“CHANGES IN NUTRITIONAL BIOMARKERS, SLEEP, PERCEIVED STRESS, AND PERFORMANCE IN DIVISION I FEMALE SOCCER PLAYERS THROUGHOUT A COMPETITIVE SEASON”

By

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ABSTRACT OF THE THESIS

CHANGES IN NUTRITIONAL BIOMARKERS, SLEEP, PERCEIVED STRESS, AND PERFORMANCE IN DIVISION I FEMALE SOCCER PLAYERS THROUGHOUT A COMPETITIVE SEASON

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Background: A balance between training stress and recovery is essential for successful athletic performance, thus the development of an evidence-based approach to monitoring changes in stress and recovery is critical. The purpose of this study was to combine analysis of nutritional biomarkers with mood, sleep, and performance assessments to examine changes in recovery and training status throughout a competitive season. **Methods**: Division I female collegiate soccer players (N=25; M_{age}=19.4 $\pm$ 1.4 yrs; M_{ht}= 167.9 $\pm$ 6.3 cm) participated in blood draws at the beginning of preseason (T1) and every four weeks after within ~18 hours following a game (T2-T4). Athletes arrived well hydrated following an overnight fast. Several performance tests were administered prior to the start of preseason (PT1) and end-of-season (PT2). These included body composition (body fat (%BF), fat free mass (FFM), and fat mass (FM), vertical jump (VJ), and VO2max. Glutamine (Glu), Taurine (Tau), Tryptophan (Trp), Phenylalanine (Phe), Iron (Fe), Vitamin B12 (VitB12), Vitamin D (VitD), and Omega-3 (n-3FA) were analyzed. Mood and sleep were assessed using the Multi-Component Training Distress Scale (MTDS), and the Pittsburgh Sleep Quality Index (PSQI), respectively. Workload was assessed using several measures including heart rate data, distance covered (Dis), caloric expenditure (kcal), caloric expenditure (kcal/kg), and training load (TL). Additionally, counter movement jump (CMJ) was assessed at the beginning of practice at each time point. RM MANOVAs with univariate follow-ups were conducted with significance at P<0.05. **Results**: Weight was maintained throughout the season despite an increase in LBM (P<.05) and a decrease in %BF (P<.05). VO_{2max} and VT significantly decreased (P<0.05). CMJ was maintained from T1-T3 but began to decline at T4. Measures related to training load decreased following T1-T2 (P<0.05; ES_{TL}=-2.86, ES_{Dis}=-1.33, ES_{kcal}=-2.22, $ES_{kcal/kg}$=-2.78) and remained depressed through T4. Total mood disturbance increased from T2-T3 (ΔMood= 6.4 ± 1.9, P<.05) and remained elevated. There was an initial decrease in perceived stress (PS) from T1-T2 (P<0.05, ES=-0.95) that was followed by an increase at later portions of the season (P<0.05). No changes in sleep quality (SQ) were seen. Sleep duration (SD) increased from T3-T4 (ΔSD=0.4±0.1, P<.05). n-3FA increased from T1-T2 (Δn-3FA= 0.5 ± 0.1 %, P<.05), then returned to baseline. VitD decreased from T1-T2 (ΔVitD= 6.8 ± 1.4 ng/mL, P<.05) and continued a downward trend. VitB12 increased from T1-T3 (ΔVitB12= 72.0 ± 18.8 pg/mL, P<.05) and remained elevated. Fe decreased from T1-T2 (ΔFe= -29.6 ± 7.9 mcg/dL, P<.05), before returning to baseline. There were no significant changes in Phe or Tau (P>.05). Trp decreased from T2-T3 (ΔTrp = -10.9 ± 4.3 umol/L, P<.05) and remained depressed. Glu increased from T1-T2 (ΔGlu = 82.1 ± 23.1 umol/L, P<.05) then returned to baseline. **Conclusions**: The highest training stress occurred during the initial training block (T1-T2) and resulted in negative changes in VitD and Fe. The greatest mood disturbance occurred at the end of the season when Trp levels also declined. Trp, a

precursor of serotonin, may provide a mechanism for understanding changes in mood typically reported with overreaching. Decreases in VO_{2max} and VT also occurred at the end of the season which corresponded to tournament play. SQ may be more important for full recovery than SD, as increased SD did not mitigate changes in mood. These results highlight the stress experienced by a collegiate athlete during a condensed season including increased physiological disruption around tournament play. Further, this holistic approach to athlete monitoring may provide greater insight to assessing accumulated stress both on and off the field by tracking physiological, nutritional, and psychological changes throughout a season.

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Chapter 1: Literature Review

I. Demands Placed on Competitive Athletes

In recent years, there has been a growing interest in recovery for sport and exercise, both in practice and in academic research. Athletes, coaches, and researchers alike recognize the importance of adequate recovery, not only for the health of an athlete but also for improvements in performance. Proper recovery is essential to reduce the risk of injury, to prevent prolonged periods of breakdown or illness, and ultimately to promote optimal health of an athlete. Similarly, adequate recovery allows an athlete to train more effectively thus enhancing fitness, technique, efficiency, and overall performance (Kellmann, 2010).

The high physical demands placed on competitive athletes can make achieving adequate recovery a challenge. For example, soccer is a popular sport worldwide that is recognized for high training load demands and a reduced time for recovery. A typical soccer match lasts about 90 minutes, during which an elite level player will cover an average distance of 10-12 km and maintain an average intensity around 80-90% of maximal heart rate (Stølen et al., 2005). Additionally, soccer players must repeatedly produce high power while maintaining intensities close to VO_{2max} (Rielly, 2001; Clark et al., 2003) and these metabolic demands are further increased by movements such as accelerating, stopping, changing pace, turning, jumping, kicking and tackling (Reilly, 1997). As a result, soccer can be classified as a power-endurance sport where athletes must maximize both their aerobic endurance and anaerobic power (Kraemer et al., 2004).

The degree of stress imposed on soccer players has been evaluated for decades. However, quantification of the physiological demands and recovery strategies has mainly been restricted to players at the professional and national team level. As a result, populations of competitive soccer players at the collegiate level are far less studied. However, college soccer players may also experience a significant amount of stress and may be worth investigating.

In college soccer, there is a short amount of time allotted to preparation for the season due to NCAA regulations. As a result, during preseason training an athlete is required to participate in frequent high intensity training sessions that are often followed by little or inadequate recovery. This has the potential to create an environment where an athlete is at risk for sustaining injury and illness even before the season begins. Inadequate recovery during preseason training may also have a lasting negative impact on later portions of the season if inappropriate recovery continues. Further, during the regular season a college soccer player will typically participate in as many as two games per week. These games are often played less than two days apart and may require extensive travel between games. As a result, the frequency of match play for college soccer differs greatly from professional athletes who will normally participate in one game per week (Vescovi & Favero, 2014). Aside from game play, college athletes also must regularly attend lengthy training sessions, team meetings, or other team activities, in addition to keeping up with their academic requirements. Therefore, it may be said that the physical demands imposed on collegiate players both on and off the field are very high and are worth investigating.

In addition to collegiate level soccer players, female soccer players in particular are another subpopulation who tend to be understudied compared to their male counterparts. Participation in women's soccer has grown significantly over the past few decades, including more opportunities to play in college since the passing of Title IX (Vescovi & Favero, 2014). Still, the bulk of available research investigating the physical demands and recovery needs of soccer players has been performed with men. From the available research performed in female players, it has been suggested that the physical demands experienced by female athletes may be similar to those experienced by male players. During a game, elite female soccer players have been found to cover similar distances compared to men (Davis & Brewer 1993). Additionally, across men and women, similar proportions of the game appear to be spent at varying intensities and women seem to tax their aerobic and anaerobic energy systems to level comparable to men (Davis & Brewer 1993). Still despite some understanding gained regarding the physical demands experienced by female players, the availability of published research on the physiological effects is still lacking. There is a lack of established criteria or standards for monitoring female players which makes evaluation of the physical capabilities and recovery needs of female soccer athletes difficult. Further, most research on females is also performed on elite level players and the availability of research investigating the demands of female collegiate players is even more scarce.

II. Normal Training Responses, Abnormal Training Responses & Potential Challenges

Although female and college athletes may be less studied compared to professional and male athletes it is still apparent that all competitive athletes endure a considerable amount of physical stress requiring adequate recovery. Researchers have been trying to understand the manifestation of stress and physiological responses to training for many years. Much of the original research adapted the ideas of Hans Selye's General Adaptation Syndrome (GAS). GAS describes the physiological response that occurs within the body due to stress, and the subsequent adjustments to reach homeostasis. This theory may be applied to a number of settings including sport performance (Halson & Jeukendrup, 2004). As with many other sources of stress, following an increase in training load the body will seek to maintain homeostasis through adaptation. In sport, the application of training stress and resulting desired adaptive response is called supercompensation. Following an increase in training load, an athlete will experience fatigue and a temporary reduction in performance. If followed by adequate rest, a positive training adaptation will occur, and performance will be enhanced compared to baseline. As a result, it can be said that a certain degree of fatigue is necessary in order to produce changes in fitness and performance (Carfagno & Hendrix, 2014; Gambetta, 2007).

The application of stress during training can be manipulated in several ways. In order to ensure adaptation, the athlete must be healthy, and the appropriate training stress must be applied. If the training stimulus is too difficult or outside an athlete's capacity for adaptation, the athlete will struggle to reach homeostasis, resulting in fatigue and underperformance. Conversely, if the training stimulus is not sufficient enough there will be little to no adaptive response or enhancement in fitness. As a result, the goal of an effective training program should be to match increases in training load and resulting fatigue with adequate rest and recovery in order to enhance performance (Carfagno & Hendrix, 2014; Gambetta, 2007).

Although necessary, matching a physically demanding training schedule with adequate rest is much easier said than done. Changes in training frequency, duration, and intensity over a long competitive season must all be considered and incorporated into scheduling appropriate rest periods in order to avoid maladaptation. This becomes even more of a challenge when working with team sports such as soccer where there is great variation regarding level of physical fitness, capacity for adaptation, and recovery needs among players (Halson, 2014). Further, fatigue by nature is highly influenced by many factors. Several of these factors may occur outside of training and therefore may be even more difficult to account for by coaches, sports medicine, and other staff. These factors may include, but are not limited to, nutritional habits, sleep habits, illness, or other lifestyle and environmental stressors that may increase the amount of fatigue within an athlete and influence the ability to recover following training (Main & Grove, 2009; Meeusen et al., 2013).

The idea that the accumulation of training-related and non-training related stress must be considered in order to fully understand manifestation of fatigue and recovery needs may be particularly important to college athletes considering that these athletes experience stress related to their athletic status as well as academic demands. For example, many college athletes experience stress outside of the pure physicality of training including injuries, conflicts with coaches or teammates, perceived pressure to win, or excessive anxiety. They are also required to manage their time efficiently as a result of busy travel schedules, regularly have to make up missed coursework, on top of being a full-time student (Wilson & Pritchard, 2005).

Due to their split demands between academics and athletics, a considerable amount of stress can occur within a college athlete that can significantly impact their ability buffer the physical demands of training. It has been reported that half of male collegiate athletes and more than half of female athletes indicate that their stress associated with being a college athlete significantly affects their mental or emotional health (Wilson & Pritchard, 2005). Further, these individuals may be more likely to engage in poor health habits that could also impact their ability to recover adequately from exercise (Wilson & Pritchard, 2005). Moreover, it is well known that athletes are at a higher risk for injury during periods of heightened stress (Williams & Andersen, 1988; Blackwell & McCullagh, 1990), regardless of whether the stress stems from competition, academics, or other life stressors (Mann et al., 2016).

As a result, the interaction of both training related, and non-training related stress presents a unique challenge for ensuring proper recovery in college athletes. Stress is a common occurrence among this population, and it is not possible to eliminate certain sources of stress. Instead, coaches and other sport staff must find ways to help their athletes cope with accumulated stress and resulting fatigue so that they can recover adequately from training. This will ultimately lead them to improved performance and success in sport. However, ensuring adequate recovery is challenging especially when dealing with team sports and many athletes with different capacities for adaptation to stress.

III. Consequences of Inadequate Recovery & The Overtraining Syndrome

As mentioned previously, utilizing supercompensation through manipulation of training load is commonly employed by many athletes and coaches to improve physical fitness. Following an increase in training load, an athlete will experience fatigue and diminished performance that will ultimately be followed by a positive training adaptation if accompanied by appropriate rest. These short-term decrements in performance are commonly referred to as overreaching (OR). Overreaching in an athlete may require several days or weeks to recover from fully, and some may even consider it to be a part of the normal training process (Meeusen et al., 2013). However, if appropriate recovery is not allowed, a disruption in adaptation to training continues and a state of overtraining (OT) may develop. Overtraining or Overtraining Syndrome (OTS) can significantly compromise an athlete's career (Meeusen et al., 2013; Halson & Jeukendrup, 2004).

Although the exact cause of OTS is unknown, it is generally agreed to result from the accumulation of training and non-training related stressors that exceed an individual's capacity for adaptation. Additionally, performance decrements associated with overreaching and overtraining may take anywhere from several days to months to recover from and may or may not be associated with additional physiological or psychological impairments (Meeusen et al., 2013). Therefore it is generally agreed upon that OTS differs from OR primarily in the amount of time required for a full recovery rather than the amount of stress, type of stress, degree of physiological impairment, or symptoms associated with the condition (Kreher & Schwartz, 2012; Carfagno & Hendrix, 2014).

Impairments that result from OR and OTS may include immune suppression, increased perception of fatigue, as well as mood and sleep disturbances (Kreider et al., 1998).

Several theories have attempted to explain the pathophysiology behind OTS, although none are definitive. A few of the current hypothesis that have received support in the literature include the hypothalamic hypothesis, glutamine hypothesis, and the central fatigue hypothesis. The hypothalamic hypothesis focuses on the hypothalamus and its role in the autonomic nervous system, including the hypothalamic-pituitary-adrenal axis (HPA) and the hypothalamic-gonadal-axis (HPG). The hypothalamus is well recognized as a major coordinating center for neuroendocrine function and involvement in the stress response. It is responsible for regulation of several stress related hormones such as cortisol, epinephrine, and norepinephrine, as well as several gonadal hormones such as testosterone and estradiol. Chronic intense training that can possibly lead to OTS is known to cause a significant amount of physiological and psychological stress in an athlete. As a result, it is likely that OTS may involve the hypothalamus due to its involvement in the stress response. With an appropriate training stimulus, stress can be buffered adequately, and the related axes are stabilized (Smith, 2000; Carfagno & Hendrix, 2014). However, significant physiological and psychological stress experienced by an athlete may result in disruptions in hormonal balance and these imbalances have been associated with overtraining athletes (Barron et al., 1985; Fry et al., 1991; Stone et al., 1991). This impaired hormonal response has been suggested to be due to chronic exposure to stress and a decreased responsiveness of the HPA axis (Cadegiani & Kater, 2017).

Although alterations in the HPA axis and subsequent imbalances in several hormones have been associated with OTS, results of studies that clearly demonstrate these changes in athletes are lacking (Lehmann et al.,1998; Urhausen et al., 1998). Additionally, these hormonal imbalances cannot account for all symptoms seen with overtraining syndrome. For example, testosterone and cortisol are two hormones under hypothalamic and pituitary control that influence the anabolic and catabolic balance, and concentrations have been shown to fluctuate with different training variables. However, changes in levels of resting cortisol, testosterone, and the ratio of testosterone to cortisol have not been well supported in overtrained athletes (Halson & Jeukendrup, 2004; Kreher & Schwartz, 2012). This conclusion could be due to the studies used misinterpreting the subjects as “overtrained” when they were most likely in a non-functional over reached state at best.

Overall changes in stress related hormones are not consistently observed in overtrained athletes, and inconsistent findings may be attributed to a number of factors including varying testing protocols, exercise capacity of individuals, and levels of other hormones (Carfagno & Hendrix, 2014). Still, alterations in the HPA axis are likely related to fatigue states observed in athletes considering that high-level training has the potential to induce significant levels of stress, both physiologically and psychologically. As a result, changes in the HPA axis should be considered and more research is needed to fully understand the role that the hypothalamus plays in the training response, particularly in a real-world setting, including whether changes in certain stress-related hormones may occur as a byproduct or a predictor of OTS (Smith, 2000).

In addition to the role of the hypothalamus, several other hypotheses have been developed to explain OTS that involve the amino acids glutamine and tryptophan. The glutamine theory was proposed by Newsholme and colleagues which suggests that reductions in blood concentrations of the amino acid may contribute to the impaired immune response and increased infection rate seen with OTS (Newsholme et al., 1991). It has been suggested that chronic intense training results in a significant decrease in blood concentrations of glutamine due to an increase in the use of glutamine for fuel by lymphocytes and macrophages (Newsholme et al., 1991; Smith, 2000). For example, decreases in glutamine have been observed in runners who were believed to be overtrained and did not normalize even after performance decrements improved (Foster & Lehman, 1997). Additionally, Rowbottom et al. (1995), looked at a wide range of biochemical, physiological, and immunological markers and found that glutamine was the only marker that was consistently blunted. As a result, glutamine may serve as an indicator of increased exercise stress and immune impairment in athletes, but it has also been suggested that it is unlikely that glutamine is the primary cause of OTS. It is more likely that glutamine plays a critical role of exercise related metabolism and may serve as a potential an indicator of OTS (Keast, 1996; Smith 2000).

Additionally, the amino acid tryptophan has received some attention in light of OTS, where decreases in circulating levels of tryptophan have been thought to reflect increased uptake by the brain. Tryptophan is the precursor for serotonin and therefore its interaction in the brain may be related to changes in mood, sleep, and appetite that have been observed with OTS (Graeff, 1997; Smith, 2000). This is known as the central fatigue hypothesis and both Newsholme et al. (1991), as well as Kreider et al. (1998), have suggested that changes in tryptophan concentrations may account for several of the behavioral changes observed in OTS. However, consistent observations of increased tryptophan uptake and resulting increases in serotonin levels have only been seen in animal research (Gastmann & Lehmann, 1998). A lack of consistent findings in human research may be due to non-standardized testing protocols or because studies were performed in an acute setting where athletes were not truly overtrained (Kreider et al., 1998).

Overall OTS is caused by an increase in accumulated stress within an athlete that is not paired with adequate recovery. OTS is also known to have severe consequences for the career of an athlete. Today, the mechanisms involved in the manifestation of OTS are not clearly understood, although many markers have been proposed as indicators of OTS. More research is still needed to identify markers related OTS so that they can be used to provide a conclusive diagnosis of OTS, or, better yet, help identify athletes who are possibly at risk for OTS. Ultimately changes in markers related to excessive training stress and inadequate recovery can be used to prevent significant damage in an athlete due to OTS before it is too late.

IV. Current Methods for Monitoring Stress and Recovery

The ability to understand fatigue experienced by an athlete and possible risk of training maladaptation is essential for both health and performance. As a result, implementation of methods for monitoring changes in training loads and physiological responses to training by coaches is essential. It will allow coaches to better understand their athletes' responses to training as well as determine their degree of readiness for competition. Further, it will prevent coaches and athletes from making the mistake of associating all performance decrements with a lack of effort or fitness, and the mentality of training harder when additional rest may be needed. Objectively monitoring athletes will allow for more individualization of training programs in team sports, allow coaches to retrospectively evaluate relationships between training load and performance, plan for future training and competition, as well allow for prevention and early detection of athletes that may be at risk for overtraining (Halson, 2014).

Several methods have been developed to monitor training load and determine appropriate recovery strategies needed following training. Although multiple measures exist, few are well supported by research and there has yet to be a single marker developed that is indicative of the stress-recovery state of an athlete. Existing methods primarily include tracking performance and physiological changes, changes in mood and sleep, as well as changes in biomarkers related to health and nutritional status.

Performance and Physiological Measures

Monitoring of athlete performance and additional physiological measures such as heart rate may be useful for tracking performance readiness of an athlete throughout a season. High amounts of fatigue typically result in the inability to sustain exercise at an intensity that could be performed previously as well as decreases in sport-specific performance. Additionally, a key feature of OTS is an unexplained decrease in performance, therefore tracking changes in performance and physiological related variables may be useful to understand an athlete's response to a training program (Meeusen et al., 2013).

Several different performance tests have been proposed including time to fatigue tests, time trials, and high-intensity exercise performance testing, however it is debated as to which test is the most effective. Sport specificity must also be considered and time trials or time to fatigue assessments may only accurately represent specificity of certain endurance type activities but not across all sports (Halson et al., 2003). Soccer players must maximize both aerobic and anaerobic fitness throughout a season; as a result, high-intensity exercise performance measures may be a more appropriate. In soccer, higher maximal oxygen consumption (VO_{2max}) is related to key components of a match including total distance covered, total distance covered at high intensity running, number of sprints performed, and number of contacts with the ball during a game (Rampinini et al., 2007). Monitoring changes in aerobic capacity of soccer players across a season and the ability to sustain exercise at a given intensity may be an appropriate reflection of response to and recovery from training throughout a season (Meckel et al., 2018). Moreover, there is a large anaerobic component to soccer. As a result, certain high intensity performance measures such as countermovement vertical jump (CMJ) may also reflect anaerobic performance capabilities in a soccer athlete throughout a season (Rampinini et al., 2007).

In addition to measures related to performance, physiological measures such as heart rate may be used to monitor physiological responses to training and may be combined with performance measures. Heart rate is a straightforward and easily accessible measure that can be used to assess internal load and performance capacity in a variety of sports (Bosquet et al., 2008). There is an ample amount of research that has demonstrated the use of heart rate monitors as valid and reliable during both physically and mentally stressful situations when compared to ECG (Seaward et al., 1990; Goodie et al., 2000). There has also since been an increase in the use of heart rate monitoring in team sports such as soccer, which require an accurate analysis of the intensity of training and means to evaluate the effectiveness of training programs (Alexandre et al., 2012).

Heart rate monitoring technology is based on the linear relationship between heart rate and oxygen utilization during steady-state exercise (Hopkins, 1991). As a result, heart rate responses during training can be used to reflect work intensity. This information is highly utilized by coaches who can use percent of maximum heart rate to both prescribe and monitor the intensity of training programs (Borresen et al., 2008). Additionally, heart rate recovery following exercise may be an indicator of fitness and function in an athlete. If the time needed for recovery during training is prolonged in a particular athlete, this may indicate that additional rest is needed. Moreover, resting heart rate may also indicate health and training status of the athlete. With increases in fitness, athletes will generally see a reduction in resting heart rate. In the same sense, increases in resting heart rate may, therefore, indicate a loss of fitness, decline in health, excessive fatigue due to training, or a combination. Further, the more recent incorporation of energy expenditure estimates may provide additional measures that can be used to assess physical demands across a season and provide insight into the recovery needs of an athlete (Achten & Jeukendrup, 2003).

Although there may be several benefits to performance and physiological monitoring of heart rate throughout a season, they are not without their limitations.

Research regarding the use of specific performance tests is still limited and several problems have been identified. For example, one limitation is that baseline measures of performance tests are not always available and therefore their use to assess decrements in performance is inadequate. Further, standardized testing conditions are necessary to detect changes but are not always replicable from one environment to another or even within the same laboratory. For example, standardized temperature and pressures can influence the results of a test but can be variable depending on environmental factors and number of tests performed. Also, if a test is not performed in a laboratory setting, environmental conditions will be even more variable, but such conditions may also be more representative of those actually seen by an athlete. This contributes to a big critique of many performance measures available, which is their ability to reflect sport performance changes outside of standard testing conditions (Meeusen et al., 2013). Additionally, there is a 6.5% daily variation in heart rate that occurs outside of any training or non-training related factor that must be considered (Borresen & Lambert, 2009). Heart rate responses are highly sensitive to a variety of factors applicable to athletes including hydration status, environmental conditions, nutrition status, and sleep (Borresen & Lambert, 2009).

Overall, changes in performance and physiological responses to training may occur as a consequence of fatigue, but not all aspects are impacted uniformly, and interpretation of data may be difficult as a result. Additionally, it is likely that other physiological changes may occur prior to changes in performance, and therefore could be detected earlier in effort to prevent detrimental changes with added rest. Similarly, if performance decrements are suspected, an athlete may already be at risk for significant consequences due to maladaptation to training stress or may be at risk for OTS. At this point it may be too late to prevent any significant performance or physiological impairment by rest alone. As a result, measures of performance should at least be combined with other types of assessments that also indicate fatigue to fully understand the state of the athlete (Achten & Jeukendrup, 2003).

Subjective Assessment of Mood and Sleep

Mood and sleep profiles have also been suggested to reflect an athlete’s readiness to perform knowing that recovery from exercise is enhanced by both optimal nutrition and sleep. With regard to mood, if periods of intense training are followed by inappropriate recovery it has been reported that an athlete may experience additional psychological disturbances. Psychological disturbances may include changes in overall mood, irritability, restlessness, anxiousness, loss of motivation, and depression. As a result, changes in mood can be monitored in an athlete to potentially suggest when training loads are adequate or excessively high (Purvis et al., 2010). Subjective responses to training and athlete wellbeing are obtained using self-report measures or questionnaires. These measures examine mood profiles, perceived stress, and recovery, physical or behavioral symptoms, or a combination of these factors (Nässi et al, 2009). Additionally, scales or items included in these measures may address symptoms that are sport specific, related to general wellbeing, or a combination (Saw et al.,2017).

Research using self-report measures began in the 1980s, where changes in mood were observed in swimmers using the Profile of Mood States (POMS) during periods of heightened training stress (Morgan et al., 1987; Saw et al., 2017). The POMS has shown to consistently respond to changes in TL across a variety of sports (Meeusen et al., 2013). Since the development of the POMS, several other subjective report measures have been developed and proven to be valid and reliable indicators of athlete wellbeing. The most supported measures include the Training Distress Scale (TDS) (Raglin & Morgan, 1994), Recovery-Stress Questionnaire for Athletes (RESTQ-Sport) (Kellmann & Kallus, 2001), Daily Analysis of Life Demands for Athletes (DALDA) (Rushall, 1990), and Multi-Component Training Distress Scale (MTDS) (Main & Grove, 2009), and the State Trait Anxiety Inventory (STAI) (Spielberger 2010; Cox et al., 2003; Saw et al., 2015).

A recent review by Saw and colleagues (2017) indicated that subjective measures are more sensitive and consistent in response to changes in training stress than several objective measures. This finding has also been reported by several other studies, and it has been concluded that subjective measures exhibit a dose-response relationship with TL (Saw et al., 2015; Meeusen et al., 2013; Urhausen & Kindermann, 2002; Hooper & Mackinnon, 1995). During acute increases in TL, subjective measures indicate impaired well-being, and improvements are seen following a reduction in training load (Halson et al., 2002; Coutts et al., 2007). Similar responses are also observed during chronic changes in training load (Raglin et al., 1996; O’Connor et al., 1989). Overall the sensitivity of subjective measures and ability to reflect changes in both acute and chronic training load may be necessary for ongoing monitoring of the degree of stress experienced by an athlete over a long season (Meeusen et al., 2013).

In addition to changes in mood and perceived stress, changes in sleep quality and duration may also reflect the ability of an athlete to recover adequately from training.

Sleep promotes many physiological, psychological, and cognitive processes that are important to competitive athletes. For example, sleep plays a role in recovery from training and is important in functioning of the immune system. Sleep deprivation has been associated with impaired cellular and hormonal functioning and may have a negative impact on tissue healing and recovery in athletes. Further, sleep plays a role in neuromuscular performance, skill learning, and mood (Venter, 2012).

Additionally, some evidence suggests that athletes may experience reductions in quality and quantity of sleep with very intense training (Halson, 2014). For example, Urhausen and colleagues found that overtrained cyclists and triathletes reported difficulty falling asleep and sleeping through the night (1997). Similarly, Hooper and colleagues reported increased levels of mood disturbance, sleep disturbance and stress following competition (1995). Further, college students are one population where reductions in sleep duration, delayed bedtimes, and increased napping has been reported (Venter, 2012). As a result, monitoring sleep quality and quantity may be a useful way to prevent significant decreases in performance and health, especially in a college athlete.

The use of self-report measures to detect changes in mood and sleep demonstrates the potential for identifying individuals at risk for maladaptation to training stress. However, there are still several limitations. Currently, there is no standard procedure for implementation of self-report measures and only general guidelines have been provided. This is problematic because the application is impacted by a variety of factors including time, frequency, and type of questionnaire administration. As a result, use of self-report measures in sport tends to favor short custom assessments over those that have been supported by research which creates a gap between research and application (Saw et al., 2017). Moreover, response distortion is another significant limitation. Response distortion includes when subjects falsely respond to items on a questionnaire, particularly those items that are more personal or sensitive. Athletes may intentionally fake responses that indicate increased well-being for fear of having playing time reduced and to present themselves positively. Conversely, athletes may intentionally fake responses which indicate they are fatigued in attempt to have their training load reduced. Response distortion may also be unintentional and influenced by the type or frequency of measurement. For example, response fatigue may be present when questionnaires are administered too frequently (Meeusen et al., 2013). Overall athlete monitoring should not be limited to use of either subjective or objective measures alone to monitor the training response. Instead, subjective and objective measures should be used in combination, in order to complement one another and provide a complete analysis of the recovery state of an athlete (Saw et al., 2015).

V. Biomarkers Related to Nutrition and Health

There has been a vast amount of research examining the biochemical, hormonal, and immunological responses to exercise training. It has been suggested that these responses may be used to monitor fatigue levels and overall health of an athlete, and thus prevent any excessive amounts of fatigue or risk of maladaptation (Halson, 2014; Urhausen et al., 1995). Some markers that have been researched extensively include creatine kinase, testosterone, cortisol, and growth hormone, as well as other markers related to hematological status and immune function. Research on biomarker monitoring in athletes is promising, but there are still several limitations. There is an abundance of biomarkers that assess very different, yet essential, aspects of athlete health. As a result, it may not be possible that a single marker may be indicative of an athlete's health status and readiness to perform. Further, the availability of research that consistently demonstrates the sensitivity of a single biomarker or group of markers to indicate fatigue in an athlete is limited. Lastly, reference ranges specifically for athletic populations are not well defined, and there is a large amount of individual variability seen with fluctuations of markers (Lee et al., 2017).

In addition to hormonal and biochemical markers, specific markers related to nutrition status and health may provide additional insight into the recovery and performance potential of an athlete. It is well understood that exercise performance and recovery are significantly impacted by nutrition status. Both can be improved when nutrition is adequate, additionally, both may be negatively affected when proper nutrition is lacking (Lee et al., 2017). Furthermore, nutrition can have a significant impact on hormonal responses to exercise, and energy deficiencies have been shown to impair specific neuroendocrine pathways involved in exercise performance (Purvis et al., 2010). Although the importance of proper nutrition is well recognized, tracking of specific nutrition related markers important to recovery and performance in an athlete has not been researched extensively. Further, disordered eating patterns are commonly seen in athletes, and are particularly higher in female athletes (Escalante, 2016). As a result, monitoring nutritional biomarkers in this population, who also experience such a high caloric expenditure, may help avoid several nutrient deficiencies are that are frequently observed, and help optimize health and adaptations to intense training.

Traditionally, the nutritional status of an athlete is performed using subjective assessments such as dietary recalls and questionnaires. However, the results of these measures are profoundly impacted by bias. Biomarker testing provides a more objective means to monitor nutritional markers and therefore eliminates any bias related to nutrition status. Also, biomarker assessment may help identify any deficiencies of specific dietary markers and allow for tracking changes throughout a season that may influence recovery or performance (Lee et al., 2017).

It is well accepted that both changes in macronutrients and micronutrients can significantly impact an athlete's ability to recover and perform at a competitive level. Micronutrients of particular interest to an athlete include Omega-3 polyunsaturated fatty acids (PUFAs), vitamin D, iron, and vitamin B12 (Lukaski, 2004). Additionally, athletes require greater protein intake to promote recovery and muscle protein synthesis. Moreover, levels of specific amino acids such as glutamine, phenylalanine, tryptophan, and taurine may provide additional insight into the ability of an athlete to recover (Lee et al., 2017).

Omega-3 fatty acids

Fat is used as an energy source at times during exercise, especially when carbohydrate sources are low. Additionally, certain types of fats have shown to have potential impacts on recovery and performance. Omega-3 fatty acids (n3FA) such as eicosapentaenoic acid (EPA) and docosahexaenoic acid (DHA) found in fish oil are well known for their cardioprotective and anti-inflammatory properties (Mickleborough, 2013). Additionally, dietary intake levels may also be important to an athlete to optimize recovery and ability to train. n3FA possess both antioxidant and anti-inflammatory properties, both oxidative stress and systemic inflammation increase acutely in response to strenuous exercise and are necessary to induce training adaptations (Mickleborough, 2013, Lee et al, 2017). However, continued increases in oxidative stress and inflammation are also observed with chronically high training volumes or exercise overtraining, and can ultimately lead to performance decrements and consequences for health (Lee et al, 2017). As a result, it may be essential to monitor n3FA status in an athlete who undergoes a rigorous training schedule (Mickleborough, 2013).

With n-3FA supplementation, previous research has shown decreases in oxidative stress and inflammation. This suggests that supplementation may be useful for managing high levels of oxidative stress and inflammation seen with high training stress (Bloomer, Larson, Fisher-Wellman, Galpin & Schilling, 2009). Similarly, supplementation has shown to attenuate muscle soreness post exercise and decrease perceptions of fatigue during exercise (Jouris et al., 2011; Tartibian et al.,2009). As a result, n-3FA may help promote recovery and training adaptations with an increase in training volume (Jouris et al.,2011; Tartibian et al.,2009). Conversely, it has been suggested that low levels of n-3FA may put an athlete at risk for maladaptation to exercise stress, where low n-3FA status may result in the inability to manage oxidative stress and inflammatory responses with training and will negatively impact the ability to recover (Bloomer et al., 2009). Further, n-3FA is also known to influence neuromuscular function, nerve conduction velocity, and neuromuscular sensitivity. Therefore, adequate levels of omega-3 may play a role in optimizing exercise performance capacity (Stiefel et al., 1999). Overall, ensuring adequate n-3FA status is essential for ensuring at least adequate recovery with intense training and may possibly facilitate enhanced recovery and subsequent adaptations to training.

In addition to changes in recovery responses, changes in n-3FA levels have shown to influence mood and sleep patterns. Low levels of n-3FA, particularly low levels of DHA, have been linked to sleep disturbance potentially due to low levels of melatonin (Peuhkuri et al., 2012). The pineal gland responsible for melatonin synthesis and contains high levels of n-3FA. There is some evidence that reductions in n-3FA may reduce nighttime melatonin secretion (Peuhkuri et al., 2012), but both sleep duration and quality have shown to improve with n-3FA supplementation (Peuhkuri et al.,2012; Montgomery et al, 2014). In reference to mood, supplementation of n-3FA has been linked to increases in vigor and decreases in negative mood profiles measured by the POMS scale (Fontani et al., 2005, Parker et al., 2006). n-3FA supplementation has also been used as a form of treatment for mood related disorders (Fontani et al., 2005, Parker et al., 2006). Overall, both disruptions in mood and sleep are observed with inappropriate increases in training volume, and these disruptions may be further increased by low levels of n-3FA. Therefore n-3FA status should be considered to assess the functioning of an athlete and adaptations to training. n-3FA may play an important role in the ability to recovery and changes in n-3FA may help explain or attenuate fatigue and reductions in performance. Further, changes in n-3FA should be explored in relation to mood and sleep disturbances that also observed with inappropriate increases in training stress.

Vitamin D

Vitamin D is an essential fat-soluble hormone that is made via the synthesis of cholecalciferol in the skin due to sun exposure. This hormone is involved in the expression of over 900 gene variants (Dahlquist et al.,2015). Despite its importance, many studies have reported a high prevalence of insufficiency and deficiency of vitamin D across all age groups throughout the world (Dahlquist et al.,2015). Vitamin D is well recognized for its importance in bone health through enhancement of calcium reabsorption and bone deposition. Additionally, recent research has suggested that low vitamin D status may be linked to various non-skeletal chronic diseases and autoimmune diseases, as well as play a role in immune function, inflammation, and muscle function (Halliday et al., 2011).

Due to the many essential roles of vitamin D in the body, it is plausible that serum vitamin D levels can have a significant impact on athlete health and performance. However, little is known about the vitamin D status of athletes or how status changes throughout a season. Of the few studies conducted, a high prevalence of insufficiency and deficiency, even among athletes who train outdoors, has been documented (Halliday et al., 2011; Maimoun et al., 2006). Deficiency in athletes may also be related to an increased requirement and greater rate of utilization via enzyme activity with exercise (Dahlquist et al., 2015). Regardless, it is likely that vitamin D status significantly impacts the overall health of an athlete and ability to train through influence on bone health, exercise related immunity, inflammation, and muscle function (Ogan & Pritchtt, 2013).

Low levels of vitamin D has been associated with an increased risk of bone fracture and bone disorders due to higher amounts of reabsorption, and as a result can lead to increased risk of bone-related injury (Ogan & Pritchett, 2013). Similar, inadequate levels may increase the risk of overuse or inflammatory injuries, in addition to increasing the risk of upper respiratory tract infection or other illness (Halliday et al., 2011). In addition to bone health and immune function, vitamin D plays an important role in muscle function. Vitamin D receptors have been identified within nearly every tissue within the body including skeletal muscle. Additionally, a common symptom of inadequate vitamin D status is muscle weakness, possibly suggesting that vitamin D influences skeletal muscle function and strength (Larson-Meyer & Willis, 2010; Cannell et al., 2009). There are two mechanisms that have been proposed to explain the influence of vitamin D on skeletal muscle. The first involves 1,25-dihydroxyvitamin D [1,25(OH)2D] and the direct role it has on vitamin D receptors within skeletal muscle. The second proposed explanation suggests that vitamin D influences the transportation of calcium into the sarcoplasmic reticulum through increasing the efficiency or number of binding sites for muscle contraction. However, this has only been examined in rat models (Ogan & Pritchett, 2013).

To date, there is little research that has investigated the role of vitamin D status in athletes or how this relates to performance. Some research has suggested that increasing serum vitamin D levels may have potential ergogenic effects, including the ability to increase aerobic capacity, muscle growth, force, and power production, and decrease recovery time following exercise (Dahlquist et al., 2015; Von Hurst & Beck, 2014). However, this effect has not been clearly demonstrated, nor have changes in vitamin D status been compared to other markers related to changes in training load, performance, or health across a season. Similarly, the influence of vitamin D supplementation on muscle function has been studied previously but not in athletic populations. Various studied have found that vitamin D supplementation can significantly improve muscle weakness, pain, and balance. However, this observation has mainly been restricted to the aging populations (Ceglia, 2008). Overall the findings related to muscle function and vitamin D suggest that vitamin D status may play an important role in muscle performance. In conjunction with the role in bone health vitamin D status may also be important for injury prevention, influencing athletic performance as a result. However, more research is needed to determine the magnitude of the effect of vitamin D status in an athlete throughout a season.

Iron and Vitamin B12

In addition to n-3FA and vitamin D, iron and vitamin B12 are two important micronutrients that are essential for synthesis of hemoglobin and subsequent oxygen transport. Deficiencies of iron and vitamin B12 can lead to increases in fatigue, anemia, cognitive impairment, and immune impairment (Lukaski, 2004). Specifically, iron is necessary for transportation of oxygen and use at the cellular level. It serves as a structural component of iron containing proteins such as hemoglobin and myoglobin and therefore has an influence on energy use during exercise. As a result, iron status can have a significant impact on an athlete (Lukaski, 2004). With or without anemia, iron deficiency has shown to impair muscle function and limit work capacity through the inability to metabolize substrates into energy. This will ultimately lead to disruptions in training adaptations and reductions in performance (Nielsen & Nachtigall 1998;

Anderson & Barkve, 1970; Haas & Brownlie, 2001). Further, iron deficiencies with anemia have shown to result in compromised immune function and increased prevalence of upper respiratory infection specifically for endurance athletes (McClung et al., 2014).

It has been reported that 22-31% of female athletes are deficient in iron (Risser et al., 1988). Low iron status most often results from inadequate intake of heme food sources and inadequate energy intake. Further, athletes are suggested to be at a greater risk for iron deficiency due to greater losses through sweat, urine, and feces with exercise. Other non-dietary factors including blood loss and menses in females may also impact iron status (Thomas et al., 2016). As a result, monitoring levels of this micronutrient may be essential in this population due to its physiological role and influence on both health and training adaptions. Athletes and coaches should consider tracking iron or other indicators of iron status such as ferritin, transferrin, and red blood cell indices to detect and prevent nutrient deficiencies early on. Female athletes are suggested to have increased iron requirements of up to 70% of the estimated average requirement (DellaValle, 2013). As a result, an individualized approach through monitoring iron status may be necessary to determine optimal intake of iron and iron status for a particular athlete to ensure optimal performance.

Similar to iron, vitamin B12 is also significant to athletes. Vitamin B12 (cyanocobalamin) functions as a coenzyme and is important for normal erythrocyte and neurological functioning. Surveys of athletic populations suggest that certain groups may not consume adequate amounts of vitamin B12 based on the RDA (Lukaski, 2004). Specifically, athletes with low energy intake or vegetarian athletes may be particularly at risk for low vitamin B12 status (Montoye et al., 1955). Interest in vitamin B12 for athletes primarily stems from the role of B12 on erythropoiesis and subsequent oxygen transport. However, there is little research on the relationship between vitamin B12 status in athletes and influence on performance. Within supplement research, it has been suggested that vitamin B12 supplementation has no performance benefit or ergogenic effect in those individuals with adequate intakes (Montoye et al., 1955; Maya-Tu, 1978). It appears that vitamin B12 supplementation only benefits performance when a deficiency is present (Lukaski, 2004). As a result, ensuring adequate vitamin B12 status in athletes may be important to prevent any decrements in aerobic performance. Over time inadequate intake may lead to macrocytic anemia, which similar to other anemias is known to negatively affect health and performance through decreases in oxygen transport and aerobic capacity. Still, more research is needed to observe any changes in vitamin B12 status that may occur within an athlete, and any subsequent influence on performance (Barr & Rideout, 2004).

Amino Acids

Athletes require increased protein intake in order to support recovery from intense training and to maximize muscle protein synthesis. Additionally, levels of certain amino acids may provide a useful indication of muscle status and training adaptation in athletes (Lee et al., 2017). Several studies have demonstrated changes in plasma free amino acids during and after exercise and that these levels are sensitive to consumption of meals with various protein contents (Parry-Billings et al., 1992; Kingsbury et al., 1998). However, there is a lack of available research that demonstrates patterns of plasma free amino acids in athletes in relation to several aspects of athletic success including fatigue, nutrition, infection, and performance (Kingsbury et al., 1998).

The amino acid taurine is not incorporated into muscle protein but is found in high concentrations in skeletal muscle and believed to play an essential role in muscle function (Spriet & Whitfield, 2015). Taurine is needed for differentiation and growth of skeletal muscle tissue, plays a role in excitation-contraction processes through interaction with calcium, contributes to cell volume, and helps with antioxidant defense to increased stress (Spriet & Whitfield, 2015; Lee et al., 2017). Researchers have studied plasma and urinary taurine concentrations in athletes following a variety of endurance events such as a marathon or 100 km run. Immediately after strenuous exercise it has been demonstrated that plasma taurine levels increase significantly from baseline and remain elevated while other amino acids levels show no change or decrease due to substrate use in gluconeogenesis. Increases in urinary excretion of taurine have also been shown to correlate with changes in plasma creatine kinase following intense exercise. As a result, researchers have interpreted elevated plasma taurine levels and increased urinary output as a potential marker of muscle damage and impaired function (Ward et al.,1999). Overall, tracking changes in plasma taurine levels may provide insight regarding changes in muscular fatigue and muscle health in an athlete throughout a season.

Tryptophan is another amino acid that has received much attention for recognizing fatigue in athletes. Plasma free tryptophan is the precursor of the neurotransmitter serotonin (5-hydroxytryptamine). Serotonin plays a vital role in the central regulation of mood, sleep, arousal and cognitive function (Graeff, 1997). Tryptophan is transported across the blood-brain barrier to be converted to serotonin but competes with branched chain amino acids (BCAA) for transport. Thus, an increase in the ratio of free tryptophan:BCAAs will favor the uptake of tryptophan and synthesis of serotonin. The ratio of tryptophan:BCAAs is known to be influenced by both diet and exercise (Blomstrand et al., 1990). At rest, the majority of tryptophan is bound to albumin and unavailable for transport. However, during exercise free fatty acids replace tryptophan and the concentration of free tryptophan for transport is increased. The concentration of BCAAs also decreases due to metabolism in the muscle. Therefore, during exercise the tryptophan:BCAA ratio increases resulting in increased transport of tryptophan across the blood-brain barrier and subsequent synthesis of serotonin (Blomstrand et al., 1988).

Newsholme et al (2016) proposed that during exercise there is an increase in brain serotonergic activity due to increases in tryptophan:BCAAs that lead to a reduction in physical and mental efficiency, this phenomenon is known as central fatigue. Within the brain, serotonin induces sleep, depresses motor neuron excitability, depresses appetite, and impacts autonomic and endocrine function (Newsholme et al., 2016). Many of these symptoms are similar to those seen with exhaustive exercise and overtraining syndrome, thus this phenomenon has been proposed to explain some of these symptoms (Smith, 1999). However, the only research that has consistently demonstrated this response has been performed in animals. In humans, results are inconsistent and only acute changes in tryptophan have been observed (Gastmann & Lehmann 1998). Chronic intense training conditions have not been studied, which may be more representative of a state of overtraining (Smith, 1999; Gastmann & Lehmann 1998; Kreider, 1998).

Within the psychology literature, changes in circulating tryptophan are known to correlate with levels in the brain (Maes et al., 1997). It is also well recognized that low tryptophan is associated with increases in psychological disturbance and depressive symptoms (Maes et al., 1997). Similarly, low circulating tryptophan has been associated with systemic inflammatory events that are also evident in clinical depression (Maes et al., 1997). As a result, it has been proposed that circulating tryptophan levels may fall with prolonged high training stress and may be related to inflammation and changes in mood observed with overtraining (Smith, 1999). However, more research is needed to observe changes in tryptophan under chronic conditions in athletes who experience a high training stress and to determine if tryptophan changes are related to variables known to change with training load such as mood and performance.

In addition to tryptophan, tyrosine and phenylalanine are two essential amino acids that serve as precursors to neurotransmitters and, therefore, may be an important consideration for athletes. If the concentration of these amino acids changes within the body, for instance due to dietary changes, the concentration and resulting function of their corresponding neurotransmitters will be impacted. These neurotransmitter changes can alter physiological functioning and can possibly have an impact on an athlete’s performance or health throughout a season. Tyrosine is a precursor to catecholamine neurotransmitters dopamine and norepinephrine. Levels of tyrosine have been demonstrated to influence a variety of behaviors and cognitive functions that are under catecholaminergic control (Hase et al., 2015). Phenylalanine also acts as a precursor to catecholamines. There are two forms of phenylalanine; L-phenylalanine can be converted to tyrosine and D-phenylalanine is thought to have a positive impact on mood (Maughan et al., 2007).

Although some have suggested it, there is currently no convincing evidence demonstrating that phenylalanine or tyrosine have an impact on exercise performance. However, it should be noted that of the available studies, few have investigated chronic training conditions, and most have been performed under an acute setting (Maughan et al., 2007; Hase et al., 2015). More information is needed to determine if monitoring plasma levels of these amino acids throughout a season due to their possible impact on cognitive function and mood. For example, a review performed by Young (1996) indicated that there are several benefits of tyrosine on cognitive task performance, fatigue, and general alertness under stressful conditions. Tyrosine was found to offset decreases in working memory, decreases in time for information processing, and increases in negative affective mood states, induced by physically and mentally exhausting conditions. This may be useful for athletes, who are regularly exposed to periods of heightened stress, asked to use fine motor skills, and make cognitive decisions simultaneously during exercise. Additionally, tyrosine levels may be related to changes in mood or have the ability to alleviate negative mood states (Hase et al., 2015). Therefore, this amino acid may be important to monitor during periods of chronic increases in training stress that can possibly result in negative affective mood states commonly observed with overtraining.

Similar to tyrosine, phenylalanine also appears to have an impact on mood. Some research supports the use of phenylalanine in treatment of clinically depressed patients, but this research appears to be equivocal (Meyers, 2000). Regardless, phenylalanine may also be worth investigating in relation to mood during periods of heightened training stress. There is some research that supports the use of phenylalanine for managing chronic pain in patients who experience diagnosed chronic pain or fatigue, who have not experienced pain relief from any other therapeutic intervention. However, research supporting this use is mixed where some studies have reported good to excellent pain relief in patients when given phenylalanine (Balagot & Ehrenpreis 1979) and others have reported no difference compared to a placebo (Walsh et al., 1986). To date, monitoring levels of phenylalanine or the use of phenylalanine for the relief of fatigue or pain during recovery from intense training has not been studied but may be worth investigating in athletes that undergo a rigorous training schedule.

In combination, tyrosine and phenylalanine are promoted for use by athletes in order to boost alertness and motivation. Although there appears to be some support for a positive impact on cognitive and psychomotor performance under stressful conditions, this effect has not been demonstrated specifically in athletes using conditions similar to training or competition. Thus, the use of tyrosine or phenylalanine supplementation in athletes is not well supported (Maughan et al., 2007). In depletion experiments, research has investigated the effects of tyrosine and phenylalanine depletion on cognition and behavior. Results of these studies have indicated that acute depletion of catecholamines following amino acid depletion leads to decreased motivation (Cawley et al., 2013), cognitive impairments (Harmer et al., 2001), and increased vulnerability to negative mood states (Hase et al., 2015). As a result, it may be important to monitor levels of these amino acids in athletes with changes in their psychological health or cognitive performance over a season.

Glutamine is another amino acid that is necessary for a variety of metabolic processes and may be important to track in athletes throughout a season. Glutamine is the most abundant free amino acid in both skeletal muscle and plasma (Gleeson, 2008; Rowbottom Keast & Morton, 1996). The role of glutamine in the body has been classified as anabolic and immunostimulatory. Glutamine is highly used by leukocytes to provide energy and support cell proliferation and rely on glutamine sources provided by skeletal muscle synthesis. Further, there has been some evidence suggesting that concentrations of intramuscular glutamine are related to the rate of protein synthesis and promoting glycogen synthesis. However, these anabolic effects are not consistently observed or clearly understood (Gleeson, 2008).

Following a single bought of high intensity work some studies have shown increases in glutamine concentrations (Babij et al., 1983) and others have shown no change (Robson et al., 1999; Walsh et al., 1998). Conversely with chronic periods of intense exercise there appears to be a decrease in the availability of intramuscular and plasma glutamine, and these decreases are suggested to impair immune function (Kreher & Schwatz 2012). As a result, extended periods of intense training that result in chronic reductions in glutamine concentrations have been proposed to be responsible for immunosuppression and increased risk of illness commonly observed in overtrained athletes, known as the glutamine hypothesis (Kreher & Schwatz 2012). However, there has yet to be sufficient evidence that supports a causal link between low plasma glutamine, impaired immune functions, and increased susceptibility to infection in athletes that undergo prolonged intense training (Parry-Billings et al., 1992).

Results from previous studies have reported that resting plasma glutamine concentrations appear to be lower in individuals who are at risk for overtraining or experience chronic fatigue when compared with sedentary individuals and with healthy athletes (Kingsbury et al., 1998). Depression of plasma glutamine levels has been observed in as little as two weeks of intense training (Mackinnon & Hooper, 1996). According to the glutamine hypothesis, decreases in plasma glutamine concentrations found in these athletes would also be accompanied by immune function impairment. However, many studies show no relationship between a chronic state of fatigue due to training, glutamine concentrations, and either number or severity of upper respiratory tract infections (Mackinnon & Hooper, 1996; Kingsbury et al., 1998). For example, Kingsbury and colleagues saw low glutamine levels in chronically fatigued athletes but no differences in levels between those who suffered from infection versus those who did not (1998). Further, with supplementation of glutamine, it is believed that an increase in plasma glutamine concentrations through increased intake would prevent any associated immune impairment seen in athletes. For example, several studies found that supplementation offset the decrease in glutamine concentrations following prolonged exercise (Rohde et al., 1998). However, supplementation did not prevent a fall in lymphocyte proliferation, activity of lymphokine-activated killer cells, or have an impact on immune function (Rohde et al., 1998; Krzywkowski et al., 2001a; Krzywkowski et al., 2001b). To date, only one study has shown improved immune function through a decrease in the occurrence of upper respiratory tract infections (Castell et al., 1996). Although there has not been a direct link established between glutamine concentrations and immune function, it is well supported that glutamine is necessary for a variety of functions for cells of the immune system. Additionally, it is well supported that glutamine levels are significantly depressed during periods of catabolism such as with prolonged intense training. As a result, monitoring concentrations of this amino acid, which is usually abundant within the body, may provide a useful indicator of prolonged periods of catabolic stress due to excessive training loads in athletes (Gleeson, 2008).

VI: Conclusions

Athletes are continuously seeking to find a competitive edge in training and performance. As a result, they will often push the limit of their physical capacity, even to the point of risking physiological impairment. Similarly, it is well known that a certain degree of fatigue is necessary to elicit adaptations to training stress. Yet excessive fatigue without allowing appropriate recovery can have significant consequences for an athlete and possibly put them at an increased risk for serious health consequences such as OTS. As a result, it is essential as both a coach and an athlete to balance fatigue with appropriate recovery strategies in order to avoid compromises in health, increased risk of injury, maladaptation to training, or possible risk of overtraining.

Many factors that occur in and outside of the training environment will impact the ability of an athlete to recover and perform optimally. The ability to recover is highly influenced by several factors that occur outside of sport including sleep, nutritional habits, lifestyle, psychological stress, and other environmental factors. As a result, ensuring appropriate training stimuli and achieving adequate recovery and in an athlete is much easier said than done, and there are several challenges to be faced especially when working with team sports. It can be said that recovery itself and the manifestation of fatigue is complex, and as a result must be monitored as such. Objectively monitoring the balance between stress and recovery in an athlete rather than relying on how an athlete "looks" during training will allow for greater insight to athlete status. Still, more research is needed to understand which measures provide the best indication of the stress-recovery state of an athlete that can be used by coaches and athletes in an applied setting. Further, these methods must be applied to the appropriate population and level of athlete. Female athletes and college athletes in particular, may be two populations for which recovery and adaptations to training have been vastly understudied compared to their professional and male counterparts. More research is also needed on these athletic populations that may differ in terms of physical capacity, type of training and non-training related stress experienced, and recovery capabilities.

Several methods for monitoring the balance between fatigue and recovery have been developed. However, no standard method has been identified that can indicate when training stress is optimal or excessively high. Monitoring changes in performance is one method that has been proposed which utilizes the fact that a decrease in performance is a universal sign for fatigue in an athlete. However, not all aspects of sport performance are impacted uniformly by fatigue. Furthermore, it is likely that other physiological changes may proceed decreases in performance. If detected early enough, physiological changes that occur prior to disruptions performance can allow a coach to intervene and prevent performance decrements before they occur. If a coach simply relies on changes in performance, it may already be "too late" where an athlete is already experiencing some form of maladaptation. As a result, additional objective measures that can potentially predict performance changes are needed.

In addition to performance measures, heart rate it a very easy and accessible measure to obtain that may reflect intensity and physical capacity of an athlete. However, there is a lack of evidence demonstrating clear and consistent changes in heart rate responses during both exercise and rest throughout periods of high training stress. Heart rate responses are also highly sensitive to outside environmental factors and there is a large daily variation within an individual. Thus, heart rate should be combined with additional objective measures that can reflect other aspects of training adaptation or lack thereof in addition to physiological responses during training.

Moreover, prolonged inadequate recovery has been associated with disruptions in mood and sleep patterns, and it has been suggested that these changes can be measured using subjective assessments. Subjective assessments related to changes in mood, perceived stress and recovery, and other behavioral or physical symptoms have been suggested to indicate positive or negative responses to training and have demonstrated a dose-response relationship with training load. It has also been suggested that these changes may precede performance decrements. However, no research using self-report measures has looked at changes in mood or sleep patterns in athletes in relation to other physiological markers known to be related to healthy mood and sleep. Additionally, it is possible that other physiological measures also impacted by training may facilitate or be related to changes seen in mood or sleep with intense training. As a result, further research is needed to understand how changes in mood and sleep occur in relation to performance changes and other physiological measures, as well as the time course of each of these.

Lastly, investigation of several biochemical markers has recently received attention regarding understanding fatigue and performance readiness in an athlete. However, no single marker has been suggested to represent recovery status adequately or has demonstrated consistent relationships with prolonged fatigue. Several nutrition related markers may provide additional insight into the recovery and performance readiness of an athlete knowing that nutrition can either enhance or inhibit both recovery and performance. Nutritional habits can also have an impact on hormonal responses with exercise and insufficient energy intake may impair certain neuroendocrine pathways related to exercise performance. As a result, monitoring changes in markers such as omega-3, vitamin D, vitamin B12, and amino acids known to be important in athletes may provide useful insight into recovery and exercise adaptation potential. However, no research has looked at changes in nutritional related markers extensively, nor have these changes been compared with other physiological or performance related measures over a competitive season.

Overall it appears that the best approach to monitoring fatigue in competitive athletes may be to use a multi-factor approach. An approach that combines the use of both objective and subjective assessments, in order to assess a variety of internal and external physiological responses to training, may provide the best representation of fatigue experienced by an athlete. There are many factors that will contribute to how fatigue manifests within an athlete. Additionally, there are many factors that will influence the ability to monitor or measure fatigue. In conclusion, the use of a variety of measures may be the best approach to account for the high amount of variability among athletes and in sport. Further, it may allow coaches and sports medicine staff to detect changes in training adaptations within an athlete that may have gone unseen if relying on only a single measure.

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Chapter II: Changes in Nutritional Biomarkers, Sleep, Perceived Stress, and Performance in D1 Female Soccer Players Throughout a Competitive Season

INTRODUCTION

Adequate recovery from training is essential to optimize athlete health and performance. However, it can be a challenge due to the high physical demands placed on competitive athletes. Soccer in particular is a popular sport worldwide, where athletes often experience a high training load and demanding season. Elite players will cover 10-12 km on average during a 90-minute match and maintain intensity near 80-90% of their maximal heart rate (Stølen, Chamari, Castagna, & Wisløffet, 2005). Additionally, these athletes repeatedly produce high power movements such as accelerating, stopping, change of pace, change of direction, jumping, and tackling (Reilly, 1997).

The physiological demands of high levels soccer players have been evaluated extensively. It has become recognized that participation in this sport is physically demanding and matching a physically demanding training schedule with adequate rest, recovery, and nutrition over a long competitive season provides a unique challenge for coaches and athletes. This is especially true when working with team sports where individual variability in recovery needs, training status, and capacity for adaptation is increased. Changes in training frequency, duration, and intensity during the season must be accompanied by appropriate rest and recovery strategies in order to avoid maladaptation associated with overreaching and overtraining (Halson, 2014).

Further, along with the physical demands of training, high-level athletes are often faced with additional life stressors occurring outside of training which may influence their ability to recover appropriately. Athletes regularly experience the external stress of travel along with other personal or psychological stressors. These sources of stress may be even more difficult to account for by coaches, sports medicine, and other staff (Main & Grove, 2009; Meeusen et al., 2013). This may be particularly important to college athletes, who experience the dual demands of academic and athletic status. Collegiate athletes often experience altered sleep and nutritional practices while transitioning to college life as well as a condensed season. However, college athletes are far less studied compared to professional or other high-level players. Still, a comprehensive monitoring approach is needed in soccer players who experience a stressful season influenced by both on-field physical stress and off field life stressors as well. This method should account for the accumulation of both on and off field stressors in order to enhance adaptation to training and avoid any consequences to health due to excessively high training loads over a long season.

Several methods of monitoring the stress-recovery state of athletes have been used previously. These methods include performance testing, on field monitoring (heart rate and GPS), mood alterations, and sleep tracking (Meeusen et al., 2013, Kreher & Schwartz 2012). Tracking changes in physical performance is one suggested method as one of the first signs of inadequate recovery tends to be a decrease in performance (Meeusen et al., 2013). However, routinely monitoring physical performance in a lab setting can be difficult to utilize during the season due to time constraints and physical stress on the athletes. Additionally, not all aspects of performance are impacted uniformly by fatigue, and it is likely that other physiological changes may occur prior to decreases in performance that may be detected earlier (Meeusen et al., 2013). In addition to performance measures, on-field monitoring using heart rate and GPS has become a widely used and accessible measure for monitoring internal (heart rate) and external (GPS) training load of athletes. However, assessment of training load only reflects the stress experienced within the training environment. As a result, this method is limited to on-field monitoring and does not encompass all other stressors that occur outside of training. In order to account for additional life stressors that may occur, tracking changes in mood and sleep through subjective questionnaires have also been utilized (Main & Grove, 2009). Disruptions in sleep patterns and increases in negative affective mood states have been thought to reflect prolonged inadequate recovery and have been shown to exhibit a dose-response relationship with training load measures (Meeusen et al., 2013). These measures may reflect the accumulated stress experienced by an athlete both in and out of training. However, the relationship between changes in mood, sleep, or perceived stress has not been previously investigated in conjunction with other physiological measures typically associated with excessive training stress.

In addition to the previously mentioned methods of on and off field athlete monitoring, there has been a growth of research examining the biochemical, hormonal, and nutritional responses to exercise training through the use of biomarker monitoring. Biomarkers are useful for monitoring chronic stress in athletes due to their objective nature and the ability to reflect accumulated stress from both training and non-training stimuli. The objective analysis of biomarkers provides valuable insight on the physiological response to training, and specific markers can indicate how training alters nutritional requirements during training. Additionally, utilizing biomarkers provides an alternative to traditional nutritional monitoring techniques such as self-reported dietary measures which have been shown to be highly flawed and can be impractical to use in a real-world team-based setting (Lee et al., 2017). Despite importance of optimal nutrition in athletes, biomarkers specific to nutritional status have not been researched extensively but may provide further insight into the performance and recovery status of an athlete. Tracking specific biomarkers such as omega-3 fatty acids (n-3FA), vitamin D (VitD), iron (Fe), vitamin B12 (VitB12), and amino acids may provide additional information on the health of an athlete (Lee et al., 2017). n-3FA fatty acids are important to an athlete to optimize recovery due to its antioxidant and anti-inflammatory properties which aid in the recovery aspect of training (Mickleborough, 2013). Similarly, VitD supports performance and recovery due to its role in bone health, immune function, inflammation, and muscle function (Halliday et al., 2011). Deficiencies of Fe and VitB12 can lead to increases in fatigue, anemia, cognitive impairment, and immune impairment (Lukaski, 2004). A less common, though potentially very useful, nutritional biomarker is the evaluation of various amino acids such as Glutamine (Gln), Taurine (Tau), Tryptophan (Trp), Phenylalanine (Phe). Athletes require greater protein intake in order to support recovery from training (Lee et al., 2017). Additionally, levels of certain amino acids have demonstrated changes in concentration during and after exercise and may also provide a useful indication of muscle status and training adaptation in athletes (Kingsbury et al., 1998).Gln is the most abundant free amino acid in skeletal muscle and plasma where its main role is anabolic and immunostimulatory (Gleeson, 2008; Rowbottom et al., 1996). Alternatively, Tau is needed for differentiation and growth of skeletal muscle tissue, plays a role in excitation-contraction processes through interaction with calcium, contributes to cell volume, and helps with antioxidant defense to increased stress (Spriet

& Whitfield, 2015; Lee et al., 2017). Trp is another amino acid that has received much attention for recognizing fatigue in athletes due to it being the precursor of the neurotransmitter serotonin which plays a vital role in the central regulation of mood, sleep, arousal and cognitive function (Graeff, 1997). Phe is also of particular importance as it acts as a precursor to catecholamines (Maughan et al., 2007). As a result, monitoring nutritional biomarkers in these athletes, who also experience a high caloric expenditure may help avoid several nutrient deficiencies are that are frequently observed, and provide insight on how to optimize health and adaptations to intense training.

Excessive stress and fatigue can have serious health consequences for an athlete and may ultimately result in decreases in performance. As a result, the development and implementation of appropriate methods for monitoring stress of training and subsequent recovery in athletes is critical. Stress and the manifestation of fatigue can be said to be multidimensional, as many factors both within and outside of the training environment will contribute to total accumulated stress in an athlete. As a result, accumulated stress should be monitored using a comprehensive approach that looks into all aspects related to stress, athletic performance, and recovery practices such as sleep and nutrition status Furthermore, the research available currently is primarily focused on the male athlete with minimal information on female athletes. Similarly, research has focused on professional level athletes, while those at lower levels such as collegiate players have not been researched extensively. Therefore, the purpose of this study was to analyze nutritional biomarkers in conjunction with mood, sleep, and performance assessments to examine changes in recovery and training status of division I female collegiate soccer athletes throughout a competitive season. It was hypothesized that there would be changes in blood-based biomarkers, performance, mood states, and sleep patterns throughout the season.

MATERIALS AND METHODS

Subjects

Division I female collegiate soccer players (N= 25; M_{age}= 19.4 $\pm$ 1.4 yrs; M_{ht}= 167.9 $\pm$ 6.3 cm) from Rutgers University were studied. The participants were free from injury, serious illness, or metabolic conditions. All athletes were cleared to participate by a physician or athletic training staff person. This research was approved, and written consent waived, by the Rutgers University Institutional Review Board for the Protection of Human Subjects in accordance with the Declaration of Helsinki.

Fitness and Performance Measures

The athletes participated in a battery of performance testing prior to the start of pre-season (PT1) and within a week of their final competition (PT2). The athletes were instructed to arrive to The Rutgers Center for Health and Human Performance (CHHP) well hydrated and fasted for two to three hours. Testing began with body composition assessment using air displacement plethysmography (BodPod, COSMED, Concord, CA, USA) (McCrory *et al.,* 1995). Subjects were tested wearing non-padded compression shorts and sports bra, and a swim cap. Total body weight, lean body mass (LBM), and percent body fat (%BF) were all measured. The error of body volume reading is roughly 0.02%, which allows for calculation of %BF with only 0.01% error. Following body composition, the athletes were instructed to warm-up on a treadmill at a self-selected pace for five minutes. Counter Movement Vertical Jump (CMJ) without arm swing was assessed following the warm-up using the Just Jump system (Probotics, Huntsville, AL, USA) (Leard *et al.*, 2007). The participants completed three attempts using each jump method and the highest value was recorded. Following CMJ a maximal graded exercise test (GXT) using a H/P-Cosmos treadmill (H/P Cosmos Nussdorf-Traunstein, Germany) was performed to measure maximal aerobic capacity (VO_{2max}) and Ventilatory Threshold (VT) by direct gas exchange. Direct gas exchange was measured via a Quark CPET (COSMED, Concord, CA, USA) utilizing a speed-based protocol with stages that were MET equated to the Bruce protocol. This protocol consisted of two min stages and a constant 2% incline. The speeds were as follows: 6.4, 7.8, 9.9, 11.7, 13.6, 15.6, 17.0, 18.1, 19.7, 21.0 (kph). Heart rate was monitored for the duration of the test to monitor effort and to obtain maximal heart rate values (HR_{max}). Heart rate was monitored using the Polar TeamPro HR transmitters (Polar Electro Co., Woodbury, NY, USA). Additionally, the athletes were asked their rating of perceived exertion (RPE) using the Borg Scale near the end of each stage of the GXT (Borg *et al.*, 1982). At least three of the following criteria were met to determine 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. Following completion of each test, VT was calculated as the point where ventilation began to increase non-linearly with VO_2 and was expressed as a percentage of VO_{2max} (Gaskill et al., 2001).

CMJ was assessed at each time-point (T1-T4) corresponding to each blood draw. CMJ was assessed at the start of practice using the Just Jump system (Probotics, Huntsville, AL, USA). The jump was performed while holding the hands on the hips; this was done in order to remove the influence of arm swinging on jump height and attempt to isolate leg power. The athletes were allowed two attempts and the best performance was recorded. The same dynamic warm-up was performed prior to each assessment at each time-point.

Monitoring and Training Load

The Polar TeamPro system (Polar Electro Co., Woodbury, NY, USA) was used during all in-season practices and games to assess workload. At the start of pre-season the athletes were provided with a heart rate monitor. The heart rate monitors were individualized to each player using the results of performance testing. Workload was assessed using several measures including heart rate data, distance covered (Dis), caloric expenditure (kcal), caloric expenditure as a function of body mass (kcal/kg), and training load (TL). Heart rate was expressed as a percent of HRmax during live play, as well as accumulated time spent in different heart rate zones (≥90%, 80-89%, 70-79%, 60-69% and 50-59% HR_{max}). Training Load was determined by an algorithm created by Polar™ based on the individual characteristics of the players (Ceesay et al., 2018).

Sample Collection and Analysis

The subjects participated in blood draws prior to the start of pre-season (T1) and every 28-days thereafter (T2-T4). The blood draw at T1 occurred prior to the start of the first pre-season practice. Blood draws in-season occurred within 18 hours following a game. The participants were instructed to arrive to the CHHP between 0700-0900, well hydrated following an overnight fast (~8 hours). The athletes provided a urine sample as soon as they arrived into the lab. Following urine collection, a ~70 ml blood sample was drawn from the antecubital vein. 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. All samples were ran in duplicate, CV for all biomarkers: 0.5-7.5%. Gln, Tau, Trp, Phe, VitB12, VitD, n-3FA, and Fe were analyzed.

Subjective Measures

At each time point, mood and sleep profiles were assessed using subjective questionnaires. Mood was assessed using the Multi-Component Training Distress Scale (MTDS) (Main et al., 2009). This scale included symptoms related to emotionality, general fatigue, concentration difficulties, physical discomfort, sleep disturbance and appetite changes. Participants were required to rate items by indicating the extent to which they have felt mood and fatigue related symptoms within the past 48 hours. Responses were recorded on a five-point scale, where a 0 represented “not at all” and a 4 represented “an extreme amount” (Main & Grove, 2009). Sleep profiles were assessed using the Pittsburg Sleep Quality Inventory (PSQI) (Buysse et al., 1989). Mood and sleep questionnaires were completed by the participants at each time point, prior to the start of practice.

Statistical Design and Analysis

Biomarker, performance, and body composition testing data were analyzed using RM ANOVA univariate analysis (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

Anthropometric and Performance Measures

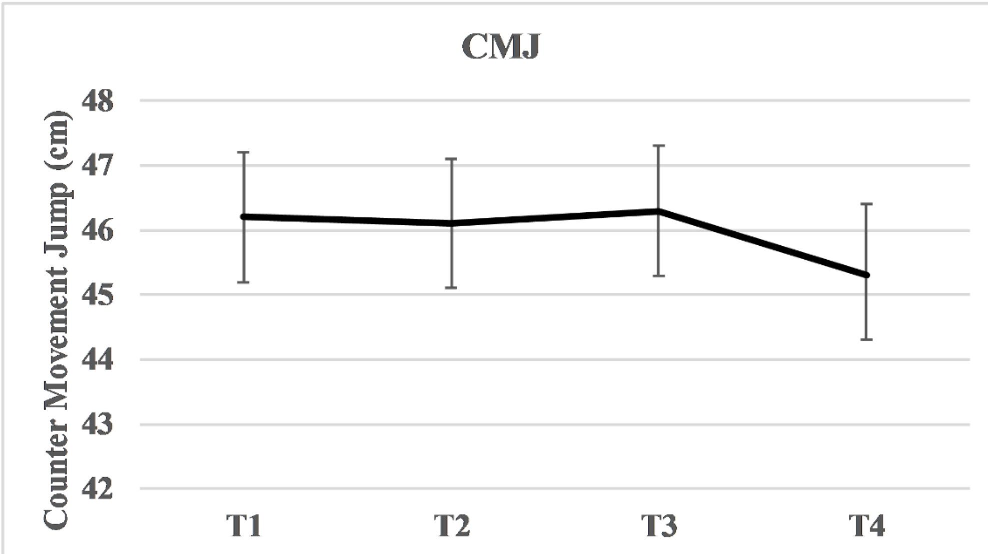
Table 1. Body Composition and Performance

Measurement	PT1	PT2	ES
Weight (kg)	65.8 ± 6.5	65.0 ± 6.2	-0.13
%BF	20.9 ± 3.5	19.2 ± 3.8*	-0.48
LBM (kg)	51.6 ± 4.9	53.1 ± 6.1*	0.28
VO2 (ml/kg/min)	50.8 ± 3.2	48.4 ± 3.3*	-0.76
VT (%)	84.1 ± 3.4	82.2 ± 2.6*	-0.63
CMJ (cm)	46.1 ± 5.0	45.3 ± 5.3	-0.17

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

Body composition and performance measures can be found in Table 1. Weight was maintained throughout the season despite a significant increase in LBM ($P<0.05$) with a decrease in %BF ($P<0.05$). VO_{2max} and VT significantly decreased ($P<0.05$). CMJ was maintained throughout the season from T1 to T3 but began to show a downward trend at T4 ($P>0.05$) (Figure 1).

Figure 1:

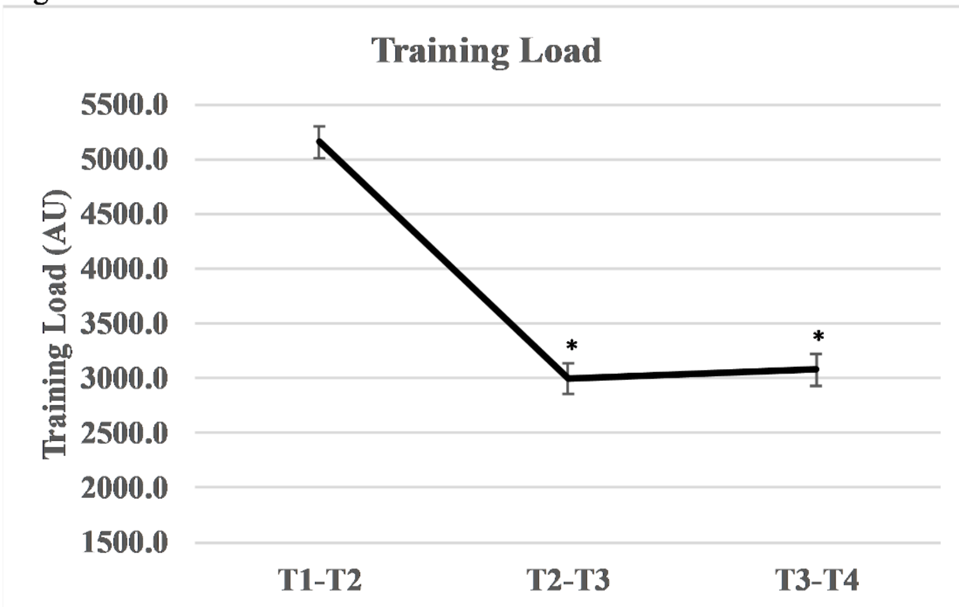


Values are Mean ± Standard Deviation
* Significant Change from T1

Training Load

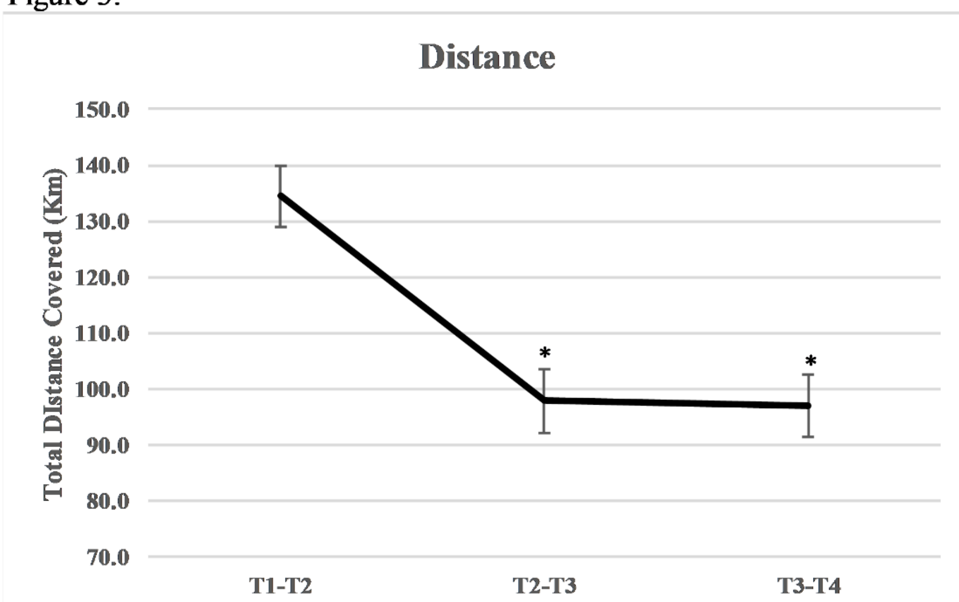
All training load data can be found in figures 2-4 representing the cumulative load of each marker between blood draws (T1-T2, T2-T3, T3-T4). There was a significant decrease in all markers following the initial training block ($P<0.05$; ES_{TL}=-2.86, ES_{Dis}=-1.33, ES_{kcal}=-2.22, $ES_{kcal/kg}$=-2.78) then remained depressed as the season progressed.

Figure 2:



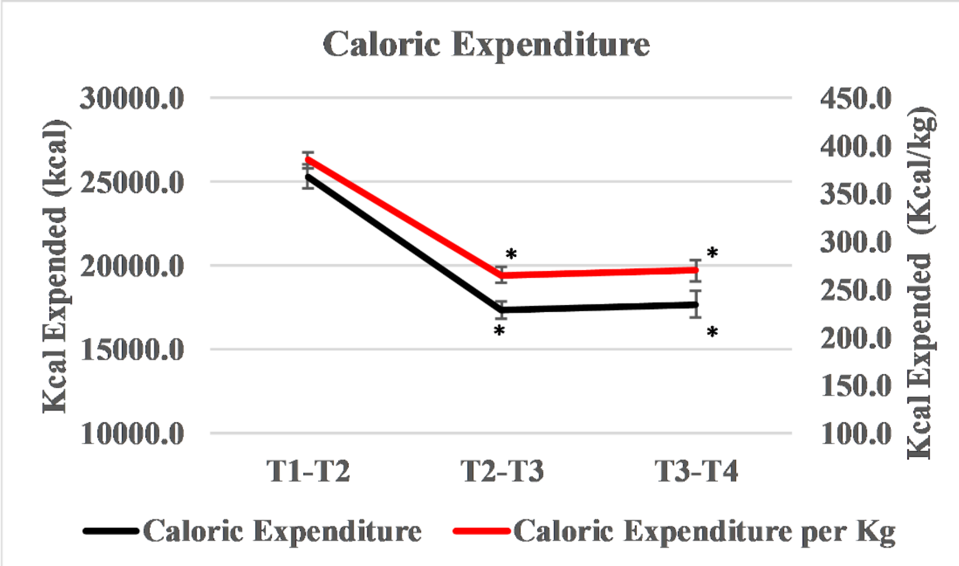
Values are Mean ± Standard Deviation
* Significant Change from Baseline (T1-T2)

Figure 3:



Values are Mean ± Standard Deviation
* Significant Change from Baseline (T1-T2)

Figure 4:



Values are Mean ± Standard Deviation

* Significant Change from Baseline (T1-T2)

Mood and Sleep Measures

Table 2. Mood and Sleep Measures

Marker	T1	T2	T3	T4
Sleep Quality	1.0 ± 0.6	1.2 ± 0.6	1.2 ± 0.6	1.1 ± 0.5
Sleep Duration	0.3 ± 0.5	0.3 ± 0.6	0.3 ± 0.5	0.7 ± 0.6*
Total Mood Disturbance	23.4 ± 10.5	20.1 ± 9.3	26.5 ± 11.3$	31.0 ± 10.3*
Depressed Mood	1.1 ± 2.5	0.4 ± 0.8	1.7 ± 2.9$	2.9 ± 3.2*
General Fatigue	4.2 ± 3.1	3.1 ± 2.7	4.9 ± 3.3$	6.1 ± 3.5*
Perceived Stress	5.0 ± 3.7	1.5 ± 2.1*	3.4 ± 3.0$	4.8 ± 3.0$

Values are Mean ± Standard Deviation

* Significant Change from Baseline (T1), $ Signifcant Change from the Previous Time Point

Changes in mood and sleep measures can be found in Table 2. Total mood disturbance significantly increased from T2 to T3 ($P<0.05$, ES=0.62) and continued an upward trend through T4. At the start of the season there was a decrease in perceived stress from T1-T2 ($P<0.05$, ES=-0.95). This was followed by a significant increase in perceived stress from T2-T3 (P<0.05, ES=0.94) and again from T3-T4 (P<0.05, ES=0.44). General fatigue scores showed no change from T1-T2 but increased from T2-T3 (P<0.05, ES=0.67) and this increase in fatigue persisted through T4. Similarly, depressive mood showed no change from T1 to T2 but significantly increased from T2 to T3 (P<0.05, ES=1.56) and continued an upward trend at T4. There were no significant changes in sleep quality throughout the season (P>0.05). However, there was a significant increase in sleep duration observed towards the end of the season from T1 to T4 (P<0.05, ES=0.75).

Nutritional Markers

Table 3. Nutritional Markers

Marker	T1	T2	T3	T4
Fe (mcg/dL)	90.2 ± 40.6	60.6 ± 32.1*	73.0 ± 34.4	90.8 ± 33.7*
VitB12 (pg/mL)	483.8 ± 168.3	522.4 ± 192.9	555.8 ± 221.4*	568.0 ± 203.1
VitD (ng/mL)	47.0 ± 15.3	40.2 ± 12.1*	39.2 ± 11.2*	40.2 ± 11.1*
n-3FA (%)	2.1 ± 0.6	2.7 ± 0.7*	2.5 ± 0.7*	2.3 ± 0.5

Values are Mean ± Standard Deviation
* Significant Change from Baseline (T1), $ Signifcant Change from the Previous Time Point

Nutritional biomarker values can be found in Table 3. There was a significant increase in n-3FA from T1 to T2 (P<0.05, ES=0.91). This was followed by a significant decrease from T2 to T3 (P<0.05, ES=-0.29) before returning to preseason values. VitD significantly decreased from T1 to T2 (P<0.05, ES=-0.44) and remained depressed through T4. VitB12 increased from T1 to T3 (P<0.05, ES=0.42) and remained elevated through T4. Fe significantly decreased from T1 to T2 (P<0.05, ES=-0.72), remained suppressed through T3, before returning to preseason values by T4.

Amino Acid Markers

Table 4. Amino Acid Markers

Marker	T1	T2	T3	T4
Glu (µmol/L)	530.3 ± 76.9	612.4 ± 105.6*	579.0 ± 86.6*	540.2 ± 93.1
Tau (µmol/L)	40.4 ± 9.6	44.0 ± 11.8	38.4 ± 12.6	38.4 ± 12.9
Trp (µmol/L)	64.8 ± 13.4	73.1 ± 22.3	62.2 ± 12.9	59.3 ± 13.6
Phe (µmol/L)	70.6 ± 9.3	66.4 ± 10.9	72.1 ± 11.0	70.7 ± 9.3

Values are Mean ± Standard Deviation
* Significant Change from Baseline (T1), $ Signifcant Change from the Previous Time Point

Changes in amino acid biomarkers can be found in Table 4. There were no significant changes seen for Tau or Phe throughout the season (P>0.05). There was a non-significant increase in Trp from T1 to T2 followed by a significant decrease from T2 to T3 (P<0.05, ES=-0.48) that remained depressed through T4. Glu significantly increased from T1 to T2 (P<0.05, ES=1.06) then continued to decrease through T4 (P<0.05, ES=-0.68).

DISCUSSION

The results of this observational study provide valuable insight on the effects of season-long stress in Division I female athletes on performance, mood, sleep, and nutritional biomarkers. To our knowledge, this is one of the first studies to evaluate biomarkers related to nutritional status over an entire season in female soccer players. Interestingly, this study found that nutritional biomarker changes appeared to preceed or coincide with mood and perceived stress throughout the season. These results suggest that changes in nutritional biomarkers may be a viable method for assessing athlete readiness throughout a season. Further, monitoring these changes can potentially be used to implement preventive dietary strategies to mitigate fatigue and disruptions in mood or sleep, which have previously been associated with decrements in performance (Purvis et al., 2010).

Training Load, Performance, and Body Composition

Results of this study demonstrate that the first training block, including two weeks of preseason training, resulted in the highest physical load and caloric expenditure compared to all other training blocks throughout the season. These results indicate that this early-season training block is a time of increased physical stress, which then leveled out as the season progressed. Following the initial decrease from T1-T2, all aspects of physical loading including training load, total distance, and caloric expenditure, showed a more consistent training demand throughout the rest of the season.

Over the course of the season, the athletes experienced an apparent body re-composition, exhibited by the maintenance of body weight while simultaneously decreasing %BF and increasing LBM. These results are unique as it is uncommon to increase LBM during the competitive season (Minett et al., 2017). The changes in LBM observed during the season may have been due to the strength training, though this was not measured. Despite these positive changes in body composition, there was a decrease in both VO_{2max} and VT, while CMJ was maintained. Given both the outcomes as well as the time-course of the measures, it is possible that performance may have been decreased due to dietary deficiencies. Total caloric intake may have been maintained to increase LBM but may have been deficient in micronutrients which ultimately negatively affected aerobic performance.

Mood and Sleep Measures

Disruptions in mood and sleep have been observed during prolonged periods of high training stress, thus tracking changes in these parameters may be useful to detect risk of breakdown or overtraining (Purvis et al., 2010; Saw et al., 2017; Main & Grove, 2009). Previous studies have looked at changes in mood or sleep in relation to changes in TL but few have looked at changes in mood or sleep in relation to other physiological measures also related to high training stress or overreaching (Meeusen et al., 2013; Raglin et al., 1996; O'Connor et al., 1989; Hooper & Mackinnon, 1995). Interestingly, perceived stress decreased over the first training block (T1 to T2), which included preseason training and therefore may have been due to a stressful anticipation of the start of the season. As the season and academic year progressed, perceived stress increased indicating the cumulative stresses of collegiate athletes. Total mood disturbance increased during the season from T2 to T3, with the greatest disturbance seen at end of the season (T4). Similarly, general fatigue and depressive symptoms were also greatest at T4. These data suggest that there was an increase in negative affective mood states towards the end of the season which coincided with tournament play. In regard to sleep, total sleep duration began to increase towards the end of the season (T3 to T4) which coincided with an increase in mood disruption. The observed increase in sleep duration may have been an attempt to buffer the stress accumulated over the course of the season. However, despite these psychological changes, there were no changes in sleep quality seen throughout the season. This may suggest that an increase in sleep duration is not sufficient enough to mitigate increases in negative mood states, fatigue, or perceived stress observed towards the end of the season around tournament play. Interestingly, the changes seen in mood and sleep occurred after the observed changes in biomarkers and coincided with the changes in CMJ. This is unique in regard to the current position stand on overtraining that states mood states precede changes in biomarkers and performance, though this conclusion was primarily made from studies using male endurance athletes and may not apply to team sports or females (Meeusen et al.2013). Still, utilizing both mood and sleep changes may provide insight for coaches and athletes to make both on- and off-field alterations to maximize performance and health throughout the season.

Nutritional Biomarkers

In addition to changes in mood, biomarker analysis can assist with monitoring health, recovery, and training status of athletes in an effort to minimize fatigue and illness. Specifically, biomarkers related to nutritional status may help determine an athlete's readiness to perform given that recovery from exercise and exercise performance is enhanced by optimal nutrition (Lee et al., 2017). Maintaining proper nutrition over the course of the season is essential for high level athletes to maintain overall health, performance, and optimize recovery during a stressful competitive season.

Due to the known anti-inflammatory and antioxidant properties of n-3FA, it is a beneficial biomarker to monitor in athletes experiencing high physiological stress of a season (Mickleborough, 2013). Interestingly, the lowest n-3FA status occurred immediately following preseason (T2) and at the very end of the season (T4), while status improved during the season. The observed decrease following the highest training load (T1-T2) may indicated that n-3FA is being decreased due to an increase in oxidative stress and inflammation associated with increased training load, though this was not assessed in this study (Mickleborough, 2013). These changes could also have been due to dietary modifications; however, this could also suggest the importance of n-3FA intake at critical points during the season. N-3FA supplementation has previously shown to attenuate muscle soreness and perceptions of fatigue (Jouris et al., 2011; Tartibian et al.,2009). As a result, omega-3 may help promote recovery and training adaptations and these results highlight an opportunity for dietary intervention during preseason and at the end of the season where higher training stress may occur. Furthermore, inadequate n-3FA status has previously been associated with increases in sleep disturbance and negative mood profiles (Peuhkuri et al., 2012; Fontani et al., 2005, Parker et al., 2006). The negative associations of mood and sleep with insufficient n-3FA may potentially influence recovery ability, however, has not been extensively researched in athletes. In the present study, changes in n-3FA status mirrored changes in total mood disturbance, depressive symptoms, and general fatigue, which improved at the start of the season and exhibited the greatest disruption at T4. These changes support the connection between n-3FA and mood profiles and may provide an opportunity to mitigate mood disruptions through dietary modification or supplementation during the season (Fontani et al., 2005, Parker et al., 2006).

Another common nutrition biomarker utilized VitD due to its essential role in immune function, inflammation, skeletal muscle function, and bone health (McClung et al., 2014). Additionally, there is a high prevalence of deficiency in athletes, even among those who train outdoors despite the synthesis of VitD occurring through sunlight (Constantini, 2010). This study demonstrated a decrease in VitD immediately following preseason (T1-T2), which remained depressed through the end of the season (T4). These changes were observed despite training and competition being performed outdoors in the late summer and fall in the Northeastern United States. Resultingly, these changes may suggest a training-induced decrease in VitD. Given the importance of VitD and its role in muscle function, enzyme activity, and bone health, the drop in VitD at the start of the season may have negative implications for performance and health such as immune function, muscle function, and protein synthesis (McClung et al., 2014). These results suggest that supplementation may be warranted in female athletes to maintain a healthy VitD status, despite being an outdoor sport.

VitB12 and Fe status are also important contributors to successful athletic performance. VitB12 is essential for metabolism of fats and carbohydrates and plays an important role in protein synthesis (Huskisson et al., 2007). Fe represents the total Fe in the blood, which is the oxygen-carrying component of hemoglobin and is essential for aerobic performance (Pedlar et al., 2017). Decreases in these markers can lead to fatigue, anemia, and cognitive and immune impairment (Lukaski, 2004). Fe deficiency has also been suggested to impair muscle function and limit work capacity through the inability to metabolize substrates into energy (Nielsen & Nachtigall 1998). Interestingly, VitB12 increased over the course of the season, which most likely reflected dietary modifications. Alternatively, Fe significantly decreased during the initial training block (T1-T2) showing a training induced reduction in Fe. For this particular biomarker and its clinical relevance, athletes that fell below clinical levels received attention from the sports medicine staff. As the season progressed Fe levels rebounded to towards preseason levels by T4. These findings support an increased iron need in female athletes during times of increased training load and supplementation may be beneficial.

Amino Acids

Periods of catabolism seen with very intense training or overtraining are known to decrease the plasma concentrations of several essential amino acids that support a variety of metabolic processes important to exercise performance (Lee et al., 2017). Several studies have demonstrated changes in multiple amino acids such as taurine, phenylalanine, and glutamine in response to exercise, however, most have been executed in acute settings and few have followed these changes over a season (Kingsbury et al.,1998; Lee et al, 2017). Similarly, changes in amino acids have not been investigated extensively in relation to other measures related to recovery or performance. The amino acids of interest were Gln, Trp, Tau, and Phe. Utilizing blood amino acid markers may provide a more structured method of determining dietary intervention strategies to maximize player health, recovery, and performance.

Interestingly, according to the Gln hypothesis of overtraining, resting concentrations of Gln may deplete in athletes due to high training stress resulting in decreases in performance and altered immune function (Kreher & Schwartz, 2012; Kingsbury et al., 1998). In the current study, there was an initial increase in serum Gln observed from T1 to T2. Levels remained the highest at T2 and T3 before returning to baseline by the end of the season (T4). These results are opposite of what is expected in reference to the Gln hypothesis of overtraining, though this study does not indicate the athletes were in an overtrained state at any point throughout the season.

In addition to Gln, Trp is of particular importance serving as a precursor to serotonin and, thus, may have an influence on mood, sleep, and cognitive function (Graeff, 1997). Normally, Trp will compete with other BCAAs for transport across the blood brain barrier, but with exercise, uptake of Trp is favored. Serotonin synthesis increases as a result and has been associated with increases in fatigue (Kreher & Schwartz, 2012). Changes in Trp and subsequent brain serotonin levels may play a role in fatigue experienced by athletes during periods of overreaching or overtraining, yet few studies have looked at changed in Trp over a full season (Smith, 1999). Further, low levels of Trp have been associated with increases in psychological disturbance and depressive symptoms also observed with overtraining syndrome (Maes et al., 1997; Smith, 1999). The present study found that Trp levels were maintained from T1 to T2 but began to decline from T2 to T3 and remained depressed at T4. Trp levels were the lowest at the end of the season (T4) where the greatest mood disturbance, depressive symptoms, and general fatigue were also observed. As a result, Trp levels may provide a mechanism for understanding increases in negative affective mood states seen during periods of high training stress such as during tournament play.

Additional amino acids that were evaluated were Tau and Phe. Unlike the previously mentioned amino acids, Tau is one of few endogenous amino acids that is not encoded by DNA and is not required for muscle protein synthesis (Spriet & Whitfield, 2015). However, Tau possesses antioxidant properties and does play an important role in skeletal muscle function (Lee et al., 2017). In the present study, there were no significant changes in Tau observed. In addition to Tau, Phe serves as a precursor to catecholamines. As a result, changes in Phe will alter availability of taurine in the brain and may influence dopamine and norepinephrine levels. Due to the influence on catecholamines, Phe has been suggested to play a role in exercise performance. However, there is currently no convincing evidence to support this claim and responses to long-term training have not been investigated (Hase et al., 2015). Similar to Tau, there were no changes seen in Phe over the course of the season. These results suggest there is a need for more research on the role of Tau and Phe and whether they play a role in mood or other aspects of health important to the athlete.

The results of this study provide insight on methods for evaluating wellness and performance readiness of Division I female athletes throughout an entire competitive season. However, there are a few limitations that are recognized within this study. First, there is an inherent risk of using subjective questionnaires when working with information that may be personal or sensitive. Athletes may intentionally falsifying responses to present themselves in a manor they feel is desirable or favorable by the coaching staff (Meeusen et al., 2013). Despite these limitations, there are several benefits of using subjective questionnaires including being cost-effective and easy to perform. Further, although nutritional biomarkers were assessed, diet was not monitored or analyzed. It is possible that the changes observed in certain nutritional biomarkers could be due to changes in the athletes' diet. However, the use of dietary recalls or food diaries to monitor nutritional intake are highly flawed and can result in unreliable data that are highly impacted by bias and knowledge base (Burke et al., 2008). Lastly, the influence of the menstrual cycle and use of oral contraceptives on performance and biomarkers was not accounted for, though the authors chose a 28-day sampling time frame as a secondary attempt to "control" for the menstrual cycle. Addressing these limitations in future studies would prove beneficial to attempt to account for diet and menstrual cycle, though this may be difficult when utilizing a free-living athlete in a real-world setting.

PRACTICAL APPLICATIONS

This holistic approach to athlete monitoring may provide greater insight to assessing the nutritional influences and accumulated stress both on and off the field by tracking both physiological, nutritional, and psychological changes in high-level female athletes. These results showed the highest training load during the preseason training block coinciding with negative changes in both VitD and Fe, which may have detrimental effects on performance. Furthermore, there was a decrease in mood state as the season progressed exhibiting the greatest disruption at the end of the season which corresponds to tournament play. Interestingly, this disruption was mirrored by changes in Trp, thus strengthening the notion of Trp in facilitating negative mood states. Cumulative effects of season-long training load also resulted in significant decreases in both VO_{2max} and VT. These decreases also corresponded to the end of the season representing tournament play, a time when it is desirable to peak or at least maintain performance. The combination of disruption of dietary biomarkers, disruption in mood states, and decreases in performance of the course of the season indicates these athletes are experiencing the greatest disruption during tournament play. These results highlight the stress of college athletes who experience high physical demands of training, academic requirements, and possible alterations of diet while in season. These results provide valuable data in free-living female athletes in a real-world setting. Given the changes observed in this study, possible supplementation of Fe, n-3FA, and VitD as well as various amino acids such as Glu and Trp may be beneficial in female athletes.

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