physiological changes seen in the biomarkers. This study provided real world data that can lead to sex specific recommendations on biomarker utilization. The concurrent analysis of both males and females under similar stress and training loads provided a unique insight on physiological response to season long stress.

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1.1 Introduction

Hans Selye developed the idea for the primary stress response and adaptation through the development of the general adaptation syndrome (GAS) in 1936 (1). This idea has evolved and been applied to many physiological processes including aspects of sports performance through adaptation to the stress of exercise. The GAS has three distinct phases which include the alarm stage, resistance stage, and the exhaustion phase (1). When the body experiences a stressor that disrupts homeostasis, i.e., exercise, the body enters the first alarm stage where there is an initial decrease in performance to mobilize resources and react to the stressor. In response to the alarm stage, comes the resistance stage where the body will increase performance to overcome the stressor. If the stressor continues for too long, the body enters the third phase of exhaustion, compromising performance and other physiological functions. This GAS forms the basic structure for utilizing exercise to increase performance as well as the downstream effects of the exhaustion phase. When GAS is applied correctly, the stress response to the exercise will cause a decrease in performance (alarm), followed by improvement in performance (resistance) (1). This process can be repeated throughout training and exercise to increase athletic performance gradually. When this technique is performed incorrectly, i.e. the training load (TL) is excessively high coupled with inadequate rest and recovery, this can transition to non-functional overreaching (NFOR) representing the exhaustion phase. Non-functional overreaching is a short-term decrease in performance that may or may not be accompanied by both physiological and psychological disruptions which can take anywhere from weeks to months from which to recover (2–4). If this inappropriate TL persists for a long duration, NFOR can develop into the overtraining syndrome (OTS). Overtraining syndrome is a prolonged maladaptation due to inadequate

rest and recovery from training stress resulting in decrements in performance along with unfavorable changes in mood and physiological response (2–4). Maladaptations include disruptions in sleep, appetite, irritability, restlessness, staleness, lack of motivation and depression. Further, perturbations of the hypothalamic-pituitary-adrenal axis (HPA axis), hypothalamic-pituitary-gonadal axis (HPG axis), sympathetic-adrenal-medullary axis, and immune system will occur (2,4). Overtraining syndrome has major physiological maladaptations that can occur, which require months to years for recovery (3,5). Interestingly, the dose-response relationship for the transition from NFOR to OTS is dependent on the individual response to a TL as well as the intensity of the TL.

1.1.1 Hypothesis

There have been several hypotheses for the primary cause of OTS. Theories include: glycogen hypothesis, central fatigue hypothesis, glutamine hypothesis, oxidative stress hypothesis, autonomic nervous system hypothesis, monotony theory, hypothalamic hypothesis, and the cytokine hypothesis (6). Of these theories, the glycogen hypothesis, glutamine hypothesis, monotony theory, hypothalamic hypothesis, and the cytokine hypothesis have received more attention in the literature (7). The glycogen hypothesis states that during times of chronically high TL, athletes are unable to maintain sufficient glycogen stores due to an inability to maintain adequate caloric intake, resulting in changes in mood and performance (6,7). Adaptations of this theory have been developing in the literature through the concept of energy availability, stating that caloric intake does not compensate for energy expenditure resulting in decreases in performance and physiological changes. Using this theory, the primary treatment for OTS would be increasing caloric intake to maintain muscle glycogen throughout the athlete's training,

though it may not account for all of the physiological changes seen. Along with the glycogen hypothesis, the glutamine hypothesis has also received consideration as a viable mechanism for OTS. This theory states that there is a decrease in circulating glutamine, which is the primary fuel source used by lymphocytes, which decreases lymphocyte function leading to increased infection and inflammation (7). While this theory provides mechanisms for increased infection seen with OTS, it does not account for many other symptoms such as the various decreases in performance and other physiological alterations. Both theories have significant gaps in the explanation of all the symptoms seen with OTS, showing the complications in finding a common mechanism for the response to inappropriate and prolonged TL.

Currently, the soundest theories on the mechanisms of OTS include the hypothalamic and cytokine hypotheses. The hypothalamic theory states that the prolonged TL with inadequate recovery results in disruptions in both the hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary gonadal (HPGn) axes, yielding changes in the physiological response to the TL and recovery (6). Alterations in hypothalamus function and feedback mechanisms result in downstream disruptions in hormones, such as cortisol, testosterone, thyroid hormones and growth hormone, which play significant roles in muscle recovery and adaptation, immune function and ultimately performance (8). Unfortunately, the data supporting this theory is inconsistent in the findings due to variations of study designs seen in the literature. The cytokine theory proposes that repetitive trauma from a prolonged TL with reduced recovery causes muscle damage (7). This muscle damage results in increases of local inflammatory factors including cytokines such as IL-1β, TNF-α, and IL-6 (7). This theory proposes the

mechanisms behind OTS are the cytokines ability to act on various organs and tissues including the liver and higher brain centers, such as the pituitary gland, that affect the hormonal responses stemming from these systems (7). The ability to act in higher brain centers also provides a possible mechanism for this theory to manifest in changes in the psychological mood profile as well. Furthermore, individuals who experience OTS have similar brain chemistry as those who have clinical depression (the interleukin hypothesis for major depression), stemming from alterations from systemic inflammation and increased circulating cytokines from high TL (7). Additionally, various cytokines including IL-1 and IL-6 can significantly alter the HPA and HPGn axis, which can prove to be a possible mechanism for many of the hormonal responses seen with OTS (7,9).

Not all the theories presented are rooted in the physiological change to a TL. The monotony theory states the psychological monotony of repetitive training can cause performance decrements, physiological changes, and the staleness seen in OTS (7). A constant TL without variation in the program is thought to lead to the symptoms of OTS according to this theory. Much debate surrounds this theory raising concerns if repetitive training can produce the physiological alterations associated with OTS (8). Additionally, this theory leaves questions of intensity and duration of the training for this theory to be considered. While all the given theories have strengths and supporting research, each has various holes in the explanation of OTS and cannot individually account for all the changes seen in OTS. Because there is an individualistic nature to each physiological response to a given TL, it is difficult for any single hypothesis to universally apply to all athletes and individuals who experience NFOR and OTS. It is more reasonable to accept

a combination of these various hypotheses that are acting to contribute to the onset of OTS.

1.1.2 Prevalence

The current research on the prevalence and which populations or sex are at the most risk for the development of OTS is sporadic with little agreement. The general finding has shown that 5-10% experience OTS, while up to 45% of athletes may experience aspects of NFOR (8,10). Furthermore, individual sports athletes such as swimmers, runners, and cyclists are at a higher risk due to the high TL and repetitive nature of these sports. Team sports have not received the same attention in respect of tracking the incidence of OTS, primarily due to the difficulty in managing a team TL rather than the individual athlete. Interestingly, males experience OTS (17%) at a higher rate than females (11%), (8).

Though NFOR/OTS is a widely accepted phenomenon, replicating these demands and developing a cheap and easily utilized tracking and prevention system has yet to be determined. There is no current recommendation for the prevention of OTS, but several techniques have been used to track the progression of functional over reaching (FOR) to NFOR and finally to OTS. These techniques include periodic performance testing, monitoring physiological TL, tracking perceptual changes, as well as the impact on biomarkers. While each method has specific strengths and weakness, the combination of all three seems to hold the most promise with an emphasis being on biomarkers to reveal predictive potential. The current retrospective analysis of various biomarkers in the evaluation of NFOR/OTS does not provide adequate bases for the development of tracking techniques to prevent NFOR/OTS from developing. It is important to note that

this retrospective analysis is not ideal, however it is the only ethical analysis of OTS in humans. Many of the laboratories that seek to "over train" subjects fail in comparison to the real-world TL. These TL cannot be ethically replicated in the laboratory setting, forcing researchers to focus on the animal model as well as the retrospective analysis of human subjects making the development of both the mechanisms of action as well as prevention guidelines a challenging task.

1.2 Performance

Monitoring the potential detrimental changes in performance associated with NFOR/OTS has the most direct implication on the athlete yet poses several challenges. Performance measures are essential for athlete monitoring but do not hold predictive characteristics, meaning if an athlete has reduced performance, they have already transitioned towards NFOR or OTS. The performance tests used range from quick field tests to a full battery of laboratory testing, depending on the sport. The current research has shown that following an intensified/overload training period, there is a decrease in time trial performance, max speed, total distance covered and vertical jump in male triathletes, cyclists, and resistance trained athletes (11–15). Many of the studies mentioned above claim to have put their athletes in an "overtrained state" in as short as one week to up to four weeks using the respected intensified training protocols (11,13,15). However, following a one to two week taper many of these studies show a supercompensation in performance, more likely showing these athletes were on the spectrum of functional OR to NFOR rather than true OTS (11,12,15). With the decreases seen in performance in these studies, which do not depict OTS, it is understandable to expect significant decrements in performance with OTS. These results, though valuable,

do not apply as readily to team sports where monitoring performance encompasses the entire team making testing problematic.

Testing for NFOR/OTS poses difficult problems outside of the current research findings. Testing is critical to have an accurate baseline and maximal performance values, as well as needing to be repeatable, reliable, and not being an unnecessary burden on the athlete. Given these needs, lab-based testing protocols are the most logical answer, but many athletes and teams do not have access to these tools. If an athlete does have access, testing should be performed several times throughout the year to track if these players are experiencing performance decrements. When considering team sports, this can be challenging, especially if the team lacks the time or resources to test throughout the competitive season, especially with a congested match schedule. If testing can only be performed pre-season and post-season, any decrements will have already happened with no opportunity for prevention, rather than merely being to identify that it did happen retrospectively. Reducing the quality of the performance test conducted minimizes the reliability of data collected. Highlighting the challenges of using performance as an indicator of the progression to NFOR/OTS, as it must be a test that is cheap, fast, reliable, repeatable, and not overly taxing on the athletes to perform throughout their training. Unfortunately, the current state of performance testing does have a practical answer to tracking the progression of NFOR and OTS due to the retrospective nature of identifying decreases in performance as NFOR/OTS. Therefore, the burden of monitoring the progression of NFOR and OTS primarily falls on tracking other indicators such as monitoring systems and physiological or psychological changes that precede the decreases in performance.

1.3 Monitoring Training Load

To achieve the possibility of preventing NFOR and OTS, TL needs to be

monitored and tracked to have a controllable and manipulatable variable that is one of the

underlying causes of the problem. Training load is quantified in a variety of different

ways, with the most common being GPS tracking and heart rate monitoring (HRM).

Heart rate monitoring has been utilized as a TL monitoring technique since its emergence

in the 1980s (16). Heart rate monitoring is a quantification of internal workload or effort

put forth by the individual to perform the given task (17). A recent meta-analytic review

assessed the capability of HRM to monitor OR and OTS which concluded that the correct

interpretation of these data should be used in conjunction with other signs, symptoms,

and tests to be meaningful (18). This conclusion provides further support for the need of

multiple analyses and evaluations to properly determine, diagnose and possibly even

prevent NFOR and OTS. Heart rate monitoring serves to track the effort of a given task,

while GPS can provide data on the physical workload. GPS provides metrics such as total

distance and distance at various speeds or speed zones (19). Recently, GPS has been

coupled with accelerometry to offer additional variables such as accelerations,

decelerations, and sprint performance to expand the scope of the physical work (19). The

combination of HRM and GPS provides a complete picture of effort and physical work

athletes are performing to give the best metric for athlete tracking.

With the recent advancements in technology, it is becoming easier to accurately

measure indicators of physiological readiness, outside of on-field TL, such as heart rate

variability (HRV). Heart rate variability indicates the autonomic balance of the heart

which shows the parasympathetic tone relative to sympathetic activity (3). Currently,

there are very few studies evaluating the effects of NFOR/OTS on aspects of HRV (3). A

meta-analysis evaluating the use of heart rate as a monitoring tool for OTS showed mixed

results. Short-term overload training decreased HRV with a higher resting HR and lower

maximal HR, while a more extended overload training did not have the same results (18).

In a study that evaluated the effects of a three week intensified training (one-week taper)

then a three week overloading period to "overreach" male triathletes, there was a decrease

in performance, HRV at rest, and HR during the exercise showing the adverse effects on

parasympathetic activity (11). It is important to note that following the overload period, a

one-week taper resulted in increased performance, HRV at rest, and HR during the

exercise indicating that athletes were more on the line of overreached, possibly NFOR,

rather than over trained. Regardless of the training state, this study shows the potential

adverse effects of NFOR/OTS on resting HRV and HR during exercise. This study did

conclude an essential notion in the measurement of HRV showing that weekly averages

of HRV are a better indicator of athlete readiness than daily measures to eliminate the

day to day variation that is seen (11). While HRV holds excellent potential for athlete

monitoring to encompass both on and off field readiness, logistics, accuracy, and

interpretation of the data is still in beginning phases and needs further development to

apply to the everyday athlete/coach in both an individual and team setting.

A new stance on athlete maintenance that is growing in popularity is the concept

of acute: chronic workload ratio. This concept involves acute workloads (i.e., workload in

a single day or multiple day average) as a ratio of chronic workloads (i.e., rolling average

of multiple week workloads) (20). The workload-injury paradox utilizes this concept

which states that when acute workloads and chronic workloads are similar, the result is a

low acute: chronic workload ratio which decreases risk for injury (22, 23). If the athlete

has a low chronic workload and begins a series of high acute workloads, this ratio

increases, as does injury risk (22, 23). However, regardless of workload ratio, the

absolute workload must also be evaluated in total athlete status. For example, if the

chronic and acute loads are too low, there will be a detraining effect. Likewise, if both

acute and chronic workloads are very high, this will result in breakdown and increased

risk of injury (22). Therefore, the "Goldilocks principle" should be applied to TL

whereby there is a point at which acute: chronic workload is in an optimal zone. In

summation, the total workload must be appropriate to accomplish the intended training

outcome (24). Though this concept has increasing applicability, it is not without

limitations aside from the "Goldilocks principle". First and foremost, this concept loses

validity when leaving the professional athlete model and moving towards a college or

youth athlete. The college athlete, for example, enters their sports preseason with an

unknown chronic TL due to NCAA restrictions on coaches' involvement and contact

during their offseason. The nature of a college preseason, which is limited to a matter of

weeks, forces coaches to utilize multiple training sessions per day, compounding the

problem of NCAA restrictions. The unknown chronic TL coupled with the inevitably

high acute TL often associated with preseason leaves the concept impractical in this

population.

1.4 Perceptual Measurements

Along with the various TL measurements, many researchers and

recommendations use perceptual well-being and sleep analysis. Overall, the recent

position stand and recommendations claim that the perceptual well-being analysis has

proven to be the most reliable way to track NFOR/OTS (21). Current research has shown that with increases in TL towards NFOR/OTS, there is a negative shift in athletes mood profiles resulting in less vigor, more mood disturbances, lack of concentration, increased anxiety, increased irritability and signs of depression (6,21). Along with the mood disturbances, there is often disruptions in sleep and resulting in restlessness and the feeling of being unrefreshed upon waking (6). Though individual studies have found success in using perceptual measurements, a meta-analysis showed inconsistent results across the OTS literature concluding that these measures are not the most applicable for OTS monitoring (22). The current research base on subjective measures of mood provides an inherent flaw in the inability to blind the subjects to an increase in TL in a lab-based setting of many studies. Additionally, this does not indicate that all athletes who experience OTS will have changes in mood and perceptual measurements (22).

With the subjective nature of measuring perceptual measures and sleep come many possibilities of error. Both require honesty and athlete buy-in to provide reliable data that genuinely reflect mood and sleep profiles. However, sleep analysis is becoming a more objective measurement with the development of wearable technology. It is reasonable to understand a change in subjects' mood and outlook on the TL with no attainable goal or desired outcome. An athlete may increase TL working towards a major competition or game, which may mask changes in mood. This goal-orientated increase in TL may have altered effects of the subjective measurements of mood making these measurements interpretation difficult.

It is important to note the iceberg profile in athletes which states that athletes typically report lower scores on tension, depression, anger, and fatigue and a higher vigor

score when compared to the population average (10). Another common issue with psychological markers is the tendency for athlete's to answer to portray themselves in a positive way to coaches, trainers, and researchers to avoid punishment (3). The opposite response, which is less common, is for athletes to falsely answer to reduce the TL, using the measures to benefit themselves (3). These issues raise the simple fact that athletes can and will lie to get what they believe are the most beneficial results, whether that be increased perception or decreased TL. While these subjective measurements are critical components for the diagnosis and prevention of NFOR/OTS, they need to be analyzed carefully regarding the possible issues and flaws that come with subjective measures.

1.5 Biomarkers

The most objective measurement to the individual response to a TL seems to be the use of various biomarkers that encompass general health markers, nutrition, performance and recovery in conjunction with another workload monitoring load to understand both the physical TL as well as the physiological response to the given TL. Biomarkers can provide an unbiased snapshot of the athlete and how they're responding to training. Furthermore, biomarkers provide a more encompassing analysis that includes the stress on the field as well as the extra cumulative stress they experience in their lives such as work, school, personal, social, dietary, and sleep. All of which have an impact on the total physiological response to training and play into the development of NFOR/OTS. To date, the current literature has used a wide variety of biomarkers including hormones, markers of general health, and nutritional markers. There is no agreed upon gold standard for a biomarker panel. More research in each marker is necessary to reveal a possible predictive biomarker or panel.

1.5.1 Cortisol

A common marker across the research in NFOR and OTS research has been cortisol, both the free and total measurements. During times of increased TL and less than optimal recovery, there is an elevation in resting cortisol due to a disruption in homeostasis and the subsequent stress response. If this progresses to OTS, the stress response can become desensitized, resulting in a decreased cortisol response (3). This disruption of the HPA axis provides support to the hypothalamic hypothesis for OTS due to disturbances in the feed forward mechanism due to excessive stress manifesting in the changes seen in cortisol (5). Athletes in the over trained state often have a decreased cortisol response to a given exercise with a significant rise in the resting values in the early stages of OTS (23). As athletes further advance into the progression of OTS, there are significant alterations to HPA function resulting in diminished levels at rest and exercise (22). Additionally, a meta-analysis showed a reduction of cortisol when compared to average values highlighting the bottoming out during OTS (22). Additional measurements of corticotropin-releasing hormone (CRH) and adrenocorticotropic hormone (ACTH) could further explain the cortisol response. Corticotropin-releasing hormone is released by the hypothalamus and stimulates the anterior pituitary to produce ACTH which is the primary regulator of cortisol secretion from the adrenal glands (9). This pathway exhibits a pulsatile activation and is regulated through a negative feedback loop stimulated by circulating cortisol (9). Increases in cortisol have also been linked to increases in circulating cytokines in response to the high TL; these cytokines stimulate the HPA axis to increase cortisol as well as catecholamine secretion from the adrenal glands (7). Evaluating all three of the hormones active in this pathway may provide a

complete evaluation of how OTS is affecting this pathway with a decreased response to exercise as well as increased resting levels of cortisol.

A recent consensus statement has suggested that resting cortisol is not a useful measurement due to seasonal rhythmicity of resting cortisol in trained men as well as 24 hour urinary free cortisol remaining in normal levels compared to aged matched sedentary induvial (3). Alternatively, this has not been evaluated in many real-world studies or in females (3). The lack of sex-specific ranges and/or the effects of menstrual disruption and oral contraceptives on the HPA-axis is not well established in the NFOR/OTS literature making the evaluation of female athletes difficult (24). The current literature base exhibits many flaws, in using different athletes, varying TL, how the measurement is being taken (saliva, urine, blood), what time the measurement is taken, and sex.

1.5.2 Sex Hormones

Testosterone is one of the most common biomarkers evaluated in association with OTS, especially in the male athlete. The anabolic effects in response to exercise are primarily controlled by the HPGn axis in the release of gonadotropin-releasing hormone (9). The actions of testosterone are critical for athlete development and management, including protein synthesis, reducing protein breakdown, glycogen replenishment and red blood cell production (25,26). Current research has proved to be contradictorily showing both decreases as well as no change in testosterone (8). In general, the standard view of the effects of OTS on testosterone is a resulting decreased circulating bioavailable testosterone indicating a reduced anabolic potential (25). Additionally, a systematic review evaluating the hormonal response to OTS found that two-thirds of individuals

who experienced OTS had reductions in testosterone, with 14.3% of functionally overreached athletes having low testosterone (27). To further evaluate the changes in testosterone, it is often used in combination with cortisol to calculate the testosterone:cortisol ratio, representing the anabolic to catabolic balance. A decrease in this ratio is often associated with inappropriate and prolonged exercise intensity and duration shifting the physiological response to favor a more catabolic state. Furthermore, changing this ratio can result in decreased performance, energy, strength, and recovery that can impair the athlete's performance and health.

Estrogen and luteinizing hormone (LH) hold significant relevance for the female athlete but have yet to be evaluated as biomarkers of OTS. Overtraining syndrome can cause the suppression of estrogen which can lead to the disruption of the menstrual cycle or the development into oligomenorrhea and amenorrhea, one of the components of the female athlete triad (24). The depression of estrogen is also affected by increased caloric expenditure, if caloric intake is inadequate there is a decrease in energy availability, which can cause the suppression of estrogen and dysregulation of menstrual function (28). Estrogen becomes difficult to measure when an athlete is on a contraceptive, which can mask the hormonal levels depending on the composition of the contraceptive. Along with estrogen, OTS can also reduce LH, which is associated with decreased body fat and often accompanied by decreases in estrogen in females (29). In males, a decrease in LH results in a reduction of testosterone in that LH is the primary regulator of testosterone production from the Leydig cells (9).

Furthermore, prolactin, which has been shown to increase in response to stress, hypoglycemia, and physical exercise, has relevance in female athletes due to its

suppressive effects on estrogen when elevated (30). Similar results have been noted in

female athletes in an acute setting, in which findings show an increase in prolactin that

correlates with an increased EE (31). These markers hold significant relevance, especially

in the advancement of research on the female athlete with OTS. More research is needed

to develop normative values as well outside influencers such as oral contraceptives and

the responses to OTS and chronically high TL.

Alternative measures include precursors such as dehydroepiandrosterone (DHEA)

as well as transporters like sex-hormone binding globulin (SHBG). DHEA is an

intermediate in the biosynthesis of both the androgen and estrogen sex steroids (32). OTS

has been shown to have a depressive effect on DHEA which reflect the downstream

hormones (25). SHBG binds to testosterone and estradiol for transport and is thought to

have a protective effect on the sex hormones (25). Very few studies have measured

SHBG, though one has shown an increase with previously over trained athletes (27).

Both alternative measures show potential in both male and female studies in that the

combination with the primary sex hormones can provide the most comprehensive

analysis of the physiological response to OTS for the primary sex hormones in both

sexes.

1.5.3 Growth Hormone and IGF-1

Growth hormone (GH) and its downstream effects of insulin-like growth factor I

(IGF-1) may also play a significant role in biomarker analysis. Growth hormone is

another anabolic hormone produced in the anterior pituitary, with its primary anabolic

function being the stimulation of IGF-1 from the liver. Growth hormone positively

affects protein synthesis, protein sparing, gluconeogenesis, and the conversion of T4 to

T3 (9,25,33). The effects of GH is the downstream activator and stimulation of the production and release of IGF-1 that provides the primary protein synthesis (33). Despite the known anabolic effects associated with GH and IGF-1, these biomarkers are rarely evaluated. With OTS, there will be a possible decrease in GH and a potential reduction in circulating IGF-1. Alternatively, it is essential to consider the stimulation of muscle derived IGF-1 that is stimulated by the exercise itself which could mask the decreases otherwise observed in GH (25,33). One of the few studies to evaluate GH in over trained cyclists showed there was no change in resting GH, but a decrease in GH secretion after exercise. Additionally, the reductions in IGF-I seen can be a result of glycogen depletion, relating more to the glycogen hypothesis of OTS (21). Further research on the effects of OTS on GH and IGF-1 can provide substantial information on the efficiency of these biomarkers as indicators of OTS in males, and more importantly females as these hormones act as the primary anabolic stimulators.

1.5.4 Creatine Kinase

Despite being labeled as a marker inadequate of indicating NFOR or OTS, creatine kinase (CK) is continuously used as a supporting marker in OTS evaluation (3). CK is an indicator of muscle damage which leaks into the plasma through damaged muscle fibers and tears in the muscle membrane (34). It has been suggested to obtain more individual values for CK to account for individual differences such as the permeability of the membrane which can cause more CK to enter the bloodstream in some individuals (34). Furthermore, it is important to note that CK is unique as a biomarker in that there are athlete specific values that represent the expected amount of muscle damage experienced with the sport (male athletes: 82-1083 U/L, female athletes:

47-513 U/L) (2). Despite the bias against using CK as a marker of NFOR and OTS, it is critical to monitor CK changes as a supporting marker because chronically high CK can indicate insufficient recovery. Acute markers, such as CK, that are chronically elevated become a chronic marker, in this case showing a constant state of muscle damage and inadequate recovery. Markers such as CK can also provide insight into the more strenuous and damaging times of the year, such as preseason, where more attention to recovery is needed.

1.5.5 Cytokines

The cytokine hypothesis for OTS has led to various cytokines being commonly evaluated in the literature. Cytokines have been shown to increase in response to muscle contraction, decreased muscle glycogen, prolonged stress, as well as to injury and muscle damage to promote an immune response (35,36). All the given mechanisms that promote increases in cytokines are indicative of exercise and are highly dependent on proper rest and recovery to mitigate an unwarranted response. Excessive muscular contraction with insufficient recovery (decreasing muscle glycogen) results in both muscle damage and an increased stress response which can promote this acute response to chronic inflammation (7). This inflammation can affect multiple tissues including higher brain centers due to their capability to cross the blood-brain barrier (7,36). The primary cytokines that are indicative of NFOR/OTS include IL-6, IL-1β, and TNF-α (36). Currently, cytokines and their systemic effects have been shown to alter physiological processes such as hypothalamic decreases in hunger (7,36). These systemic effects can in turn decrease glycogen stores, disrupt sleep, increase depressive symptoms, HPA activation, adrenal

stimulation, increased prostaglandins, granulocyte proliferation and lymphocyte activation (7,36).

Of the primary cytokines, IL6 and TNF-α have received the most attention. IL-6 and TNF-α are proinflammatory cytokines that modulate both local and systemic inflammation by the capability of being expressed in almost every cell and tissue type (7). Along with the proinflammatory actions, IL-6 also stimulates the expression of hepcidin in the liver, which has been shown to decrease iron absorption, further compounding the effects of cytokines of the body during times of NFOR/OTS which collectively results in decreases in performance (37). Additionally, increases of hypothalamic TNF-α has been shown to induce food restriction which can be a cause of the decrease muscle glycogen stores associated with OTS and the glycogen hypothesis (36). Interestingly, IL-6 can also alter sleep, which is yet another symptom of NFOR/OTS such as poor sleep quality and increased sleep disturbances (38). Furthermore, chronic increases in cytokine concentration can alter pathologies manifesting in sleep disturbances (38). In the animal model, it has been well established that with OTS, there is a subsequent increase in IL-6 and TNF-α (39). When evaluating the human model, the results are less clear. The results of the immunological response to overreaching in male cyclists show that there was no change in IL-6 and TNF-α (40). It is important to note this study only used a two week intensified training (six weeks total) which may be insufficient to induce changes in the cytokine response (40). A similar study utilizing male tri-athletes found that IL-6 increased following a two week intensified training period (four week total) (41). Similar results have been shown in elite female rowers, resulting in increased IL-6 and TNF-α during times of increased TL and

decreased when there were sufficient rest and recovery (42). These results indicate a

dose-response relationship between various cytokines and increases in TL (42).

Cytokines hold great potential as markers of OTS, but further research is needed to

develop time course recommendations along with more stringent sampling and TL

comparison in conjunction with these markers.

1.5.6 Thyroid Hormones

Thyroid hormones are rarely evaluated in the contexts of OTS but may have

significant implications with increased relevance for females. Thyroid hormones are

influenced by energy balance and contribute to performance and recovery by regulating

metabolism (43). There is a reduction of thyroid hormones with increased exercise

resulting from a negative energy balance and availability (44). Additionally, thyroid

imbalances seem to be more prevalent in female athletes due to their increased incidence

of low energy availability (28). To thoroughly evaluate the effects of OTS on thyroid

function, a full analysis needs to be performed including TSH, T3, T4 as well as free T3

and T4. When considering conditions of OTS, there is a clear connection to similar

symptoms of hypothyroidism, decreased thyroid function, including fatigue, altered

mood, and decreases in performance showing a possible relationship despite the lack of

research (44). Interestingly, the regulation of TSH can be disrupted and altered by

cortisol, leptin, insulin, and cytokines such as IL-6, all of which has been shown to be

disturbed by OTS (43). With OTS, the overload can alter not only the HPA axis activity

but also the HPA axis as well resulting in possible hypothyroidism and its associated

symptoms of fatigue, decreased metabolic rate, and a loss of strength (43). Despite the

general connection of altered thyroid function and OTS, there has only been one study to

evaluate this in a possible overtraining population. This study assessed a thyroid panel in female endurance runners and found no change, despite these findings, the authors hypothesized that thyroid hormone concentrations move too slow for monitoring OTS status (44). As with the majority of research on OTS, these negative results may be a by-product of the athletes not being over trained as it is unethical to put athletes through an OTS protocol. The observational studies performed are the most applicable but do not guarantee OTS is achieved. Despite these findings, an evaluation of thyroid function should be strongly considered for future research in evaluating OTS in both males and females.

1.5.7 Catecholamines

Catecholamines (epinephrine and norepinephrine) have been commonly evaluated as biomarkers for OTS, though few conclusions have resulted from the current research. Catecholamines are primarily produced in the adrenal glands along with spillover from neurotransmitters and act in the bodies "fight or flight" response to metabolize fuels and prime the body for exercise (9). Catecholamines hold particular importance for OTS in that the secretion during exercise has significant effects on performance while altered resting levels can affect several physiological processes and even affect sympathetic innervation altering HRV. Results indicated that with incidences of OTS, there is an increase in resting catecholamines, particularly norepinephrine (8,45). Though, not all of the findings are in agreement with several studies being unable to replicate the improvements seen in the resting levels as well as studies showing decreases as well (2,8,21,46). Unfortunately, catecholamine measurements have been inconsistent making the results of the various studies difficult to interpret. The most valid measure for

catecholamine secretion is a 24-hour measurement, though that is generally too invasive

for a competition athletic population leaving a snapshot of urinary or serum/plasma

catecholamine for evaluation (8,21). Despite the challenges of measuring and interpreting

catecholamines response to OTS, these markers hold promise as biomarkers for

diagnosing or predicting NFOR/OTS.

1.5.8 Oxidative Stress

Markers of oxidative stress are relatively understudied due to the complexity of

the analysis and the disjointed view on the implication of oxidative stress on OTS to

determine if it is a cause or a by-product. The evaluation of oxidative stress is challenging

because there is a necessary amount of reactive oxygen species that needs to be released

in response to exercise due to its regulatory factors for cellular repair and inflammatory

response (6,14). Additionally, several markers of oxidative stress can be measured such

as superoxide, hydrogen peroxide, thiobarbituric acid reactive substances (TBARS),

superoxide dismutase, glutathione, and catalase activity, making the literature difficult to

interpret (14,47). Current research is very minimal with few studies evaluating the animal

model and even less in the human model. In the animal model, no effects were observed

on markers of oxidative stress or lipid peroxidation in mice during six weeks of

overtraining (48). Alternatively, another study concluded there was a link between

oxidative stress and performance in the over trained mice with six and eight weeks of

training (49). Furthermore, there were significant increases in oxidative stress markers

during a six-week overreaching protocol in resistance trained men (14). Interestingly, this

protocol resulted in a decrease in performance of vertical jump while all other strength

measurements were maintained, raising the questions of if the athletes were overtrained

(14). These results show that markers of oxidative stress can hold promise as a supporting

marking in a larger panel. More research is required in this area evaluating alternative

modes of exercise as well as population used.

1.6 Nutritional Concerns

Along with the various physical changes, performance decrements, and hormonal

responses, there are also different nutritional concerns that arise with NFOR/OTS. These

nutritional alterations may have a significant role in both athlete health as well as

performance. These factors include hydration, hematological status, vitamin D, omega

three alterations, along with challenges of energy availability. While none of these

markers are predictive of NFOR/OTS, they could be added to various biomarker panels

to help mitigate the potential decreases in performance. Other nutritional factors such as

hydration and energy availability may be better addressed through proper education and

the development of healthy habits due to the difficulty of assessing these variables. The

strong impact hydration can have on performance is well established, but it can become

difficult to measure due to the day to day variation as well as the responsibility of

rehydration put on the athlete (50). Education and providing adequate resources may be a

better investment rather constant testing and assessment.

Similar to hydration is maintaining sufficient caloric intake to support energy

availability. Interestingly, many of the underlying causes associated with OTS can be

linked to relative energy deficiency in sport (RED-S). The main component of RED-S is

low energy availability which describes an imbalance between energy intake and energy

expenditure which leaves the athlete with low energy to maintain essential physiological

functions that are needed to support general health and performance (28). Relative energy

deficiency in sport has significant implications in athletes with particular emphasis on female athletes. These implications include hormonal alterations, oligomenorrhea or amenorrhea, increased soreness, illness, iron deficiency, impaired cognition, and altered mood, all of which parallel the effects of OTS (28). Unfortunately, similarly to OTS, there is no practical way to measure low energy availability or RED-S, making the prevention falling primarily on raising awareness. Alternatively, there are possible hormones that may better indicate RED-S such as leptin, adiponectin, ghrelin as well as various cytokines that may provide a biomarker based solution (21,28). Education, providing adequate resources, and encouragement of healthy habits may be the most effective tool in this case.

Primary nutritional concerns that are common and treatable, especially in female athletes, are hematological deficiencies such as iron deficiency depletion and anemia. Hematological issues are prevalent in 16-57% of female athletes and 1-31% of male athletes, with high incidence in primarily aerobic and aesthetic sports (51). Iron (Fe) status consists of total Fe in the blood as well as the amount stored as ferritin (Fer), which is mobilized in times of decreased Fe (52). Transferrin status incorporates total iron binding capacity (TIBC) which represents the capacity of Fe to bind to transferrin while percent saturation (%Sat) represents the amount of occupied iron binding sites on transferrin (53,54). Changes in these markers can indicate a shift towards a training-induced Fe deficiency or anemia. The adverse changes in iron status are well established and result in significant decreases in performance and overall exercise capacity (e.g., reduced VO_{2max}) (25). Preventing these adverse hematological changes requires nutritional monitoring to address changes in diet during competition and possible

supplementation to combat decreases if changes in diet are insufficient. Hepcidin has also

been used to evaluate Fe status which serves as a biomarker (51). Hepcidin is a peptide

hormone that can be increased through increased inflammation, which in turn

downregulates the absorption of Fe (51). Hepcidin could be a possible biomarker to be

added with hematological markers as a possible precursor leads to disruptions in Fe

status.

Other nutritional concerns should be the evaluation of possible vitamin

deficiencies, with increased attention to those that are treatable with dietary intervention

or supplementation. These deficiencies can range from vitamins (A, B6, B12, C, D, and

E) to minerals (copper, iron, manganese, selenium and zinc), macronutrients

(carbohydrate, fat, and protein), and the essential amino acids and fatty acids (55,56). Of

these markers, Vit-D and omega-3 (25) have significant effects for athletes with

performance implications including bone health, muscle damage, and inflammation.

Females are at a higher risk for Vit-D deficiencies, which has added implication due to

the symptom of low bone density associated with RED-S (24,57). Adding assessment of

these markers may not add to the diagnosis of NFOR/OTS, but they can provide

supporting factors to combat the potential decrements in performance and health with

NFOR/OTS. Supplementation may have a likely impact on the recovery status of the

athletes and provide a more favorable physiological environment to maximize recovery

during these times of increased training load (58).

1.7 Limitations in the Literature

Given the current research on OTS performed over the past few decades, there are

still several substantial limitations. As previously mentioned, the vast majority of studies

claiming to be inducing an over trained state in the athletes analyzed are, at best, achieving a non-functional overreaching state. The achievement of a functional overreaching state (possibly NFOR) is evident in the several studies to utilize a taper following the intensified exercise resulting in an explicit super compensation, indicating the athletes were not over trained. The lack of an over trained state is primarily due to the unethical circumstances that would be required to induce OTS involving not only an exceptionally high TL but increased psychological stress (personal, professional, travel, etc.) of over trained athletes. Lab-based settings cannot adequately represent the cumulative stress that is the driving force of OTS. Despite this significant limitation, athletes who are active in competition may provide the needed data on the use of the various measures discussed and the effectiveness.

Along with the ethical issues associated with OTS and the current literature base, there is a major inconsistency with the use and measurement of the various techniques ranging from performance, perceptual, as well as the biomarkers. These inconsistencies make interpretation and comparison of the multiple results challenging to form a single conclusion and recommendation. Creating a single conclusion would require a set recommendation for each individual technique including biomarkers (serum, plasma, saliva, or urine), performance markers (what test to use, when/ how that test is administered), and perceptual (what is used, when/how it is administered). Additional inconsistencies include the almost sole inclusion of males in the literature with the very scarce addition of females. The primary inclusion of males commonly leads to the misappropriation of data found in the male athlete as the bases for the recommendation

for the female athletes. Further research is needed in the female athlete to create sex-specific norms and changes associated with each athlete's sex.

Another major limitation of the current research and recommendations for OTS are being able to create a viable tracking/monitoring method for different athletes. A comprehensive approach for all athletes needs to include all aspects of OTS tracking, monitoring, and prevention. Development of these principles must be able to use to individual and team sports across many disciplines and skill levels. Additionally, this approach must incorporate a technique that easily performed, affordable, reliable, and not overly invasive or disruptive to the athletes, providing a significant challenge.

1.8 Conclusion

Considering the complexity of OTS, the individualistic nature of the response, and time course, recent advancements have begun to shed light on the possibilities of tracking and prevention. To track the development of OTS several aspects need to be measured throughout the athletes training and competition. The first aspect must be monitoring TL to be assessed continuously during athletes training and competition. Training load is the controllable variable that can be manipulated to increase or decrease the physical stress put on the athlete. With the TL, performance must be tracked and evaluated in some form to identify any potential changes and address the changes promptly to mitigate further decreases. Additional measurements can be incorporated to gain further insight into the cumulative importance the athletes face in training and outside of training that includes perceptual, sleep, diet, and the incorporation of biomarkers. Of these additional tests, biomarkers hold the most promise in the prevention and tracking of OTS. Biomarkers indicative of stress, performance, recovery, and health

can be tracked and monitored for adverse changes that precede the changes in

performance. Despite the limitations, the combination of testing, tracking, and

biomarkers provides the most complete analysis for maximizing athlete health and

performance.

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Title: Early Season Hormonal and Biochemical Changes in Division I Field Hockey Players: The Fit Athlete Paradox

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Abstract

The purpose of this study was to evaluate the changes in hormonal and biochemical markers as a result of the accumulated stress in Division-I female field hockey players over the initial training block. Women's field hockey players (N=22; M_{age}=19.7±1.1yrs) were tested prior to the start of preseason (A1) and monitored over four-weeks including two-weeks of preseason and two-weeks of the season (A2). At A1, a battery of tests were administered, including body composition (%BF), vertical jump (VJ), and VO_{2max}. Prior to A1, blood draws were conducted assessing creatine kinase (CK), iron (Fe), hemoglobin (HGB), hematocrit (HCT), percent saturation (%sat), total cortisol (TCORT), free cortisol (FCORT), Interleukin-6 (IL-6), sex-hormone binding globulin (SHBG), prolactin (PRL), vitamin-D (vitD), and thyroxine (T_3). Blood draws were repeated four-weeks later (A2). Energy expenditure (Kcal) was monitored via heart rate monitors. There were significant perturbations in TCORT, FCORT, T3, CK, Fe, and SHBG ($P<0.05$) from A1-A2. VO_{2max} accounted for 31% ($P<0.05$) of the variance in TCORT and %BF accounting for an additional 20.1% ($P<0.05$). VO_{2max} accounted for 32.7% ($P<0.05$) of the variance in FCORT. Percent BF accounted for 48.9% ($P<0.05$) of the variance in T_3. Kcals were positively correlated with VO_{2max} ($P<0.05$) and negatively correlated with %BF ($P<0.05$).

- Athletes experienced significant alterations of hormonal and biochemical markers over this initial timeframe.
- Athletes with higher VO_{2max} and lower %BF are capable of higher work output and therefore are more likely to experience increased physical stress during

training, reinforcing the importance and difficulty of managing players at an

individual level.

Keywords: Biomarkers, Training load, Preseason, Female Athlete, Field Hockey, Fit

Athlete Paradox

INTRODUCTION

Athletes strive to attain a competitive edge over their opponents through improvements in performance. The off-season is a critical time for athletes to improve their physical and physiological characteristics. Unfortunately, direct supervision of college athletes by the coaching staff during the off-season is limited by NCAA rules, placing the onus on the athlete to arrive fully prepared for preseason. This presents a unique challenge for Fall sport athletes, as preseason begins approximately two weeks before the first competitive game, which is remarkably condensed compared to professional sports preseason (Gamble 2006). During preseason, it is imperative for athletes to attain "full-readiness" for their fitness to maximize physical capabilities and minimize injury risk (Heidt et al. 2000).

To overcome this limited timeframe and maximize team development, coaches will maintain a higher workload in the weeks immediately following the preseason despite the increased injury rate seen in preseason of college sports (Anderson et al. 2003; Agel et al. 2013). This is possible because this period typically occurs before classes start, thus allowing coaches to continue to utilize multiple practices per day (Hootman et al. 2007). The unique scenario presented warrants further investigation to the effects of this early season time block on the athletes.

Increased workload and intensity coupled with reduced recovery time is further compounded by stressors inherent to student-athletes including changes in housing, environment, diet, and sleep patterns (Killen et al. 2010; Mann et al. 2015). Athletes who experience increased stress, including physical stress in training and psychological stress outside of training, have been shown to have a higher injury rate compared to athletes

with lower stress (Mann et al. 2015). The preseason training period accounts for the

highest practice injury rate and may produce adverse effects on the athletes that last

throughout their season (Gabbett 2004; Brooks et al. 2005). In fact, women's field

hockey had the fifth highest preseason:in-season injury ratio amongst collegiate sports,

highlighting the challenges of this training period and need for further evaluation of

athlete response (Agel et al. 2013; Dalton et al. 2015). The typical structure of NCAA

Fall sports preseason may put athletes at a heightened risk of overreaching (OR), non-

functional overreaching (NFOR), or overtraining syndrome (OTS), with females being

particularly susceptible (Meeusen et al. 2012).

Current athlete monitoring tools employ the use of heart rate [HR], global

positioning systems [GPS], or both (Halson 2014). Wearables are limited because they

only capture workload during training sessions and competitions, which may not

adequately account for other off-field stressors (Duclos 2008). Blood biomarkers can be

utilized as a complimentary monitoring technique because they yield a complete picture

of the cumulative stress athlete's experience (Meyer and Meister 2011; Heisterberg et al.

2013; Silva et al. 2014). Unfortunately, much of the research in this area has been

performed in males, which may lead to the erroneous application of findings when

working with female athletes.

The stress of preseason training for collegiate athletes increases the need for an

encompassing monitoring approach. Therefore, the first purpose of this study was to

evaluate changes in hormonal and biochemical markers resulting from the accumulated

stress in Division 1 female field hockey players over a four-week training block including

two weeks of preseason and the first two weeks of the competitive season. The second

purpose was to identify physical and performance characteristics that predict changes in hormonal and biochemical markers related to stress and recovery.

METHODS

Subjects

Twenty-two Division I Big Ten conference female field hockey players free of major injuries or known metabolic conditions were included. Subjects continued game and daily schedules with no dietary intervention throughout the duration of this study. Subjects were asked not to change their diet over this period. All subjects performed testing as part of routine team activity and participated in all team actives during this period. This study was approved, and consent forms waived, by the Institutional Review Board at Rutgers University in accordance with the declaration of Helsinki.

Table 1. Descriptive Characteristics

Measurement	T1
Age (yrs)	19.7 ± 1.10
Height (cm)	166.32 ± 3.40
Weight (kg)	64.3 ± 7.40
% BF	26.1 ± 6.70
VO_2 (ml/kg/min)	45.9 ± 5.70
VT (% VO_2)	76.8 ± 2.90
VJ (cm)	51.1 ± 2.90

Table 1: Descriptive Characteristics

Values are Means ± Standard Deviations.

Design

Training load variables were monitored for the first four weeks of the competitive season. This period represents the highest accumulated load in collegiate fall sports encompassing the preseason training (two weeks) and the first two weeks of the season prior to the start of academic requirements. Biomarkers were analyzed before the start of this training block and immediately after to evaluate the effects of accumulated stress of training on biomarkers representing performance, recovery, and general athlete health. Additionally, fitness variables were examined as potential predictors of physiological change.

Performance Testing

Subjects reported to the Rutgers Center for Health and Human Performance (CHHP) prior to the start of preseason (A1) to complete a battery of fitness testing. Subjects arrived euhydrated and at least 2 hours fasted. Body composition was measured via air-displacement plethysmography (BOD POD, COSMED, Concord, CA) for Lean Body Mass (LBM), Fat Mass (FM), and percent body fat (%BF) (Dempster and Aitkens 1995). Then, athletes performed a five-minute systemic warm-up after which a counter-movement vertical jump (VJ) (with arm swing) was performed using the "Just Jump" system with the highest jump recorded from 3 attempts (Probotics, Inc., Huntsville, AL) (Nuzzo et al. 2011). Following this, VO_{2max} was measured via direct gas exchange with a TrueOne 2400 metabolic cart (ParvoMedics, Salt Lake City, Utah) using a MET equivalent modification of the Bruce protocol. This protocol utilizes three min stages that increase in both speed (2.7, 4.0, 5.5, 6.7, 8.0, 8.6, 9.6, 11.9 KPH) and grade (10, 11, 12, 13, 14, 15, 15, 15%) (Golem and Arent 2015). Participants ran until volitional fatigue. At the end of each stage, the athlete was asked to give their rating of perceived exertion

(RPE), using a 6-20 scale (Borg 1982). At least three of the following criteria had to be met for 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 $\geq$18. Heart rate was continuously monitored using a Polar S610 HR monitor (Polar Electro Co., Woodbury, NY, USA) to obtain an accurate maximum heart rate (HR_{max}) and heart rate at ventilatory threshold (VT). VT was calculated after the completion of the test as the point at which ventilation began to increase non-linearly with VO_2 and was expressed as a percentage of VO_{2max} (Gaskill et al. 2001).

Sample Collection and Analysis

Subjects reported to the CHHP on a day prior to performance testing in a euhydrated condition for blood draws immediately prior to the start of preseason (A1) and 28 days later at a time corresponding to 36 hours after training (A2). This four-week timeframe served as a secondary "control" for the menstrual cycle. All blood draws were taken at the CHHP between 0700 and 0830 hours following an overnight fast. Blood samples were processed on site and centrifuged for 10-minutes at 4,750 rpm (Allegra x-15R Centrifuge, Beckman Coulter, Brea, CA) and stored at -80°C or 1.6°C prior to shipping. All samples were shipped same day to Quest Diagnostics via Quest Diagnostic's pick-up delivery services for duplicate analysis via LC-MS/MS-based assays. Biomarkers measured included creatine kinase (CK), iron (Fe), hemoglobin (HGB), hematocrit (HCT), percent saturation (%sat), total cortisol (TCORT), free cortisol (FCORT), Interleukin 6 (IL-6), sex-hormone binding globulin (SHBG), prolactin (PRL), vitamin D (vitD), and thyroxine (T_3) (Lee et al. 2017).

HR Monitoring

Athletes were monitored during all practices and games using the Polar Team[2] system (Polar Electro Co., Woodbury, NY, USA) to determine individual training load (TL) and energy expenditure (kcal) via HR analysis (Ceesay et al. 2018). Values obtained from performance testing were programmed into the Polar Team[2] system to obtain accurate TL and kcal values specific to each player. TL for each player was determined via algorithm generated by Polar™ utilizing physiological attributes of the player and physical work load (e.g., time spent in different HR zones defined as 55-65, 66-75, 76-85, 86-95, and 96-100 %HR_{max}). Daily team average TL and kcal can be found in Figure 1.

Figure 1: Daily Training Load

Statistical Analysis

Repeated measures ANOVA were used to analyze biomarker changes over time. For each univariate analysis, the Huynh–Feldt epsilon was examined for the general

model to evaluate sphericity. If the Huynh–Feldt epsilon exceeded 0.75, sphericity was considered to have been met and the unadjusted statistic was used. If epsilon was less than 0.75, the adjusted Huynh–Feldt statistic was used to test significance. Pearson-product moment correlations and hierarchical multiple regression with stepwise variable entry were performed to examine relationships between fitness and biomarker changes. R^2_{change} was assessed at each step of the regression. Data are expressed as mean $\pm$ SD and statistical significance was set at the $P \leq 0.05$ level. Effect size (ES) were calculated for between-groups differences using Cohen's *d*. Using Cohen's conventions, ES of 0.20, 0.50, and 0.80 were considered indicative of small, medium and large effects, respectively (Thalheimer and Cook 2002).

RESULTS

Biochemical and Hormonal Responses

A significant time effect was seen for markers of muscle damage, physiological stress, and hematological changes. TCORT, FCORT, T_3, SHBG, and CK increased significantly from A1 to A2 ($P<0.05$). Fe, HGB, HCT, and %sat all decreased significantly from A1 to A2 ($P<0.05$). There was no significant difference in IL-6, VitD, or PRL over the four-week period. Biochemical and hormonal marker values as well as ES can be found in Table 2.

Biomarker	A1	A2	ES
Creatine Kinase (U/L)	88.73 ± 34.99	142.86 ± 51.15*	1.54
Total Cortisol (nmol/L)	699.6 ± 272.4	783.80 ± 325.7*	0.31
Free Cortsiol (nmol/L)	17.1 ± 9.6	21.8 ± 8.5*	0.49
SHBG (nmon/L)	90.68 ± 60.61	98.05 ± 63.53*	0.12
Prolactin (µg/L)	14.02 ± 9.38	13.80 ± 5.73	-0.02
T_3 (nmol/L)	1.81 ± 0.3	2.09 ± 0.58*	0.93
IL-6 (pg/mL)	1.06 ± 0.41	1.75 ± 3.87	1.68
Vitamin D (ng/mL)	53.45 ± 19.51	55.18 ± 16.15	0.08
Iron (µmol/L)	23.35 ± 6.87	14.03 ± 6.37*	-1.36
Hematocrit (%)	44.17 ± 2.89	42.86 ± 2.86*	-0.45
Percent Saturation (%)	32.11 ± 11.22	19.86 ± 10.77*	-1.09
Hemoglobin (g/dL)	14.32 ± 0.89	13.73 ± 0.94*	-0.66

Table 2: Biomarkers

Values are Means ± Standard Deviations.

* Significant Change from A1.

Predictive Measures

Kcals expended in preseason were positively correlated with VO_{2max} (r=0.76, P<0.05) and negatively correlated with %BF (r=-0.48, P<0.05). VO_{2max} accounted for 31% (β=0.56, P<0.05) of the variance in TCORT and %BF accounted for an additional 20.1% (β=-0.71, P<0.05). VO_{2max} also accounted for 32.7% (β=0.57, P<0.05) of the variance in FCORT and 16.3% of the variance in SHBG, which approached significance (β=-0.40, P=.063). In addition to the variance accounted for in TCORT, %BF also accounted for 48.9% (β=-1.11, P<0.05) of the variance in T_3 and 14.8% of the variance in PRL, which approached significance (β=0.52, P=.074). Finally, VJ accounted for 32.8%

of the variance in IL-6 (β=0.95, P<0.05). There were no variables that predicted changes in CK or Fe.

DISCUSSION

The results of the present study revealed, as expected, alterations in biochemical and hormonal alterations throughout the first four weeks of the initial training block. Furthermore, to the authors' knowledge, this study was the first to use athletes' fitness attributes to account for changes in markers of physiological perturbation and increased stress in female athletes. The data show that a "fit athlete paradox" presents itself during preseason training period. The more fit field hockey athletes (i.e. higher VO_{2max}, lower %BF, and higher VJ) had the most substantial stress response and showed the greatest indications of physiological disruption from the TL experienced during this training block encompassing the preseason training and the first two-weeks of in-season play. It is hypothesized that because more fit athletes have a higher capacity for work, they compensate for the lower workload of the less fit to maintain the pace of play during training (Rampinini et al. 2007). The current hypothesis is supported by the fact that energy expenditure was positively correlated with VO_{2max} and negatively correlated with %BF indicating an increased work output. Furthermore, the hypothesized compensation by the more fit athletes is especially prominent during the first training block as there is still a high level of competition for starting positions (Hootman et al. 2007). The increased ability to perform more work coupled with the high volume/intensity of training may put the more fit athletes at a higher risk of overreaching and injury as the season progresses if not addressed adequately (Dalton et al. 2015).

The challenges mentioned above in the collegiate preseason for Fall sports marked with high TL, repeated sessions, and compromised recovery time creates an environment that appears to be conducive to revealing the fit-athlete paradox (Agel et al. 2013). In the current study, this resulted in physiological alterations reflected in biomarkers of all athletes with more fit athletes showing indication of greater strain. As shown in Figure 1, 7-8 of the preseason days were at or above a game load. While the high TL observed may not necessarily elicit immediate injuries or performance decrements in the athletes, the changes in blood biomarkers show a clear impact of this high load on their physiological response. The combination of physiologic challenge from high initial TL set the stage to begin the season with elevated physiological strain without adequate recovery. If this persists, the physiological disruption may not only result in impaired performance but also the increased likelihood of NFOR (Meeusen et al. 2013).

Significant increases in both TCORT and FCORT may be indicators of early signs of OR (Urhausen et al. 1995). It has been suggested that cortisol typically rises during periods of NFOR before decreasing and "bottoming out" during the athlete's transition into OTS (Meeusen et al. 2012). Despite being only four-weeks in duration, this study demonstrates notable changes in these markers across the team. Additionally, this increased stress response was more pronounced in the more fit athletes, with VO_{2max} and %BF together accounting for over 50% of the variance in TCORT. VO_{2max} also accounted for 37% of the variance in FCORT. Unexpectedly, the more fit players experienced a more significant stress response to the first training block, rather than a mitigated one, thus supporting the notion of the fit athlete paradox.

It is important to note the relatively high levels of cortisol seen before training began suggesting the athletes entered preseason with an already heightened level of stress. One hypothesis is the players attempted to rapidly improve fitness levels in the final few weeks of the off-season to prepare for the upcoming preseason. Similar results have been seen in Division I male soccer players due to a late summer push to prepare for the season (Kraemer et al. 2004). Another explanation for the increased cortisol before the start of the preseason training is a possible difference in normative cortisol values for female athletes compared to their male counterparts, which may be further compounded by oral contraceptive use. However, these explanations were not measured and are not necessarily mutually exclusive, illustrating the need for further research on the female athlete.

SHBG was used to provide indirect insight into the anabolic hormones (Lee et al. 2017). SHBG binds to testosterone and estradiol for transport and is thought to have a protective effect on the sex hormones (Lee et al. 2017). SHBG increased throughout the first training block, with VO_{2max} accounting for 16.3% of the variance. Given the TL observations and other biomarker changes, there is likely a discrepancy between energy intake and energy expended during this period, which may lead to an increase in SHBG (Longcope et al. 2000). With the link observed between TL and fitness, this energy imbalance may be more pronounced in the more fit athletes explaining why VO_{2max} accounted for a portion of the change in SHBG (Longcope et al. 2000). Without dietary intake information throughout this study, a definitive conclusion cannot be drawn. Similarly, increases in SHBG have been seen in an overreaching study involving cadets

during military training (Tanskanen et al. 2011). It appears that SHBG holds promise as an indicator of training status.

CK has been commonly used as a marker of muscle damage as well as a marker of overtraining (Meeusen et al. 2012). The results of the present study show the athletes experienced a significant increase in CK after this training block regardless of fitness and body composition. It is noteworthy that CK is one of the few biomarkers that has athlete specific values taking into consideration normal muscle damage experienced with a sport (female athlete reference range: 47-513 U/L) (Mougios 2007). Given the athlete specific reference ranges, there was not necessarily an "excess" of muscle damage because of this TL. It is, however, essential to monitor CK changes in comparison to baseline levels as chronically high CK indicates insufficient recovery.

IL-6 is an underutilized biomarker that can give insight to an athlete's readiness. It has been shown to increase in response to muscle contraction, decreased muscle glycogen, as well as to muscle damage and injury in order to promote an immune response (Pedersen and Febbraio 2005). A likely reason for the observed increase in IL-6 is the increase in muscle contraction and a concomitant decrease in muscle glycogen because of greater TL. Interestingly, VJ accounted for 32.8% of the variance in IL-6, which could indicate that the athletes who can produce more powerful contractions had an increase in muscle-derived IL-6 production providing further support for the "fit-athlete paradox" (Febbraio and Pedersen, Bente 2002). Another possibility is the rise in IL-6 in this study indicates an increase in the inflammatory response due to the training stimulus itself. The rise in IL-6 mirrors the increases for cortisol, which together may play a significant role in the immune response to exercise and likely indicate NFO (Wyatt

et al. 2013). Much like cortisol, IL-6 has been suggested to increase during NFO before declining significantly as an athlete begins to experience OTS (Meeusen et al. 2012; Wyatt et al. 2013).

The increase in T_3 throughout this training block is indicative of an increased metabolic response to the workload. Surprisingly, almost half of the variance in T_3 was accounted for by %BF, with leaner athletes demonstrating the most substantial increases in T_3. The increase in T_3 was a unique finding in that a decrease (or no change) in T_3 is often seen with an increase in energy expenditure with exercise in order to provide an "energy sparing" effect (Steinacker et al. 2005). One possible explanation may be that increased metabolism was required to meet the energy demand of the increased workload during this training block and represented resource mobilization. Additionally, the body may sense less of an "energy reserve" with the leaner athletes, thus enhancing this compensatory response. Overall, the preseason workload produced significant changes in the hormonal biomarkers, indicating notable homeostatic disturbance.

Significant decreases in all hematological (Fe, HGB, HCT, and %sat) markers were observed after this training block. This suggests a training-induced iron deficiency. It is well documented that decreases in iron and the related markers are more commonly seen in females and have significant effects on performance (Ostojic and Ahmetovic 2008). The current study shows a 40% decrease in Fe with a 39% decrease in %sat along with significant decreases in both HCT and HGB with no relation to fitness or body composition. These findings suggest the increased need for Fe in female athletes during periods of heightened workloads. Supplementation strategies may be required if dietary changes to improve bioavailability are not sufficient.

While the strengths of this study include the real-world setting (thus providing a non-contrived stressor), the high training and performance level of the athletes, and the nature of the assessments used, there are also inherent limitations that must be acknowledged. First, diet was not controlled nor was it assessed. Given the free-living approach to the study, dietary control was not feasible. Future research should consider practicable methods of dietary assessment that can be utilized over extended periods of an athletic season without being an unnecessary burden on the athlete (Mountjoy et al. 2018). We should also recognize that the menstrual cycle and the use of contraceptives were not assessed. However, to "account" for cycle influences on biomarkers, blood draws were taken in a 28-day interval. This also represents a more realistic approach in women's team sports when matches and training are on a fixed schedule. In this regard, "control" of the menstrual cycle is neither feasible nor realistic. Furthermore, the authors recognize the potential insights that an additional mid-point blood sample may have provided to represent just the preseason. However, an additional blood draw (as well as additional performance testing) was not logistically feasible due to coaching concerns over burden on the athletes as well as timing related to practice and games. This highlights practical considerations that must be considered when studying high-level athletes, particularly when done in-season. Finally, additional workload due to supplemental training (i.e., weightlifting) was not monitored or provided to the researchers, though this was extremely limited during this part of the season.

Coaches and performance staff need to approach the early-season training block with caution to best manage their players. When dealing with a heterogeneous team from a fitness and capability standpoint, it is critical to recognize the need to prevent the more

fit players from compensating for their less fit teammates. This potentially preserves the well-prepared athlete while simultaneously providing additional training stress essential for athletes in need of improvement. To stratify athletes, it is vital to include systematic fitness testing in addition to monitoring TL. This study also demonstrates the potential utility of employing biomarker assessment as part of the overall monitoring and performance plan for athletes. Unique insights from biomarkers may allow coaches, managers, and players to be aware of physiological changes that are occurring and possibly prevent adverse events. The combination of proper player and training management during the early part of the season, adequate preseason duration, and use of various monitoring tools provides opportunity to maximize athlete health and performance. Because of the high intensity nature of preseason training and the range of fitness levels within a team, it is important for coaches and trainers to manage players at an individual level to optimizing performance (Gabbett 2004; Killen et al. 2010).

CONCLUSIONS

Overall, this study demonstrates that preseason for an NCAA women's Fall sport (i.e., field hockey) induces considerable physiological stress on the athlete and results in notable changes to associated biomarkers, including those related to the stress response, muscle damage, and hematological status. Based on previous research, it is likely that a longer preseason would be beneficial to the athletes' health and performance (Killen et al. 2010). An unexpected outcome was that the more "fit" athletes incurred greater perturbation throughout the preseason training. This appears to be primarily due to their greater workload in relation to their less fit teammates. This is not to suggest that it is better to be less fit coming into preseason. Instead, it emphasizes the importance of all

players reporting in peak condition in order to adequately share workload as well as provide inoculation against injury risk and breakdown (Killen et al. 2010).

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References: 32

Abstract

Purpose: Evaluate effects of training load (TL) on performance and biomarkers of health, performance, and recovery in Division I female soccer players throughout a competitive season. **Methods:** Participants (N=25, M_{age}=20±1.1yrs) were monitored prior to the start of preseason and every four-weeks thereafter (T1-T5). A battery of performance tests was administered prior to the start of preseason (P1) and end-of-season (P2), including body composition (body fat (%BF), fat free mass (FFM), and fat mass (FM)), vertical jump (VJ), and VO_{2max}. Blood draws were conducted at every time point (T1-T5) to assess free and total cortisol (CORTF, CORTT), prolactin (PRL), T_3, IL-6, creatine kinase (CK), sex-hormone binding globulin (SHBG), omega-3 (n-3FA), vitamin-D (Vit-D), iron (Fe), hematocrit (HcT), ferritin (Fer), percent saturation (%Sat), and total iron binding capacity (TIBC). Daily exercise energy expenditure (EEE) and TL were determined. **Results:** There were significant declines in VO_{2max}, VJ, weight, and %BF from P1-P2 (p<0.05) with no significant differences in FFM. TL and EEE significantly decreased from T1-T3 (p<0.05). Significant increases were seen in CORTT, CORTF, PRL, T_3, IL-6, CK, and TIBC throughout the season (p<0.05). Significant decreases were seen in n-3FA, Fe, Fer, %Sat, and Hct throughout the season (p<0.05). **Discussion:** Female athletes experience significant physiological changes following high TL and EEE associated with preseason and appear to be further exacerbated by the cumulative effects of the season. **Practical Applications:** Unique insights provided by biomarkers enable athletes and coaches to be cognizant of the physiological changes that are occurring throughout the season.

Key Words: Performance, Stress Response, Training Load, Athlete Monitoring

INTRODUCTION

Soccer is the most popular sport in the world with 265 million participants across all ages, sexes, and skill levels competing in the game (14). Female soccer players represent a growing portion of this population; however, research related to the physiological changes that occur in females because of soccer-specific training demands is lacking. A recent review by Datson et al. (2014) evaluated the current literature on the demands that high level female soccer players experience in games and found on average, these athletes cover about 10 km, perform 76 skill involvements (passing, dribbling, headers, and shooting), and experience 1350-1650 changes in activity during competitive play. Additionally, these athletes maintain an average body fat percentage of 14.6-20.1% and a VO2max of 49.4-57.6 mL/kg/min (7). Thus, to maintain this high level of play, coaches and athletes must optimize training to elicit the greatest performance benefits and maximize physiological attributes. In order to do this, systematic athlete monitoring has become increasingly common.

Athlete tracking and monitoring approaches have made recent technological advancements to encompass internal physiological markers (heart rate, heart rate variability, biomarkers) (17). Heart rate monitoring is a commonly used technique to monitor on-field training load (TL), which represents internal "effort" to complete a physical task (4). This effort is quantified as TL via algorithms based on heart rate response specific to each athlete or as exercise energy expenditure (EEE) (4,6). Unfortunately, many TL monitoring techniques (including heart rate monitoring) only account for what is happening on the field and are unable to capture off-field stressors.

Implementation of additional monitoring tools, such as blood biomarkers, can give insight regarding athlete health, performance, and recovery status by encompassing both on and off the field stressors. Blood biomarkers can provide a comprehensive analysis of the physiological and biochemical response to TL that would otherwise be undetected through the more traditional monitoring techniques (15). Markers such as cortisol, testosterone, creatine kinase, sex hormones, cytokines, hematological panels, and nutritional markers have been used to assess athletes' response to TL (11,12,15,18,26). In research, however, the use of biomarkers has been far more prevalent in male athletes with far less emphasis on female athletes despite known sex differences that could impact performance and recovery. This lack of diversity in the research is primarily driven by an unwillingness to work with female athletes due to hormonal variations associated menstrual cycle and use of oral contraceptives, though it seems counterproductive to exclude a large portion of the athletic population due to these factors.

Utilization of monitoring techniques allows for athlete management to optimize performance as well as to potentially prevent injury and long-term decrements to accumulated TL and stress, which may manifest as non-functional overreaching (NFOR) or overtraining syndrome (OTS). NFOR is defined as an accumulation of stress, physical workload, and psychological strain, resulting in short-term performance decrements without physiological and psychological maladaptation, while OTS includes both physiological and psychological maladaptation (17). Not only are NFOR/OTS detrimental to athletic performance and overall health, but full recovery may take months to years (17,25,33).

Applying these methods to team sports presents a unique challenge of assessing the team as a group and as individuals, in addition to considering the external stressors athletes are facing (10). Accounting for the individualized response to TL and accumulated stress provide coaches, trainers, and sport scientists the ability to tailor the athletes' workload and required recovery individually. Adequate monitoring is especially vital in collegiate athletes who experience increased physical and psychological stress due to the combination of a condensed season, TL, travel, academic requirements, changes in the environment, diet, and sleep patterns, which may all interact to inhibit athletic performance and recovery (16). Therefore, the purpose of this observational study was to evaluate the cumulative effects of season-long TL in conjunction with changes in performance and blood-based biomarkers associated with health, performance, and recovery in high-level Division I female soccer players. It was hypothesized there would be alterations in blood-based biomarkers, performance, and body composition over the course of the full season.

METHODS

Experimental Approach to the Problem

This observational study sought to evaluate the season long effects of training on various biomarkers in a real-world setting utilizing high level female athletes. Performance testing was performed prior to the start of the season and four to six days after the final match to observe any performance changes. Training load variables were monitored throughout the duration of the competitive season including preseason, regular

season play, and tournament play in NCAA Division I female soccer players. Biomarkers were analyzed prior the start of preseason (T1) and every four-weeks approximately 18-36 hours after a game thereafter to evaluate the effects of accumulated stress of training on biomarkers representing performance, recovery, and general athlete health.

Subjects

Twenty-five Division I female soccer players (M_{age} =20 ± 1.1yrs) were included. Descriptive and baseline performance data are presented in Table 1. All participants performed testing as part of regular team activity associated with their sport science program. Subjects were asked not to change their diet over this period. 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.

Table 1. Body Composition and Performance

Measurement	P1	P2
Weight (kg)	63.42 ± 6.11	62.41 ± 6.36*
LBM (kg)	49.40 ± 5.31	49.63 ± 5.33
% BF	22.07 ± 4.17	20.43 ± 3.76*
VO_2 (ml/kg/min)	48.36 ± 3.51	45.09 ± 4.30*
VT (% VO_2)	79.68 ± 3.35	79.42 ± 4.24
VJ (cm)	58.09 ± 6.35	56.31 ± 6.17*

Values are Mean ± Standard Deviation.
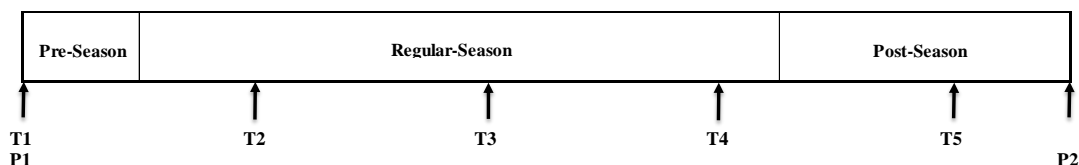* Significant Change from Baseline (T1)

Procedures

Performance Testing

The testing timeline is presented in Figure 1. Athletes reported to the Rutgers
University Center for Health and Human Performance (CHHP) prior to the start of
preseason (P1) and four to six days following the final competitive match (P2) to
complete a battery of three fitness tests in one session. Subjects were instructed to arrive
euhydrated, at least two-hours fasted, and without having trained 24 hours prior to
testing.

Body composition was assessed by air displacement plethysmography via the
BodPod (BOD POD, COSMED, Concord, CA) (8) in order to determine percent body fat
(%BF), fat free mass (FFM), and fat mass (FM). Following a general systemic warm up,
subjects were given three attempts for maximal single counter movement vertical jump
with arm swing (VJ) using the Just Jump system (Probotics, Huntsville, AL, USA), with
the highest jump recorded. Following this, a maximal graded exercise test was used to
measure maximal aerobic capacity (VO_{2max}) and ventilatory threshold (VT) via direct gas
exchange measured by a TrueOne 2400 Metabolic Measurement System using a
modified Bruce protocol (Parvo Medics, Sandy Utah). 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 heart rate max, a respiratory
exchange ratio greater than 1.10, and/or an RPE $\geq$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). VT was calculated after the completion of each test as the point where ventilation begins to increase nonlinearly with VO_2, which is expressed as a percentage of VO_{2max}(9).

Figure 1: Testing Timeline

Pre-Season	Regular-Season	Post-Season

T1
P1   T2   T3   T4   T5   P2

Season Training Monitoring

All practices and games were monitored using the Polar Team[2] system (Polar Electro Co., Woodbury, NY, USA). Resistance training, though minimal throughout the season, was not monitored and details not consistently provided to the researchers. Full season training load can be found in Figure 2. The Team[2] system monitored each player's individual workload, energy expenditure, and time spent at percentages of HR_{max} (55-65, 66-75, 76-85, 86-95, and 96-100). The quantification of an individual player's workload was estimated by total Kcal expenditure (EEE) and training load (TL), the latter being calculated via an algorithm developed by Polar[TM] based on physiological attributes of the player obtained from laboratory testing, which was entered for each player (height, weight, HR_{ma}, VO_{2max}, VT), and physical workload measured (6).

Figure 2: Season Long Training Load

Sample Collection and Analysis

The players reported to the CHHP for blood draw samples during five-time points throughout the season. Preseason samples were drawn one day prior to the first day of practice, with players having refrained from training for 36 hours (T1); subsequent blood draws were conducted every four-weeks approximately 18-36 hours after a game until the last competitive match (T2-T5). Athletes arrived at least eight hours fasted and euhydrated between 0700–0900 hrs. Blood samples were centrifuged for 10 minutes at 4,750 rpm (Allegra x-15R Centrifuge, Beckman Coulter, Brea, CA) and were shipped to Quest Diagnostics for analysis via LC-MS/MS-based assays. Biomarkers analyzed include free and total cortisol (CORTF, CORTT), prolactin (PRL), T_3, IL-6, creatine kinase (CK), sex-hormone binding globulin (SHBG), omega-3 (n-3FA), vitamin-D (Vit-D), iron (Fe), hematocrit (HcT), ferritin (Fer), percent saturation (%Sat), and total iron binding capacity (TIBC).

Statistical Analysis

Biomarker, performance, and body composition testing data were analyzed using RM MANOVAs with RM ANOVA univariate follow-ups (IBM SPSS v23). Planned simple contrasts were conducted using the baseline values as the comparison term. Pairwise contrasts were included in the case of significant univariate findings using the least significant difference method. The null hypothesis was rejected when $p<0.05$. Cohen's *d* was used to calculate effect sizes (ES).

RESULTS

Performance and Training Load

Body composition and performance values can be found in Table 1. Weight and %BF decreased from P1 to P2 ($p<0.05$, ES=-0.17; $p<0.05$, ES=-0.39) with no significant difference in FFM. VO_{2max} and VJ decreased from P1 to P2 ($p<0.05$, ES=-0.93; $p<0.05$, ES=-0.28, respectively) with no significant difference in VT.

TL was evaluated as the total sum during the 4-week training block between time points and can be found in Figure 2. T1-T2 had 18 practices (six double sessions and two exhibition matches) and 4 games, T2-T3 had 15 practices and 6 games, T3-T4 had 13 practices and 7 games, T4-T5 had 11 practices and 7 games (including the first three rounds of the NCAA tournament). All subsequent training blocks were significantly lower ($p<0.05$) than the initial preseason training block (T1-T2) (see Figure 3). Following preseason, there was a substantial decrease in TL in the second training block (T2-T3) (ES=-1.35) followed by a further reduction in the third training block (T3-T4) (ES=-0.94) before normalizing through the last training block through the NCAA tournament.

Figure 3: Training Load Accumulated

EEE was also evaluated as the total sum during the 4-week training block between time points (see Figure 4). All subsequent training blocks were significantly lower (p<0.05) than the initial preseason training block (T1-T2). Following preseason, there was a substantial decrease in EEE in the second training block (T2-T3) (ES=-5.44) followed by a further reduction in the third training block (T3-T4) (ES=-3.79) before normalizing through the last training block.

Figure 4: Caloric Expenditure

Biomarker Responses

All biomarker data can be found in Table 2. Compared to T1, CORTT was significantly higher at T3-T5 (p<0.05). There was an initial increase at T3 (ES=0.61) followed by a second larger increase at T5 (ES=0.91). Similarly, CORTF was significantly higher at T3-T5 compared to T1 (p<0.05). There was a similar pattern showing an initial increase at T3 (ES=1.36), followed by a second increase at T5 (ES=1.0). Compared to T1, PRL significantly decreased at T2 (p<0.05, ES=-0.38) before significantly rising at T3 (p<0.05, ES=1.63) and remained elevated through T4 and T5 (p<0.05). CK significantly increased at T2 (p<0.05, ES=0.85) before returning to baseline values at T3 and T4. CK increased again and reached its highest value at T5 (p<0.05, ES=1.08). IL6 remained at baseline values before significantly increasing at T5 (p<0.05, ES=5.73). T_3 significantly increased at T2 (p<0.05, ES=1.15) and remained significant through T3 before returning to baseline values at T4 and T5. N-3FA was significantly

lower at all time points (T2-T5) compared to T1 ($p<0.05$). Compared to baseline, Vit-D significantly decreased at T4 ($p<0.05$, ES=-0.44) and remained significantly lower through T5 ($p<0.05$). There were no significant changes seen in SHBG.

Table 2. Biomarkers

Marker	T1	T2	T3	T4	T5
CortT (nmol/L)	637.00 ± 276.50	683.92 ± 322.09	826.62 ± 275.17*†	727.81 ± 281.52*†	1108.69 ± 757.89*†
CortF (nmol/L)	19.87 ± 6.90	19.59 ± 10.76	34.22 ± 12.42*†	27.32 ± 9.66*†	36.71 ± 11.86*
SHBG (nmol/L)	89.40 ± 56.07	89.96 ± 60.27	85.96 ± 52.03	88.88 ± 60.61	87.00 ± 63.85
Prl (µg/L)	13.58 ± 6.11	11.24 ± 5.48*†	20.18 ± 10.77*†	17.64 ± 8.75*	16.48 ± 7.43*
CK (U/L)	137.96 ± 148.28	263.84 ± 224.00*†	162.60 ± 133.45†	130.56 ± 86.64	306.45 ± 373.60*†
IL-6 (pg/mL)	1.18 ± 0.80	1.32 ± 0.81	1.74 ± 1.41◊	1.04 ± 0.38†	3.16 ± 4.10*†
T3 (ng/dL)	1.49 ± 0.34	1.88 ± 0.41*†	1.63 ± 0.36*†	1.54 ± 0.31	1.57 ± 0.38
OMG3 (%)	4.06 ± 3.04	2.02 ± 0.59*†	2.54 ± 0.47*†	2.06 ± 0.54*†	2.21 ± 0.53*†
VitD (ng/mL)	49.16 ± 12.23	48.56 ± 9.20	49.88 ± 12.60†	44.36 ± 13.03*†	44.40 ± 13.52*

Values are Mean ± Standard Deviation.
* Significant Change from Baseline (T1). † Significant Change from the Previous Time Point

All hematological values can be found in Table 3. Compared to T1, Fe was significantly lower at T2-T5 ($p<0.05$). There was an initial decrease at T2 (ES=-0.83) which remained stable before a second decline happened at T5 (ES=-0.75). Similarly, Fer was significantly lower at T2-T5 compared to T1($p<0.05$) with an initial decrease at T2 (ES=-0.76) which continued through T5 with little deviation. Compared to baseline, TIBC was significantly higher at T3-T5 ($p<0.05$), while Hct was significantly lower at all time points (T2-T5) ($p<0.05$).

Table 3. Hematological Makers

Marker	T1	T2	T3	T4	T5
Iron (µmol/L)	21.52 ± 10.59	12.40 ± 4.84*†	14.67 ± 8.93*	15.02 ± 7.07*	9.63 ± 5.06*†
Ferritin (µg/L)	35.64 ± 13.20	25.52 ± 11.46*†	23.88 ± 11.08*	22.84 ± 10.86*	23.48 ± 10.93*
%Sat (%)	32.57 ± 13.33	17.70 ± 8.42*†	22.24 ± 13.14*	22.24 ± 12.26*	13.34 ± 6.70*†
TIBC (µmol/L)	67.36 ± 9.56	70.34 ± 11.96	70.15 ± 12.42*	71.24 ± 11.60*	73.96 ± 12.64*†
Hct (%)	43.26 ± 2.71	41.77 ± 2.51*†	42.14 ± 2.28*	40.57 ± 3.03*†	40.53 ± 2.95*

Values are Mean ± Standard Deviation.
* Significant Change from Baseline (T1). † Significant Change from the Previous Time Point

DISCUSSION

The results of this study provide a comprehensive evaluation of the cumulative stress of a season incorporating TL, EEE, performance, and biomarkers of health, performance, and recovery in collegiate female athletes. This study exhibited the highest TL and EEE during preseason which corresponded with several physiological perturbations, that persisted throughout the season. The authors observed a first hormonal disruption occurring at week-8 (T3) with a second hormonal disruption at week-16 (T5). To our knowledge, this is the first study to track TL, EEE, and biomarkers in female team sport athletes through the preseason, competitive season, and tournament play.

Performance and Training Load

The TL and EEE found in this study show the high metabolic demand these athletes encounter throughout a college soccer season, which is notable for its congested match fixture and short preseason. Both TL and EEE were significantly higher during preseason training compared to the rest of the season. The TL during the first block (T1-T2) corresponded to notable physiological changes, primarily seen in dietary and hematological markers. These changes may be a byproduct of NCAA restrictions on athlete monitoring during the summer months. This restriction shapes the nature of the collegiate preseason itself. It is a very short, intense, two-week period which utilizes multiple practices per day in conjunction with the stress of competition along with team, academic, and administrative meetings, thus compromising optimal recovery. These results indicate the potential negative impact of a short preseason on the athletes if not adequately managed.

Though TL exhibited a steady decline as the team progressed through the season and entered tournament play, the decrease in weight, %BF, VO_{2max}, and VJ observed at P2 depict the cumulative stress of the collegiate soccer season. Along with season-long TL, student-athletes experience the challenges and stressors of doing both athletics and academics creating increased overall stress that is often not accounted for (16). The changes in fitness found in this study have also been observed in other studies in female collegiate soccer players where significant decreases in VO_{2max} were observed over the course of a season (32). However, the changes in body composition were unique regarding the decrease in total body weight and %BF without any change in FFM. This could also be due to the minimal resistance training performed throughout the season, though this cannot be confirmed due to the inability to monitor athletes during these activities. It is notable that, despite the maintenance of FFM, aerobic capacity and power still declined, and biomarker perturbation was still evident. These results show that during the most competitive phase of the season (tournament play), athletes are at their lowest fitness levels, possibly due to the underlying physiological response to the accumulated TL, EEE, or insufficient recovery.

Biomarkers

Along with changes in anthropometric and performance variables, biomarkers can provide further insight into the physiological changes that athletes experience during the season. One of the more common biomarkers used is cortisol, a primary hormone released in response to stress. During times of increased TL and less than optimal recovery, an elevation in resting cortisol can be seen due to a disruption in homeostasis

and the subsequent stress response (2,17). If this inappropriate TL continues, the stress response can become desensitized, resulting in a decreased cortisol response (17). It is important to note that is has been suggested resting cortisol is not a useful measure due to the lack of change seen in male endurance athletes (17). However, a recent review indicated these notions may be overstated due to a contradictory literature base with varying results, thus highlighting the need for further investigation (2). Furthermore, these recommendations may not apply to female power endurance athletes who experience a more physical (contact oriented) TL rather than the more aerobic based male endurance athletes that are often studied (17). The nature of the college soccer season with congested match fixtures may further exacerbate this. The current study revealed significant elevations in both CORTT and CORTF at T3 with the second inflection at T5, indicating an accumulated stress response throughout the season. It is important to note that at all five time points, CORTT values were above the clinical range. The authors believe these chronically high cortisol levels could be due to the lack of sex-specific ranges or due to the effects of menstrual disruption and oral contraceptives on the hypothalamic pituitary adrenal (HPA) axis (21).

Somewhat surprisingly, there were no changes in cortisol immediately following the highest TL (T1-T2), which may indicate a potential delayed disruption of the HPA-axis or other compensatory adjustments (17). However, the increased cortisol response evident at T3 and T5, suggests the total cumulative stress may begin to alter HPA-axis activation. These results are contrary to the lack of change or even decreased cortisol

response that has been seen in both collegiate and professional male soccer players,

providing additional support for the need for more female-specific ranges and data (26).

Testosterone is commonly measured in conjunction with cortisol in males to show

the anabolic:catabolic balance of the athlete. Though testosterone was not evaluated due

to the low resting values in females, secondary indicators such as SHBG and PRL were

used as exploratory alternatives. SHBG acts as a transport vessel for sex hormones and

has been shown to increase in both males and females with exercise (15). PRL, which has

been shown to increase in response to stress, hypoglycemia, and physical exercise, has

relevance in female athletes due to its suppressive effects on estrogen when elevated (31).

Similar to other observed hormones, PRL increased during the middle of the season (T3)

and remained elevated throughout the rest of the season, while SHBG showed no change.

The similar responses of PRL and CORT provide additional support for the likelihood of

the cumulative effects of TL and stress on endocrine perturbation. Further, an

HPA/hypothalamic pituitary gonadal (HPG) axis disruption occurs with inappropriate TL

(13), potentially affecting PRL which may play a role in reproductive cycle dysfunction

and contribute to the female athlete triad(21). PRL appears to be an important biomarker

in female athletes, though more research is needed along with the addition of markers

such as estrogen. A lab system error at baseline resulted in the lack of completion of the

estrogen analysis that was beyond the control of the researchers.

Along with the changes in the HPA/HPG responses, there were also changes in

markers of muscle damage as reflected by CK (18). The initial increase in CK was

associated with the high TL in the preseason. It is important to note that all markers remained in the athlete- and sex-specific ranges (female athlete reference range: 47-513 U/L) (20). Similar results have been seen in both collegiate and professional soccer players, with an increase in CK following preseason training, though within the normal athlete ranges (11,18,26). The results of high CK may indicate strenuous periods within the season where additional recovery strategies should be implemented.

IL6 was evaluated as a marker of inflammation, as it responds to decreased muscle glycogen and muscle contraction, along with muscle damage and injury in order to facilitate an immune response (22). With systemic inflammation resulting from chronic intense exercise, cytokines, such as IL6, activate the HPA-axis (29). Not surprisingly then, IL6 followed a similar trend as other hormones linked to the HPA-axis such as CORTT. There was a notable elevation of IL6 at T3 and again at T5, representing the highest state of physiological disruption. These results, specifically the elevation at T5, are consistent with the cytokine hypothesis of overtraining which posits that the increased systemic inflammation can be a major disruptor of the HPA-axis (29). Along with the effects mentioned above, IL6 also stimulates the expression of hepcidin in the liver, which has been shown to decrease iron absorption, further exacerbating the changes in iron observed in this study (27). The physiological alterations observed at T3 and T5 may result from a multi-faceted hormonal response to the cumulative stress of the condensed collegiate season.

Thyroid hormones are influenced by energy balance and contribute to performance and recovery by regulating metabolism (30). The results of this study show an increase in metabolically-active T_3 after the preseason which returned towards baseline as the season progressed. The initial increase at T2 was a unique finding in that a decrease (or no change) in T_3 is often seen with an increase in EEE in order to provide an "energy-sparing" effect (30). These results are contrary to what was found in collegiate female rowers who displayed decreased thyroid markers during periods of high EEE over a 20-week in-season training block (3). Future studies may benefit from the use of a combination of TSH, T_3, and T_4 to get a complete profile of thyroid function.

Dietary biomarkers can also provide additional information for athlete readiness. Vit-D and n-3FA have significant effects on female athletes with performance implications including bone health, muscle damage, and inflammation (15). In this study, an immediate and sustained decrease in n-3FA following preseason was found, indicating that there may be insufficient dietary compensation to account for resource recruitment. Furthermore, Vit-D decreased towards the end of the season which also coincides with the change of seasons: games and practices are often transitioned to indoor settings or more clothes are worn in November in the northeastern United States. In light of the observed decreases in Vit-D and n-3FA, supplementation may have an impact on the recovery status of the athletes and provide a more favorable physiological environment to maximize recovery (19).

The hematological markers evaluated in this study represent both the Fe and transferrin statutes of these athletes (23,24,28). Fe status consists of total Fe in the blood as well as the amount stored as Fer, which is mobilized in times of decreased Fe (23). Transferrin status incorporates TIBC which represents the capacity of Fe to bind to transferrin while %Sat represents the occupied iron binding sites on transferrin (24,28). Changes in these markers show a shift towards a training-induced Fe deficiency or anemia. After the preseason training block (T1-T2), there was a negative change Fe and transferrin status, which persisted throughout the season. Fe reached its lowest values at T5 (56% lower than the initial baseline values). Similarly, Fer demonstrated a 35% decrease by this timepoint, while, TIBC and %Sat decreased by 9% and 40%, respectively. While these values never went below the "clinically normal" range (Fe: 8.95-31.32 µmol/L, Fer: 10-154 µg·dL^{-1}, TIBC: 44.75-80.55 µmol/L, %Sat: 11-50 %) (15), the change from baseline likely represents a sub-optimal range for performance given the magnitude of change. Additional measurements of Hct, showed decreases following preseason. It is well known that negative changes in iron status results in significant decreases in performance and overall exercise capacity, e.g. reduced VO$_2$ max (15). The results found in this study are consistent with previous research indicating a high number of female athletes experience declines in hematological values during the season (1). Further, it is important to recognize differences between clinical ranges vs. optimal ranges for athletic performance. Clinical ranges are generalized and concrete numbers for diagnosis which do not take into consideration changes from the athlete's baseline.

This study provides a comprehensive evaluation of TL, EEE, and biomarkers in high-level female soccer players throughout the season and during tournament play. We recognize a few limitations with the study, some of which are inherent to working with high-level athletes in a real-world setting. Expectations and demands on the athletes and coaches related to the research must be balanced with the reality of the season. First, the athlete's diet was not measured throughout the season. However, research has indicated that self-reported dietary measures can be highly flawed and can be unreliable and impractical to measure in this population during an already demanding season (5). Secondly, this study did not account for the menstrual cycle or the use of oral contraceptives, though a 28-day period between blood draws was used to provide a degree of "control" for the menstrual cycle. It is the authors' views that because the athletes cannot control for the menstrual cycle during competition, it is essential to evaluate their response in a holistic and real-world analysis. Additionally, sleep quantity/quality was not evaluated before blood draws. Though this was impractical to attempt to control these factors when working with this team, future researchers may consider reasonable ways to practically assess this. Future studies would benefit from tracking diet, menstrual cycle, mood disturbances, sleep quality, and additional biomarkers including estrogen, testosterone, and a more comprehensive thyroid panel when possible. It is important to note that including a degree of control as suggested for future studies may prove problematic in free-living athletes if it becomes too invasive and disruptive for coaches and athletes.

PRACTICAL APPLICATIONS

Despite the limitations, this study provides much needed observational data on high-level female athletes in a real-world setting. The results of this study show female athletes experience a culmination of decreased performance and significant physiological disruptions in conjunction with increased cumulative TL/EEE and external stressors during the collegiate competitive season. The preseason/early season training block resulted in a negative change in dietary and hematological markers that persisted throughout the season. As the season progressed, there appeared to be a delayed hormonal response associated with the cumulative stress which may indicate the onset of both NFOR. These results emphasize the importance of tracking TL and biomarkers throughout a full season to show the cumulative effects to the on and off-field stressors placed on collegiate athletes. Biomarkers, in conjunction with TL monitoring, provide a more complete profile on athlete readiness and overall health, allowing for better player management. Periodic evaluations provide several opportunities to intervene and potentially mitigate the performance decrements as seen in this study. Possible supplementation of Fe, n-3FA, and Vit-D may prove beneficial for many female athletes to maintain performance throughout the entire season. Monitoring techniques can be utilized to make in-season adjustments to maximize player performance.

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Title: Workload, Energy Expenditure, and Biomarker Differences in Division I Male and

Female Soccer Players

Submission Type: Original Investigation

Running Head: Biomarker Response in Collegiate Soccer Players

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ABSTRACT

PURPOSE: To compare work load and biomarker changes in male and female Division I college soccer players through preseason and the first half of the competitive season. METHODS: Male (MS) (N=24; Mage= 20± 1.23yrs) and female (WS) (N=25; Mage= 19 ± 1.38yrs) DI college soccer players preformed a battery of performance tests was administered prior to the start of preseason (P1) and end-of-season (P2), including body composition (body fat (%BF), fat free mass (FFM), and fat mass (FM)), vertical jump (VJ), and VO_{2max}. Athletes participated in blood draws prior to preseason (T1) and every 28 days thereafter (T2-T4). Athletes arrived fasted in the morning. T2 and T3 draws occurred ~18-36 h after a game. Free and total cortisol (CORTF, CORTT), creatine kinase (CK), iron (Fe), ferritin (Fer), percent saturation (%Sat), and total iron binding capacity (TIBC), thyroid-stimulating hormone (TSH), total triiodothyronine (T_3), total thyroxine (T_4), free and total testosterone (TESTF, TESTT), estradiol (E2), sex-hormone binding globulin (SHBG), prolactin (PRL), growth hormone (GH), insulin-like growth factor-1 (IGF-1), interlukin-6 (IL-6), omega-3 (N-3FA), vitamin D (Vit-D) were assessed. Workload (training load (TL), distance (DIS) and kcal/kg) was monitored using the Polar Pro system. RESULTS: Significant differences between WS and MS in weight, %BF, LBM, VO_{2max}, VJ, VJ_{HOH}, TL, Dis and kcal/kg (P<0.05). Both teams had a significant decrease in VO_{2max} while WS had an additional decrease in VJ and VT along with an increase in LBM (P<0.05). Both teams had a significant decrease in training variables following the first time block (T1-T2) (P<0.05). There was a significant difference between WS and MS in CORTT, CK, T4, SHBG, TESTT, TESTF, E2, Prl, GH, and IGF-1 (P<0.05). Significant alterations in biomarkers were seen in CORTF, CK,

Fe, Fer, %Sat, T4, TESTT, TESTF, IL-6, GH, IGF-1, Vit-D, and n-3FA in WS (P<0.05).

Significant alterations in biomarkers were seen in Fer, T_3, T_4, TESTT, E2, SHBG, IL-6,

IGF-1, Vit-D, n-3FA in MS (P<0.05) CONCLUSIONS: The results of this study revealed

both male and female athletes experienced a decrease in aerobic performance throughout

the season. Similarly, both teams experienced the highest TL, DIS, and EEE during the

initial preseason training block that decreased throughout the year. This initial training

block resulted in several hormonal, biochemical, and nutritional changes in both teams

with WS experiencing altered hematological values.

INTRODUCTION

The sport of soccer is one of the most played and studied sports throughout the world. Nevertheless, the challenges to which these athletes are exposed, both on and off the field, are substantial at all levels of competition. Soccer players are often faced with a short and intense season consisting of multiple games per week. These stressors are especially prevalent in collegiate soccer players who experience a short preseason and season along with congested match fixtures, travel, and academic requirements[1]. If these athletes are not properly managed throughout the season, they are put at risk for decreases in performance, along with increased risk of injury and non-functional overreaching[2,3]. To maximize performance and minimize the risk for non-functional over reaching, athletes must be adequately monitored throughout the entirety of the season. This is typically accomplished through monitoring an aspect of on-field training load.

Training load (TL) has been quantified in a variety of different ways, with the most common methods being heart rate monitoring (HRM) and GPS tracking. HRM is a quantification of internal workload or effort put forth by the individual to perform a given task [4]. A recent meta-analytic review assessed the capability of HRM to assess overreaching and overtraining and concluded that the correct interpretation of these data should be used in conjunction with other measures to be meaningful [5]. GPS can provide data on physical workload through metrics such as total distance traveled and distance at various speeds or speed zones [6]. Recently, GPS has been coupled with accelerometry to measure additional variables such as number of accelerations, decelerations, and numbers of sprints to expand the scope of the physical work measurements [6]. The combination of

HRM and GPS provides a complete picture of physiological effort and physical work that athletes are performing to give the best metric for on-field athlete tracking.

While monitoring workload through HRM and GPS is essential, it is limited to evaluating athletes during team activities (e.g., practices, weight training, and games). This does not account for off-field aspects of life that may negatively affect athletic performance. To account for these off-field aspects, other monitoring systems should be utilized to provide a more complete and accurate analysis of athlete readiness and health. Recent advancements in blood biomarker analysis have shown that markers of athlete health, performance, and recovery status provide unique insight by offering a more complete athlete monitoring profile [7]. Markers of stress (e.g., cortisol), sex hormones (e.g., testosterone, estrogen, prolactin), muscle damage (e.g., creatine kinase), inflammation (e.g., cytokines), and hematologic status (e.g., iron, iron binding capacity, hemoglobin, percent saturation) have been used to assess athletes' status (e.g., recovery, performance, overreaching/over training)[7,8]. A complete biomarker panel can provide an unbiased snapshot of the athlete and the chronic physiological responses to training. Furthermore, when used in conjunction with an on-field TL monitoring system (e.g., HRM and GPS), both the acute TL as well as the chronic physiological response to this TL can be evaluated. Ultimately, biomarkers provide a wholistic analysis that includes the on-field demands along with the extra cumulative stress experienced in athletes' lives.

While the physical aspects of soccer and training load have been investigated in both men and women extensively, the season long physiological response (hormonal and biochemical) to that given TL has almost exclusively been studied in males[9,10]. Recent investigations utilizing biomarkers in athletes have only studied male athletes, with few

being high-level soccer players. Further, females are seldom studied in this context due to the hormonal variations accompanied with the menstrual cycle, though the athletes themselves cannot control for menstruation[9]. Female athletes must perform during all phases of the menstrual cycle, suggesting that evaluating the cumulative response to TL may be more appropriate for free-living, actively competing athletes[9]. Therefore, the purpose of this study was to compare workload and biomarker changes in male and female Division I college soccer players throughout an entire competitive season. The authors hypothesize there will be changes in the various biomarkers measured throughout the competitive season as well as differences between males and females.

METHODS

Subjects

Twenty-four Division I male soccer players (MS) ($M_{age}= 20\pm 1.23$ yrs) and twenty-five Division I female soccer players (WS) ($M_{age}= 19\pm 1.38$yrs) participated in this study. Descriptive and baseline performance data are presented in Table 1. All participants performed testing as part of regular team activity associated with their sport science program. Subjects were asked to not make any dietary changes over this period. 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 consent forms waived, by the Rutgers University Institutional Review Board for the Protection of Human Subjects in accordance with the Declaration of Helsinki.

Performance Testing

Athletes reported to the Rutgers University Center for Health and Human Performance (CHHP) prior to the start of preseason (PT1) and again within one week

following the last competitive match of the season (PT2) to complete a battery of three

fitness tests in one session. Subjects were instructed to arrive euhydrated, at least 2 hours

fasted, and without having exercised 24 hours prior to testing.

During the testing sessions, body composition was assessed via air displacement

plethysmography (BOD POD, COSMED, Concord, CA, USA) [11]. Height was measured

using a stadiometer, and the BODPOD measured body mass and body density to

determine percent body fat (%BF), fat free mass (FFM), and fat mass (FM). Prior to

testing, all equipment was calibrated according to the manufacturer's instructions.

Following a self-selected warm up, subjects were given three attempts for maximal

counter movement vertical jump with arm swing (VJ) and maximal counter movement

vertical jump without arm swing (VJ_{HOH}) using the Just Jump system (Probotics,

Huntsville, AL, USA); the highest jump was recorded. Following this, a maximal graded

exercise test was used to measure maximal aerobic capacity (VO_{2max}) and ventilatory

threshold (VT) via direct gas exchange measured by a COSMED Quark CPET metabolic

measurement system using a speed-based protocol (COSMED, Concord, CA)[12]. The

speed-based protocol used was designed with stages that were MET-equated to the Bruce

protocol. This protocol included 2-min stages at a constant grade of 2%. The speeds of

this protocol are as follows: 6.43, 7.88, 9.97, 11.74, 13.67, 15.61, 17.06, 18.18, 19.79,

21.08 (KPH). The women's team began the test at 6.43 KPH while the men's team began

at 7.88 KPH. 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 heart rate max, a respiratory exchange ratio greater than 1.10,

and/or an RPE above 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). VT was calculated after the completion of each test as the point where ventilation begins to increase nonlinearly with VO_2, which is expressed as a percentage of VO_{2max}[13].

Season Training Monitoring

All practices and games were monitored using the Polar TeamPro system (Polar Electro Co., Woodbury, NY, USA). The TeamPro system utilizes heart rate, GPS, and accelerometry technology. The TeamPro system monitored each player's individual workload, energy expenditure, time spent at percentages of HR_{max} (55-65, 66-75, 76-85, 86-95, and 96-100), total distance covered, total distance spent at various speeds (2.99-7.00, 7.00-11.01, 11.01-15.00, 15.00-19.01, >19.01 KPH). The quantification of an individual player's workload was estimated by total Kcal expenditure (EEE), training load (TL), and total distance covered (DIS). TL was calculated via an algorithm developed by Polar[TM] based on physiological attributes of the player obtained from laboratory testing and physical workload measured encompassing heart rate, GPS, and accelerometry [14]. To equate for body mass, Kcal expended per kg (kcal/kg) was calculated.

Sample Collection and Analysis

The players reported to the CHHP for blood draw samples during four time points throughout the season. Preseason samples were drawn 24 hours prior to the first day of practice starting in early August (T1); subsequent blood draws were conducted every four weeks until the last competitive match ending in November (T2-T4). Athletes arrived

following an 8-hour fast in a euhydrated state approximately 18 hours after a game between 0700-0900. All athletes were informed to maintain dietary intake throughout the duration of this study. Blood samples were processed on site and centrifuged for 10 minutes at 4,750 rpm (Allegra x-15R Centrifuge, Beckman Coulter, Brea, CA, USA) and stored at -80°C or 1.6°C prior to shipping. All samples were shipped same day to Quest Diagnostics via Quest Diagnostics pick up services for analysis via LC-MS/MS-based assays. Biomarkers analyzed include free and total cortisol (CORTF, CORTT), creatine kinase (CK), iron (Fe), ferritin (Fer), percent saturation (%Sat), and total iron binding capacity (TIBC), thyroid-stimulating hormone (TSH), total triiodothyronine (T_3), total thyroxine (T_4), free and total testosterone (TESTF, TESTT), estradiol (E2), sex-hormone binding globulin (SHBG), prolactin (PRL), growth hormone (GH), insulin-like growth factor-1 (IGF-1), interlukin-6 (IL-6), omega-3 (N-3FA), vitamin D (Vit-D).

Statistical Analysis

Biomarker, performance, and body composition testing data were analyzed using RM MANOVAs with RM ANOVA univariate follow-ups (IBM SPSS v23). Planned simple contrasts were conducted using the baseline values as the comparison term. Pairwise contrasts were included in the case of significant univariate findings using the least significant difference method. The null hypothesis was rejected when $p < 0.05$. Cohen's *d* was used to calculate effect sizes (ES).

RESULTS

Performance and Training Load

Body composition and performance values can be found in Table 1. There was a significant difference between WS and MS in weight, %BF, LBM, VO_{2max}, VJ and VJ_{HOH} (P<0.05). There was a significant time by sex interaction in VT (P<0.05), and there was a trending interaction for LBM (P=0.057) and VO_{2max} (P=0.068) with no significant differences in weight, percent body fat, VJ, or VJ_{HOH}. WS had significant decreases from PT1 to PT2 in percent body fat, VO_{2max}, VT in VJ (P<0.05) with a significant increase in LBM (P<0.05). There were no changes in weight or VJ_{HOH} in WS. MS had a significant decrease from PT1 to PT2 in VO_{2max} (P<0.05) with no significant changes in any other body composition or performance variables.
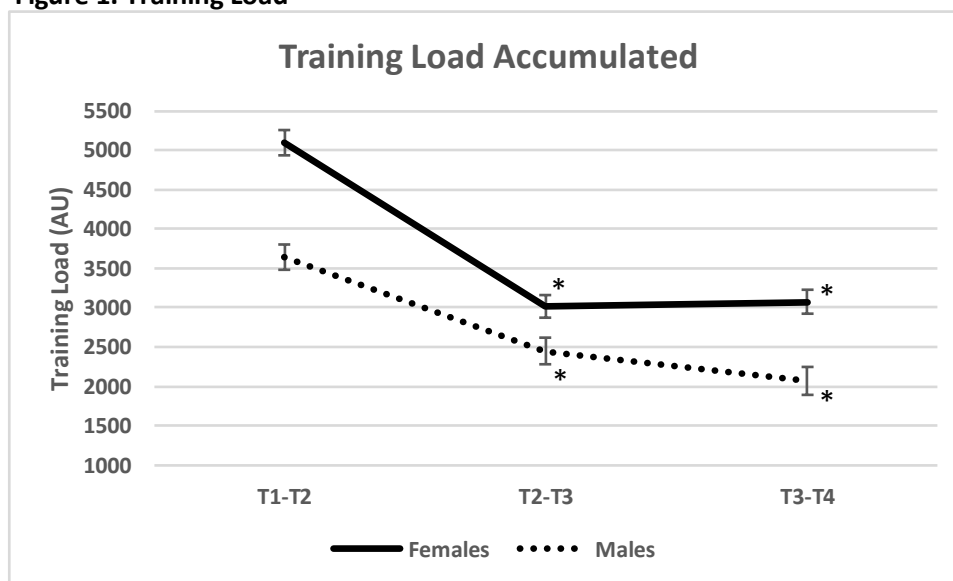
Table 1. Body Composition and Performance

Measurement	Sex	PT1	PT2	ES
Weight (kg)	Female	65.36 ± 5.61	65.30 ± 5.98	-0.01
	Male	78.58 ± 8.35	78.32 ± 8.38	-0.03
%BF	Female	20.69 ± 3.27	19.35 ± 3.77*	-0.4
	Male	11.88 ± 3.32	11.36 ± 3.78	-0.15
LBM (kg)	Female	51.69 ± 4.73	53.08 ± 5.88*	0.29
	Male	69.30 ± 7.17	69.41 ± 6.85	0.01
VO_2 (ml/kg/min)	Female	50.55 ± 4.12	48.07 ± 4.17*	-0.6
	Male	56.54 ± 4.99	52.22 ± 5.74*	-0.86
VT (%)	Female	84.20 ± 3.38	81.84 ± 2.83*	-0.69
	Male	83.66 ± 2.71	83.79 ± 4.13	0.04
VJ (cm)	Female	53.50 ± 6.40	50.63 ± 6.61*	-0.44
	Male	61.88 ± 15.43	63.31 ± 8.81	0.09
VJHOH (cm)	Female	46.07 ± 5.09	45.77 ± 5.43	-0.05
	Male	52.27 ± 12.61	54.73 ± 7.27	0.19

Values are Mean ± Standard Deviation
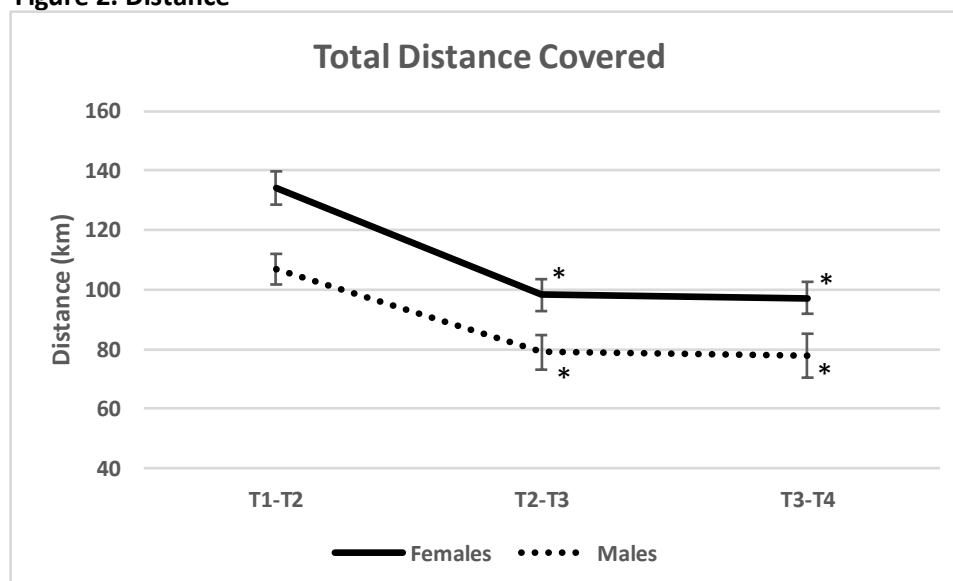
* Significant Change from Baseline (PT1)

TL, Dis, EEE, and kcal/kg were evaluated as the total sum during the 4-week training block between blood draws (28 days) and can be found in Figures 1-4. There was a significant difference between WS and MS in TL, Dis (Figure 2), and Kcal/Kg (Figure

4) (P<0.05) with EEE (Figure 3) trending towards significance (P=0.079). There was a significant time by sex interaction in TL (P<0.05) with no other interactions in training load variables. In WS, all subsequent training blocks were significantly lower (p<0.05) than the initial preseason training block (T1-T2). Following preseason, there was a substantial decrease in TL in the second training block (T2-T3) (ES= -2.58) which normalized through the last training block (T3-T4) (ES=0.08). The same pattern of significant decreases (P<0.05) from (T1-T2) was seen in DIS (ES=-6.35), EEE (ES=-10.2) and Kcal/kg (ES=-12.19) before leveling out throughout the season. In MS, a similar trend was seen as all subsequent training blocks were significantly lower (P<0.05) than the initial preseason training block (T1-T2) in TL (ES=-7.55), DIS (ES=-5.27), EEE (ES=-9.79), and Kcal/kg (ES=-8.23). Unlike WS, TL in MS had a continued significant decrease in TL from (T2-T3) to (T3-T4) (P<0.05) (ES=-2.24), while all other training load markers normalized and did not change from (T2-T3) to (T3-T4).

Figure 1. Training Load

Training Load Accumulated

Values are Mean ± Standard Deviation

* Significant Change from Baseline (T1-T2)

Figure 2. Distance

Total Distance Covered

Values are Mean ± Standard Deviation

* Significant Change from Baseline (T1-T2)

Figure 3. Caloric Expenditure

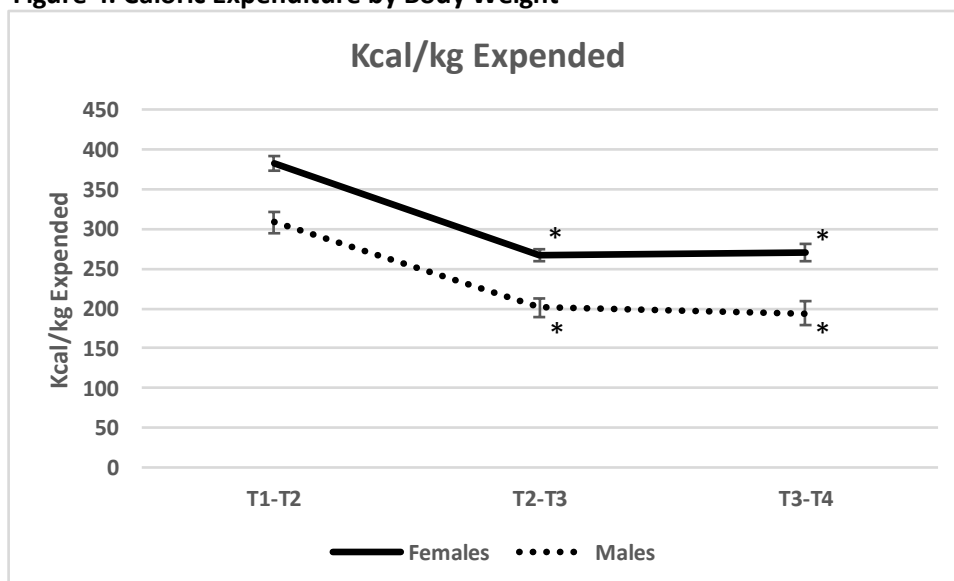Values are Mean ± Standard Deviation

* Significant Change from Baseline (T1-T2)

Figure 4. Caloric Expenditure by Body Weight

Values are Mean ± Standard Deviation

* Significant Change from Baseline (T1-T2)

Biomarker Responses

All biomarker data can be found in Table 2. There was a significant difference

between WS and MS in CORTT, CK, T4, SHBG TESTT, TESTF, E2, Prl, GH, and IGF-

1 (P<0.05). There was a significant time by sex interaction in CORTT, Vit-D, TSH, T4, GH, IGF-1, and N-3FA (P<0.05).

Table 2. Biomarkers

Biomarker	Sex	T1	SD	T2	SD	T3	SD	T4	SD
CORTF (nmol/L)	Female	1.05 ± 0.07		1.35 ± 0.10*†		1.28 ± 0.07*		1.07 ± 0.06†	
	Male	1.13 ± 0.06		1.21 ± 0.09		1.16 ± 0.10		1.02 ± 0.05	
CORTT (nmol/L)	Female	25.48 ± 2.39		24.75 ± 1.50		25.44 ± 2.03		21.41 ± 1.91†	
	Male	18.13 ± 0.81		19.16 ± 0.96		17.93 ± 0.73		18.48 ± 0.77	
CK (U/L)	Female	144.56 ± 19.82		377.40 ± 92.06*†		216.72 ± 28.40*†		217.68 ± 28.16*	
	Male	241.69 ± 42.23		827.04 ± 337.171$		359.17 ± 61.22$		388.00 ± 79.39	
TESTF (nmol/L)	Female	2.62 ± 0.36		4.67 ± 0.44*†		3.46 ± 0.38†		3.18 ± 0.49	
	Male	101.64 ± 5.64		102.63 ± 6.40		108.99 ± 6.69		102.10 ± 6.99	
TESTT (nmol/L)	Female	31.64 ± 2.24		70.92 ± 16.44*†		40.20 ± 5.67		30.88 ± 3.02	
	Male	629.39 ± 34.54		592.00 ± 29.37		602.26 ± 31.94		567.82 ± 36.99*	
ESTROGEN (pmol/L)	Female	215.09 ± 24.80		200.28 ± 22.49		177.81 ± 14.72		211.92 ± 23.66	
	Male	190.58 ± 8.54		144.11 ± 7.37*†		132.45 ± 4.47*		127.64 ± 6.47*	
PROLACTIN (µg/L)	Female	17.48 ± 1.79		20.58 ± 2.50		18.60 ± 1.55		19.10 ± 1.67	
	Male	13.79 ± 1.24		15.46 ± 1.41		12.74 ± 0.91†		14.55 ± 0.89†	
SHBG (nmol/L)	Female	81.72 ± 12.24		83.92 ± 12.66		79.72 ± 12.39		76.04 ± 11.13	
	Male	36.95 ± 2.63		30.52 ± 2.20*†		32.00 ± 2.18*		31.08 ± 2.17*	
GH (µg/L)	Female	4.60 ± 0.93		2.092 ± 0.42*†		1.96 ± 0.67*		1.79 ± 0.49*	
	Male	0.11 ± 0.02		0.17 ± 0.07		0.10 ± 0.00		0.10 ± 0.01	
IGF1 (ng/mL)	Female	300.24 ± 18.97		274.44 ± 18.75		244.80 ± 13.02*†		279.80 ± 15.24†	
	Male	215.86 ± 7.72		230.91 ± 10.49		261.73 ± 12.65*†		238.95 ± 13.01	
IL6 (pg/mL)	Female	1.82 ± 0.26		1.79 ± 0.20		2.62 ± 0.48		2.62 ± 0.27*	
	Male	1.65 ± 0.14		2.24 ± 0.19*†		2.06 ± 0.35		2.86 ± 0.35*†	
TSH (mIU/L)	Female	2.71 ± 0.27		2.94 ± 0.28		2.30 ± 0.22†		2.44 ± 0.23	
	Male	3.15 ± 0.24		2.79 ± 0.23		3.27 ± 0.26		2.66 ± 0.20†	
T3 (ng/dL)	Female	114.12 ± 6.08		118.92 ± 6.22		114.92 ± 5.26		120.72 ± 4.92†	
	Male	107.17 ± 2.26		116.91 ± 3.61*†		105.60 ± 2.30†		110.30 ± 2.13	
T4 (nmol/L)	Female	1.09 ± 0.02		1.17 ± 0.02*†		1.02 ± 0.02*†		1.10 ± 0.02†	
	Male	1.11 ± 0.03		1.27 ± 0.02*†		1.21 ± 0.03*†		1.11 ± 0.02†	
OMG (%)	Female	2.16 ± 0.11		2.62 ± 0.13*†		2.42 ± 0.14*†		2.30 ± 0.09	
	Male	2.29 ± 0.16		2.26 ± 0.10		1.98 ± 0.11*†		2.16 ± 0.11†	
VITD (ng/mL)	Female	46.44 ± 3.07		39.92 ± 2.42*†		38.88 ± 2.23*		39.84 ± 2.22*	
	Male	39.47 ± 2.15		37.34 ± 1.99*†		36.78 ± 1.81*		31.43 ± 1.38*†	

Values are Mean ± Standard Deviation
* Significant Change from Baseline (T1)
†Change from the previous time point. $Trend

In WS, there was significant increase following the preseason in CORTF at T2 (P<0.05, ES=4.29) which remained significant through T3 before returning to preseason values at T4 (P<0.05, ES=-2.92). CORTT remained consistent from T1-T3 before significantly decreasing at T4 (P<0.05, ES=-1.98). CK significantly increased at T2

(P<0.05, ES=11.74) and remained elevated through T4 despite a decrease at T3 (P<0.05, ES= -1.74). There was no significant change from preseason values in TSH despite significant changes from T2-T3 (ES=-2.3) and T3-T4 (P<0.05, ES=0.62). T_3 remained stable then significantly increased from T3-T4 (P<0.05, ES=-2.36). T_4 significantly increased following preseason at T2 (P<0.05, ES=3.58) before falling significantly below preseason values at T3 and normalizing at T4. There was a significant increase in both TESTT (ES=17.51) and TESTF (ES=5.68) at T2 before returning to preseason values at T3 and T4 with no change in SHBG, E2, or Prl. GH decreased at T2 (P<0.05, ES=-2.70) then remained depressed through T4. IGF-1 significantly decreased above preseason values at T3 (P<0.05, ES=-3.08) before returning to baseline levels at T4. There was a significant increase at T4 in IL6 (P<0.05, ES=3.16) despite a non-significant increase at T3. There was a significant decrease in Vit-D at T2 (P<0.05, ES=-2.12) that remained depressed through T4. There was a significant increase in N-3FA following the preseason at T2 (P<0.05, ES=4.05) which remained significant before returning towards preseason values at T4.

In MS, there were no significant changes in CORTT or CORTF throughout the season. Following the preseason, there was a trend for increased CK (P=0.079, ES=2.42) which remained trending at T3 (P=0.061) and returned towards preseason values at T4. TSH exhibited no change, but there was a significant increase in T_3 at T2 (P<0.05, ES=4.3) before returning to preseason values. T_4 had a significant increase at T2 (P<0.05, ES=3.58) which remained increased through T3 before returning to preseason values at T4. There was a steady decline in TESTT over the season that became significant at T4 (P<0.05, ES=-1.07) with no changes in TESTF. In addition, there was an observed

significant decrease in SHBG at T2 (P<0.05, ES=-2.44) that remained depressed through T4. There was a significant decrease in E2 at T2 (P<0.05, ES=-5.44) that remained depressed through T4 with no change in Prl. GH and IGF-1 had no changes throughout the season. There was a significant decrease in Vit-D at T2 (P<0.05, ES=-0.99) that remained depressed through T4. There was a significant decrease in N-3FA at T3 (P<0.05, ES=-1.91) before returning to preseason values at T4.

The hematological markers can be found in Table 3. There was a significant difference between WS and MS in %sat, Fer, and TIBC (P<0.05) with no time by sex interaction observed. In WS, there was a significant decrease in Fe following the preseason at T2 (P<0.05, ES=-3.43) before trending toward baseline at T3 (P=0.073, ES=1.60) and returning to baseline at T4. Fer significantly decreased at T2 (P<0.05, ES=-2.4) that remained depressed through T4. There was a significant decrease following the preseason at T2 (P<0.05, ES=-3.22) in %Sat before returning to baseline at T3 (ES=0.94) and stabilizing through T4. There was no change in TIBC throughout the season. In MS, there was a significant decrease following the preseason at T2 (P<0.05, ES=-1.25) in Fer which significantly decreased again at T4 (P<0.05, ES=-0.63). There were no changes in MS for any other hematological marker.

Table 3. Hematological Markers

Biomarker	Sex	T1	SD	T2	SD	T3	SD	T4	SD
PCTSAT (%)	Female	23.00 ± 2.20		15.90 ± 1.9*		17.70 ± 1.60		22.70 ± 1.8†	
	Male	24.20 ± 2.20		23.60 ± 1.70		23.20 ± 1.60		24.20 ± 1.80	
IRON (µmol/L)	Female	89.44 ± 8.17		61.40 ± 6.24*		71.36 ± 7.06		86.36 ± 6.69†	
	Male	82.52 ± 6.95		79.08 ± 5.80		77.86 ± 4.82		82.73 ± 5.61	
FERRITIN (µg/L)	Female	26.84 ± 2.93		19.80 ± 1.83*		17.72 ± 1.36*		17.96 ± 1.08*	
	Male	73.91 ± 9.06		62.52 ± 6.42*		64.43 ± 7.05		59.95 ± 6.74*	
TIBC (µmol/L)	Female	401.56 ± 14.10		400.24 ± 10.86		403.20 ± 11.89		386.60 ± 12.50†	
	Male	345.34 ± 8.46		338.34 ± 8.10		342.00 ± 10.16		346.17 ± 8.78	

Values are Mean ± Standard Deviation
* Significant Change from Baseline (T1)
†Change from the previous time point

DISCUSSION

This study demonstrates the cumulative stressors of the competitive season through measuring TL, DIS, EEE, physical performance, and biomarkers indicative of health, performance, and recovery in collegiate male and female athletes. This study found that the highest TL, DIS, and EEE occurred during preseason in both MS and WS, which resulted in several physiological and nutritional alterations. To the authors knowledge, this is the first study to track training load variables, performance variables, and season long biomarkers in male and female team sport athletes through the entire season encompassing both the preseason and tournament play at the same university experiencing similar physical and environmental stresses.

Performance and Training Load

The TL, DIS, and EEE found in this study revealed the high metabolic demand that both male and female soccer athletes encounter throughout a college soccer season. Collegiate soccer seasons incorporates a short preseason, congested match fixture throughout the season, along with team, academic, and administrative meetings. Both MS and WS had the highest TL, DIS, and EEE during the first training block (T1-T2), which reflected a very short two-week period consisting of multiple high intensity practices per day along with athletic and academic meetings creating a high stress environment. The high TL during this first training block corresponded to several physiological changes in both MS and WS. Interestingly, WS had a significantly higher TL, DIS, and kcal/kg at every training block when compared to MS despite the known game demands showing MS covering more ground than WS [15]. The authors hypothesize that this difference in TL,

DIS, and kcal/kg is a product of coaching style and practice management showing how player management can affect overall training load throughout the season.

Throughout the competitive season and entering tournament play, MS and WS exhibited a steady decline in TL. Despite this decline, both MS and WS experienced a significant decrease in VO_{2max} at P2 showing the cumulative stress these athletes experience throughout the season. Interestingly, MS maintained all body composition factors as well as VJ while WS increased LBM leading to a decrease in %BF while VJ decreased. These results are unique in that WS had a higher TL throughout the year but experienced an increase in LBM with a subsequent decrease in performance. This increase in LBM may also be contributed to resistance training performed during the season, though all resistance training sessions were not quantified. These results indicate that athletes finish the season with decreased fitness values which corresponds to the most competitive phase of the season during tournament play. The decreased fitness variables could be a byproduct of the underlying physiological response to the accumulated TL, DIS, and kcal/kg. Furthermore, in addition to season-long TL, student-athletes experience combined challenges and stressors of athletics, academics, and travel which can increase the overall stress of the athletes that is often overlooked by coaches and athletes [1].

Biomarkers

Along with the changes in both body composition and physical performance variables, biomarkers were utilized to evaluate the physiological changes that the athletes experience throughout the season. One of the primary biomarkers assessed was cortisol, which is the primary stress hormone. Recent research suggests that during times of

increased TL coupled with reduced recovery, an increase in resting cortisol can be seen due to an increase in the stress response resulting in disruption in homeostasis [16,17]. If this inappropriate training continues, the stress response can become desensitized and down regulated, resulting in a chronically decreased cortisol response [17]. Alternative views suggest that resting cortisol may not a useful due to minimal changes seen in male endurance athletes in various overreaching studies [17]. However, a recent review suggested these alternative views may be overstated due to contradictory findings within the literature with varying results warranting the need for further investigation in both males and females [16]. Furthermore, these recommendations have been primarily based around studies utilizing male endurance athletes in a lab-based setting which may not apply to free-living, collegiate power-endurance athletes who experience a more physical (contact-oriented) TL [17]. In fact, in more extreme cases such as military training which is marked by intense exercise with restricted sleep and food, cortisol has been seen to significantly increase during this time of high stress [18]. In the current study, the nature of the NCAA college soccer season may further exacerbate this due to the condensed schedule coupled with travel and academic requirements. The current study revealed significant elevations in WS in CORTF at T2 immediately following the highest TL (T1-T2) and remained elevated through T3 before returning to baseline at the end of the season. These results show there was a significant disturbance in WS following preseason suggesting these athletes having an altered response to the TL when compared to MS who had no change in CORTF. Interestingly, there was no change in CORTT for either team throughout the season. Though, it is important to note that all CORTT values were above the clinical range in WS. The authors hypothesize these high cortisol levels in WS

could be due to the lack of sex-specific ranges, the possible effects of menstrual

disruption indicating hypothalamic-pituitary-adrenal (HPA) axis and hypothalamic-

pituitary-gonadal (HPG) axis disruption, or possible alterations due to the effects of oral

contraceptives on the HPA axis, though these theories need to be investigated further [19].

These results for MS are consistent to what have been reported in the literature showing

the lack of change or even decreased cortisol response that has been reported in both

collegiate and professional male soccer players [20]. The results found in this study provide

additional support for the need for more female-specific ranges and data specifically on

the female athlete and highlight the use of cortisol as a useful biomarker in this

population.

In addition to the hormones associated with the HPA-axis, hormones associated

with the HPG-axis are often measured as indicators of stress overtraining. Testosterone

and estradiol are the primary sex hormones in males and females, respectively.

Testosterone is the primary male sex hormone and serves as the main androgen to

promote muscle growth[7]. Estradiol is the primary female sex hormone responsible for

female sex characteristics and has been shown to decrease with increased TL coupled

with low energy availability [21]. Not surprisingly, there was a large difference between

MS and WS in both TESTF and TESTT. MS showed no change in TESTF with a steady

decline in TESTT over the course of the season. This is consistent with the findings of no

change or a slight decrease in testosterone in overreached and over trained male athletes

[22]. Kraemer et al. (2004) found the opposite trend of an increase in TESTT over the

course of a collegiate soccer season in males, though it is important to note the current

study exhibited higher levels of TESTT throughout the entire season when compared to

these results [23]. Interestingly, WS had an increase in both TESTF and TESTT following the highest TL in the first training block before returning towards preseason levels throughout the season. This could be due to a possible increase in adrenal stimulation during the increased TL in the first training block as reflected by CORTF, though there is no current research on the testosterone response in females during increased TL indicating a possible novel finding in this population. When evaluating the female-specific sex hormone, E2, there was no change for WS, yet MS had a significant decrease following the first training block, which persisted throughout the year. The lack of change in WS is similar to previous meta-analytic findings that concluded there was no change in E2 with exercise [21]. It is important to note these results could be influenced by the use of oral contraceptives, though this was not measured in this study [21]. The lack of change in E2 supports the notion of the female athletes remaining in a favorable energy balance throughout the season, though further dietary analysis would be able to support this notion. The decreases in E2 seen in MS was unexpected but may be due to the increased levels at the preseason time point, which were considered high for a college-aged male. One possible explanation for this increase in E2 in MS could be the increased use of plastic water bottles in the heat of the summer time which can leak estrogenic chemicals; this may be masked in the females due to naturally higher levels or the use of oral contraceptives though this notion needs to be investigated further [24]. Similar to the testosterone response in females over the course of a season, the estradiol response in males has not been researched much due to the low levels in the opposite sex.

To further evaluate the sex hormone changes throughout the season, SHBG and PRL was evaluated. SHBG acts as a transport vessel for male and female sex hormones,

such as testosterone and estrogen, and has been shown to increase in response to exercise in both males and females [7]. PRL is a sex hormone released from the pituitary gland with the primary action of stimulating milk production in females with its main regulator being dopamine [25]. Outside of milk production, PRL has also been shown to increase in response to physical exercise, hypoglycemia, stress, and has particular relevance in female athletes due to its suppressive effects on estrogen [26]. SHBG exhibited no change in WS, while there was an immediate decrease in MS following the initial training block. Interestingly, the decrease following the first training block in MS is contrary to what is often found with exercise in both men and women, as previous literature has shown an increase in SHBG [21,27]. The decrease in MS could be linked to the decrease in E2 as it followed a similar trend, though there is no current research on the SHBG response as it relates to E2 during prolonged training. This study found no change in PRL in either team throughout the season. It is important to note that, similar to cortisol, PRL was consistently above clinical ranges in WS suggesting the need for a possible sex-specific range. Alternatively, these data suggest there may be altered HPA/HPG-axis activity in female athletes when compared to males, though this notion needs to be investigated further.

In addition to the sex hormones, GH and IGF-1 were also evaluated to get a more complete picture of anabolic status in both males and females. GH is a pulsatile anabolic hormone that is secreted throughout the day and night following a circadian rhythm, with the most potent stimulators being sleep and exercise [28]. GH's anabolic function is exhibited primarily on the downstream activation of IGF-1 which is produced in the liver and has anabolic characteristics [28]. Not surprisingly, there was no change in GH in MS,

primarily due to the increased levels of testosterone in males which acts as the primary

anabolic hormone. WS had an increased level of GH as it acts as the primary anabolic

hormone in females; GH exhibited a decrease after the first training block which

remained suppressed throughout the season. This response in resting GH could be due to

a reduced sensitivity with increased acute stimulation through high intensity exercise that

is performed throughout the season and thought to be a potential adaptation of training

[7,28,29]. It is important to note that a single resting measurement of GH may not accurately

represent total GH secretion due to the pulsatile secretion as well as the increases due to

training each day; thus, IGF-1 may be a better indicator. Interestingly, MS and WS had

an opposite response in IGF1 with MS increasing through T3 before returning towards

preseason values. In WS, IGF1 decreased through T3 before returning to preseason

values. Research has shown that there is a decrease in IGF-1 as athletes transition

towards a overreached state [7,17]. Furthermore, it is important to note that IGF-1 can be

decreased as a result of a negative energy balance, which makes the interpretation of

IGF-1 difficult due to the lack of dietary control in this study. Future studies should

analyze energy availability to better understand the effects of TL on GH and IGF-1 [30].

     Along with the HPA/HPG related hormones, inflammation was evaluated through

IL-6. IL-6 is an interleukin that acts as a pro-inflammatory cytokine that is upregulated

with muscle damage and injury in order to facilitate an immune response, decreased

muscle glycogen, and muscle contraction [31]. With chronic exercise, there can be

accompanying systemic inflammation and muscle contraction resulting in increasing

cytokines, such as IL-6, which can activate the HPA-axis and play a major role in the

cytokine hypothesis of overtraining [32]. Interestingly, the inflections seen in both MS and

WS did not correspond to other biomarkers associated with the HPA-axis such as cortisol. This modest increase in MS following the first training block may be associated with the increased muscle damage and contraction, as reflected in the increases in CK. This could indicate the differences in the physical play in MS compared to WS. Furthermore, both teams experienced a slight increase in IL-6 as the season progressed suggesting a possible cumulative effect of the season increasing basal levels of IL-6 due to possible chronic inflammation.

To further evaluate the possible muscle damage associated with training, CK was evaluated [33]. The first observed increase in CK occurred during the preseason which exhibited the highest TL in both teams, with MS having consistently higher levels. Similar results of increased levels of CK in males compared to females have been found in several studies due to an increase in muscle mass[33]. It is important to note that all CK values remained in ranges that are specific to both male and female athletes due to the normal muscle turnover and damage that is associated with participation in sport (male athlete reference range: 82-1083 U/L; female athlete reference range: 47-513 U/L) [34]. Similar results to this study have been observed in soccer players at both collegiate and professional levels, showing an increase in CK following preseason training, though these did not exceed what is considered normal athlete ranges [20,33,34]. The results of increased CK indicate the high stress and strain on athletes during the preseason and highlight strenuous and more damaging periods during the season where coaches and athletes can implement additional recovery strategies to promote optimal repair.

To evaluate the effect of season long TL on the regulation of metabolism, an evaluation of thyroid hormones was employed. Thyroid hormones are the primary

regulator of metabolism and contribute to performance and recovery which are influenced by energy balance [35]. The results of this study provide a complete evaluation of thyroid function over the course of a season. Throughout the season, there was no change in TSH for both teams. Despite these results in TSH, there was an increase in T_3 after the preseason in MS before returning towards baseline as the season progressed while no change was observed in WS. These results are consistent with what has been seen in the research of a decrease or no change in T_3 which is often shown with an increases in EEE [35]. Interestingly, T_4 increased in both teams before returning to baseline as the season progressed. This could be a result of the increased TL but difficult to assess without dietary information. It is important to note that all values remained within the clinical range which could indicate these athletes maintain sufficient energy availability.

In addition to hormonal and biochemical markers, dietary biomarkers can also provide unique information regarding athlete readiness and health status beyond the subjective nature of dietary recalls. Two dietary biomarkers of importance are Vit-D and n-3FA, which have been shown to have performance implications which include bone health, muscle damage, and inflammation, all of which can have significant effects on athletes [7]. In this study, there was in increase in n-3FA in WS following the initial training block before steadily declining towards the initial levels while MS remained stable through the first training block before declining. It is important to note that all values are below what is considered "optimal" but above high-risk levels. Given the sub-optimal levels along with high TL, these athletes may have insufficient dietary compensation to account for resource recruitment during the season. Furthermore, Vit-D decreased in both MS and WS immediately following the first training block.

Interestingly, MS had a subsequent decrease at T4 while WS maintained level during this time. This final decrease in Vit-D in MS occurred towards the end of the season which also coincides with a change in the seasons in the northeastern United States where practices will often be transitioned to indoor settings or more clothes are worn when playing outside. Given the changes in Vit-D and n-3FA during the season, supplementation may have a favorable impact on the recovery status of the athletes [36].

The hematological biomarkers evaluated in this study represent a well-rounded evaluation of Fe status throughout the season [37,38]. Total Fe status represents total Fe in the blood, while Fer represents stored Fe, which, in times of decreased Fe, is mobilized to maintain the oxygen carrying capacity of hemoglobin [37]. In addition, transferrin represents the main protein that binds to Fe and transports it throughout the body which is measured through TIBC, showing the capacity of Fe to bind to transferrin and %Sat indicating the occupied iron-binding sites on transferrin [38,39]. The changes in total Fe status indicates a shift towards a training-induced anemia or Fe deficiency in WS while MS remained unchanged but having lower than expected values. These results show there is a strong sex-specific response to the high TL seen in the first training block which decreased Fe, Ferritin, and %Sat in WS and approached clinical levels for anemia. This shift towards training-induced Fe deficiency or anemia after the initial training block containing preseason indicates the highest TL, plus the inability to monitor athletes in the summer months resulting in unknown training status entering the preseason, has severe negative implications for female's Fe status. The negative performance implication for iron deficiency and anemia have been well established and marked with decreases in performance, most importantly resulting in reduced VO_2 max[7]. The decreases seen in WS

are consistent with previous research in female athletes showing a decline in hematological values during the season in athletes[40]. The current study revealed a training-induced decrease in the hematological values that remained depressed until the final draw where it returned to preseason values. While these results did remain in the clinical ranges, the change from the preseason values represents a major change in these values that may not be optimal for athletic performance despite remaining "clinically" normal. This notion leads to evaluation of changes in athletes to optimize performance rather than focusing on clinical diagnosis. The lack of changes in MS are consistent with minimal changes being seen in male soccer players, which has been well-established [41]. Due to the lack of changes found in male athletes, hematological markers are rarely used in team biomarker panels, despite the significant disruptions in female athletes with high TL.

Overall, this study provides a unique comprehensive assessment of TL, DIS, EEE, and biomarkers in high-level male and female soccer players throughout the duration of a competitive season. It is important to recognize a few limitations with the study, though many of these limitations come with the reality of working with collegiate athletes in a real-world setting. Throughout the duration of this study, the athletes' diets were not controlled or measured. Despite the importance of dietary control, it is a challenging task to track nutrient intake for an entire team over the course of a season in student athletes. Furthermore, research has suggested that self-reported dietary measures can be highly flawed, impractical, and unreliable when being used with free-living team-sport athletes which is more difficult when using college athletes who already experience a very demanding season [42]. In addition to dietary markers, sleep quantity and quality were not

evaluated before blood draws. It is essential to keep expectations and demands on the athletes and coaches balanced with the reality of the season when compared to research requirements. When considering the changes in WS, it is essential to highlight that this study did not control for the menstrual cycle or the use of oral contraceptives. In a loose degree of control for the menstrual cycle, a 28-day period between blood draws was used. In hope to maintain the evaluation of these athletes in a real-world setting, it was the authors' view that this should not be controlled due to the fact that the female athletes themselves cannot control their menstrual cycle. While the authors recognize the importance of these factors, it was impractical to attempt to control all aspects of a free-living and actively-competing team in season, though it should be considered in future research to practically assess these issues. Future studies may also have difficulty in applying a stringent degree of control when the research becomes to invasive and disruptive to team activities, coaches, and players. Despite the limitations, this study provided a representation of free-living athletes at the same intuition.

PRACTICAL APPLICATIONS

These results provide valuable, real-world free living, observational data on high-level athletes comparing male and female athletes experiencing a similar season at the same institution. The results of this study revealed both male and female athletes experienced a decrease in aerobic performance throughout the season. Similarly, both teams experienced the highest TL, DIS, and EEE during the initial preseason training block that decreased throughout the year. This initial training block resulted in several hormonal, biochemical, and nutritional changes in both teams with WS experiencing altered hematological values. This study not only highlights the known sex differences

between males and females, but also sheds light to the different physiological responses of each sex to training. These results emphasize how the combination of both on-field TL along with biomarker analysis throughout a full season can provide additional information to the cumulative effects of not only the on-field stressors but the additional off field-stressors placed on collegiate athletes. The combination of biomarkers and TL monitoring provide a more complete profile on athlete health, readiness, and recovery. This can provide coaches and players additional information to maximize player management to optimize performance. Furthermore, the use of biomarkers can help develop sex-specific recommendations for female athletes who are inappropriately grouped with males' recommendations due to the lack of female-specific evaluation. Utilizing periodic testing and evaluation of biomarkers can provide opportunities to intervene and alter training to possibly mitigate the performance decrements seen in both teams at the end of the season. Furthermore, possible supplementation of both n-3FA and Vit-D in both males and females may aid in recovery and maintaining performance and heath throughout the entire season as well as supplementation of Fe in female athletes to reduce the negative changes in the beginning of the season. This study highlights the importance of utilizing monitoring techniques to maximize player performance.

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