

**CHANGES IN MARKERS OF STRESS, RECOVERY, TRAINING LOAD AND PERFORMANCE  
DURING A WOMEN'S DIVISION I FIELD HOCKEY SEASON**

By

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## ABSTRACT OF THE THESIS

### Changes in Markers of Stress, Recovery, Training Load and Performance During a Women's Division I Field Hockey Season

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Understanding how the health and physiology of female collegiate athlete changes in response to training is critical to optimizing performance and maintaining fitness over a long competitive season. Evaluating these changes using blood-based biomarkers along with fitness tests and training activities may help maximize outcomes and improve athlete management. **PURPOSE:** To evaluate changes in fitness and blood-based biomarkers over a Women's Division I Field Hockey season. **METHODS:** Field hockey players (N= 23;  $M_{age}=19\pm 1.09$  yrs;  $M_{ht}=166.05\pm 3.33$  cm;  $M_{wt}=64.49\pm 7.39$  kg;  $M_{\%BF}=26.14\pm 6.52$ ) were monitored for changes in biomarkers prior to the start of pre-season (T1) and at four week intervals thereafter (T2, T3, T4). Athletes arrived fasted and euhydrated prior to the first pre-season practice and 36 hours after a game for T2, T3 and T4. Blood was used to assess biomarkers correlated with stress, oxygen carrying capacity, metabolism and nutritional status. On a separate visit from T1 and T4 athletes reported for performance testing including body composition (%BF), vertical jump (VJ) and a maximal graded exercise test to ( $VO_{2max}$ ) via direct gas exchange. All players were monitored for training stress using the Polar Team<sup>2</sup> System throughout training. **Results:** There was no significant ( $P>0.05$ ) change in total cortisol but, significant differences

( $P < 0.05$ ) were found in markers associated with training induced stress. Significant differences ( $P < 0.05$ ) were found in all metabolic, nutritional and hematological markers over the course of the season. Fitness tests were found to be significantly correlated with changes in stress, metabolism and Kcal expenditure during the pre-season (T1 – T2). **Conclusions:** These results show significant changes in biomarkers and training stress over the course of a competitive field hockey season. Biomarkers were shown to significantly change throughout the season but most of this change occurred during intense pre-season training (T1-T2). Continued elevation of these markers showed athletes never truly recovered throughout the season despite significant decreases in training volume. Despite showing no significant change over the season performance markers were shown to be predictive of the pre-season response to training. Incorporating blood-based biomarkers into an established monitoring program can help coaches better evaluate team training stress and recovery.

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**Chapter 1**

**Methods of Analyzing and Evaluating an Athlete's Response to Training**

By

SEAN P. CONWAY

## Introduction

The use of technology to monitor physical training load placed on athletes has become standard across many levels of sport. For example, the use of heart rate (HR) monitors and global positioning system (GPS) technology has become commonplace and the efficacy of these tools has received considerable support (Alexandre *et al.*, 2012). However, these technologies only provide an isolated piece of the “puzzle” in understanding how the body responds to training stressors. Using a panel of meaningful biomarkers has been proposed as a means of better evaluating an athlete’s response to training and clarify recovery needs while potentially detecting early warning signs of non-functional overreaching (NFO) and overtraining syndrome (OTS) (Urhausen *et al.*, 2002; Meeusen *et al.*, 2006; Hinrichs *et al.*, 2010). Research has shown that, depending on level of play and sport, biomarkers associated with stress, nutrition, inflammation and recovery have the potential to change significantly over the course of a season (Baird *et al.*, 2012; Hinrichs *et al.*, 2010; Purvis *et al.*, 2010). Through understanding these changes, coaches and athletes can better evaluate performance, modify training, identify nutritional deficiencies, and advocate for rest. Historically, research has focused on the response of collegiate and elite level male athletes to immediate and chronic stress of training. This has left female athletes largely underrepresented in understanding unique responses and adaptations to training and competition. Chapter 1 will discuss the physical demands placed on athletes while defining parameters of NFO and OTS, current methods of evaluating athlete performance, the potential use of

biomarkers associated with performance, and their role in managing the female athlete throughout the season.

### **Physical Demands on an Athlete**

The hallmark of excellent coaching is the ability to manage a balance between training stress and recovery for their athletes. Avoiding a combination of high training load with insufficient recovery is key to optimizing athlete health over long competitive seasons. Athletes in power-endurance sports, such as field hockey, soccer, rugby, etc., must be able to repeatedly generate high power output and perform at intensities close to  $VO_{2max}$  intermittently over the course of a match or practice (Reilly, 2010; Clark *et al.*, 2003; Sell *et al.*, 2016). Work output, change of direction and physiological stress have been assessed through HR and GPS technology to show total physical load on players. For example, elite level soccer and field hockey players have been shown to travel between 8 and 14 km over the course of a match, expend between 8 and 20 Kcal/kg/min, spend >80% of time at or above 75% of  $HR_{max}$  and engage in repeated sprints at speeds between 5.5 and 7.5 m/s (Boyle *et al.*, 1994; White *et al.*, 2013; Scott *et al.*, 2013). Athletes are asked to repeat this intensity several times per week in the form of training and during matches over a several-month-long competitive season. Duration and intensity of this caliber requires an optimal training and recovery plan to minimize the risk of athlete breakdown and overtraining.

### **Non-functional Overreaching and Overtraining**

The purpose of any successful training program should be to optimize performance and achieve a peak in physical ability during critical periods of the

competitive season. Intervals of intense training are usually followed by a decrease in training volume in order to allow the body to “rebound” to a higher level of performance. When done correctly, these planned periods of high training stress which push or even slightly exceed an athlete’s capacity are referred to as functional overreaching (FO). This can also occur when a coach unintentionally pushes athletes beyond their capabilities in an effort to get the athlete more fit or correct negative outcomes by increasing training volume. This typically has the opposite effect resulting in decreased performance, otherwise known as non-functional overreaching (NFO). NFO is defined as short periods of accumulated training and non-training stress that results in performance decrements which may or may not illicit physiologic changes (Meeusen *et al.*, 2006). Typically, athletes are able to recover from a state of NFO with anywhere from several days to several weeks of rest depending on the duration and magnitude of the stressors. Unfortunately, the decreases in performance are often met with the natural inclination to “train harder” rather than recognizing the role of insufficient recovery in the development of the problem. This further compounds the issue and delays recovery from NFO. If this cycle continues, it may develop into overtraining syndrome (OTS) a condition characterized by physiologic and psychological abnormalities (Meeusen *et al.*, 2006). Unlike NFO, athletes suffering from OTS take significantly longer to regain performance, and some may never fully recover, thus jeopardizing their athletic career and health. For these reasons, it is imperative that athletes and coaches are conscious of training and recovery status. Utilizing technology

to uncover early warning signs of NFO and OTS can become an invaluable resource at all levels of sport for maintaining athlete health and wellbeing.

### **Heart Rate Monitoring**

The coach of a team sport must understand the fine-line between overtraining, undertraining and optimal training in order to manage the individuals that comprise the team for a successful season. Up until the last decade or so, this was accomplished by a coach assessing how well a player “looked” at practice and the amount of perceived effort or fatigue a player displayed. More recently, coaches and players have turned to research and technology in order to better manage players both in and off season (Reilly, 2010; Konarski *et al.*, 2010; White *et al.*, 2013). HR monitoring has technically been used to examine player performance since the late 1960’s (Seliger, 1968). However, the monitors today allow input of performance measures including  $VO_{2max}$ , ventilatory threshold (VT), maximum heart rate ( $HR_{max}$ ) and more, giving athletes the ability to accurately evaluate their training. The past decade has also seen the rise of whole team HR monitoring systems to allow coaches to better manage players in practices and games (Konarski *et al.*, 2006). Monitoring on this scale has provided coaches the means to assess team performance, recovery time, calorie expenditure and training volume. Despite the advances in HR monitors and incorporating GPS technology, it remains limited to assessing the physical stress of training while an athlete is wearing the monitor (Alexandre *et al.*, 2012). Sport is multifaceted and places more than just a cardiovascular demand on the body. While acceleration, environmental stress, hydration status, etc. can influence HR, measuring athlete physiology and

changes in blood chemistry over time allows a more comprehensive assessment of player condition.

### **Blood-Based Biomarkers**

The use of meaningful biomarkers sensitive to training, stress, nutrition, and recovery have become a new avenue in evaluating athlete health and training status. Research has linked many of these biomarkers to athlete performance and suggested others as early warning signs for NFO and OTS (Risser *et al.*, 1988; Urhausen *et al.*, 1995, 2002; Kraemer *et al.*, 2005; Heisterberg *et al.*, 2013; Kanda *et al.*, 2014). Traditional markers associated with the stress of training include cortisol (CORT), sex hormone binding globulin (SHBG), creatine kinase (CK), lactate dehydrogenase (LD), prolactin (PRL) and interleukin-6 (IL-6). (Urhausen *et al.*, 1995; Kraemer *et al.*, 2005; Ispirlidis *et al.*, 2008; Tankkanen *et al.*, 2011). Each of these markers has been associated with an element of athlete breakdown including the stress response, muscle damage, and inflammation. Alterations in metabolism have also been tied to the stress of training due to changes in thyroid stimulating hormone (TSH), thyroxin (T<sub>4</sub>) and triiodothyronine (T<sub>3</sub>) (Meeusen *et al.*, 2010; Budgett *et al.*, 2010). Deviations in thyroid hormones result in altered energy availability and whole body metabolism. Markers of oxygen binding and transport such as iron (Fe), hemoglobin (Hb), ferritin (FER), total iron binding capacity (TIBC), percent saturation (%Sat), hematocrit (HCT) and mean corpuscular hemoglobin concentration (MCHC) have been shown to fluctuate with training and respond differently between genders (Risser *et al.*, 1988; Di Santolo *et al.*, 2008; Heisterberg *et al.*, 2013). An athlete's ability to transport and utilize oxygen during

exercise is key to maintaining performance. Markers of nutritional status, including vitamin D (VitD) and the omega 6:3 ratio (OMG63), have been implicated with overall athlete health (Larson-Meyer *et al.*, 2010; Buonocore *et al.*, 2015). Changes in nutritional markers can be used to evaluate the quality of food an athlete eats and identify deficiencies that may ultimately lead to impaired recovery. Current research has provided insight to how each of these biomarkers are individually influenced by acute and chronic training, recovery and as markers of OTS in male athletes.

***Stress and Recovery Markers.*** Training an athlete requires repeated, high intensity training that places a great deal of physical strain on the body. Biomarkers linked with anabolism (recovery) or catabolism (breakdown) indicate how well the body manages exceptional stresses (Urhausen *et al.*, 1995). Examining these markers of catabolism in non-overreached and overreached athletes identifies changes with a disordered response. Biomarkers of particular interest are CORT, SHBG, CK, LD, PRL and IL-6 because of their relationship with the stress response and inflammation (Urhausen *et al.*, 2002; Kreher *et al.*, 2012; Balsalobre-Fernández *et al.*, 2014; Meeusen *et al.*, 2010; Lehmann *et al.*, 1992). Monitoring deviations in catabolic marker concentrations from resting baseline values provides a deeper understanding of how the athlete responds to training.

Regulatory markers of stress define the magnitude of catabolism after either single or repeated training sessions. The amount of CORT in plasma has been established as a quantitative measure of evaluating the body's response to training (Kraemer *et al.*, 2005; Tankkanen *et al.*, 2011). Some research has demonstrated

decrease of ~25% in resting CORT in athletes on the verge of NFO or OTS (Synder *et al.*, 1995; Urhausen *et al.*, 2002; Kreher *et al.*, 2012). Suppressed CORT has been implicated with alterations in the hypothalamic-pituitary-adrenal (HPA) axis; however, contradicting research has made determining the patterns of these changes difficult. Other research has shown increase of ~18% in resting CORT accompany feelings of staleness typically associated with OTS (Kraemer *et al.*, 2004; Budgett *et al.*, 2010; Silva *et al.*, 2014; Balsalobre-Fernández *et al.*, 2014). In these studies, CORT was correlated with decreases in performance markers (vertical jump,  $VO_{2max}$ , sprint time), increased training loads, and elevated ratings of perceived exertion (RPE). Discrepancies in the literature on CORT response may be due to mode of training, insufficient intensity, or a lack of reaching a state of NFO or OTS. Kraemer *et al.* (2004) reported that CORT levels in starting male soccer players were classified as “high normal” and non-starters were measured above clinical range at baseline; these levels persisted throughout the season contributing to a decrease in physical performance. These results are indicative of a persistent catabolic state and were associated with acute endurance overtraining during the pre-season (Kraemer *et al.*, 2004). The fact that acute overreaching can result in season long elevations to CORT in male power-endurance athletes makes repeated evaluations critical for managing athlete performance. Given this, further research is needed to evaluate whether similar changes occur in female athletes participating in these types of sports.

Prolactin (PRL) is typically associated with milk production in females but recent research has implicated it in decreased athletic performance (Budgett *et al.*, 2010).

Research by Budgett *et al.*, (2010) has shown PRL to increase after an acute bout of intense exercise for up to 150 minutes before trending toward baseline in elite athletes assigned to control and OTS groups. In this study, the OTS group exhibited significantly higher PRL at rest and at 150minutes post when compared to non-OTS controls. Further research using male OTS and NFO athletes demonstrated a notable difference in PRL response to two maximal exercise bouts 4 hours apart (Meeusen *et al.*, 2010). After the first exercise bout, the percent change in PRL between both groups was similar although the OTS group exhibited higher PRL throughout the test. After the second maximal session, there was little to no change in the OTS group while the percent change of the NFO group was greater than the previous exercise bout (Meeusen *et al.*, 2010). The reason for this change has been reported as a hypersensitivity in the glucocorticoid receptors of NFO and insensitivity in OTS athletes following the second bout of exercise (Meeusen *et al.*, 2010). The research by Budgett *et al.* (2010) and Meeusen *et al.* (2010) demonstrates the importance of evaluating PRL as both a marker and a differentiator of NFO and OTS. While measuring PRL within the first 2.5 hours has been shown to be ideal for monitoring a peak in male athletes, comparing resting, 24+ hours post-training PRL has been shown to effectively evaluate athletes training status (Budgett *et al.*, 2010). Future research is needed to understanding how PRL changes in response to season long training.

Maximizing recovery between exercise bouts requires an anabolic state to be achieved quickly and maintained until the next training stress is applied. This process requires increased release of the sex hormones, testosterone and estrogen, and their

carrier protein SHBG. Research has established the sensitivity of the sex steroids to the stress of training and their elevated response after an exercise condition (Kreher *et al.*, 2012; Kraemer *et al.*, 2005; Urhausen *et al.*, 1995). This elevation in sex hormone production should be met with a similar rise in SHBG to facilitate binding. Research has been mixed, with reported acute elevations in SHBG after resistance training but no significant change following several weeks of strenuous exercise in different environmental conditions (Kraemer *et al.*, 2005; Hug *et al.*, 2003). These findings imply that fluctuations in SHBG concentration are dependent on the presence of sex hormones in plasma. This is contradictory to research by Tanskanen *et al.* (2011) that demonstrated significant changes in SHBG levels in males after 8 weeks of strenuous military basic training with no significant change in testosterone. Furthermore, utilizing SHBG as a marker of health status in female athletes has been shown to identify menstrual irregularities (Bermon *et al.*, 2014). Bermon *et al.* showed elite female athletes not using oral contraceptives and reporting as oligo- and amenorrheic had SHBG levels above clinical norms when compared with their peers. While SHBG is a useful marker for determining androgen status and ultimately anabolism, its role as an identifying factor of amenorrhea in female athletes may be vital to reproductive health and an important monitoring consideration. To date, little research has been done on evaluating SHBG response to chronic training in females and future research should focus on understanding how SHBG levels fluctuate in response to a competitive athletic season.

Markers specifically associated with micro trauma to the muscle cell have been shown to be effective for assessing training stress. Resistance training and rapid athletic movements on the field result in these micro traumas, allowing for the release of proteins like CK from the cell (Kanda *et al.*, 2014; Urhausen *et al.*, 2002; Baird *et al.*, 2012; Kraemer *et al.*, 2005). CK is typically only found in muscle tissue acting as a key enzyme in energy production; therefore, changes in CK plasma concentration have been associated with degree and frequency of training stress. Though the use of CK as a biomarker for catabolism or muscle damage has been disputed in the literature (Karvonen, 1992; Hartmann *et al.*, 2000; Ispirlidis *et al.*, 2008; Baird *et al.*, 2012; Meister *et al.*, 2013) because of its long biological half-life and inconsistent results in classifying OTS. Recent contradicting research has suggested that CK is a viable marker for categorizing a catabolic state (Baird *et al.*, 2012; Silva *et al.*, 2014). Silva *et al.* (2014) demonstrated that CK is related to training-induced stress in male soccer players. CK peaked during the pre-season training period and fell to near baseline levels by the end of season. Ispirlidis *et al.* (2008) identified a rise in CK up to 72 hours post exercise that did not return to baseline values until 120 hours post exercise. The long half-life of CK should be given consideration when selecting sampling times for repeated measures (Silva *et al.*, 2014; Nabhan, 2015). With standardized test times, CK may be a viable marker in assessing athlete breakdown and should be considered when evaluating an athlete for OTS.

The high physical load experienced during training is associated with the increase of CK and LD in plasma despite each marker having been shown to have a wide range of

interindividual variability (Ispirlidis *et al.*, 2008). LD is found in most cells of the body, but fills a critical role in muscle of interconverting lactate and pyruvate for energy production. Like CK, the increase in LD peaks by 48 hours post exercise and returns to baseline within 96 hours (Ispirlidis *et al.*, 2008; Baird *et al.*, 2012). This has lead researchers to draw similar, mixed, conclusions regarding the use of LD as an indicator of OTS. Ispirlidis *et al.* (2008) argues that while LD indicates elevated levels of tissue damage post-exercise, it is hard to determine from where that LD is being derived. In this case, changes in LD may be a reliable measure of muscle damage within the first 48 hours post-exercise as they precede the rise in CK (Ispirlidis *et al.*, 2008). While changes in LD can be associated with the stress of training within the first 48 hours, it may be more useful in detecting overall tissue damage because of its presence in a variety of other tissues (Baird *et al.*, 2012). Current research has yet to show a direct relationship between CK, LD and OTS potentially due to never assessing athletes who were truly overtrained. However, these markers have been implicated in decreased performance in male athletes (Ispirlidis *et al.*, 2008). When measuring markers of muscle damage in athletes, short periods of rest between training sessions makes evaluating CK and LD difficult because of their delayed response. Therefore, any assessment of these markers should be preceded by 24 hours without training and performed at a similar time interval from the previous training session. Further research is needed in order to evaluate if season changes in these markers occur in females similar to their male counterparts.

Besides elevating levels of CK and LD, the micro trauma the the muscle caused by training also results in a local inflammatory response. Inflammation is an important regulatory process that allows the body to mobilize resources, fight off disease, and signal for repair. During the prolonged stress of training, continual stimulation of the inflammatory response can perpetuate a catabolic state associated with OTS. The pro-inflammatory cytokine IL-6 has been shown to be particularly sensitive to the stresses associated with exercise training and is released by the muscle during and immediately after intense exercise (Nieman *et al.*, 2000; Kasapis *et al.*, 2005; Ispirlidis *et al.*, 2008; Peeling *et al.*, 2008). Research by Ispirlidis *et al.* (2008) has shown a brief increase in IL-6 for 450% immediately post-exercise that returns to baseline within 24-hours. However, habitual training has been shown to produce prolonged elevation in IL-6, implicating it as a marker of OTS (Kreher *et al.*, 2012; Henning *et al.*, 2013). Research by Henning *et al.* (2013) found that soldiers completing Army Ranger School presented with symptoms of OTS, including elevated resting levels of IL-6 of ~217% from baseline. Therefore, monitoring athlete performance in conjunction with season changes in IL-6 should act as an indicatory of prolonged breakdown and an early warning sign of OTS. While chronic changes in IL-6 have been shown as a viable marker of breakdown in males, their impact on female athlete performance has yet to be demonstrated.

**Nutritional Markers.** Imbalances between training and recovery over time can cause detrimental changes in athlete physiology resulting in decreased performance, which can be compounded if athletes do not meet their nutritional needs. Matching calorie intake with training volume and educating athletes on meal selection can

improve training and recovery. However, research has shown that the quality of the source can have just as much impact as excess training or deficient recovery (Buonocore *et al.*, 2015). The OMG63 ratio determines the quality of fat an athlete is ingesting and therefore reflects the nutritional value of the meal, with omega-6 (OMG6) generally indicating poor quality source and omega-3 (OMG3) indicating higher quality (Buonocore *et al.*). The ratio between OMG6 and OMG3 demonstrates the balance between the two with a high value (high OMG6 and/or low OMG3) being less desirable than a low value (low OMG6 and/or high OMG3). As a biomarker, OMG63 can also act as an indicator of inflammation and other health detriments. Buonocore *et al.* describe an elevated OMG63 as being correlated with an increased risk for metabolic disease and increased levels of pro-inflammatory cytokines (IL-6); conversely, a low OMG63 has the opposite effect by decreasing pro-inflammatory cytokines and acting as an anti-oxidant. Low OMG63 due to a high OMG3 has been shown to provide a series of performance benefits including decreased CORT, reduced inflammation and improved psychological health (Jazayeri *et al.*, 2010). Managing OMG63 for an athlete becomes particularly important as IL-6 and other pro-inflammatory cytokines have been shown to increase with prolonged training (Nieman *et al.*, 2000; Ispirlidis *et al.*, 2008). Research by Jazayeri *et al.* (2010) has also shown a low OMG63 due to high OMG3 to improve mood and psychological health of an individual. Minimizing this effect through foods with low OMG6 and high OMG3 can help manage the rise in inflammation that typically occurs over the season keep an athlete in the game when they are needed most.

Providing healthy foods as options and educating athletes on the connection between nutrient rich and poor foods with performance is critically important at all levels of sport. Simply matching calorie intake with expenditure is not enough; changes in micronutrient status have been shown to directly impact on-field performance (Larson-Meyer *et al.*, 2010; Buonocore *et al.*, 2015). Particular micronutrient markers of interest are those related to bone health (VitD) and oxygen binding; especially in female athletes. VitD is a regulator marker of calcium concentration making it particularly important in maintaining bone health, inflammatory response, and muscle function (Larson-Meyer *et al.*, 2010). Larson-Meyer *et al.* states that the VitD status of athletes varies as a function of time-of-year of the sport and gender: It tends to be higher in summer sports than winter sports and higher in males than females. The lower VitD status in female athletes is of particular importance in regard to its role in the female athlete triad. The female athlete triad is a physiological condition that arises as female athletes strive to achieve an unrealistic body image resulting in dysregulation of several systems (Otis *et al.*, 1997). Regulation of calcium by VitD in female athletes experiencing or on the verge of entering this disorder can contribute to decreases in bone mineral density and osteoporosis, defining characteristics of the condition (Otis *et al.*, 1997). Helping female athletes understand how changes in VitD occur using biomarkers can minimize or avoid these conditions through early detection, improved nutritional intake and VitD supplementation.

Deficiencies in micronutrients often produce rapid decreases in performance due to their relatively low concentrations. Fe is required for many physiologic processes but

is most notably the key oxygen binding element of hemoglobin. Research has shown Fe deficiency to result in significant decreases in aerobic capacity for this reason (Risser *et al.*, 1988; Hinrichs *et al.*, 2010). This makes Fe status critically important for athletes, particularly those relying on prolonged aerobic performance and oxygen uptake/delivery. Research by Karl *et al.*, (2015) has shown Fe status to be significantly impacted in both male and female soldiers following military training. While a significant change was found in both sexes from pre to post-training, female soldiers showed significantly lower Fe than their male counterparts (Karl *et al.*, 2015). The work by Karl *et al.* (2015) further supports previous research in showing a higher prevalence for low or clinically deficient (anemia) Fe status in female athletes (Risser *et al.*, 1988; Hinton *et al.*, 2000; Di Santolo *et al.*, 2008). Evaluating these changes in female athletes is critically important as it allows for early intervention to either maintain or regain performance. Research by Hinton *et al.* (2000) showed that supplementing Fe in non-anemic physically active, but not trained, females to significantly increase Fe concentration and aerobic capacity from baseline. The subjects also showed a significant difference in the Fe supplemented group opposed to the control group. This research supports the use of Fe supplementation for females as part of a healthy routine, particularly if they are frequent exercisers or trained athletes. It is important to consider the role of Fe on other hematological markers as its deficiency can have widespread performance implications.

***Hematologic Markers.*** An athlete's Fe status influences several other hematologic variables that have been shown to directly impact performance (Risser *et*

*al.*, 1988). FER regulates Fe concentration by storing and releasing Fe in response to dietary intake and physiologic demand. The current literature related to Fe and FER status on performance is mixed depending on the gender being assessed (Risser *et al.*, 1988; Hinrichs *et al.*, 2010; Heisterberg *et al.*, 2013). Risser *et al.* (1988), Di Santolo *et al.* (2008) and Hinrichs *et al.* (2010) found that female athletes have a higher incidence of low Fe and FER prior to beginning training (with further losses after training) than their male counterparts. These studies also found that female athletes were more susceptible to other signs of anemia including low %Sat and elevated TIBC (Di Santolo *et al.*, 2008; Hinrichs *et al.*, 2010). Conversely, Fe and FER status in males does not seem to reach clinical significance as often, although a decrease in Fe has been noted with training (Heisterberg *et al.*, 2013; Karl *et al.*, 2015). The research with female athletes has shown that supplementation improves Fe and FER status, significantly improves  $VO_{2max}$  and shortens 15-km run time (Friedmann *et al.*, 2001; Hinton *et al.*, 2000; Hinrichs *et al.*, 2010). While the incidence of Fe deficiency may be lower in males, a decrease in Fe has the potential to impact performance.

Training and Fe status have also been shown to cause changes in Hb, HCT and MCHC (Brocherie *et al.*, 2015; Heisterberg *et al.*, 2013; Hinrichs *et al.*, 2010). Slight decreases in Hb and HCT are expected in athletes due to increases in plasma volume associated with training, a condition commonly termed “sports anemia” (Heisterberg *et al.*, 2013). Sports anemia is typically transient and does not affect performance to the extent of clinical anemia. A diagnosed anemia is characterized by clinical changes in hematologic markers and decreases in aerobic capacity. Several researchers have

correlated hematologic parameters (i.e., Hb, HCT and MCHC) to decreases in  $VO_{2max}$  and scores on the Yo-Yo Intermittent Recovery Level 2 test (YYIR2) (Brocherie *et al.*, 2015; Heisterberg *et al.*, 2013). Brocherie *et al.* (2015) found absolute Hb concentration and MCHC were moderately correlated with YYIR2 while athletes with lower mass Hb showed a decreased time to exhaustion. Hematologic parameters in professional male soccer players demonstrated significant fluctuations in HCT and MCHC during the competitive season compared to post-season; despite the variations in parameters none of the values were outside clinical reference ranges (Heisterberg *et al.*, 2013). Variation in these markers identifies the potential for an imbalance in hematologic status leading to performance decrements. Due to the demands of high-level sport, even these small fluctuations can result in meaningful changes when it comes to efficiency of training and the outcomes of games.

The current body of research has established the efficacy of using markers associated with hematologic status to measure the impact of training and recovery on athletes. Decreases in Fe and FER have been correlated with decreases in aerobic capacity and introducing an Fe supplement has been shown to reverse this decrement (Friedmann *et al.*, 2001; Hinton *et al.*, 2000; Hinrichs *et al.*, 2010). Deviations in Fe status occurs more often in female than male athletes suggesting that the female athlete would benefit more from Fe supplementation while training. The use of s Hb, HCT and MCHC in identifying training status has been similarly conclusive. Small changes in Hb and HCT are associated with normal plasma volume shifts indicating sports anemia but continued changes in hematologic markers are risk signs of clinical anemia or may result

in meaningful performance changes. Research in elite male soccer players has shown the potential for hematologic factors to change in response to a competitive season resulting in an associated decrease in  $VO_{2max}$  (Heisterberg *et al.*, 2013). Given these results, future research should evaluate season changes in hematologic status of female athletes participating in power-endurance sports.

**Metabolic Markers.** Training for sport places a huge energy demand on the athlete's body requiring an increased metabolic output. Changes in metabolic rate should result in increased TSH signaling of  $T_4$  and  $T_3$  to meet these needs. Studies have shown a disruption in TSH,  $T_4$  or  $T_3$  associated with training but have not been able to correlate those changes to altered performance (Urhausen *et al.*, 1995; Kraemer *et al.*, 2004; Henning *et al.*, 2013). A review by Kraemer *et al.* (2004) outlines previous research showing a mixed result in TSH,  $T_4$  and  $T_3$  responses to a week of intense resistance training; some individuals showed significant decreases while other evidenced no change. Dietary intake is a key moderator of thyroid hormones and research has shown reductions in calorie intake to result in decreased  $T_4$  and  $T_3$  secretion (Henning *et al.*, 2013; Williams *et al.*, 2001). This is due to a negative feedback on TSH secretion that ultimately reduces  $T_4$  and  $T_3$  synthesis and slows metabolism. Henning *et al.* demonstrated that a considerable energy deficit resulted in a significant rise in TSH after 8 weeks of intense military training with no significant change in  $T_4$  or  $T_3$ . The rise in TSH may be due to the prolonged demand for an increased metabolic rate coupled with stimulatory signaling from low  $T_4$  and  $T_3$  overriding the feedback signal of decreased dietary intake on TSH. The result of this would be an increase in TSH, similar to that

shown by Henning *et al.* (2013) and elevated  $T_4$  and  $T_3$  synthesis. Given the current research, significant changes in TSH may require consistent monitoring for an extended period of time.

### **Considerations for Female Athletes**

Special considerations should be made when evaluating performance in female athletes. Currently, most of the research in markers of training load, physical stress, physiologic response and recovery have been performed in males. This is partially due to the ease in controlling hormonal variation in males compared to females. However, some researchers have begun to bridge the knowledge gap between male and female athletes. Sell *et al.* (2016) demonstrated training loads, average HR,  $HR_{max}$  and times in HR zones to be similar in female field hockey players as in males during competitive matches. While relative stress from training may be similar, the physiologic response of female athletes to the demands of a competitive season may differ from their male counterparts (Clark *et al.*, 2003; Di Santolo *et al.*, 2008). Female athletes are typically associated with a host of physiologic concerns that may otherwise remain unnoticed without frequently assessing changes in meaningful biomarkers.

The female athlete triad is a condition that occurs in physically active women and is characterized by disordered eating, amenorrhea and osteoporosis (Otis *et al.*, 1997). This condition typically arises in the pursuit of an unrealistic low body composition and eventually leads to decreases in performance. Female athletes have been shown to under-eat while training, resulting in decreased metabolic rate, poor recovery, and micronutrient deficiencies (Clark *et al.*, 2003). Research has shown that

exercising in a chronic caloric deficit decreases metabolic rate through inhibition of the thyroid hormones, eventually leading to a decrease in performance (Henning *et al.*, 2013). Measuring these hormones in female athletes can be used to assess metabolic activity and identify the potential for calorie deficiency. Under-eating in female athletes increases their risk of micronutrient deficiencies, such as Fe, and more susceptible to developing chronic conditions like clinical anemia causing a decrease in aerobic capacity (Clark *et al.*, 2003). By measuring Fe, FER and other markers associated with oxygen transport, deficiencies can be averted through changes in diet and supplementation. VitD has been correlated with bone health as it is a key regulator of calcium and phosphorus (Larson-Meyer *et al.*, 2010). Utilizing VitD as a biomarker can help assess calcium status indicating high or low rates of absorption that influence the rate of bone reabsorption. This is of particular concern for clinicians and coaches assessing a female athlete for osteoporosis as part for the female athlete triad. Altered menstrual patterns can potentially be identified by biomarkers like SHBG if they are not assessed directly. Female athletes may not readily notice the signs of oligo- or amenorrhea or may even believe it indicates “optimal” training (Otis *et al.*, 1997). Elevated levels of SHBG can be used as an early indicator of amenorrhea before other reproductive health concerns arise (Bermon *et al.*, 2014). Assessing a panel of biomarkers over a training period or longer may help better understand the relationship between each leg of the triad.

### **Conclusion**

As discussed in this review, a coach’s ability to apply appropriate physical stress in order to optimally train a team of individuals while avoiding NFO and OTS is critical to

the team's success. Measuring an athlete's physical output using HR or GPS technology is an easy way of monitoring the stress of training but is limited as a "snapshot" of that particular training session and accumulated training load over weeks or months. Though this is critical information, it does not assess the systemic responses over time (including during what should be recovery periods) and may benefit from concurrent application with more comprehensive measures of physiological strain. Research has shown measuring biomarkers associated with stress, recovery, and nutrition to be potentially useful tools for evaluating the physical and non-physical stress of training. The current literature not only shows these markers to be effective tools to measure acute exercise but also season-long changes in male athletes. However, this research has primarily focused on a single group of closely associated markers (stress, metabolic, nutritional etc.) rather than assessing a panel to gauge total physiological response. Even so, a complete physiological response in male athletes can be inferred based on past research; this is not the case for females. There is a serious knowledge gap between the male and female response to training. While some research has been performed assessing the impact of training load on performance in female athletes, there is relatively little information on their endocrine and biochemical response to prolonged training. Therefore, the objective of this study was to use a panel of meaningful blood-based biomarkers to explore the response of a female athlete's physiology to the demands of a competitive season.

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**CHAPTER 2**

**CHANGES IN MARKERS OF STRESS, RECOVERY, AND TRAINING LOAD DURING A  
WOMEN'S DIVISION I FIELD HOCKEY SEASON**

By

SEAN P. CONWAY

## Introduction

Female NCAA field hockey athletes undergo intense physical training to prepare for the demands of a long competitive season. Coaches typically have a two-week pre-season to prepare athletes for 10-weeks or more of match play. This makes understanding the the fine balance of applying training stress and optimizing recovery critical to maintaining athlete health and performance. Research has shown not managing this balance appropriately can lead to a chronic state of elevated stress, mental fatigue, and increased risk of injury associated with overtraining syndrome (OTS) (Urhausen *et al.*, 2002). Using technology to evaluate the physical stress of training gives coaches a means of better managing their team to avoid a decrease in performance. Using a heart rate (HR) monitoring system to quantify physical training load, though remarkably useful, is limited to the time that an athlete is actually wearing a monitor. In other words, it cannot account for compounding stress throughout the season, including time off the field. Research suggests measuring and evaluating changes in physiologic biomarkers of training, stress, and recovery from blood as a better tool for managing an athletic team (Budgett *et al.*, 1990). Studies have historically looked at biomarkers as isolated clusters, though a more encompassing approach may be more appropriate for understanding the response to training (Silva *et al.*, 2013). Regardless of how these markers are assessed, female athletes have been largely underrepresented in the current literature. Understanding the female athlete's response to the training stress applied across a competitive season through the use of biomarkers has implications for maintaining athlete performance and physical health.

Field sports such as soccer, field hockey, rugby, etc. are considered “power-endurance” sports and are characterized by their physical demand requiring athletes to achieve and maintain a high level of fitness to excel. While each sport has its own unique technical requirements, all of the above noted sports require high aerobic capacity ( $VO_2$ ), high anaerobic threshold (VT), power, speed, and agility (Reilly, 2010). The sport of field hockey is played on a pitch slightly smaller than a soccer field but requires similar multidirectional movements, coordination and physical ability. Beyond these characteristics and unique to field hockey, athletes must also exhibit proficient stick skills to competently move the ball around a field (Boyle *et al.*, 1994). This requires the athlete to spend a substantial amount of time in a semi-crouched position dribbling, passing, and contesting the ball between bouts of high intensity upright running (Boyle *et al.*, 1994). Research by Astorino *et al.* (2004) has shown that the physical requirements placed on female collegiate field hockey players produced significant changes to several key areas of performance including body composition (%BF) and  $VO_{2max}$ . Changing critical fitness criteria, either positively or negatively, will directly impact field hockey athlete output throughout the season, thus making athlete conditioning a critical consideration during all periods of the training calendar.

Preparing athletes for the demands of the competitive season is one of the primary goals of the coaching staff. However, there is a fine balance between training stress and recovery that a coach must manage during a short, intense pre-season to prepare a team of individuals for optimal performance. If this balanced is mismanaged, a coach can push individual players or an entire team beyond their capacity resulting in

an elevated stress response and underperformance. This state of decreased performance, referred to as non-functional overreaching (NFO), is characterized by changes to an athlete's resting physiology that can take several weeks to fully recover (Meeusen *et al.*, 2006). If the athlete is continued to be pushed performance detriments, physiologic dysregulation and psychological abnormalities develop into the overtraining syndrome (OTS) requiring several months for recovery (Meeusen *et al.*). Incidence of NFO and OTS are typically characterized by decreases in player performance, negative physiologic changes, and psychological deviations. Measuring changes in fitness has traditionally been used to assess an athletes condition and gauge recovery.

Utilizing performance measures to gauge athlete fitness at the start of the season is imperative to identifying "readiness" and establishing a baseline for detecting detrimental changes that may lead to OTS. It is the responsibility of a coach to manage player skill while it should be the role of a strength coach or exercise physiologist to measure player physical capacity (Reilly, 2010). However, many coaches are forced to fill all of these roles, and are typically ill-equipped to do so. Regardless, reliable and valid methods for evaluating physical fitness should be standard practice in athletics. These methods can be performed either in a lab-based setting or in the field with great accuracy (Reilly, 2010). Physical parameters that have relevance in power-endurance sports include  $VO_{2max}$ , VT, vertical jump (VJ) and percent body fat (%BF). These measures can be used in order to develop an individual athlete profile where changes can be tracked at regular intervals during the season or over a calendar year. Beyond this,

evaluating the stress of daily training provides immediate feedback for coaches to quickly manage an athlete's needs or adjust the team's training going into competition.

Monitoring the demand of practices and games has become a vital element for evaluating athlete performance in sport. Several diagnostic tools are currently being used to monitor athletes from a more "low-tech" rating of perceived exertion (RPE) scale to high-tech HR and GPS monitoring (Scott *et al.*, 2013). Utilizing HR and GPS technology provides measurable changes in physical output that can be used to calculate calorie expenditure and training stress. A review by Borresen *et al.* (2009) found HR and GPS to be a viable measure of training stress in athletes. These benefits become more pronounced when they are used in conjunction with fitness tests to provide a complete athlete profile. Despite their accuracy and usefulness during training, HR monitoring is only capable of measuring a finite period of time and does not account for other stressors placed on the athlete (psychological stress, sleep, nutrition, etc.). Utilizing meaningful blood-based biomarkers can fill in the gaps between training session and allow for training effectiveness to be further evaluated along with HR and GPS.

Biomarkers have been used in research to detect acute and chronic hormonal, biochemical, and hematological changes due to exercise in athletes and the general population. Historically, research has focused on clusters of specific markers associated with catabolism, metabolic changes, hematologic status, or nutritional standing. Scientists have typically assessed each of these clusters individually rather than evaluating the entire physiological response at once. Using similar study populations,

researchers like Heisterberg *et al.* (2013) and Meister *et al.* (2013) have used elite male soccer players to measure the season changes in hematologic status and muscle damage, respectively. Performing these studies in a similar population allows a physiologist to draw inferences in total response but the imposed stress of training may not be similar, therefore altering the adaptive or regulatory response. Until recently, it has been the job of review articles, like that of Purvis *et al.* (2010), to provide a comprehensive assessment of the relationship between biomarkers and stress from practices. Currently research like that of Silvia *et al.* (2014) is starting to take a broader approach in assessing several clusters of biomarkers in an attempt to evaluate whole body response to training in male athletes. This more inclusive approach provides a better understanding of how these biomarkers influence each other and change in response to training stress. Unfortunately, research assessing a panel of biomarkers has been limited to collegiate and elite-level male athletes, leaving females disproportionately underrepresented.

Female athletes pose unique considerations in assessing changes in biomarkers in response to training due to a variety of physiological concerns. The menstrual cycle is characterized by a series of hormonal, biochemical, and metabolic changes. However, Jonge *et al.* (2003) has suggested that these fluctuations do not impact performance or biomarker reliability unless the athlete presents with a dysfunctional or absent cycle. Ironically, biomarkers may be the exact tool needed to assess this. Hematologic status, iron deficiencies, and anemia have also been strongly associated with the female athlete. Research by Di Santolo *et al.* (2008) found that female athletes were three

times more likely than non-athletic controls to exhibit low serum iron and have a non-significantly increased risk for anemia. Changes in bone mineral density have been reported in female athletes due to reduced caloric intake (Otis *et al.*, 1997). The combination of these three physiological concerns have been reported as the female athlete triad. The use of biomarkers in understanding how the female athlete changes over a competitive season can not only help detect this condition early but also improve its treatment. Given these considerations, there is still a great deal that research has not addressed with regard to the female athlete compared to her male counterparts. The wealth of knowledge on the male athlete is encompassing and has helped guide training decisions for over 20 years. Understanding how the female athlete responds to the imposed demands of training and tracking that response over the course of a competitive season would identify key areas of deficiency, changes in performance, and the physical health of the athlete.

Few studies have focused on how female athletes adapt to training over a competitive season. Therefore, the current research is designed to use a discovery-based approach to assess how the female athlete responds to training and begin to define other external influences that may be promoting or hindering these changes. The main purpose of this study is to evaluate the fitness and blood-based biomarker changes that occur in female Division I field hockey players in response to the 12-week competitive season. The goal of this study was to discover how these markers changed over the season and whether athlete fitness could be used as a predictive measure of changes in these markers during the most physically demanding portion of the season.

## Materials and Methods

**Subjects.** Female athletes from the Rutgers University Division I field hockey team ( $N= 23$ ;  $M_{\text{age}}= 19 \pm 1.09$  years;  $M_{\text{ht}}= 166.05 \pm 3.33$  cm;  $M_{\text{wt}}= 64.49 \pm 7.39$  kg;  $M_{\%BF}= 26.14 \pm 6.52$  %;  $VJ= 51.43 \pm 7.09$  cm;  $VO_{2\text{max}}= 46.63 \pm 5.52$  mlO<sub>2</sub>/kg/min;  $VT= 76.84 \pm 2.91$ %) were recruited for this study. Testing was carried out as a normal part of the team's fitness assessment program. The Rutgers University Institutional Review Board reviewed and approved all methods and procedures.

**Performance Testing.** Subjects participated in performance testing prior to the start of pre-season (PT1) and within a week of the final competitive match (PT2). The athletes were instructed to arrive at the Rutgers Center for Health and Human Performance normally hydrated, at least 2 hours fasted, and without having exercised 24 hours prior to testing. Upon arrival, the testing protocol was explained before body weight (BW), body composition (%BF) and lean body mass (LBM) were assessed using air displacement plethysmography (BodPod, COSMED, Concord, CA, USA). The BodPod has been shown to be valid and reliable for measuring %BF compared to hydrostatic weighing (McCrorry *et al.*, 1995). The %CV in our lab is <2.3%. Subjects were then instructed to warm-up for a 5-minute period on a treadmill at a self-selected pace. Following the warm-up, subjects performed three attempts to achieve a maximal vertical jump (VJ) using the Just Jump system (Probotics, Huntsville, AL, USA). Validity and reliability of the Just Jump system for measuring VJ has been previously established by Leard *et al.*, 2007 demonstrating an  $r$  value of 0.967 when compared to a 3-camera motion analysis system. The %CV in our lab is <2.0%. A Track Master treadmill (JAS

Fitness Systems, Carrollton, TX, USA) was used to perform a maximal graded exercise test (GXT) to measure maximal aerobic capacity ( $VO_{2max}$ ) and ventilatory threshold (VT) by direct gas exchange using a TrueOne 2400 Metabolic Measurement System (ParvoMedics Inc., Sandy, Utah, USA). A Modified Bruce Protocol was selected as the GTX; the protocol contains 3 minute stages with speed and grade increasing at the start of each new stage. Players with a %BF <30% began the GXT at 2.5 mph and 11% grade while those players with %BF  $\geq$ 30% began the test at 1.7 mph and 10% grade. Subsequent stages had an increased speed to 3.4, 4.2, 5.0, 5.5 and 6 mph respectively and an increase in grade by 1% until a maximal grade of 15% was reached at the 5.5 mph stage. Subjects continued to perform the test with encouragement from the lab staff until voluntary exhaustion. At the end of each test, subjects were asked to provide their rating of perceived exertion (RPE) using a Borg scale from 6 to 20 (Borg *et al.*, 1982). Heart rate was continuously monitored using the Polar Team<sup>2</sup> HR transmitter to accurately obtain their maximal heart rate ( $HR_{max}$ ) (Polar Electro Co., Woodbury, NY, USA). Each subject's VT was calculated after the completion of each test as the point when ventilation increases non-linearly with  $VO_2$  and was expressed as a percentage  $VO_{2max}$ .

**Monitoring.** All in-season practice and game monitoring was performed using the Polar Team<sup>2</sup> system (Polar Electro Co., Woodbury, NY, USA). The Team<sup>2</sup> system was used to monitor each player's individual workload, energy expenditure, and time spent at percentages of  $HR_{max}$  ( $\geq$ 95%, 94-85% and 84-75%  $HR_{max}$ ). Quantifying an individual player's workload is done by Kcal expenditure and by a training load (TL) algorithm

developed by Polar based on work rate and physical characteristics determined during lab testing. Changes in training volume and intensity were left up to the field hockey coaching staff. Daily, weekly and monthly changes in TL were recorded between sample collections (T1/2, T2/3 and T3/4).

**Sample Collection.** Over the course of the season, the athletes reported to the Rutgers Center for Health and Human Performance for blood draws. Athletes were told to arrive in a post-absorptive state following an overnight fast (~8 hours) and euhydrated approximately 36 hours after a game. Pre-season samples were drawn the day prior to the first pre-season practice day (T1); subsequent blood draws were conducted every 4 weeks from T1 until 36 hours after the last competitive match (T2, T3 and T4 respectively). Upon arrival, subjects were asked to provide a urine sample. Following this, a total of ~70mL of blood was drawn from the antecubital vein of each player. Samples were collected into unchilled vacutainer tubes (five 7.5mL serum separator tubes, one 10mL serum tube, two 6mL trace element EDTA tubes and two 4mL EDTA tubes) and a single chilled vacutainer tube (4mL EDTA tube). Processing of all samples was performed at the Rutgers Center for Health and Human performance immediately after all samples were taken and analysis of all blood samples was performed by Quest Diagnostics™ using their Blueprint for Athletes™ diagnostic panel. As part of the Blueprint for Athletes™ panel, recommended “normal ranges” were provided for each of the markers and specific time sensitive ranges were recommended for diurnal hormones like CORT.

**Statistics.** Data were analyzed using repeated measures ANOVA (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). The ES were considered small between 0 and 0.29, medium between 0.3 and 0.79 and large  $> 0.8$ . Step-wise multiple regressions were conducted to examine whether fitness ( $VO_{2max}$ , VT, and %BF) at the beginning of the season predicted any biomarker changes seen in the preseason period.

## Results

**Anthropometric and Performance Measures.** Outcomes of pre- and post-season performance testing for 19 subjects are described in Table 1. Subjects excluded in these results (N=4) were not cleared by sports medicine staff to participate in maximal performance testing at one of the two testing times. There was no significant difference in BW, %BF, VJ or  $VO_{2max}$  from pre to post-season. Significant changes ( $P < 0.05$ ) were detected in LBM and VT ( $\Delta LBM = 0.53 \pm 0.22$  kg;  $\Delta VT = 2.3 \pm 0.9$  %).

**Training Measurements.** The changes in TL over the season can be seen in Figure 1 and the associated ES-values are located in Table 2. There was a significant difference ( $P < 0.05$ ) between each training period: T1/2 to T2/3 ( $\Delta TL_{T1/2-T2/3} = -1217.1 \pm 105.13$  pts;  $P < 0.05$ , ES=-1.73), T1/2 to T3/4 ( $\Delta TL_{T1/2-T3/4} = -1549.41 \pm 108.16$  pts;  $P < 0.05$ , ES=-2.17) and from T2/3 to T3/4 ( $\Delta TL_{T2/3-T3/4} = -332.2 \pm 65.43$  pts;  $P < 0.05$ , ES=-0.40). T1/2 (the pre-season period) was characterized by the highest accumulated TL which proceeded to decrease as the season continued. Assessing TL alone shows a decrease in

training volume from T1 to T2 and decreased intensity from practices between T2 and T3 given that frequency and intensity of games remained the same.

A similar change in Kcal can be seen in Figure 2 and ES-values can be found in Table 2. There was a significant decline ( $P < 0.05$ ) between all training periods following the pre-season: T1/2 to T2/3 ( $\Delta Kcal_{T1/2-T2/3} = -9238.9 \pm 558.97$  kcal;  $P < 0.05$ ,  $ES = -2.71$ ), T1/2 to T3/4 ( $\Delta Kcal_{T1/2-T3/4} = -11651.7 \pm 603.64$  kcal;  $P < 0.05$ ,  $ES = -3.56$ ) and from T2/3 to T3/4 ( $\Delta Kcal_{T2/3-T3/4} = -2412.8 \pm 353.39$  kcal;  $P < 0.05$ ,  $ES = -0.80$ ). The highest Kcal expenditure occurred during the pre-season period as players improved condition and practiced skills during daily multi-sessions. The decline in Kcal, like TL, indicates a decrease in training volume assuming games remained consistent.

### **Stress and Recovery Markers**

**Cortisol.** Changes in CORTT are shown in Figure 3 and the ES-values are described in Table 2. There were no significant differences ( $P > 0.05$ ) between any time points in CORTT and only a small/medium effect size of 0.30 between T1 and T3; all other effect sizes were found to be  $< 0.30$ . This may be due to a wide range of interindividual variability on the team. Despite no significant change in CORTT throughout the season, the team average at T1 ( $26.10 \pm 10.03$  mcg/dL), T2 ( $28.01 \pm 11.67$  mcg/dL), T3 ( $29.09 \pm 12.10$  mcg/dL) and T4 ( $28.71 \pm 11.55$  mcg/dL) were all above the normal range of 4.6 – 20.6 mcg/dL provided by Quest Diagnostics™ Blueprint for Athletes™ (BFA). Having elevated CORTT at baseline may have resulted in a ceiling effect.

Figure 4 shows the changes in CORTF and the ES-values are described in Table 2.

There were significant changes ( $P < 0.05$ ) in CORTF from T1 to T2 ( $\Delta\text{CORTF}_{\text{T1-T2}} = 0.15 \pm 0.06$  mcg/dL;  $P < 0.05$ ,  $\text{ES} = 0.45$ ), T1 to T3 ( $\Delta\text{CORTF}_{\text{T1-T3}} = 0.26 \pm 0.08$  mcg/dL;  $P < 0.05$ ,  $\text{ES} = 0.74$ ), T1 to T4 ( $\Delta\text{CORTF}_{\text{T1-T4}} = 0.38 \pm 0.07$  mcg/dL;  $P < 0.05$ ,  $\text{ES} = 1.11$ ), T2 to T3 ( $\Delta\text{CORTF}_{\text{T2-T3}} = 0.10 \pm 0.05$  mcg/dL;  $P < 0.05$ ,  $\text{ES} = 0.33$ ), T2 to T4 ( $\Delta\text{CORTF}_{\text{T2-T4}} = 0.23 \pm 0.07$  mcg/dL;  $P < 0.05$ ,  $\text{ES} = 0.74$ ) and from T3 to T4 ( $\Delta\text{CORTF}_{\text{T3-T4}} = 0.13 \pm 0.05$  mcg/dL;  $P < 0.05$ ,  $\text{ES} = 0.40$ ). The continual increase in CORTF indicates an increasingly catabolic state. Unlike CORTT, the team average for CORTF did not exceed the clinical range of 0.07 – 0.93 mcg/dL until T4 ( $1.0 \pm 0.35$  mcg/dL).

**Sex Hormone Binding Globulin.** Figure 5 depicts the changes in SHBG over the season and ES-values can be found in Table 2. There were no significant changes ( $P > 0.05$ ) in SHBG between any time points and all effect sizes were  $< 0.15$  indicating a small effect. Large standard deviations for each sample average (T1= $95.87 \pm 64.22$  nmol/L; T2= $99.35 \pm 62.38$  nmol/L; T3= $90.48 \pm 63.21$ ; T4= $91.52 \pm 70.02$ ) indicate large interindividual variability. Looking at Figure 5a there are four players consistently above the team averages and the BFA range of 17 – 124 nmol/L.

**Creatine Kinase.** The graph for values of CK can be found in Figure 6 and the associated ES-values are located in Table 2. CK displayed a significant increase ( $P < 0.05$ ) between T1 to T2 ( $\Delta\text{CK}_{\text{T1-T2}} = 54.78 \pm 8.69$  U/L;  $P < 0.05$ ,  $\text{ES} = 1.59$ ); T1 also showed a significant difference from T3 ( $\Delta\text{CK}_{\text{T1-T3}} = 39.39 \pm 6.55$  U/L;  $P < 0.05$ ,  $\text{ES} = 1.14$ ). CK fell between T2 and T3 but did not reach significance ( $P > 0.05$ ). However, it did show a small/moderate effect (-0.30). CK reached a significant difference ( $P < 0.05$ ) again

between T2 and T4 ( $\Delta\text{CK}_{\text{T2-T4}} = -33.17 \pm 12.38$  U/L;  $P < 0.05$ ,  $\text{ES} = -0.66$ ). This fluctuation in CK shows an escalation in the pre-season period (T1 to T2) that follows training load and kcal in a downward trend thereafter. Team average CK only rose above the BFA range of  $< 143$  U/L at T2 ( $144 \pm 50.51$  U/L) further indicating a state of physical catabolism.

**Lactate Dehydrogenase.** Figure 7 displays the changes that occurred in LD over the course of the season and Table 2 denotes the ES-values. There was a significant rise in LD from T1 to T2 ( $\Delta\text{LD}_{\text{T1-T2}} = 93.31 \pm 9.34$  U/L;  $P < 0.05$ ,  $\text{ES} = 2.74$ ) and remained significantly elevated from T1 at T3 and T4 ( $\Delta\text{LD}_{\text{T1-T3}} = 90.95 \pm 10.51$  U/L;  $P < 0.05$ ,  $\text{ES} = 2.67$ ;  $\Delta\text{LD}_{\text{T1-T4}} = 69.46 \pm 10.17$  U/L;  $P < 0.05$ ,  $\text{ES} = 2.05$ ). Between T2 and T3 there is a non-significant ( $P > 0.05$ ) change in LD. However, as LD continued to decrease through T4 a significant ( $P < 0.05$ ) change was found from T2 to T4 and T3 to T4 ( $\Delta\text{LD}_{\text{T2-T4}} = -23.86 \pm 4.1$  U/L;  $P < 0.05$ ,  $\text{ES} = -0.99$ ;  $\Delta\text{LD}_{\text{T3-T4}} = 21.50 \pm 3.33$  U/L;  $P < 0.05$ ,  $\text{ES} = -0.81$ ). Similar to CK, LD demonstrated a large initial increase between T1 and T2 before a slow trend downward through T4 but never returning to baseline. Average LD comes close to, but does not cross, the upper clinical threshold of 200 U/L at T2 ( $183.14 \pm 23.95$  U/L) and T3 ( $180.77 \pm 26.09$  U/L), potentially due to the shorter half-life of LD and the interval from the previous game.

**Interleukin-6.** Table 2 indicates the ES-values and Figure 8 demonstrates the changes that occur in IL-6 over the season. IL-6 showed a significant difference ( $P < 0.05$ ) between T1 and T4 ( $\Delta\text{IL-6}_{\text{T1-T4}} = 0.47 \pm 0.15$  pg/mL;  $P < 0.05$ ,  $\text{ES} = 1.05$ ). While there was no significant difference ( $P > 0.05$ ) between the other averages the effect size from T1 to T2 and T1 to T3 were large ( $\text{ES}_{\text{T1-T2}} = 1.35$ ;  $\text{ES}_{\text{T1-T3}} = 0.81$ ). The team averages remained within

clinical norms of 0.31 – 5.00 pg/mL for the entire season. Graphically, the change in IL-6 from T1 to T2 was found to have a large effect ( $>1.0$ ) indicating a meaningful increase in inflammation associated with the pre-season before falling from T2 to T3 and ultimately increasing again between T3 and T4.

**Prolactin.** Figure 9 illustrates these changes and Table 2 denotes the ES-values for PRL. There was no significant change in PRL between any time point; but there was a trend toward significance ( $P<0.1$ ) between T2 and T3 ( $\Delta\text{PRL}_{\text{T2-T3}}=1.575 \pm 1.58$  U/L;  $P<0.1$ ,  $\text{ES}=0.28$ ). This increase from T2 to T3 was maintained. The average team PRL did not exceed BFA ranges for females 3.2 – 20.0 ng/ml. The increase in PRL occurred as other markers of breakdown begin to decrease, perhaps suggesting a delayed or more chronic effect.

### Nutritional Markers

**Omega 6:3 Ratio.** Figure 10 illustrates changes that occurred with OMG63; associated ES-values can be found in Table 2. OMG63 increased significantly ( $P<0.05$ ) during the pre-season period and remained different from baseline through T4 ( $\Delta\text{OMG63}_{\text{T1-T2}}=7.89 \pm 0.38$  %;  $P<0.05$ ,  $\text{ES}=5.44$ ;  $\Delta\text{OMG63}_{\text{T1-T3}}=8.27 \pm 0.55$  %;  $P<0.05$ ,  $\text{ES}=5.51$ ;  $\Delta\text{OMG63}_{\text{T1-T4}}=7.81 \pm 0.36$  %;  $P<0.05$ ,  $\text{ES}=5.15$ ). There was no further significant finding between T2, T3 or T4 and effect sizes were found to be small ( $<0.2$ ). OMG63 was found to be slightly elevated above BFA ranges (1.3 – 12.0) at T2 ( $12.68 \pm 2.36$ ), T3 ( $12.78 \pm 2.81$ ) and T4 ( $12.25 \pm 2.02$ ).

**Vitamin D.** Changes in VitD are outlined in Figure 11 and ES-values are located in Table 2. Significant changes ( $P<0.05$ ) were found from T1 to T4 ( $\Delta\text{VitD}_{\text{T1-T4}}=-10.73 \pm 2.04$

ng/mL;  $P < 0.05$ ,  $ES = -0.54$ ), from T2 through T4 ( $\Delta \text{VitD}_{T2-T3} = -6.36 \pm 1.35$  ng/mL;  $P < 0.05$ ,  $ES = -0.39$ ;  $\Delta \text{VitD}_{T2-T4} = -11.86 \pm 1.42$  ng/mL;  $P < 0.05$ ,  $ES = -0.73$ ) and between T3 and T4 ( $\Delta \text{VitD}_{T3-T4} = -5.50 \pm 1.04$  ng/mL;  $P < 0.05$ ,  $ES = -0.42$ ). The pre-season period (T1 – T2) showed a marginal increase in VitD before the team average significantly fell through the remainder of the season. Average values did not deviate the BFA range (30 – 100 ng/mL) indicating no concern of deficiency.

**Iron.** Differences in Fe are depicted in Figure 12 and the corresponding ES-values are identified in Table 2. The difference between T1 and all other assessments was found to be significant ( $P < 0.05$ ). Fe decreased from T1 to T2 ( $\Delta \text{Fe}_{T1-T2} = -50.04 \pm 8.01$  mcg/dL;  $P < 0.05$ ,  $ES = -1.30$ ) remaining significantly different at T3 and T4 compared to T1 ( $\Delta \text{Fe}_{T1-T3} = -54.44 \pm 10.05$  mcg/dL;  $P < 0.05$ ,  $ES = -1.42$ ;  $\Delta \text{Fe}_{T1-T4} = -54.39 \pm 11.13$  mcg/dL;  $P < 0.05$ ,  $ES = -1.42$ ). There was no significant difference ( $P > 0.05$ ) in Fe between any other time points ( $ES < 0.15$ ). Team averages remained within normal ranges (27 – 167 mcg/dL).

### Hematologic Markers

**Ferritin.** Table 2 shows the ES-values for FER and Figure 13 illustrates the differences that occurred during the observation period. T1 was found to be significantly different ( $P < 0.05$ ) from all other time points ( $\Delta \text{FER}_{T1-T2} = -11.22 \pm 2.01$  ng/mL;  $P < 0.05$ ,  $ES = -0.60$ ;  $\Delta \text{FER}_{T1-T3} = -6.74 \pm 1.87$  ng/mL;  $P < 0.05$ ,  $ES = -0.36$ ;  $\Delta \text{FER}_{T1-T4} = -5.70 \pm 1.76$  ng/mL;  $P < 0.05$ ,  $ES = -0.31$ ). There is also a significant difference between T2 and both T3 and T4 ( $\Delta \text{FER}_{T2-T3} = 4.48 \pm 1.73$  ng/mL;  $P < 0.05$ ,  $ES = 0.41$ ;  $\Delta \text{FER}_{T2-T4} = 5.52 \pm 1.37$  ng/mL;  $P < 0.05$ ,  $ES = 0.50$ ). FER follows a similar pattern as it almost decreased by half from T1 to T2

during the pre-season period. FER rebounded from T2 through T4 but never reaches baseline values. There was no deviation from the established BFA norms (6 – 67 ng/mL) for FER over the season.

**Total Iron Binding Capacity.** Changes in TIBC can be found in Figure 14 and the ES-values can be found in Table 2. TIBC decreased from T1 through T4 with a trend toward significance between T1 and T3 ( $\Delta\text{TIBC}_{\text{T1-T3}} = -22.39 \pm 10.99$  mcg/dL;  $P < 0.1$ ,  $\text{ES} = -0.50$ ) and a significant difference from baseline by T4 ( $\Delta\text{TIBC}_{\text{T1-T4}} = -22.65 \pm 7.97$  mcg/dL;  $P < 0.05$ ,  $\text{ES} = -0.50$ ). A significant change also occurred from T2 to T4 ( $\Delta\text{TIBC}_{\text{T2-T4}} = -16.57 \pm 7.49$  mcg/dL;  $P < 0.05$ ,  $\text{ES} = -0.31$ ). Initially, TIBC was close to the upper BFA range (271 – 448 mcg/dL) but fell into the midrange by T3 and T4. These findings deviate from the reported inverse relationship between TIBC and Fe/FER.

**Percent Saturation.** Changes in %Sat have been outlined in Figure 15 and the ES-values between time points can be found in Table 2. %Sat demonstrated a significant difference ( $P < 0.05$ ) between T1 and all other time points ( $\Delta\% \text{Sat}_{\text{T1-T2}} = -11.76 \pm 1.89$  %;  $P < 0.05$ ,  $\text{ES} = -1.04$ ;  $\Delta\% \text{Sat}_{\text{T1-T3}} = -12.04 \pm 2.57$  %;  $P < 0.05$ ,  $\text{ES} = -1.07$ ;  $\Delta\% \text{Sat}_{\text{T1-T4}} = -11.20 \pm 3.05$  %;  $P < 0.05$ ,  $\text{ES} = -0.99$ ). No differences were found between T2, T3 and T4 and all effect sizes were found to be  $< 0.1$ . The team average for %Sat was never outside the BFA range of 8 – 45%. The significant change in %Sat follows a similar degree of change to Fe and FER during the pre-season period (T1-T2), illustrating the relationship between these markers.

**Hemoglobin.** Hb changes throughout the season are depicted in Figure 16 and associated ES-values can be found in Table 2. There was a significant decrease ( $P < 0.05$ )

from T1 to T2 ( $\Delta\text{Hb}_{\text{T1-T2}} = -0.578 \pm 0.17 \text{ g/dL}$ ;  $P < 0.05$ ,  $\text{ES} = -0.64$ ) that persisted through T3 and T4 ( $\Delta\text{Hb}_{\text{T1-T3}} = -0.552 \pm 0.17 \text{ g/dL}$ ;  $P < 0.05$ ,  $\text{ES} = -0.61$ ;  $\Delta\text{Hb}_{\text{T1-T4}} = -0.530 \pm 0.17 \text{ g/dL}$ ;  $P < 0.05$ ,  $\text{ES} = -0.58$ ). Much like the other hematologic markers, following the Hb decrease at T2 there was no significant ( $P > 0.05$ ) change between the other measurement times and the effect sizes were determined to be  $< 0.1$ . Hb did not deviate from clinical norms (11.5 – 15.3 g/dL) despite the initial decrease in Hb.

**Hematocrit.** The fluctuation of HCT over the course of the season is shown in Figure 17 and associated ES-values are defined in Table 2. There was a significant decrease ( $P < 0.05$ ) in HCT from T1 through T3 ( $\Delta\text{HCT}_{\text{T1-T2}} = -1.25 \pm 0.59 \%$ ;  $P < 0.05$ ,  $\text{ES} = -0.43$ ;  $\Delta\text{HCT}_{\text{T1-T3}} = -1.69 \pm 0.17 \%$ ;  $P < 0.05$ ,  $\text{ES} = -0.58$ ) before trending ( $P < 0.1$ ) slightly upward between T3 and T4 ( $\Delta\text{HCT}_{\text{T3-T4}} = 0.783 \pm 0.12 \%$ ;  $P < 0.1$ ,  $\text{ES} = 0.28$ ). Average team HCT did not lie outside established norms (34.0 – 46.0%) at any time.

**Mean Corpuscular Hemoglobin Concentration.** Changes in the MCHC marker are demonstrated in Figure 18 and their associated ES-values can be found in Table 2. There were significant differences ( $P < 0.05$ ) between T1 and T2 ( $\Delta\text{MCHC}_{\text{T1-T2}} = -0.44 \pm 0.1 \text{ g/dL}$ ;  $P < 0.05$ ,  $\text{ES} = -0.84$ ), T1 and T4 ( $\Delta\text{MCHC}_{\text{T1-T4}} = -0.54 \pm 0.11 \text{ g/dL}$ ;  $P < 0.05$ ,  $\text{ES} = -1.03$ ) and between T3 and T4 ( $\Delta\text{MCHC}_{\text{T3-T4}} = -0.40 \pm 0.19 \text{ g/dL}$ ;  $P < 0.05$ ,  $\text{ES} = -0.47$ ). T3 did not differ significantly from either T1 or T2, however there was a large effect was found between T2 and T3 ( $\text{ES}_{\text{T2-T3}} = 0.73$ ). The team averages did not deviate from clinical norms (31.0 – 36.0 g/dL).

### **Metabolic Markers**

**Thyroid Stimulating Hormone.** Figure 19 illustrates the fluctuations in TSH that were measured over the competitive season and Table 2 displays the ES-values. A significant increase in TSH ( $P < 0.05$ ) occurred from T1 to T3 ( $\Delta\text{TSH}_{\text{T1-T3}} = 1.029 \pm 0.23$  mIU/L;  $P < 0.05$ ,  $\text{ES} = 1.20$ ) and between T2 and T3 ( $\Delta\text{TSH}_{\text{T2-T3}} = 0.858 \pm 0.15$  mIU/L;  $P < 0.05$ ,  $\text{ES} = 1.00$ ) before a significant decrease within the T3 – T4 interval ( $\Delta\text{TSH}_{\text{T3-T4}} = -0.823 \pm 0.18$  mIU/L;  $P < 0.05$ ,  $\text{ES} = -0.65$ ). TSH did not exceed the BFA range of 0.50 – 4.30 mIU/L, with the team average peaking at T3 ( $2.9 \pm 1.26$  mIU/L). This rise in TSH is in contrast to a decrease in  $T_3$  over the same interval and  $T_4$  reaching its lowest value.

**Thyroxine.** Changes in thyroxine can be found in Figure 20 and the ES-values are shown in Table 2. T1 was significantly different ( $P < 0.05$ ) compared to all other time points ( $\Delta\text{thyroxine}_{\text{T1-T2}} = -0.148 \pm 0.02$  ng/dL;  $P < 0.05$ ,  $\text{ES} = -1.08$ ;  $\Delta\text{thyroxine}_{\text{T1-T3}} = -0.191 \pm 0.02$  ng/dL;  $P < 0.05$ ,  $\text{ES} = -1.40$ ;  $\Delta\text{thyroxine}_{\text{T1-T4}} = -0.130 \pm 0.02$  ng/dL;  $P < 0.05$ ,  $\text{ES} = -0.96$ ) for thyroxine. There was a trend toward significance ( $P < 0.1$ ) from T2 to T3 ( $\Delta\text{thyroxine}_{\text{T2-T3}} = -0.043 \pm 0.2$  ng/dL;  $P < 0.1$ ,  $\text{ES} = -0.53$ ) as thyroxine fell further from baseline values. Thyroxine significantly increased ( $P < 0.05$ ) between T3 and T4 ( $\Delta\text{thyroxine}_{\text{T3-T4}} = 0.061 \pm 0.02$  ng/dL;  $P < 0.05$ ,  $\text{ES} = 0.46$ ), likely in response to the aforementioned spike in TSH between T2-T3. There was no deviation from the normal 0.9 – 1.4 ng/dL range for thyroxine as the highest value occurred at T1 ( $1.30 \pm 0.14$  ng/dL) and lowest at T3 ( $1.10 \pm 0.13$  ng/dL).

**Triiodothyronine.** An illustration of triiodothyronine is found in Figure 21 and the ES-values can be seen in Table 2. There was a significant rise ( $P < 0.05$ ) in triiodothyronine between T1 and T2 ( $\Delta\text{triiodothyronine}_{\text{T1-T2}} = 16.65 \pm 5.86$  ng/dL,  $\text{ES} = 0.77$ ) followed by a

significant decline below baseline values from T2 to T3 ( $\Delta$ triiodothyronine<sub>T2-T3</sub> =  $-19.65 \pm 4.11$  ng/dL, ES = -0.53) that remained significantly different through T4 ( $\Delta$ triiodothyronine<sub>T2-T4</sub> =  $-20.65 \pm 4.99$  ng/dL, ES = -0.56). There was no significant difference ( $P > 0.05$ ) between any other time points (ES < 0.2) and triiodothyronine did not deviate from clinical norms (76 – 181 ng/dl). The abrupt rise and fall of triiodothyronine may be responding to the increased physical demand that is seen during pre-season training.

***Predicting Pre-Season Biomarker Changes.*** Step-wise multiple regressions were conducted to examine whether fitness ( $VO_{2max}$ , VT, and %BF) at the beginning of the season predicted biomarker changes seen in the preseason period. Results indicated that  $VO_{2max}$  accounted for 16.3% of the variance in SHBG, trending toward significance ( $\beta = -0.40$ ,  $P < 0.1$ ).  $VO_{2max}$  also accounted for 31% ( $\beta_{CORTT} = 0.56$ ,  $P < 0.05$ ) and 32.7% ( $\beta_{CORTF} = 0.57$ ,  $P < 0.05$ ) of the variance in CORTT and CORTF, respectively. In addition, %BF accounted for another 20.1% ( $\beta = -0.71$ ,  $P < 0.05$ ) of the variance in CORTT. %BF also accounted for 48.9% ( $\beta = -1.11$ ,  $P < 0.05$ ) of the difference seen in the significant change ( $P < 0.05$ ) in  $T_3$  from T1 to T2 ( $\Delta T_3 = 18.9 \pm 5.9$  mg/dL;  $P < 0.05$ ). Changes in Kcal during the preseason were positively correlated with  $VO_{2max}$  ( $r = 0.76$ ,  $P < 0.05$ ) and negatively correlated with %BF ( $r = -0.48$ ,  $P < 0.05$ ).

## Discussion

The results of this study show significant changes in markers associated with increased catabolism, altered metabolic rate, hematologic factors, and nutrition indicating a large systemic response to the stress of a collegiate field hockey season.

This result supports previous research by Kraemer *et al.* (2004), Ispirlidis *et al.* (2008) and Heisterberg *et al.* (2013) who demonstrated chronic changes in biomarkers in male athletes. Similar to our results, Kraemer *et al.* (2004) did not identify significant change in CORT; however, results from the current study did find a significant increase in CORTF that was not reported by Kraemer *et al.* (2004). Research by Ispirlidis *et al.* (2008) reported elevated markers of tissue damage (CK and LD) for several days following a men's soccer game, which were comparable to those described in this study. The reported decrease in HCT and MCHC is more pronounced but coincides with the changes in HCT and MCHC shown by Heisterberg *et al.* (2013). When utilizing biomarkers, special consideration should be given to the time and frequency of sampling to detect meaningful changes (Silva *et al.*, 2013). While using HR monitors has been shown effective in evaluating the acute responses to training, measuring chronic changes in blood-based biomarkers allows for a better evaluation of season long response to training. The results of the current study can be used to identify the differences in response to chronic training between the sexes and illustrate the prolonged impact of an intense pre-season.

The outcome of the current study did not find meaningful changes in %BF,  $VO_{2max}$  or VJ from pre- and post-season. Field hockey athletes were tested prior to the first pre-season practice and within a week of the last competition. The mean change in  $VO_{2max}$  from P1 to P2 ( $\Delta VO_{2max} = -0.20 \pm 0.74 \text{ mL/kg/min}$ ;  $P > 0.05$ ,  $ES = -0.04$ ) demonstrates aerobic fitness was maintained throughout the season. This is in contrast to previous research which showed collegiate female soccer and field hockey players increased

$VO_{2max}$  over a competitive season (Clark *et al.*, 2003; Astorino *et al.*, 2004). Similarly, non-significant changes in BW ( $\Delta BW = -0.09 \pm 0.47\text{kg}$ ), %BF ( $\Delta \%BF = -0.6 \pm 0.47\%$ ) and VJ ( $\Delta VJ = -0.33 \pm 0.25\text{cm}$ ) contrast the results of Clark *et al.* (2003) and Astorino *et al.* (2004). The current study found significant increases in LBM and VT, which may be explained by the implementation of a strength training program around mid-season and increased time spent training at or above VT throughout the season. The absence of positive change in markers of performance is in contrast with previous research in female athletes (Clark *et al.*, 2003; Astorino *et al.*, 2004). The lack of performance change may be due, in part, to a reduction in training load by the coaches as the season progressed; similar patterns have been shown to occur in a men's soccer season (Filaire *et al.*, 2003). This allowed the athletes an opportunity to recover from the stress of intense pre-season and illustrated in the changes in biomarkers. Another possibility is that they came into preseason camp in top form. As such, any changes in an upward direction would be less likely. Given that these were Division I players in a Big 10 program, this is possible.

A collegiate field hockey pre-season is characterized by a two-week period typically consisting of several days of double practices. As a result, the highest TL and Kcal were found in the pre-season (TL=3591.77±725.16 points; Kcal=24973.32±35.16.42 kcal). This investigation showed a significant reduction in TL and Kcal as the season progressed. However, this contradicts research by Scott *et al.* (2013) who described some fluctuations in TL over a men's soccer season but no significant decrease. Fluctuations in total training volume and calorie expenditure allow for more recovery

going into critical periods of the season (i.e. playoffs and tournaments). While the trend in the current study suggest an emphasis on recovery as markers associated with training stress (CORTT, CK and LD) began decline to baseline, research has shown undulating training volume to be an effective means of managing athletes (Scott *et al.*, 2013). This study shows the reduction in training volume came too late and/or was insufficient to correct the stress applied during the pre-season.

The current study found CORT to be an effective marker of training stress and identifier of a prolonged catabolic state. CORTT was not found to have significantly changed from pre-season to the end of the season; however, the team entered pre-season with CORTT above normal BFA ranges. It is worth noting that CORTT did increase slightly from T1 to T2 and remained elevated above baseline through T4 but the effect size for this increase was relatively small ( $ES_{T1-T4}=0.26$ ). Research by Budgett *et al.* (1990) and Balsalobre-Fernandez *et al.* (2014) identified elevated resting CORTT as an indicator of increased risk of OTS in males. If this is true in females, the high CORTT at pre-season would indicate the athletes came into pre-season in a state of heightened stress from which they never recovered. Changes in CORTF were found to be significant ( $P<0.05$ ) at every point of the season despite non-significance of CORTT. Changes in CORTF independent of CORTT have been implicated in the dysregulation of the hypothalamic-pituitary-adrenal axis (HPA) in females more than males (Pruessner *et al.*, 1997). This previous research showed that females tend to have a greater CORTF response to physical and psychological stress. These results help account for the rise in CORTF given a non-significant rise in CORTT. An increase in CORT production beyond the saturation

rate of its carrier protein (cortisol binding globulin) has also been associated with elevations in CORTF (Gozansky *et al.*, 2005). This previous study found that salivary CORTF has a more robust response to exercise than traditional CORTT measures. Results from these studies help explain the rise in CORTF despite maintenance of CORTT (Pruessner *et al.*, 1997; Gozansky *et al.*, 2005). The steady and significant rise ( $P < 0.05$ ) in CORTF shown in the current study may be the result of these mechanisms working in concert. A saturation of CORT binding protein with the prolonged stress and inflammatory response of the season indicate a continual increase in CORT production that was detected as a significant ( $P < 0.05$ ) change in CORTF rather than CORTT.

Assessing the balance between catabolism and anabolism is critical to evaluating the physical state of female athletes. The current study indirectly measured anabolism using SHBG to assess androgen levels. SHBG has been shown to be a viable alternative to directly measuring the androgens (Bermon *et al.*, 2014). There was no significant change ( $P > 0.05$ ) in SHBG detected for any time points in the current study. However, there was a large variation between subjects resulting in several athletes consistently inflating the team average. Elevated SHBG has been associated with high levels of androgens, oral contraceptive use, and amenorrhea in female athletes (Bermon *et al.*, 2014). In a study of several hundred elite female athletes, Bermon *et al.* (2014) identified females not taking oral contraceptives and identifying as amenorrheic had higher SHBG levels than their peers. Amenorrhea has been implicated with decreased bone density and disordered eating as part of the female athlete triad (Otis *et al.*, 1997). Although use of oral contraceptive and menstrual regularity were not used in the

current study due to regulations by the athletic department, these results show the potential for biomarkers associated with each of these conditions to identifying risk factors for development of the female athlete triad.

Markers identifying muscle damage in the current study showed a significant increase ( $P < 0.05$ ) during pre-season training before returning back towards baseline by the end of the season. Similarly, there was a significant rise ( $P < 0.05$ ) in LD during pre-season that remained significantly different from baseline through T4. Research has shown CK and LD to be valuable markers in assessing training status and in identifying breakdown in both male and female athletes (Kanda *et al.*, 2014; Baird *et al.*, 2012; Kraemer *et al.*, 2004; Urhausen *et al.*, 2002). The rise in CK and LD demonstrated in the current study coincides with high pre-season TL and Kcal. These markers suggest that the increase in repeated physical stress that occurs during this initial training period results in chronic stress if insufficient recovery is not permitted. After this initial period, CK began to fall but remained significantly different ( $P < 0.05$ ) from baseline until T4. LD however remained significantly elevated compared to baseline throughout the season. The difference in recovery rate between CK and LD contradicts the findings from a study by Ispirlidis *et al.* (2008) who found LD returned to baseline 24 hours prior to CK. The difference in recovery or clearance time in the current study may be due to increased inflammation, nutritional status or other factors not assessed in the current study. Kraemer *et al.* (2004) showed that pushing male soccer players hard in the pre-season resulted in more harm than good as the season progressed and player performance declined. The current study shows a similar result, in that a significant increase ( $P < 0.05$ )

in CK and LD in the pre-season was followed by a mid-season decrease in performance. These results show that managing an athlete's current fitness, rather than pushing them to become more fit, in the pre-season may be the more effective strategy for long-term success.

This investigation found statistically significant changes ( $P < 0.05$ ) in inflammatory response over the competitive field hockey season. The large ( $ES = 1.35$ ) non-significant rise in IL-6 between T1 and T2 mostly the result of a single player suffering an injury prior to the T2 assessment. With this outlier removed, IL-6 is shown to moderately ( $ES = -0.40$ ) decrease between T1 and T2 before climbing and becoming significantly higher ( $P < 0.05$ ) than baseline at T4. Research has shown measuring IL-6 to be very time sensitive as inflections after exercise typically return to baseline within a few hours (Nieman *et al.*, 2000; Ispirlidis *et al.*, 2008). Therefore, an elevation in IL-6 beyond several hours has been associated with a prolonged immune response to chronic intense training (Kreher *et al.*, 2012). IL-6 and other pro-inflammatory cytokines are robust stimulators of the HPA axis and chronically high levels have been shown to enhance CORT secretion and the stress response (Kreher *et al.*, 2012; Smith, 2000). The current study shows a steady, and ultimately significant ( $P < 0.05$ ), increase in IL-6 as the season progressed but CORTT remains relatively stable. However, the stimulatory effect of IL-6 on the HPA may be seen more in the steady and significant rise in CORTF throughout the season.

Another marker that has been implicated in the response to the stress of chronic training is PRL. While this investigation did not find a significant change ( $P > 0.05$ ), there

was a trend toward significance at mid-season (T2-T3). Research by Budgett *et al.* (2010) assessed PRL as a marker of overtraining in Olympic athletes meeting the criteria for diagnosed unexplained underperformance syndrome (UUPS) and found that it rose for 150 minutes after maximal exercise. Elevated PRL several days after training has been shown to correspond with decreased performance in elite athletes (Meseusen *et al.*, 2010). This previous study found elevated PRL at rest in athletes classified as having OTS by a sports physician (underperforming for >1 year). This does not explain the trend toward significance ( $P < 0.1$ ) in the current study as the field hockey players maintained fitness over the season. Instead, the increase in PRL may be linked to the inflammatory response detected as a significant change in IL-6. Research by Gomez-Merino *et al.* (2006) showed triathletes had a substantial increase in PRL correlated to a rise in cytokines following prolonged periods of intense training. PRL response to changing cytokines is a more likely explanation for the increasing trend in the current study than players entering a state of OTS.

Nutrition is a critical component to an athlete's ability to perform, recover, and maintain optimal health. The current study evaluated VitD, OMG63 and Fe as nutritional markers because of their association with skeletal-muscular health, inflammation and performance. The current study found a significant rise ( $P < 0.05$ ) in OMG63 above clinical reference ranges occurred during the pre-season and persisted through the end of the season. However, it is unclear if the change in the OMG63 ratio is due to increase in OMG6 or OMG3 intake or a combination of the two. Bounocore *et al.* (2015) found that an elevated OMG63 corresponded to increased inflammation and decreased

performance. While no significant changes ( $P>0.05$ ) in performance markers were detected in the current study, chronically high OMG63 may have impacted the recovery of the other markers of stress and inflammation. The significant increase in OMG63 also coincided with the player returning to campus. Although dietary intake was not assessed, the changes in OMG63 and decrease in free-time suggest athletes were more inclined to eat less healthy, “grab-and-go” type foods. Helping athletes select healthier foods or meal prep for the week can help decrease OMG63 potentially improving athletic performance and recovery by reducing a source of chronic inflammation and stress (Jazayeri *et al.*, 2010). This research has shown that increasing OMG3 intake can reduce CORT, inflammation and symptoms of depression all which have been shown to negatively impact athlete performance (Budgett *et al.*, 2010). Using the OMG63 ratio as an initial tool to better understanding what an athlete’s nutritional status can then be used by the athlete to make better dietary selections.

Utilizing nutritional markers is of particular importance with collegiate female athletes who often do not receive proper nutritional information and not match calorie intake (Clark *et al.*, 2003). In the current study, VitD at the end of the season (T4) was found to be significantly different ( $P<0.05$ ) from all other time points. Other significant changes occurring between the end of pre-season (T2) and midseason (T3). The decrease in VitD during this time may be due to the change in seasons and athletes spending more time indoors for classes. Larson-Meyer *et al.* (2010) reported similar changes in VitD status noting that it varied based on the time of year an athlete competed due to sun exposure. If this is the case, the current findings suggest that

athletes are not achieving optimal VitD intake to compensate for the decreased time outdoors; however, dietary intake was not addressed in the current study. Although the current levels of VitD did not deviate from the BFA range, decreased VitD has been implicated in changes in calcium absorption and bone health (Larson-Meyer *et al.*, 2010). This previous research describes the rate of calcium absorption from the gut as 10-15% in VitD deficient individuals compared to >30% in those athletes with normal VitD. Maintain VitD for bone health is of particular importance in female athletes. The female athlete triad is characterized by a decrease in bone mineral density resulting in osteoporosis (Otis *et al.*, 1997). VitD plays a role in the development of this condition as it regulates calcium reabsorption from bone. The changes in VitD in the current study did not indicate health concerns but illustrate the potential for VitD deficiency if athletes enter the season with low VitD.

Significant changes in micronutrients are known to have direct impact on athlete performance and are particular concern in female athletes. In the current study, Fe status significantly decreased ( $P < 0.05$ ) during the pre-season and remained below baseline throughout the remainder of the season. Similar decreases in Fe have typically been shown to result in decreased aerobic capacity (Hinrichs *et al.*, 2010; Heisterberg *et al.*, 2013). This was not found to be the case in the current study as no change in  $VO_{2max}$  was found between pre- and post-season. The maintenance of performance markers may be due to the change in Fe not decreasing below clinical norms. It is unclear if the significant change in Fe seen in the current study is due to a deficiency in the diet because dietary intake was not assessed. However, any significant change in a

micronutrient should be addressed. Research by Hinton *et al.* (2000) has shown supplementing Fe to significantly increase ( $P<0.05$ ) Fe concentration and improve aerobic capacity in non-Fe deficient women. The findings by Hinton *et al.* (2000) illustrate the importance of Fe and how small changes in Fe status can result in significant changes without clinical deficiency. Given these results, it is unclear performance measures would have been shown to improve if Fe status was maintained closer to baseline values.

The importance of Fe goes beyond its nutritional intake as it is a regulator of hematologic status as well. The significant decrease ( $P<0.05$ ) in Fe from T1 to T2 was followed by similar decreases in FER and %Sat that remained depressed throughout the remainder of the season as well. These changes are consistent with findings of Di Santolo *et al.* (2008) and Hinrichs *et al.* (2010) who showed female athletes display a decrease in Fe and FER at the onset of training and typically have a lower %Sat than male counterparts. Like Fe, these markers also typically associated with a decrease in performance not witnessed in the current study. This may be due to each of the markers not deviating from their clinical range and remaining normal enough to maintain aerobic capacity.

An interesting finding of the current study is an uncharacteristic decrease in TIBC given the changes in Fe, FER and %Sat. TIBC decreased from baseline throughout the study that reached significance ( $P<0.05$ ) by the end of the season (T1-T4). This finding contradicts previous research that has found TIBC to increase due to significant decreases ( $P<0.05$ ) in Fe, FER and %Sat (Di Santolo *et al.*, 2008; Hinrichs *et al.*, 2010).

These results suggest that the decrease TIBC is resulting from a different stimulus. TIBC has been shown to decrease in response to exercise related inflammation in elite runners (Fallon, 2001). Fallon found TIBC fell over each day of a 6-day running event in relation to increases in inflammatory markers. The significant decrease ( $P < 0.05$ ) observed in TIBC in the current study may be resulting from the chronic elevation in markers associated with stress and inflammation (CK, IL-6, OMG63).

The current research also monitored several markers associated with transporting oxygen through the body. Plasma volume shifts associated with training defined as “sports anemia” are characterized by change in Hb, HCT, and MCHC which typically results in decreased performance (Heisterberg *et al.*, 2013). The pre-season changes of this study show a significant decrease ( $P < 0.05$ ) in Hb, HCT and MCHC seemingly characteristic of sports anemia; however, these changes did not result in significant ( $P > 0.05$ ) performance changes. The difference may result from the significant decrease in Fe found in the current study. As discussed earlier, female athletes are particularly sensitive to decreases in Fe status that would impact Hb and other hematologic markers negatively (Hinrichs *et al.*, 2010). Brocherie *et al.* (2015) found that Hb and MCHC status were correlated with performance on the Yo-Yo intermittent recovery test showing the importance of these markers in maximal aerobic performance. Despite significant changes in hematologic status in the current study, the maintenance of aerobic capacity contradicts the results of Brocherie *et al.* (2015).

Measuring changes in thyroid hormones has been suggested as a way of assessing metabolic function in response to exercise (Henning *et al.*, 2013). Significant

changes ( $P < 0.05$ ) were detected in all three hormones in the present study. After the initial pre-season training period, the field hockey athletes exhibited a significant increase ( $P < 0.05$ ) in triiodothyronine ( $T_3$ ) and a significant decrease ( $P < 0.05$ ) in thyroxine ( $T_4$ ). By the mid-season values of  $T_3$  had returned to baseline and were maintained throughout the remainder of the season.  $T_4$  continued to decrease, trending toward significance ( $P < 0.1$ ) until the third assessment before rising significantly ( $P < 0.05$ ) by the end of the season. The change in  $T_4$  and  $T_3$  after the pre-season period suggest an increase conversion of  $T_4$  to active  $T_3$  to meet increase metabolic demand with further decreases in  $T_4$  needed to maintain baseline values. These results contrast the findings of Henning *et al.* (2013) who did not show a significant change ( $P > 0.05$ ) in  $T_4$  or  $T_3$  in males after 8-weeks of military boot camp. The differences in findings between the previous study and the current study may due to the frequency of draws, dietary intake and gender differences. Henning *et al.* (2013) only examined pre- and post-boot camp values 8-weeks apart which may have missed the 4 week rise and fall of  $T_3$  found in the current study. Female athletes have been shown to not match calorie intake during periods of strenuous training (Clark *et al.*, 2003). The male soldiers in the Henning *et al.* (2013) study were shown to be working in a chronic caloric deficit throughout the 8-week study. Calorie deficiency has been identified as a negative feedback mechanism on thyroid hormone secretion explaining why only a brief increase in  $T_3$  was detected in the current study and no change was found in the study by Henning *et al.* (2013). The current study did show a significant rise ( $P < 0.05$ ) in TSH comparable to the one observed by Henning *et al.* (2013) after a similar 8-weeks of intense training. These

observations may indicate an overriding of the negative feedback calorie intake places on thyroid hormones secretion. The continued demand for a high metabolic output in both studies seems to have resulted in an overriding of this negative feedback, detected as an increase in TSH. In the current study this significant increase ( $P < 0.05$ ) was followed by a significant rise in  $T_4$  with no change in  $T_3$ .

Many of the significant changes ( $P < 0.05$ ) seen in the current study began during the pre-season training period (T1 to T2). In order to better understand these changes several markers were evaluated against the results of the athlete's pre-season performance tests. Of the markers evaluated,  $VO_{2max}$  and %BF were predictive of changes in CORTT, CORTF, SHBG and  $T_3$ . These findings show that players coming into the pre-season with a higher  $VO_{2max}$  and lower %BF are more likely to experience higher training stresses than their less fit teammates. This is most likely due to fit players spending more time practicing in high intensity drills and perhaps even compensating for the less fit players. The results of the current study may be related to the ability of the higher fit female athletes to perform more frequently at an elevated intensity, illustrated by the positive correlation between  $VO_{2max}$  and Kcal expenditure. Coupled with less rest during the pre-season, the intense training during this time set the players up for a persistent stress response throughout the remainder of the season.

### **Conclusions**

The current study has shown the use of biomarkers to not only be a viable option in evaluating player health and performance but a vital one. Monitoring the physical load placed on athletes during the actual training session or games only shows part of a

much more complex response demonstrated here. The heavy training loads during the pre-season resulted in a prolonged stress response that only started to taper as the season concluded despite significant decreases ( $P < 0.05$ ) in TL and Kcal throughout the season. This contributed to an atypical maintenance of  $VO_{2max}$ , VJ or %BF compared to previous research (Clark *et al.*, 2003; Astorino *et al.*, 2004). Evaluating the often significant changes in markers throughout the season shows trends toward an increased stress response, inflammation, decreased oxygen carrying capacity, and possible nutritional deficiencies. Monitoring these changes in some cases is necessary for overall health beyond simply performance and may have gone unnoticed if not for the systematic evaluation of blood-based biomarkers. This study has also found a strong correlation between fitness tests ( $VO_{2max}$  and %BF) and an increased stress response during an intense pre-season training period. This was the result of the more fit players experiencing higher TL and increased Kcal expenditure compared to their less fit teammates.

Future research should focus on establishing upper and lower ranges clinical ranges specific for female athletes. The current study showed that some of the current acceptable ranges for females provided by Quest Diagnostics™ may not be applicable for the female athlete. The current study found the average team value for CORTT to be consistently elevated about the BFA range provided. Previous research in male soccer players did not find CORT to rise above 23.5 mcg/dL (Kraemer *et al.*, 2004). Given this research, it may be that female athletes typically have higher circulating levels of CORT in response to training. Continuing research to understand how the female athlete

differs from the general female population and athletic males is necessary in evaluating some of these biomarkers.

Limitations of the current study include not monitoring diet, sleep, mood and menstrual regularity over the competitive season. Utilizing diet logs in conjunction with a panel of biomarkers in assessing the response to chronic training would help better evaluate changes in markers associated with metabolism and nutrition. Previous research by Clark *et al.* (2003) showed dietary changes and calorie deficits in female soccer players were related to changes in performance during a competitive season. Understanding how an athlete is sleeping and whether their amount of sleep is changing over a season can also provide valuable insight into change in blood-based biomarkers. Research has shown that college athletes tend to sleep less several nights before competition (Savis *et al.*, 1997). Changes in sleep or a lack of quality sleep would result in reduced recovery and a bolstered stress response (Urhausen *et al.* 2002; Budgett *et al.*, 2010). Irregular sleep patterns, decreased caloric intake and decreased physical performance have also been implicated in mood and personality changes (Armstrong *et al.*, 2009; Bodner *et al.*, 2005; Budgett *et al.*, 2010). Research by Bodner *et al.* (2005) has shown that among college aged females decreased caloric intake resulting in micronutrient deficiency has been associated with increased risk of depression. It would be insightful to have each of these logs to evaluate how changes in an athlete's disposition are influenced by or influence changes in biomarkers. Being able to evaluate where a female athlete is in their menstrual cycle would be beneficial in evaluating individual differences between time points. Although research de Jonge *et al.* (2003) has

shown menstrual cycle to not change female athlete performance, it should still be considered given that dysregulation of the menstrual cycle is a primary component of the female athlete triad.

Evaluating changes in the sex steroids would also provide valuable information in regard to changes in recovery status. Knowing the testosterone/cortisol ratio has been shown to be an effective measure of how well a male athlete is or is not recovering to strenuous training (Kraemer *et al.*, 2004; Henning *et al.*, 2013). Evaluating changes in the sex steroids can also demonstrate menstrual irregularity in female athletes if using menstrual logs is not a possibility (Bermon *et al.*, 2014). While the sex steroids were not evaluated in the current study, they have been shown to be impacted by changes in training and should be evaluated by future research.

Having true off-season comparison points when performing future research would help evaluate whether the values taken prior to pre-season were truly baseline values or if they were inflated as athletes made a late push to get fit. Evaluating an athlete over an entire calendar year would provide a better baseline for in-season training response and evaluate the effectiveness of off-season training. Kraemer *et al.* (2004) has stated this as a consideration in his work with male soccer players as the pre-season value can reflect self-induced stress as an athlete pushes themselves to prepare for the season. Future research should focus on monitoring athletes over a year to help establish individual norms that a player can be compared back to during different periods of training. Using individual data, compared to the team averages used in the current study, may provide greater insight into which players (starters/non-starters,

fit/less-fit, etc.) are changing over the season and what their “normal” might be compared to the rest of the team or the clinical ranges provided. This study was designed as an exploratory first step toward better understanding how female athletes respond to the chronic stress of training over a season using blood-biomarkers. Future research with female athletes should focus on incorporating previously discussed suggestions (dietary logs, menstrual assessment, etc.) building on the evaluation of the current study.

Understanding the response to training in field hockey athletes requires an evaluation of physical training load and physiological response. In order to evaluate the latter, the current study utilized biomarkers detectable in plasma. Almost all of the markers evaluated showed at least some significant change ( $P < 0.05$ ) over the field hockey season. The use of biomarkers has been established as a quantitative measure of training stress in male athletes (Kraemer *et al.*, 2004; Heisterberg *et al.*, 2013; Henning *et al.*, 2013). Evaluating biomarker fluctuation over a field hockey season illustrates the dynamic state of stress and recovery occurring as the season progresses. The current study found the use of fitness tests and biomarkers as compelling tools for evaluating the response of female Division I field hockey players to a 12-week competitive season. Athlete fitness was also found to be highly predictive of changes in biomarkers related to the stress of training after intense pre-season training. While this study successfully demonstrated the use of biomarkers in evaluating the female athlete’s response to training, more research is necessary to further understand what is causing these changes in the female athlete’s physiology.

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Table 1

<b>N=19</b>	<b>PT1</b>	<b>PT2</b>	<b>P Value</b>
<b>Age (yrs)</b>	19 ± 1.09	-	
<b>Height (cm)</b>	166.05 ± 3.33	-	
<b>Weight (kg)</b>	63.88 ± 7.03	63.8 ± 6.54	0.858
<b>%BF (%)</b>	25.9 ± 6.54	25.31 ± 5.55	0.229
<b>LM (kg)</b>	47.04 ± 4.57	47.57 ± 4.40	0.025*
<b>VJ (cm)</b>	51.43 ± 7.09	51.12 ± 6.39	0.632
<b>VO2 (ml/kg/min)</b>	46.63 ± 5.52	46.43 ± 4.83	0.794
<b>VT (%)</b>	76.84 ± 2.91	79.16 ± 3.02	0.019*

**Table 1: Pre- and Post-Season Fitness Changes** – Shows the difference in team fitness from pre-season (PT1) to post-season (PT2). There was no significant change ( $P > 0.05$ ) in weight, body composition (%BF), vertical jump (VJ) or aerobic capacity ( $VO_{2max}$ ). Significant changes ( $P < 0.05$ ) were detected in lean body mass (LM) and ventilatory threshold (VT) between the beginning and end of the season.

\*Significant difference ( $P < 0.05$ ) between time points

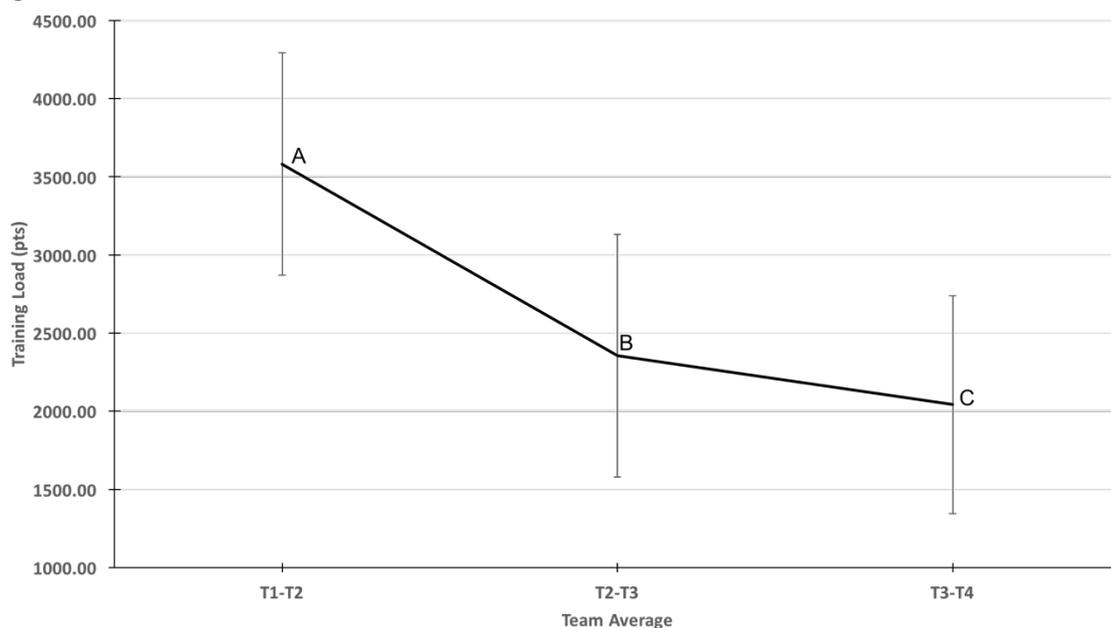
Table 2

N=19	T1-T2	T1-T3	T1-T4	T2-T3	T2-T4	T3-T4
TL (pts)	-1.73*	-2.17*		-0.40		
Kcal (kcal)	-2.71*	-3.56*		-0.80*		
CORTT (mcg/dL)	0.19	0.30	0.26	0.09	0.06	-0.03
CORTF (mcg/dL)	0.45	0.74	1.11*	0.33	0.74	0.40
SHBG (nmol/dL)	0.05	-0.08	-0.07	-0.14	-0.13	0.02
CK (U/L)	1.59*	1.14*	0.63	-0.30	-0.66	-0.34
LD (U/L)	2.74*	2.67*	2.05*	-0.11	-0.99*	-0.81*
IL-6 (U/L)	1.35*	0.81*	1.05*	-0.06	-0.04	0.08
PRL (U/L)	-0.03	0.28	0.27	0.51	0.49	-0.02
Omega6:3 (%)	5.44*	5.51*	5.15*	0.04	-0.18	-0.19
VitD (ng/dL)	0.07	-0.25	-0.54	-0.39	-0.73	-0.42
FE (mcg/dL)	-1.30*	-1.42*	-1.42*	-0.13	-0.12	0.00
FERR (ng/dL)	-0.60	-0.36	-0.31	0.41	0.50	0.06
TIBC (mcg/dL)	-0.13	-0.50	-0.50	-0.30	-0.31	0.00
%Sat (%)	-1.04*	-1.07*	-0.99*	-0.03	0.05	0.09
HGB (g/dL)	-0.64	-0.61	-0.58	0.03	0.05	0.02
HCT (%)	-0.43	-0.58	-0.31	-0.16	0.12	0.28
MCHC (g/dL)	-0.84*	-0.26	-1.03*	0.73	-0.24	-0.47
TSH (mU/L)	0.20	1.20*	0.24	1.00*	0.04	-0.65
T4 (ng/dL)	-1.08*	-1.40*	-0.96*	-0.35	0.14	0.46
T3 (ng/dL)	0.77	-0.14	-0.18	-0.53	-0.56	-0.04

**Table 2: ES-Values for Training Load, Calorie Expenditure and Blood-Based Biomarkers**

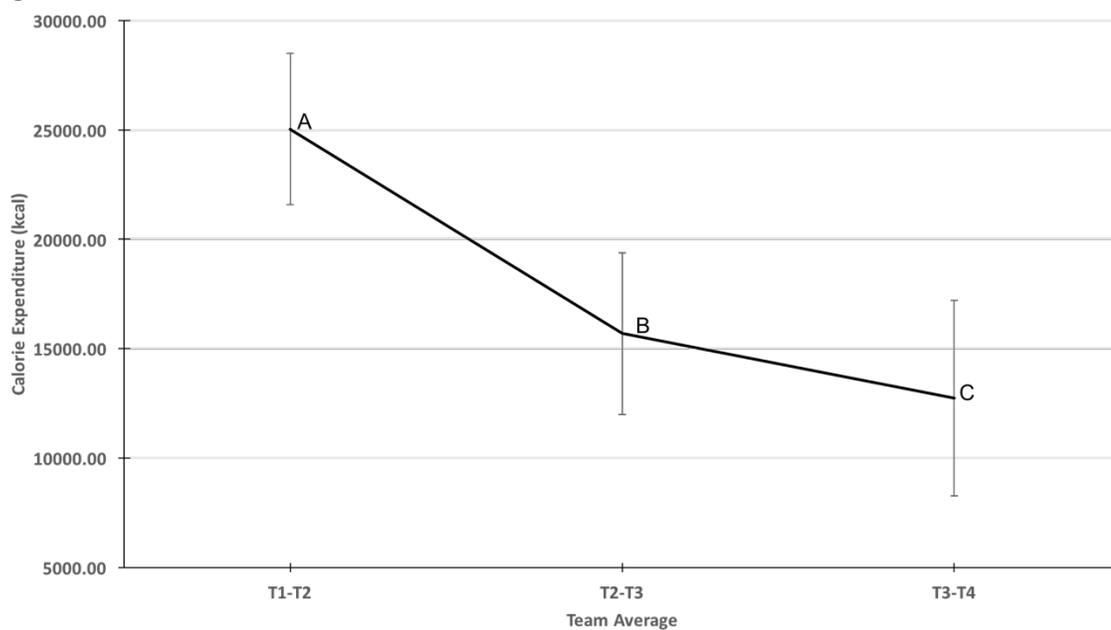
– This table illustrates the effect size of all assessed markers between each time point.

\* Large effect size (ES>0.8)

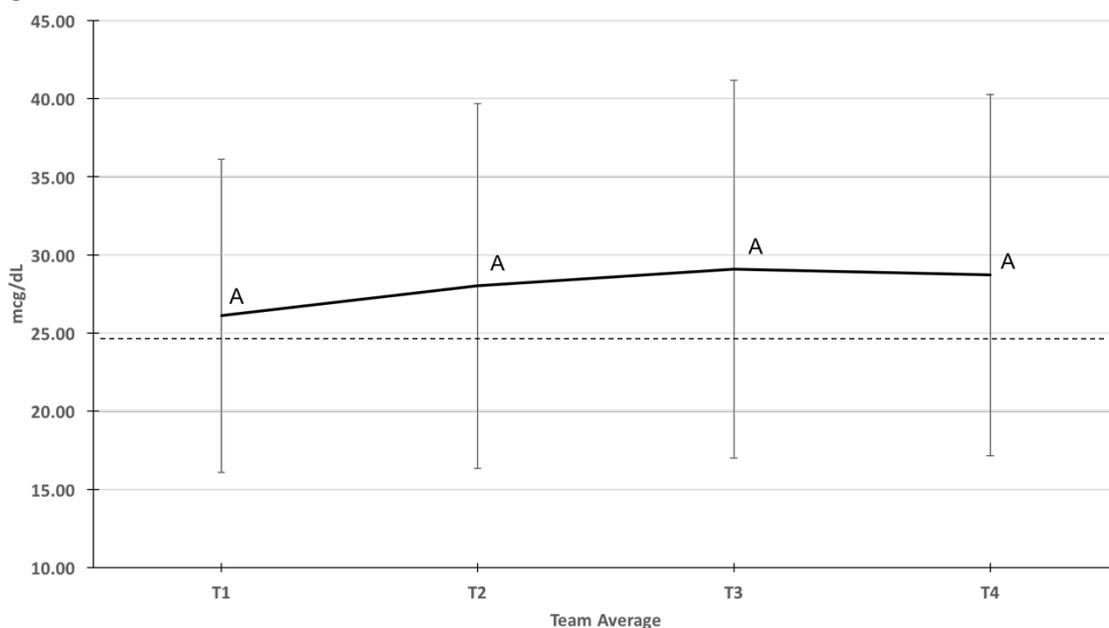
**Figure 1**

**Figure 1: Season Changes in Average Training Load** – There was a significant decline ( $P < 0.05$ ) in training load between each time point.

Time points sharing the same superscript were not found to be significantly different ( $P > 0.05$ ).

**Figure 2**

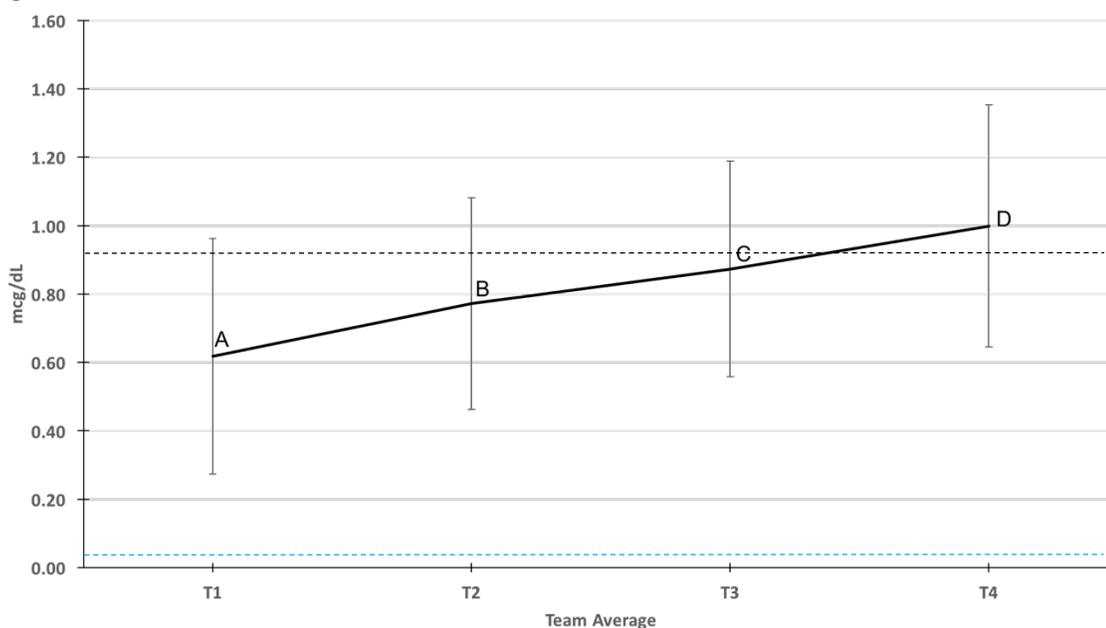
**Figure 2: Season Changes in Average Calorie Expenditure** – There was a significant decrease ( $P < 0.05$ ) in calorie expenditure between each time point. Time points sharing the same superscript were not found to be significantly different ( $P > 0.05$ ).

**Figure 3**

**Figure 3: Season Changes in Average Total Cortisol** – There was no significant difference ( $P>0.05$ ) in CORTT throughout the season.

Time points sharing the same superscript were not found to be significantly different ( $P>0.05$ ).

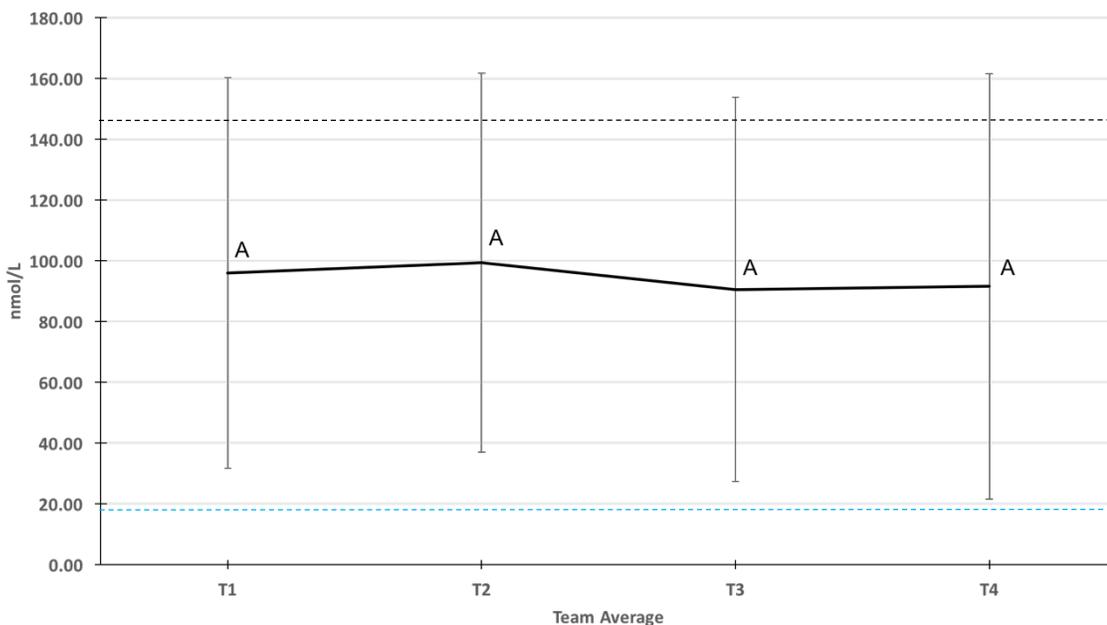
Broken lines indicate upper and lower “normal range” provided by the Quest Diagnostics™ Blueprint for Athletes™ (4.6 – 20.6 mcg/dL).

**Figure 4**

**Figure 4: Season Changes in Average Free Cortisol** – A significant difference ( $P < 0.05$ ) in CORTF was observed between each assessment.

Time points sharing the same superscript were not found to be significantly different ( $P > 0.05$ ).

Broken lines indicate upper and lower “normal range” provided by the Quest Diagnostics™ Blueprint for Athletes™ (0.07 – 0.93 mcg/dL).

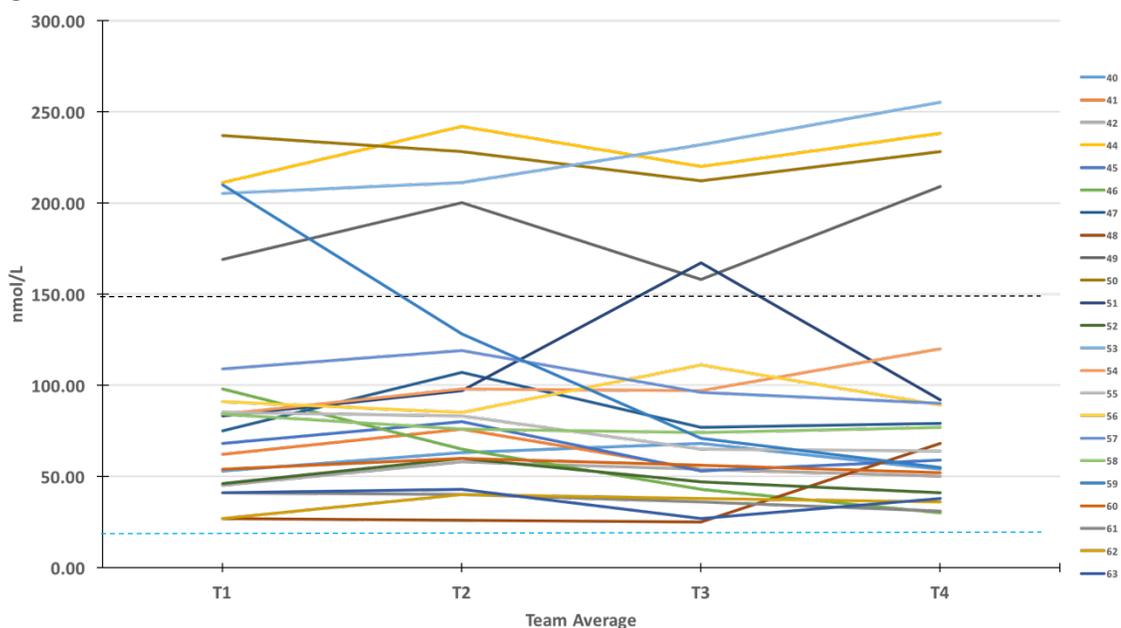
**Figure 5**

**Figure 5: Season Changes in Average Sex Hormone Binding Globulin** – There was no significant difference ( $P>0.05$ ) in SHBG throughout the season.

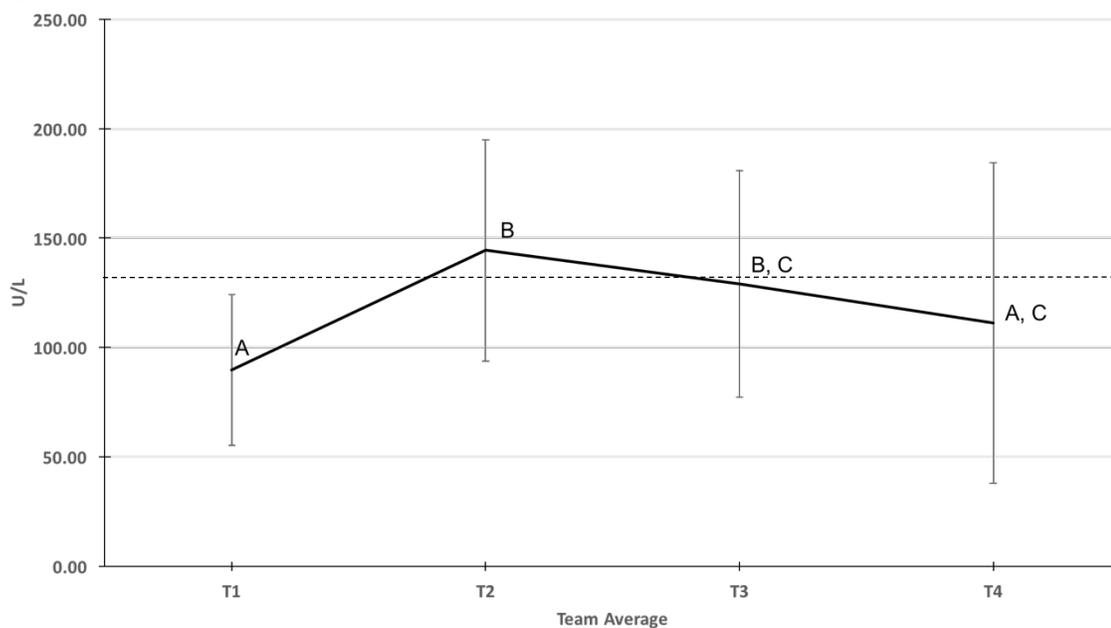
Time points sharing the same superscript were not found to be significantly different ( $P>0.05$ ).

Broken lines indicate upper and lower “normal range” provided by the Quest Diagnostics™ Blueprint for Athletes™ (17 – 124 nmol/L).

Figure 5a



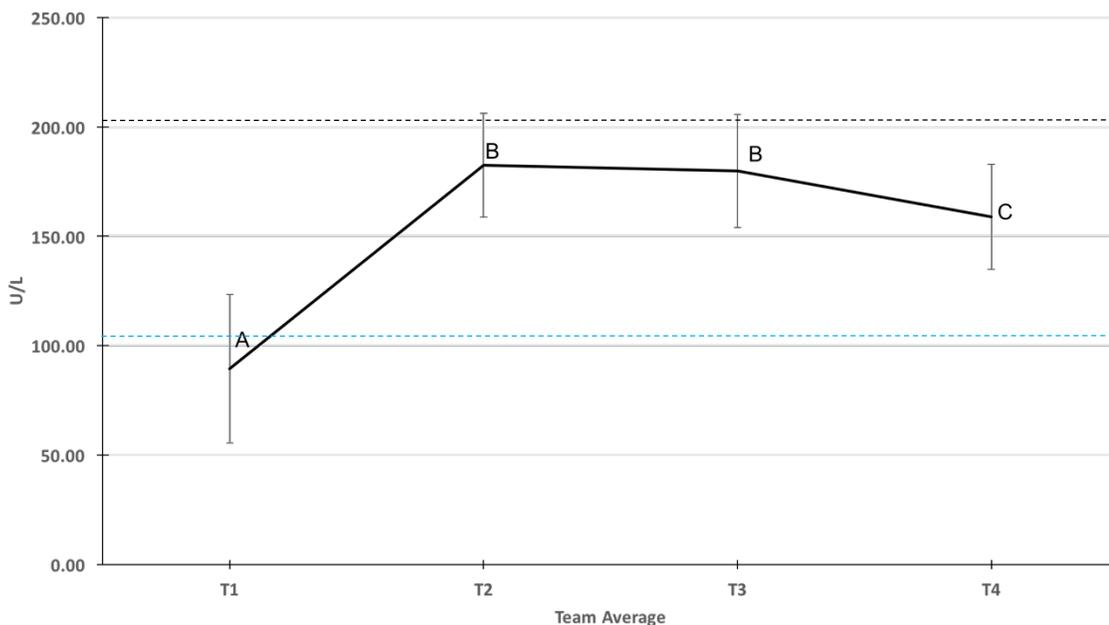
**Figure 5a: Changes in Athlete Sex-Hormone Binding Globulin** – A large standard deviation was the result of four subjects with uncharacteristically high SHBG. Broken lines indicate upper and lower “normal range” provided by the Quest Diagnostics™ Blueprint for Athletes™ (17 – 124 nmol/L).

**Figure 6**

**Figure 6: Season Changes in Average Creatine Kinase** – A significant difference ( $P < 0.05$ ) in CK was observed from T1 to T2 and T1 to T3. T4 was only found to be significantly different ( $P < 0.05$ ) from T2.

Time points sharing the same superscript were not found to be significantly different ( $P > 0.05$ ).

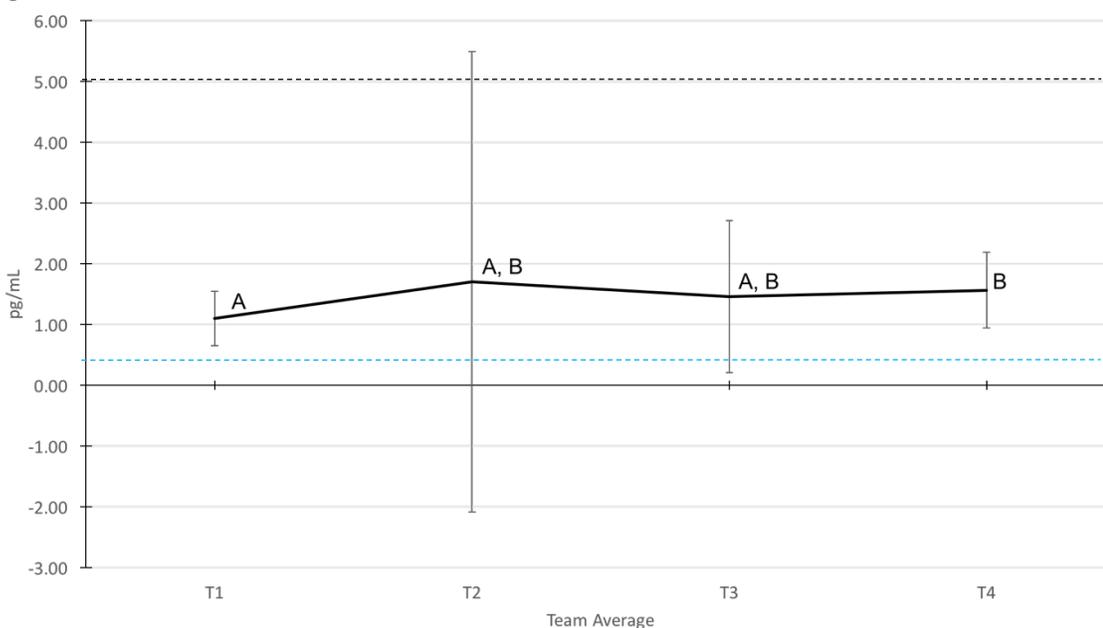
Dashed line indicates upper “normal range” provided by the Quest Diagnostics™ Blueprint for Athletes™ (<143 U/L).

**Figure 7**

**Figure 7: Season Changes in Average Lactate Dehydrogenase** – There was a significant difference ( $P < 0.05$ ) in LD measured between T1 and all other time points. T4 was also found to be significantly different from either T2 or T3. No significant difference ( $P > 0.05$ ) was found between T2 and T3.

Time points sharing the same superscript were not found to be significantly different ( $P > 0.05$ ).

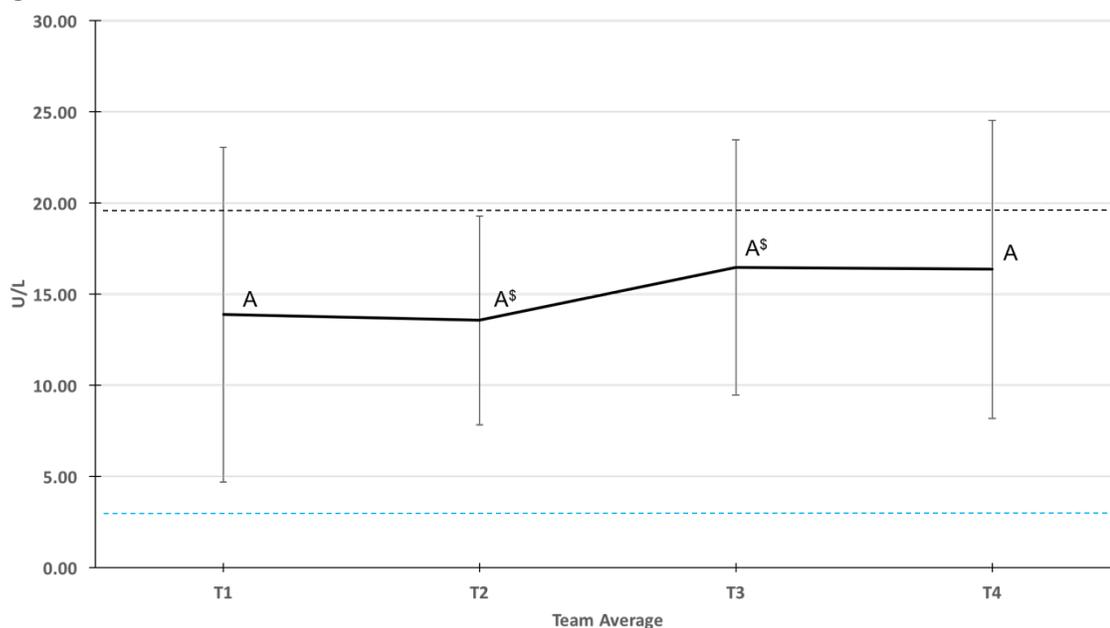
Broken lines indicate upper and lower “normal range” provided by the Quest Diagnostics™ Blueprint for Athletes™ (100 – 200 U/L).

**Figure 8**

**Figure 8: Season Changes in Average Interleukin-6** – There was a significant difference ( $P < 0.05$ ) in IL-6 between T1 and T4. There was no significant difference ( $P > 0.05$ ) between T1 or T4 with either of the other two time points (T2 and T3). Large standard deviation at T2 is a result of a single individual.

Time points sharing the same superscript were not found to be significantly different ( $P > 0.05$ ).

Broken lines indicate upper and lower “normal range” provided by the Quest Diagnostics™ Blueprint for Athletes™ (0.31 – 5 pg/mL).

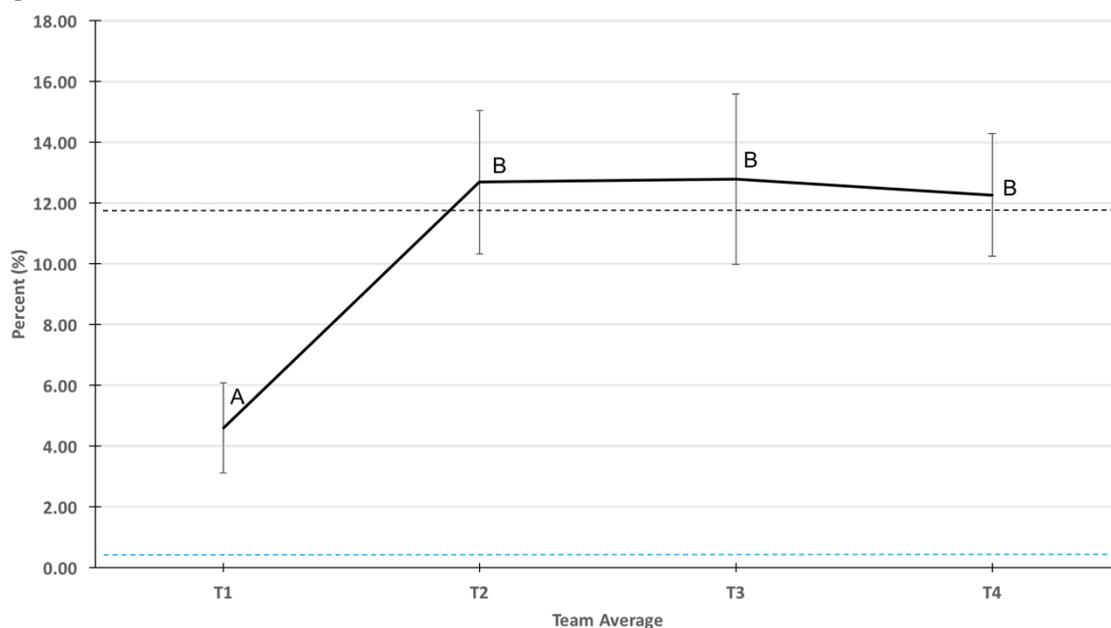
**Figure 9**

**Figure 9: Season Changes in Average Prolactin** – There was no significant change ( $P > 0.05$ ) in PRL between any two time points. However, there was a trend toward significance ( $P < 0.1$ ) between T2 and T3.

Time points sharing the same superscript were not found to be significantly different ( $P > 0.05$ ).

Broken lines indicate upper and lower “normal range” provided by the Quest Diagnostics™ Blueprint for Athletes™ (3.2 – 20.0 U/L).

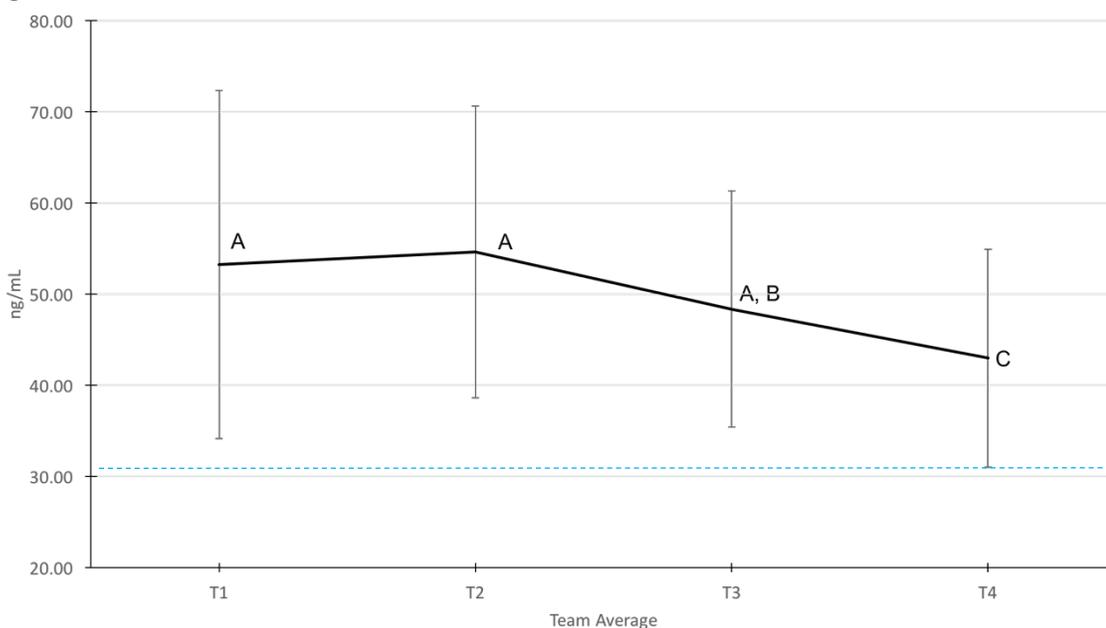
<sup>§</sup>Indicates a trend toward significant difference ( $P < 0.1$ ) between time points.

**Figure 10**

**Figure 10: Season Changes in Average Omega 6:3 Ratio** – A significant difference ( $P < 0.05$ ) was detected between T1 and all other time points for OMG63. No other significant difference ( $P > 0.05$ ) was detected.

Time points sharing the same superscript were not found to be significantly different ( $P > 0.05$ ).

Broken lines indicate upper and lower “normal range” provided by the Quest Diagnostics™ Blueprint for Athletes™ (1.3 – 12 %).

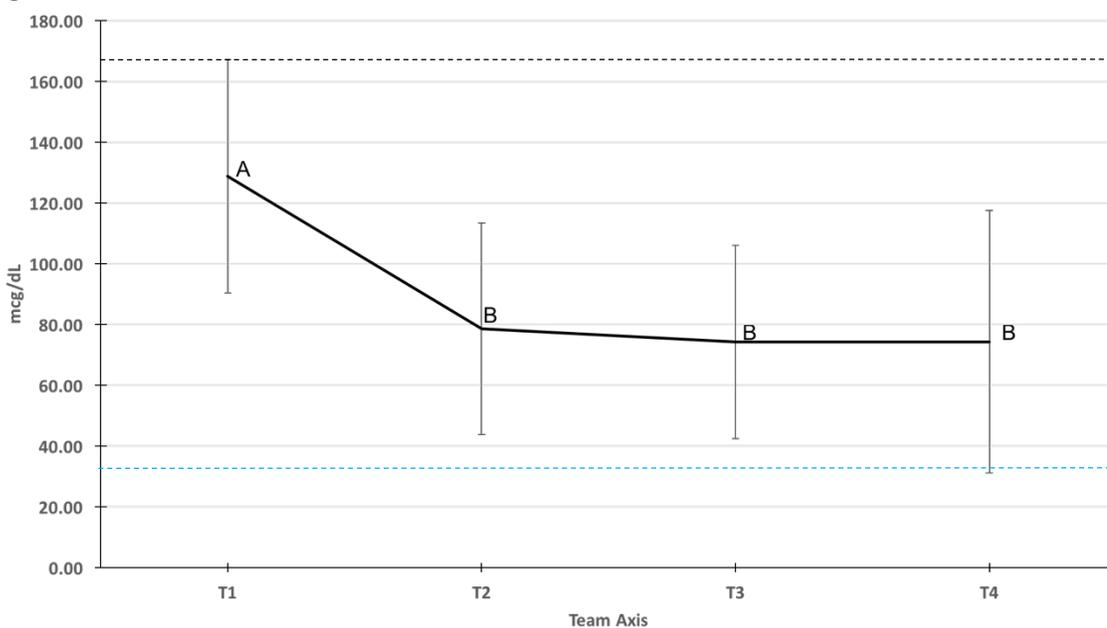
**Figure 11**

**Figure 11: Season Changes in Average Vitamin D** – A significant difference ( $P < 0.05$ ) in VitD was detected between T4 and all other time points. A significant difference was also shown between T2 and T3. No significant difference ( $P > 0.05$ ) was found between T1 and either T2 or T3.

Time points sharing the same superscript were not found to be significantly different ( $P > 0.05$ ).

Broken line indicates lower “normal range” provided by the Quest Diagnostics™ Blueprint for Athletes™ (30.0 ng/mL).

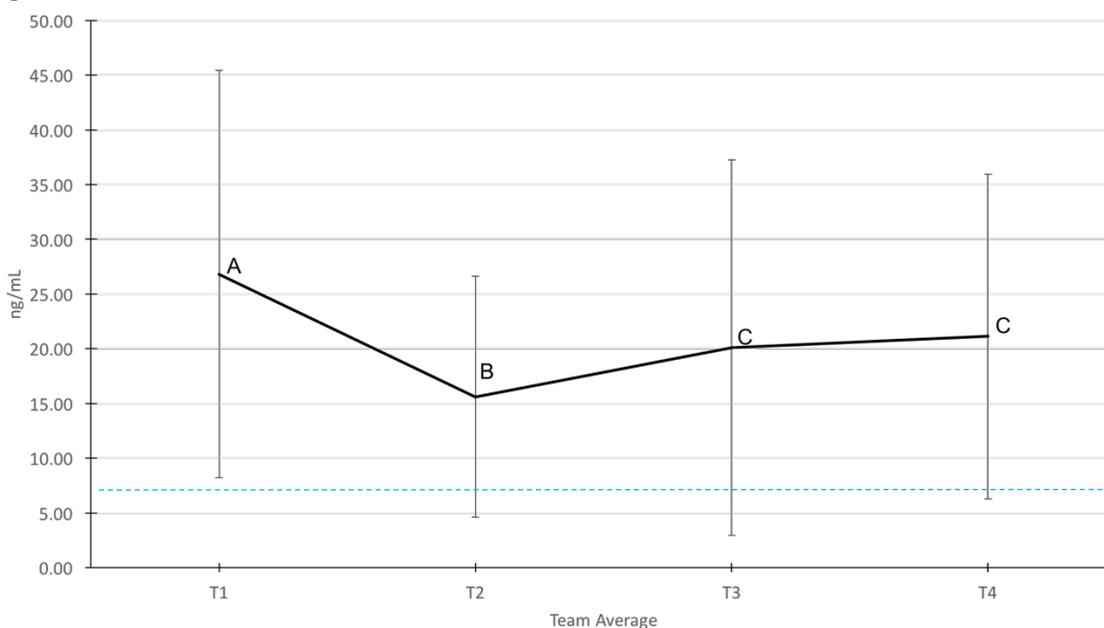
Figure 12



**Figure 12: Season Changes in Average Iron** – A significant difference ( $P < 0.05$ ) in Fe was detected between T1 and all other time points. There was no other significant difference ( $P > 0.05$ ).

Time points sharing the same superscript were not found to be significantly different ( $P > 0.05$ ).

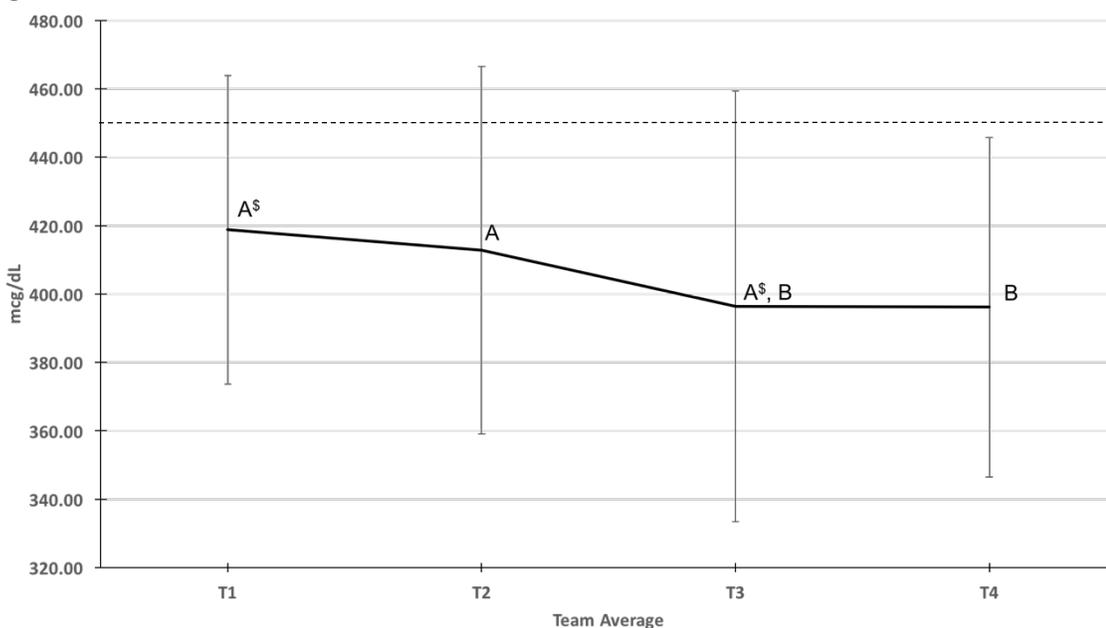
Broken lines indicate upper and lower “normal range” provided by the Quest Diagnostics™ Blueprint for Athletes™ (27.0 – 164.0 mcg/dL).

**Figure 13**

**Figure 13: Season Changes in Average Ferritin** – A significant difference ( $P < 0.05$ ) in FER was found between both T1 and all other time points. T2 was also found to be significant different ( $P < 0.05$ ) than T3 and T4. No significant difference ( $P > 0.05$ ) between T3 and T4 was found.

Time points sharing the same superscript were not found to be significantly different ( $P > 0.05$ ).

Broken line indicates lower “normal range” provided by the Quest Diagnostics™ Blueprint for Athletes™ (6.0 ng/mL).

**Figure 14**

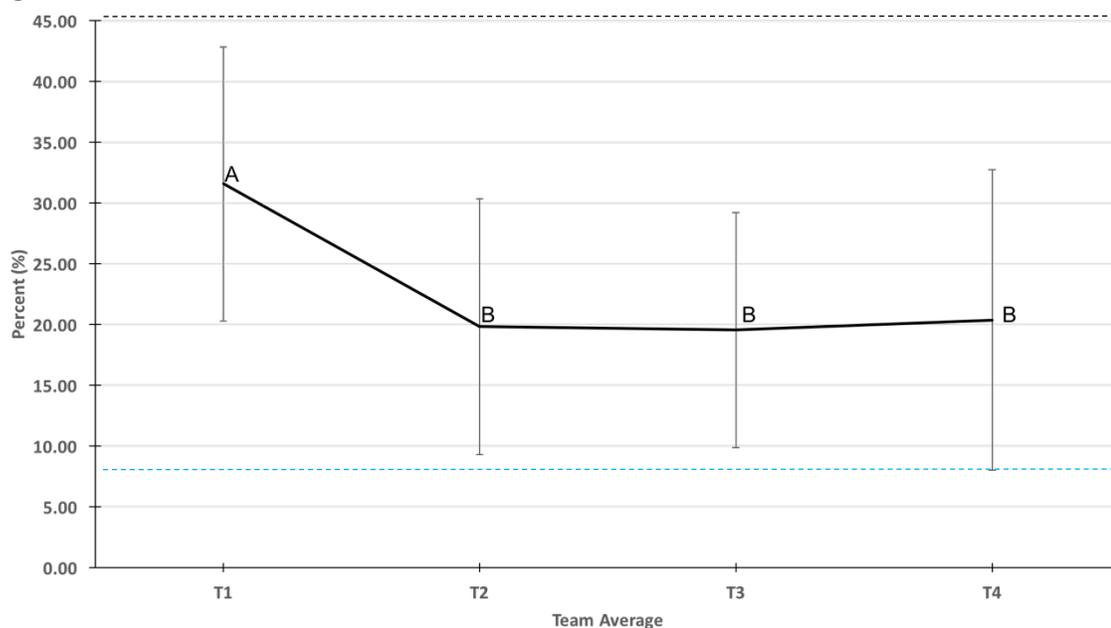
**Figure 14: Season Changes in Average Total Iron Binding Capacity** – A significant difference ( $P < 0.05$ ) in TIBC was found between T4 and both T1 and T2. A trend toward significance ( $P < 0.1$ ) was found between T1 and T3. There was no significant difference ( $P > 0.05$ ) between T2 and T3.

Time points sharing the same superscript were not found to be significantly different ( $P > 0.05$ ).

Broken line indicates upper “normal range” provided by the Quest Diagnostics™ Blueprint for Athletes™ (448 mcg/mL).

<sup>§</sup>Indicates a trend toward significant difference ( $P < 0.1$ ) between time points.

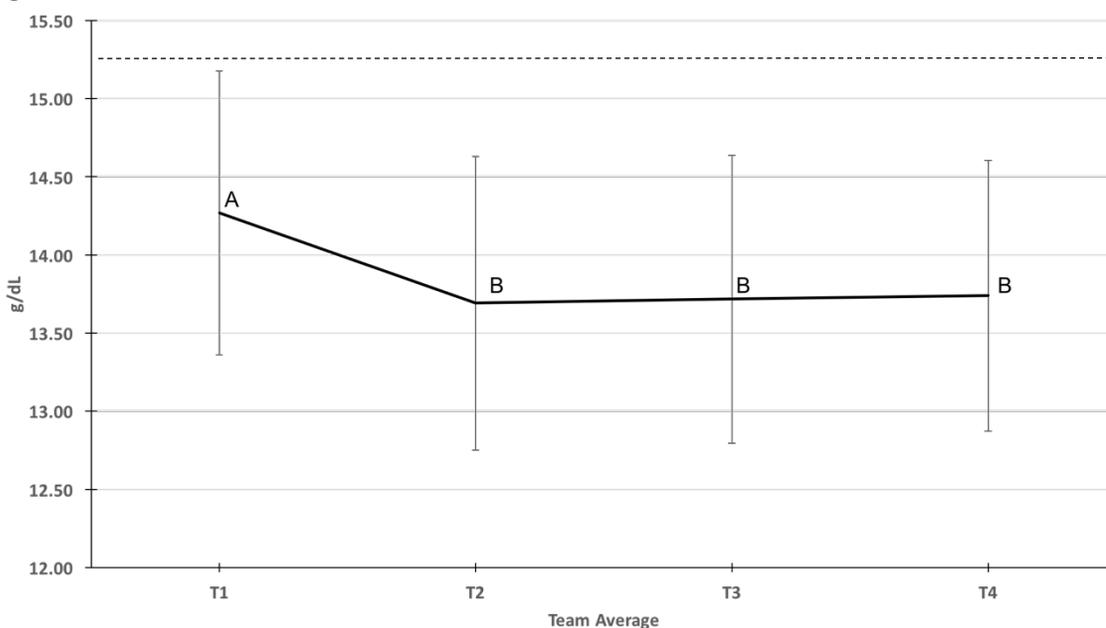
Figure 15



**Figure 15: Season Changes in Average Percent Saturation** – A significant difference ( $P < 0.05$ ) was detected for %Sat between T1 and all other time points. No other significant difference ( $P > 0.05$ ) was reported.

Time points sharing the same superscript were not found to be significantly different ( $P > 0.05$ ).

Broken lines indicate upper and lower “normal range” provided by the Quest Diagnostics™ Blueprint for Athletes™ (8 – 45 %).

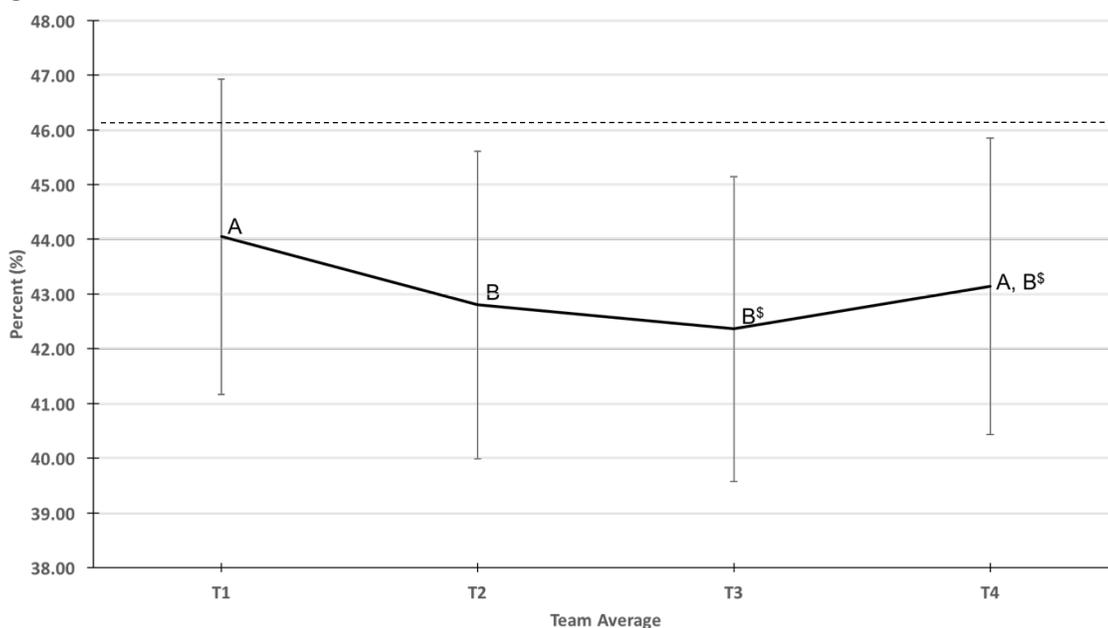
**Figure 16**

**Figure 16: Season Changes in Average Hemoglobin** – A significant difference ( $P < 0.05$ ) was detected between T1 and all other time points for Hb. No other significant difference ( $P > 0.05$ ) was reported.

Time points sharing the same superscript were not found to be significantly different ( $P > 0.05$ ).

Broken line indicates upper “normal range” provided by the Quest Diagnostics™ Blueprint for Athletes™ (15.3 g/dL).

Figure 17

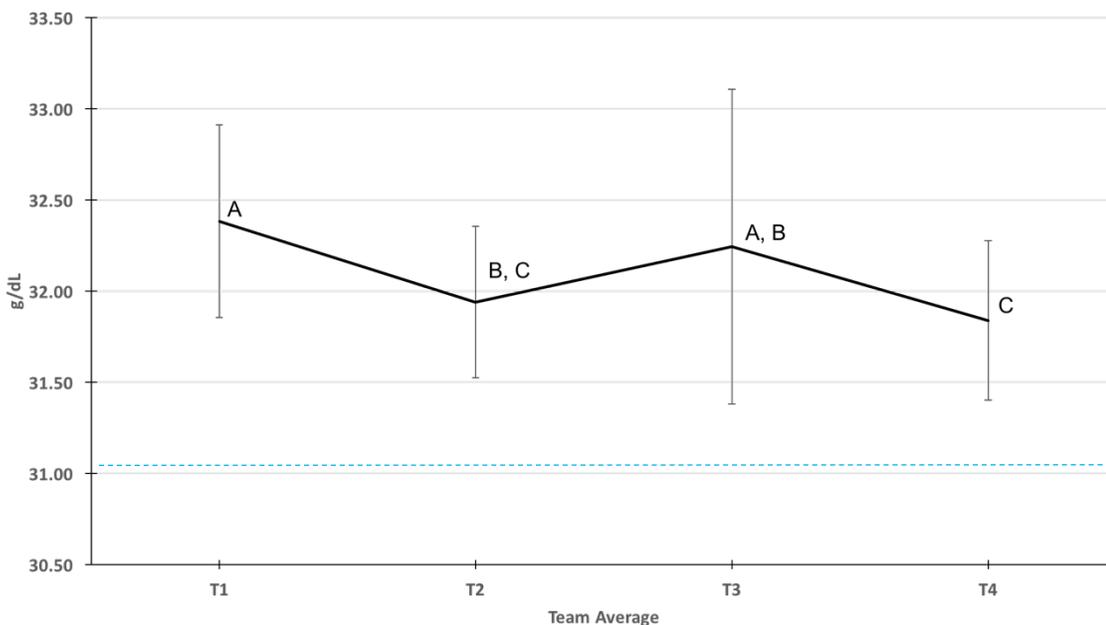


**Figure 17: Season Changes in Average Hematocrit** – There was a significant difference ( $P < 0.05$ ) in HCT between T1 and both T2 and T3. No significant difference ( $P > 0.05$ ) was found between T2 and T4. A trend toward significance ( $P < 0.1$ ) was observed between T4 and T3.

Time points sharing the same superscript were not found to be significantly different ( $P > 0.05$ ).

Broken line indicates upper “normal range” provided by the Quest Diagnostics™ Blueprint for Athletes™ (46 %).

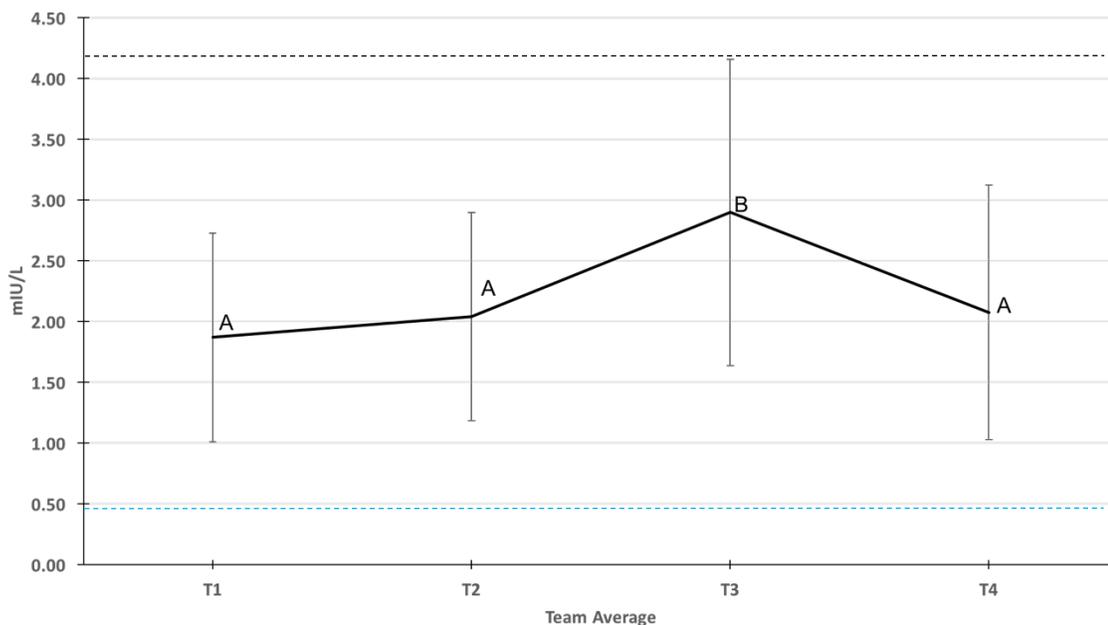
<sup>\$</sup>Indicates a trend toward significant difference ( $P < 0.1$ ) between time points.

**Figure 18**

**Figure 18: Season Changes in Average Mean Corpuscular Hemoglobin Concentration –** Significant difference ( $P < 0.05$ ) was detected in MCHC from T1 and both T2 and T4. T3 was found to be significantly different ( $P < 0.05$ ) than T4. No difference ( $P > 0.05$ ) was detected between T2 and T3 or T4.

Time points sharing the same superscript were not found to be significantly different ( $P > 0.05$ ).

Broken line indicates lower “normal range” provided by the Quest Diagnostics™ Blueprint for Athletes™ (31.0 g/dL).

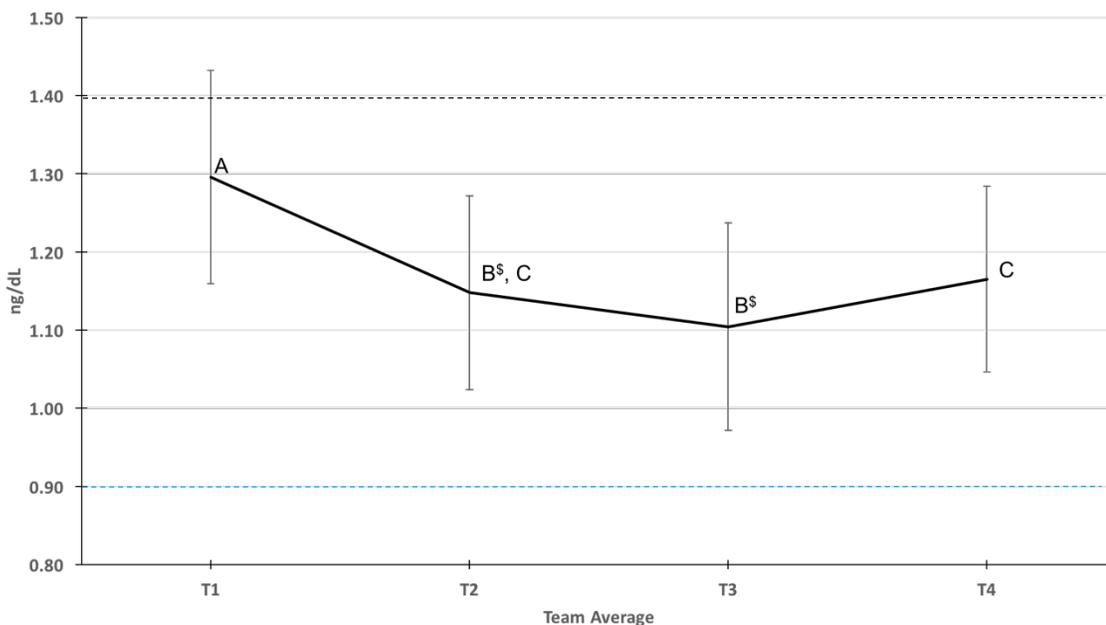
**Figure 19**

**Figure 19: Season Changes in Average Thyroid Stimulating Hormone** – A significant increase ( $P < 0.05$ ) in TSH was observed between T2 and T3 which differed from all other time points. No other significant difference ( $P > 0.05$ ) was reported.

Time points sharing the same superscript were not found to be significantly different ( $P > 0.05$ ).

Broken lines indicate upper and lower “normal range” provided by the Quest Diagnostics™ Blueprint for Athletes™ (0.5 – 4.3 mIU/L).

Figure 20

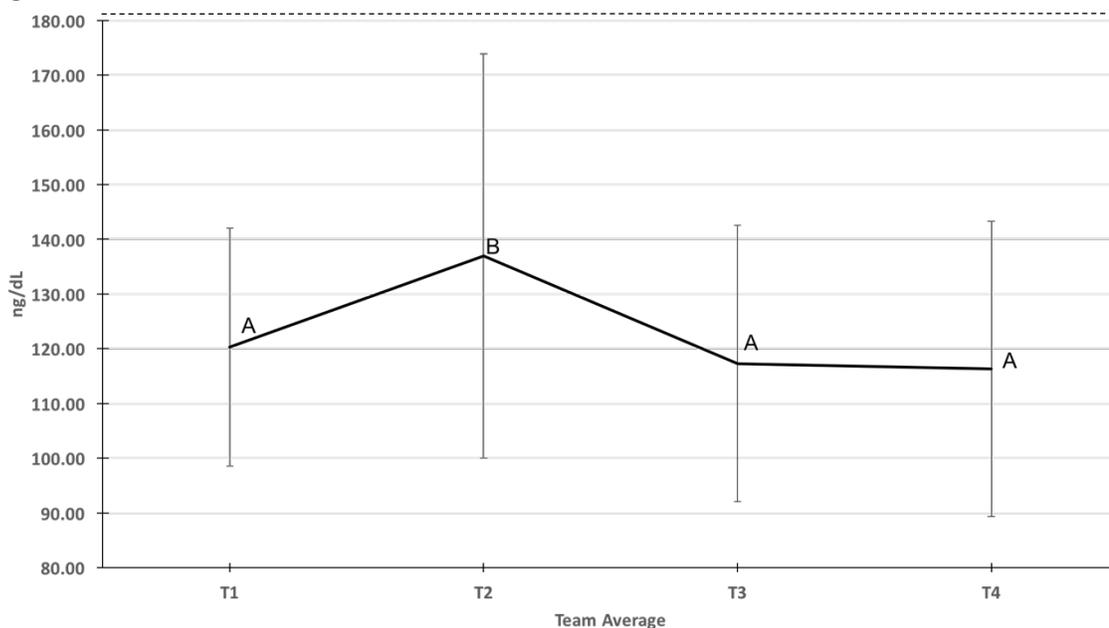


**Figure 20: Season Changes in Average Thyroxine** – There was a significant difference ( $P < 0.05$ ) in  $T_4$  between  $T_1$  and all other time points.  $T_3$  was shown to be significantly different than  $T_4$ . No significant difference ( $P > 0.05$ ) was found between  $T_2$  and either  $T_3$  and  $T_4$ .

Time points sharing the same superscript were not found to be significantly different ( $P > 0.05$ ).

Broken lines indicate upper and lower “normal range” provided by the Quest Diagnostics™ Blueprint for Athletes™ (0.9 – 1.4 ng/dL).

§ Indicates a trend toward significant difference ( $P < 0.1$ ) between time points.

**Figure 21**

**Figure 21: Season Changes in Average Triiodothyronine** – A significant difference ( $P < 0.05$ ) was found between T<sub>2</sub> and all other time points for T<sub>3</sub>. No other significant difference ( $P > 0.05$ ) was shown.

Time points sharing the same superscript were not found to be significantly different ( $P > 0.05$ ).

Broken line indicates upper “normal range” provided by the Quest Diagnostics™ Blueprint for Athletes™ (181 ng/dL).