PSYCHOLOGICAL AND PHYSIOLOGICAL CHANGES IN RESPONSE TO THE CUMULATIVE DEMANDS OF A WOMEN'S DIVISION I COLLEGIATE SOCCER SEASON

Bridget A. McFadden[1], Alan J. Walker[2], Brittany N. Bozzini[1], Morgan Hofacker[3], Mark Russell[4], Shawn M. Arent[1,4]

[1]Department of Exercise Science, The University of South Carolina

[2]Department of Exercise Science, Lebanon Valley College

[3]Center for Health and Human Performance, Rutgers, The State University of New Jersey

[4]School of Social and Health Sciences, Leeds Trinity University

Corresponding Author:

Shawn M. Arent, Ph.D., CSCS*D, FISSN, FACSM, FNAK

Professor and Chair, Department of Exercise Science

University of South Carolina

921 Assembly Street Columbia, SC 29208

Email: sarent@mailbox.sc.edu

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JSCR-08-15647R2

ABSTRACT:

This study sought to determine the effects of a women's collegiate soccer season on psychological markers, biomarkers, sleep, and performance. Athletes participated in maximal countermovement vertical jump height (CMJ) assessments and biomarker monitoring in conjunction with subjective measures of psychological wellness and sleep questionnaires prior to preseason (S1) and every four weeks following (S2, S3, S4). Training was monitored during practices and games using global positioning satellite systems and heart rate technology. Total training load was highest from S1-S2, decreased from S2-S3 (effect size [ES]=-2.5; $p<0.001$), and remained stable S3-S4. CMJ declined at S2 (ES_{1-2}=-0.51; $p=0.001$) and returned to baseline at S3. Increases from S1-S2 were seen for creatine kinase (ES_{1-2}=1.74), free testosterone (ES_{1-2}=1.27), total testosterone (ES_{1-2}=3.5) and free cortisol (ES_{1-2}=0.88) ($p<0.03$), before returning to baseline by S3 and S4 (free cortisol). Total cortisol was elevated throughout the season before declining at S4 (ES_{1-4}=-0.41; $p=0.03$). Iron declined from S1-S2 (ES_{1-2}=-0.73; $p=0.01$) and returned to baseline values at S4, whereas growth hormone declined at S2 (ES_{1-2}=-0.50; $p=0.01$) and remained depressed. Interleukin-6 increased at S4 (ES_{1-4}=0.71; $p=0.02$). Total training distress decreased from S1-S2 (ES_{1-2}=-0.38; $p=0.02$), returned to baseline by S3 and increased by S4 (ES_{1-4}=0.57; $p=0.01$). No changes were observed in markers of sleep ($p>0.05$). Biomarkers showed notable changes following the highest workload period (S1-S2), which coincided with CMJ decrements. Biomarker perturbations preceded declines in subjective psychological wellness (S4) which occurred in the latter half of the season, indicating an accumulation of fatigue as the season progressed.

Keywords: Vertical Jump, Performance, Biomarkers, Sleep, Mood

INTRODUCTION:

Adequate recovery from stress is an essential aspect of an athlete's training program. Strenuous training and match demands may increase an athlete's psychological and physiological stress (36); while collegiate soccer players, in particular, are further burdened with additional stressors that include a short preseason (<3 weeks), multiple games per week, frequent travel, and academic requirements (14, 41). These demands may have cumulative effects throughout the competitive season which can exacerbate recovery needs. Insufficient recovery can present in the form of psychological and physiological changes that may progress to performance decrements (16, 27, 31). Accordingly, methods of assessing and profiling recovery status and readiness to train/compete are valuable when seeking to optimize training and prevent chronic declines in performance. Research is warranted to assess the utility of multiple athlete-monitoring tools, particularly in women's team sports, to discover how athletes respond to the stress of the competitive season. Little has been done to examine pertinent physiological and psychological challenges and changes that occur in this population, particularly for those competing at a high level.

Early detection and prevention of excessive fatigue in athletes should be the goal of monitoring techniques in order to avoid non-functional overreaching and overtraining syndrome from developing (30). Athlete monitoring methods have emerged as a tool to prevent declines in performance stemming from inappropriate training loads and inadequate recovery (16). While no single reliable monitoring technique has been established (19), various methods of tracking an athlete's internal load (i.e. physiological response) and external load (i.e. work completed by the athlete) in conjunction with psychological assessments may prove beneficial (16, 42). Load monitoring systems include technologies, such as global positioning satellite systems (GPS), accelerometry, and continuous heart rate (HR) measurements, which provide the ability to track both on-field external and internal loads throughout a competitive season (16). While tracking on-field training load is an important step to gauge the athlete's training demands, other factors may also play a role in determining readiness to perform. The use of blood-based biomarkers has the capacity to provide an objective evaluation of the athlete's physiological response to training as well as other aspects of *overall* health, recovery, metabolic and nutritional status (23). Monitoring relative changes in biomarkers related to stress, inflammation, anabolism, muscle breakdown/turnover, metabolism, nutrition, and reproductive health, are important means of evaluating training status and fatigue.

In addition to monitoring physiological responses, changes in psychological states have been established as an important indicator of training distress and may be linked to decrements in performance (15). The study by Morgan and colleagues monitoring mood states in competitive swimmers showed mood disturbances increase during times of high training volumes in a dose-response manner (31). Questionnaires and surveys designed to assess athlete's psychological wellness have the ability to provide subjective information as to how the athlete is adapting to the imposed training load (16). Further, surveys that track athletes' sleep habits may help provide important information to coaches and support staff. One of the most commonly reported methods for managing fatigue and enhancing recovery is related to sleep (20, 37). Adequate sleep may act to buffer the negative effects of increased training demands, while inadequate sleep may lead to a worsened state of fatigue with significant negative effects on performance, perceived effort, and cognition as well as other biological functions (17). As a result, evaluating sleep quality and duration may help to better understand the athlete's overall readiness to perform.

Previous research has shown an imbalance between training demands and recovery can result in physiological, biochemical, and psychological/behavioral alterations that may occur before or in conjunction with deteriorations in performance (18, 39). Tracking changes in these metrics may help to elucidate the effects of the competitive season in women's team sport athletes as well as help to determine the point at which training may become maladaptive. The purpose of this study was to monitor the physiological and psychological profile of National Collegiate Athletic Association's (NCAA) Division I women's soccer players to determine the time course of change in various markers of fatigue in conjunction with fluctuating training demands throughout the course of a season. It was hypothesized that an increase in training load metrics would be accompanied by perturbations in biomarkers, psychological wellness, and sleep, as well as declines in performance.

METHODS:

Experimental Approach to the Problem: This observational study sought to determine the effects of a competitive collegiate soccer season on biomarkers, psychological athlete wellness, sleep, and performance outcomes in high-level women collegiate athletes. Maximal aerobic capacity tests in conjunction with body composition assessments were performed prior to the start of the season to accurately program the on-field athlete monitoring system for each player. Training variables (i.e. training load [TL], exercise energy expenditure [EEE], time spent at a percentage of HR maximum [HR_{max}], total distance covered [DIS], and distance covered in high speed running) were monitored during all practices and games. Biomarkers, maximal counter-movement vertical jump height (CMJ), subjective psychological wellness, and sleep were assessed before the start of preseason and every 4-weeks following to evaluate the effects of the accumulating demands during the competitive season.

Subjects: Twenty-five NCAA DI women's soccer players (Age = 19 ± 1 yrs; Height= 167 ± 6 cm; Weight= 65.1 ± 1.6 kg) participated in monitoring and assessments during a competitive season. All participants performed testing as part of the regular team activity associated with their sports science program. All subjects received clearance by the University sports medicine staff prior to testing. This research was approved, and written consent waived, by the University's Institutional Review Board (#16-050) for the Protection of Human Subjects. All procedures performed were in accordance with the 1964 Declaration of Helsinki and its later amendments.

Performance Testing: Athletes reported to the laboratory prior to the start of the collegiate fall preseason in late July to complete performance and body composition testing. Subjects were instructed to arrive euhydrated, 2 hours fasted, and having abstained from strenuous exercise 24 hours prior to testing. All athletes with prior participation on this team had previous experience in testing procedures. All incoming athletes were familiarized with the testing procedures through instruction and demonstration by the researchers prior to testing. Body composition was assessed by air displacement plethysmography (11) (BOD POD, COSMED, Concord, CA) to determine percent body fat (%BF), and fat free mass (FFM), using the Brozek formula (4). The error of body volume reading is roughly 0.02%, which allows for the calculation of %BF with only 0.01% error (11). Following a self-selected warm-up, a maximal graded treadmill exercise test was used to measure aerobic capacity ($\dot{V}O_{2max}$) and ventilatory threshold via direct gas exchange measured by a COSMED Quark CPET (COSMED, Concord, CA). The error of the Quark CPET has been shown to be in the 4-12% range (2, 8). A speed-based protocol was used with stages that were metabolically equivalent (MET) to the Bruce protocol. This protocol included two-minute stages at a constant 2% incline. The speeds were as follows: 6.4, 7.9, 10.0, 11.7, 13.7, 15.6, 17.1, 18.2, 19.8, 21.1 $km \cdot h^{-1}$ (29). Subjects continued the test with encouragement from the laboratory staff until volitional fatigue. $\dot{V}O_{2max}$ was determined using breath-by-breath analysis consisting of 30 second rolling time averages. At least three of the following criteria were met verifying the attainment of $\dot{V}O_{2max}$: a leveling off or plateauing of $\dot{V}O_2$ with an increase in exercise intensity, attainment of age predicted maximal heart rate (HR_{max}), a respiratory exchange ratio greater than 1.10, and/or an RPE ≥18. Heart rate was continuously monitored to obtain HR_{max} (Polar H7; Polar Electro Co., Woodbury, NY, USA). Subject's ventilatory threshold was calculated after the completion of each test as the point where ventilation increased nonlinearly with $\dot{V}O_2$, which is expressed as a percentage of $\dot{V}O_{2max}$. Body composition and performance data are presented in *Table 1*.

INSERT TABLE 1 ABOUT HERE

Biomarker Collection and Analysis: Athletes were instructed to report for blood draws following an overnight fast in a normally hydrated state. Athletes were instructed to drink water *ad libitum* before arrival. Blood draws occurred prior to the start of pre-season (S1), and every four weeks following, (S2) (S3) & (S4). S4 occurred 25 days prior to the last competitive game of the season as logistics prevented biomarker assessments post season. All blood draws were taken between 0700-0900 hours. Blood draws at S2, S3, and S4 occurred ~18-36 hours following a game.

Blood samples were obtained (antecubital fossa, 21G, BD Vacutainer, Safety-Lok) from the antecubital vein in the arm by an experienced phlebotomist while subjects were seated. Whole blood was collected and left to clot at room temperature. Samples were drawn into collection tubes consisting of anticoagulant (EDTA), or clot activator (gel free and Serum Separator Tubes). Following collection, blood was centrifuged for 10 minutes at 4,750 revolutions·min^{-1} (Allegra x-15R, Beckman Coulter, Brea, CA, USA). Plasma and serum samples were shipped (in containers designed to maintain approximately 20°C, 4°C, or -20°C depending on the analyte) to a Clinical Laboratory Improvements Amendment (CLIA) certified processing facility (Quest Diagnostics, Inc., San Juan Capistrano, CA, USA) for analysis. Samples were run in duplicate and the coefficient of variation for all biomarkers were between 0.5 – 10.0 %. Biomarkers in the analysis included markers of stress (free cortisol [FCORT], total cortisol [TCORT]) muscle breakdown/turnover (creatine kinase [CK]), inflammatory markers (interleukin-6 [IL-6]), markers of anabolism (growth hormone [GH], insulin-like growth factor-1 [IGF-1], total testosterone [TTEST], free testosterone [FTEST]), markers of reproductive health (total estrogens [E], prolactin [PRL], sex hormone binding globulin [SHBG]), markers of metabolism (tri-iodothyronine [T_3] , thyroxine [T_4]) and nutritional markers (iron [Fe], tryptophan [TRP], glutamine [GLN], phenylalanine [PHE], and taurine [TAU]) (23).

Season Training and Monitoring: All practices and games were monitored using the Polar TeamPro system (Polar Electro Co., Woodbury, NY, USA) which utilizes HR, GPS, and accelerometry technology (21). Physiological attributes of the player obtained from laboratory testing (i.e., player age, height, weight, sex, $\dot{V}O_{2max}$, HR_{max}, and HR at ventilatory threshold) were used to program each player's monitor through the Polar TeamPro online platform.

Training metrics were assessed during all practices and games and included total DIS, EEE (9, 13), time spent in HR zones expressed as a percentage of HR_{max}, (HR_{Z1}: 50-59%; HR_{Z2}: 60-69%; HR_{Z3}: 70-79%; HR_{Z4}:80-89%; HR_{Z5}: 90-100%) and distance covered in speed zones (DIS_{Z1}=3.0-6.99 km/h; DIS_{Z2}= 7.0-10.99 km/h; DIS_{Z3}=11.0-14.99 km/h; DIS_{Z4}=15.0-18.99 km/h; DIS_{Z5}= ≥ 19 km/h) based on similar speed zones used by female athletes (3). A total TL score was also determined for each practice and game which was calculated via an algorithm developed by Polar^{TM} based on the quantification of an individual player's output. GPS signal acquisition is confirmed for each practice and game from a mobile tablet containing the Polar TeamPro software. Confirmation required a minimum of four satellites for the athlete's unit number to appear on the tablet.

Subjective Psychological Wellness and Sleep: Prior to the start of preseason (S1), and every four weeks following (S2, S3, S4) paper-based surveys were administered and completed by the athletes before the start of practice. These surveys included the Multi-Component Training Distress Scale (MTDS) to monitor subjective athlete wellness (26) and the Pittsburgh Sleep Quality Index to determine sleep quality (SQ) and duration (SD) (6). The MTDS included the composite score: total training distress (TTD), and the six subscales: depressed moods, vigor, physical signs and symptoms, sleep disturbances, perceived stress, and general fatigue. The MTDS consisted of 22-item questionnaire in which responses were formed from a Likert scale with 0 corresponding to "Not at all" and 5 corresponding to "extremely". For all subscales, scores were calculated based on the sum of the questions (Q) for each category (e.g. depressed moods: 5Q, vigor: 4Q, physical signs and symptoms: 3Q, sleep disturbances: 3Q, perceived stress: 4Q, and general fatigue: 3Q). All questions regarding vigor were positively weighted in the questionnaire and therefore responses were reverse scored when included in the composite (TTD) score and when reporting (i.e. higher scores represent greater vigor). Higher scores on the Pittsburgh Sleep Quality Index were representative of adverse sleep.

Countermovement Vertical Jump Height: Survey completion was followed by a 10-15 min generalized dynamic warmup and subsequent maximal CMJ height assessments. Subjects were given two attempts at CMJ assessed using a digital contact mat (Just Jump system, Probotics, Huntsville, AL, USA) with the highest jump height recorded. Athletes maintained their hands on their hips for all jumps. Athletes were given 30-45 seconds between jump attempts. If form was incorrect, athletes were instructed to repeat attempt after guidance from the researcher. The error of vertical jump height for females has been reported with a standard error of the mean of 1.7 cm and a coefficient of variation of 4.4% (33). Following S1, this protocol was continued every 4 weeks during the first practice of the week, consistent with the same schedule as blood draws (S2, S3 & S4).

Statistical Analysis: Repeated measures (RM)-MANOVAs were performed with univariate follow-ups to assess changes in biomarkers, CMJ performance, and training metrics (TL, DIS, EEE) throughout the season. For each univariate analysis, the Huynh-Feldt epsilon was examined for the general model to evaluate sphericity. If the Huynh-Feldt epsilon exceeded 0.75, sphericity was considered to have been met, and the unadjusted statistic was used. If epsilon was less than 0.75, the adjusted Huynh-Feldt statistic was used to test significance. 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. Friedman's ANOVAs were performed for all non-parametric data, including the PSQI and MTDS metrics. In the case of significant findings, the Wilcoxon signed rank test was used with S1 values as the comparison term. A reliability analysis was carried out on the MTDS questionnaire comprising 22 items. Cronbach's alpha showed the questionnaire to reach acceptable reliability, $\alpha = 0.77$. In addition, all subscale constructs included reached acceptable reliability (e.g. depressed moods $\alpha = 0.87$, vigor $\alpha = 0.93$, physical signs and symptoms $\alpha = 0.90$, sleep disturbances $\alpha = 0.80$, perceived stress $\alpha = 0.77$, and general fatigue $\alpha = 0.90$). All analyses were conducted using SPSS Statistical Software (SPSS version 26; IBM) with significance set at $p \leq 0.05$. Cohen's *d* was used to calculate effect sizes (ES) from baseline. Using Cohen's conventions, ES of 0.20, 0.50, and 0.80 considered indicative of small, medium, and large effects, respectively (10).

RESULTS

Training Load Metrics*:* Between time points, the total sum of TL DIS, and EEE were used as indications of athlete workload over the 4-week periods. S1 to S2 consisted of 22 practices and 4 games, S2 to S3 consisted of 13 practices and 6 games, and S3 to S4 consisted of 14 practices and 7 games. The greatest total TL, DIS, and EEE was seen at the initial training block, which included preseason (S1-S2), compared to all subsequent training blocks ($p<0.001$). Following S1-S2, a substantial decrease in TL occurred at S2-S3 (ES=-2.5; $p<0.001$), before stabilizing from S3-S4. DIS was highest at S1-S2 ($p<0.001$), decreased S2-S3 (ES= -1.19; $p<0.001$) and stabilized from S3-S4. Following the initial training block, there was a substantial decrease in EEE (S2-S3: ES= -2.08; $p<0.001$), before normalizing through the last training block (S3-S4). TL, EEE, and DIS accumulated at practices and games during the four-week time periods are represented in Figures 1-3. Time spent in HR zones (1-5) and distances covered in high-speed running zones (1-5) during the four-week time periods are presented in Figures 4 & 5.

INSERT FIGURES 1-5 ABOUT HERE

Biomarker Response: All biomarker data are presented in *Table 2*. FCORT increased from S1-S2 (ES= 0.88; p=0.03) and returned to baseline by S4. Compared to S1, there was a decline in TCORT at S4 (ES= -0.41; p=0.03), but no significant differences at S2 or S3 (p>0.05). CK increased from S1 to S2 (ES= 1.74; p=0.03), then returned to baseline by S3 and remained steady through S4. IL-6 significantly increased above baseline by S4 (ES= 0.71; p=0.02). FTEST increased from S1 to S2 (ES=1.27; p<0.001). Although not significant, FTEST remained moderately elevated above baseline at S3 (ES=0.51; p=0.057) before returning to baseline by S4. TTEST followed a similar pattern with an increase from S1 to S2 (ES=3.5; p=0.02) and a return to baseline by S3. No statistically significant changes were seen in SHBG; however, modest declines were observed from S1 to S4 (ES= -0.13; p=0.07). There were no differences from baseline values throughout the season for E, PRL, or T_3 (p>0.05). T_4 increased from S1 to S2 (ES=0.81; p=0.003), declined below baseline at S3 (ES=-0.65; p=0.001) and then returned to baseline at S4. Compared to baseline, GH significantly decreased at S2 (ES= -0.50; p=0.01) and remained depressed for the remainder of the season (ES_{S3}=-0.56; p=0.001; and ES_{S4}=-0.60; p=0.002). IGF-1 significantly declined from S1 to S3 (ES=-0.54; p=0.02), before returning to baseline at S4. Fe declined from S1 to S2 (ES= -0.73; p=0.001). Although not significant, Fe remained moderately suppressed at S3 (ES=-0.42; p=0.09) before returning towards baseline values at S4. GLN increased at S2 (ES= 1.07; p=0.002) and remained elevated at S3 (ES= 0.63; p=0.03) before returning to baseline at S4. The moderate increase in TRP from S1 to S2 approached significance (ES=0.62; p=0.08), followed by a steady decline which approached significance at S4 (ES=-0.39; p=0.09). There were no significant changes in PHE and TAU from S1 throughout the season (p>0.05).

INSERT TABLE 2 ABOUT HERE

Psychological Response: All changes in scores represent the increases or decreases in subscales construct. TTD declined from S1 to S2 (ES=-0.38; p=0.02) and increased from baseline by S4 (ES=0.57; p=0.01). Compared to baseline values, depressed mood became worse at S4 (ES=0.62; p=0.03). Vigor decreased from S1 to S2 (ES= 0.48; p=0.03) and continued to decline below baseline values (ES_{S3}=1.28, ES_{S4}=1.47; p<0.001). No changes in physical signs and symptoms or as sleep disturbances were observed. Perceived stress decreased from S1 to S2 (ES=-0.89; p<0.001) before returning to baseline at S3 (p>0.05). Although not significant, reported general fatigue moderately increased at S3 (ES=0.52; p=0.056) compared to baseline before increasing above baseline at S4 (ES=0.61; p=0.01). See *Table 3* for the full change in MTDS subscales.

INSERT TABLE 3 ABOUT HERE

Sleep Quality and Duration Response: No changes were seen in SQ or SD from baseline values (P>0.05).

Performance Response: CMJ declined from S1 to S2, (ES=-0.51; p=0.001) before returning to baseline values at S3. Although not significant, there was a small decline in CMJ from baseline at S4 (ES=-0.22, p=0.079). See Figure 6 for full changes in CMJ throughout the season.

INSERT FIGURE 6 ABOUT HERE

DISCUSSION:

Monitoring the demands of a women's competitive collegiate soccer season with various assessment methods, including both objective physiological data and subjective psychological wellness evaluations, may provide a more holistic picture of the stress incurred by the athlete. In the current study, the greatest workloads and energetic demands occurred during the initial training block (S1-S2). For collegiate soccer athletes this period consists of a short, condensed preseason period comprised of high training volumes and often two training sessions per day combined with the first two weeks of the regular season (40, 41). The greatest changes in biomarkers occurred following this period along with declines in both CMJ height and vigor, despite improvements in TTD and perceived stress. Several biomarker perturbations, such as decreased GH and increased FCORT, in addition to reduced vigor persisted well into the season, which may indicate that the athletes never fully recovered from the stress incurred during the initial training block. Further, elevated IL-6 along with increased TTD, fatigue, depressed moods, and modest reductions in TRP and CMJ from baseline occurred in the latter half of the season (S3-S4). These later changes occurred despite workload remaining relatively stable. As such, time course of change in various biomarkers may point to an accumulation of fatigue that occurred as the season progressed. These findings highlight the need for physiological monitoring techniques in conjunction with subjective psychological measures, as taken together a multitude of assessment techniques may better indicate the progression of fatigue and may provide warning signs for overreaching.

Comprehensive biomarker monitoring can provide insight into the physiological changes athletes experience throughout a season (40). Chronic elevations in resting cortisol can indicate a maladaptation of the HPA-axis (30) and an impaired ability to recover from training (23). Significant elevations in the metabolically active FCORT were apparent at S2, immediately following the preseason time block, and persisted into S3. It is important to note that although TCORT levels were maintained followed by a decline at S4, TCORT values were above the normal clinical reference ranges at all time points (127-569 nmol/L when assessed 0800-1000h). The above range values may indicate a persistent catabolic state in these athletes throughout the season. CK, an indicator of muscle damage (23), increased nearly 56% from S1 values after the initial training block. Although within athlete-specific reference ranges, the increased CK seen at S2 in conjunction with increases in FCORT, indicates the need for enhanced recovery strategies during this time block to combat the increase in training related stressors. Interestingly elevations in IL-6, a pro- and anti-inflammatory cytokine, occurred at S4, in conjunction with increases in TTD, fatigue, depressed moods, reduced vigor, and modest declines in CMJ height. Although IL-6 has been shown to increase in response to decreased muscle glycogen, muscle contraction, and muscle damage in order to activate an immune response (34), it has also been implicated in the production and enhancement of negative mood states, sleep disturbances, and fatigue (24, 27).

Testosterone, often used in conjunction with cortisol to provide a relative indication of an anabolic status in male athletes (22, 23), may play a role in helping to maintain performance by reducing protein breakdown and promoting protein synthesis (23). FTEST and TTEST significantly increased following the preseason training block. We speculate that this upregulation in testosterone may have been a physiological response to combat the stress of the first training block, although more research is warranted regarding testosterone's response to overload training, specifically in women. Other biomarkers that changed in response to the stress of the season include the anabolic hormones, IGF-1 and GH, both of which are involved in muscle protein synthesis and the regulation of muscle mass (23) and may play a significant role in the anabolic response in women. Previous research evaluating acute GH responses to exercise have found blunted effects in overreached compared to healthy subjects (7). Relative declines in IGF-1 and chronic reductions in GH seen throughout the season may impact muscular adaptations to training (23). In addition, IGF-1 has been shown to be associated with energy status, as inadequate energy intakes to match high volumes of training have been shown to reduce IGF-1 (12).

Additionally, thyroid hormones respond to energy demands and may act to maintain metabolic function during training in order to accommodate the increased EEE. Conversely, thyroid hormones may act by reducing metabolic function in times of high EEE to provide an "energy sparing" effect contingent on training demands and energy intake (40). Free T_4 increased following S2 before declining below baseline at S3, which may be reflective of the high caloric expenditures experienced in these athletes. Alterations in free T_4, which is converted to the biologically active form, T_3 may be reflective of the variations in workload. Previous research has shown that chronic elevations in cortisol suppress the conversion of T_4 to the active T_3 (28), which may have contributed to the lack of changes observed in T_3 throughout the season. Examining the overlap between the HPA- and hypothalamic-pituitary-thyroid (HPT)-axes during prolonged periods of high workloads and energy expenditures may aid in understanding changes in metabolic markers throughout the season.

Other nutritional markers may play a role in evaluating athlete recovery and fueling strategies. Total Fe declined significantly after the first training block before returning to baseline values. Although not clinically classified as "iron-deficient", this decrease represents a ~32% decline from baseline. Monitoring relative changes in Fe stores throughout the season may provide an opportunity to intervene before deficiencies can occur and impact performance. The amino acid GLN has been studied in association with overreaching for its role in immune system function (32), with decreased concentrations of GLN reported during prolonged chronic exercise and heavy training (19, 35). Interestingly, GLN increased throughout the middle of the season which may suggest immune system compensation. Tracking GLN in conjunction with rates of infection, illness, and select immune markers throughout the season may help clarify the utility of this marker as an indicator of recovery status. Of additional importance is the amino acid TRP, the precursor for serotonin, which in conjunction with IL-6 may provide a mechanism for understanding changes in mood typically reported with overreaching (39). Increased brain levels of serotonin are believed to result in mood and behavioral changes such as inducing sleep and reducing appetite, both behaviors evident in overreaching and overtraining (39). TRP displayed a moderate effect for an increase at S2 when TTD, perceived stress, depressed moods, physical signs and symptoms and fatigue were the lowest. Meanwhile, the small decline in TRP at S4 corresponded to elevations in general fatigue, depressed mood, and TTD. Although PHE and TAU may provide an indication of protein turnover in athletes (23), no significant changes were seen throughout the season despite elevations in CK. Monitoring nutritional markers is important to assess overall athlete health or to provide justification for the implementation of nutrition education programs, particularly for athletes that experience high energetic demands.

In addition to tracking physiological response, monitoring athletes' psychological state and perceived wellness can identify athletes who are negatively responding to training (30), with low vigor and high ratings of fatigue and depression indicative of a maladaptive training response (31). In the current study, the greatest perturbation in athlete wellness occurred at S4, which included significant increases in TTD, fatigue, depressed moods and declines in vigor. Similar results have been found using the Profile of Mood States throughout professional, university and recreational soccer seasons (25). Researchers found the greatest change in negative mood states occurred for professional players as the seasons progressed, suggesting psychological changes may be associated with the total demands of a highly-competitive soccer season (25). Interestingly in the current study, compared to baseline values, the lowest scores for TTD, perceived stress, physical signs and symptoms, fatigue, and depressed moods occurred after the period of highest workload. These positive psychological changes occurred in conjunction with declines in CMJ performance, indicating factors other than training load alone may be driving psychological responses.

Subjective measures, while helpful in determining athletes' perceptions of wellness, may also be reflective of environmental stressors. In these athletes, the greatest perceived stress (at S1) may have been in anticipation of the upcoming season, and therefore may have allowed for improvements as the season progressed and team dynamics became established. For example, the high perceived stress could be a result of the upcoming physical demands of the condensed preseason, the need to perform for a starting position, or related to new experiences inherent to a collegiate environment. Although speculative, this could aid in explaining the improvements observed in several measures of athlete wellness (TTD, perceived stress) that occurred after the first training block, despite physiological perturbations and downturns in CMJ performance. Alternatively, certain subscales may not have been sensitive enough to detect changes along with workload fluctuations. It is important to note, psychological questionnaires rely on self-report data that can be manipulated either intentionally or unintentionally. Although confidentiality of survey responses was emphasized to the athletes, survey recording occurred in the team environment. It may be players fear their honest responses will negatively reflect playing time or make them look "weak" in the eyes of coaches or teammates. Further, the frequency of administration and length of the survey also affects reporting accuracy (38). For these reasons, it may be beneficial to use subjective measures of athlete readiness alongside objective measures of physiological responses to training, especially in collegiate team sports.

In addition to subjective psychological surveys, questionnaires designed to evaluate sleep are also an important means of assessing recovery (37). Although no reported changes in sleep disturbances, quality, or duration occurred throughout the season, this could be influenced by the sensitivity of subjective measures to detect SQ changes in a real-world setting using actively training athletes. Future research should consider alternative, objective methods of tracking sleep, as these methods may be advantageous to determining SQ in response to fluctuating workloads in collegiate athletes.

Previous research has shown CMJ height, an effective measure of lower limb muscular power ability, to be related to soccer performance across a season (1, 43). Following the highest training load block, CMJ height declined, but then returned to baseline before beginning to modestly decline at S4 (which approached significance). This final decline occurred when the athletes were entering the most critical time of the competitive season: tournament play, which consisted of post-season appearances in a division tournament final and the NCAA tournament. It appears CMJ may have been sensitive enough to detect changes in athlete readiness throughout the season. Therefore, evaluating changes in performance outcomes, such as vertical jump, that can be rapidly tested without adding additional fatigue to the athletes may help coaches to better prepare training sessions and recovery strategies, particularly upon entering tournament play.

This study sought to evaluate the effects of a competitive collegiate season on various markers of fatigue in an applied setting within a team environment; however, there are certain limitations that are inherent to the study. First, a post-season blood draw in conjunction with maximal performance testing would have been ideal to shed light on the physiological state of these athletes following tournament play. Unfortunately, this was not possible due to the tournament structure and travel logistics. In addition, diet and nutritional supplementation, which may influence biomarker responses as well as overall recovery status, was not assessed during the season. While a limitation, the feasibility and accuracy of self-reported dietary intakes make assessments difficult to achieve in a team setting (5). Further considerations specific to female athletes such as hormonal contraceptive use and menstrual status may further influence several performance and biomarker measures over the course of the season. Given the nature of the applied setting, researchers sought to evaluate typical conditions affecting a women's competitive soccer season in an effort to increase the generalizability of the study. As such, although no changes were seen in E, SHBG, or PRL throughout the season, these reproductive hormones may be affected by menstrual status and hormonal contraceptive use and thereby impact the physiological adaptations to training seen throughout the study. Further research is warranted to determine the influence of hormonal contraceptives and menstrual status on these markers.

Despite these limitations, this study sheds a novel light on the effect of a collegiate soccer season on various physiological and psychological responses. The time course of change in markers of athlete readiness revealed the greatest physiological disruptions and performance decrements occurred following the highest workload period. Yet, the findings of this study also point to an accumulation of fatigue as the season progressed and stress the need to determine optimal loading and appropriate periodization by coaches and training staff during the collegiate preseason time block. While no one measure was shown in isolation to indicate fatigue, a combination of monitoring techniques may better aid in the evaluation of athlete health and readiness throughout a women's competitive soccer season.

PRACTICAL APPLICATION:

Appropriately structured training is essential to athlete recovery and performance throughout a competitive season. Tracking workload is an important first step to determine the on-field training stress imposed on the athlete. However, the cumulative training stress is not always reflected in a ~2-hour training session. Therefore, the use of multiple monitoring techniques may provide better clarity regarding individual player's response to the season demands. When selecting a monitoring technique, it is important to consider the efficacy and sensitivity of that method to detect fatigue and insufficient recovery, while also minimizing the burden on the athlete. While subjective measures may be helpful in determining how athletes respond to training and environmental stressors, tracking changes in a multitude of biomarkers may give a more comprehensive and objective assessment of the athlete's physiological load as well as insight into recovery status and overall athlete health. Therefore, a combination of subjective and objective monitoring techniques may be most useful to combat maladaptive training responses from the accumulated stress of a competitive season and may afford an opportunity for intervention before declines in performance can occur.

ACKNOWLEDGEMENTS

Funding Provided by Quest Diagnostics

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Figure Legends:

Figure 1: Changes in Training Load throughout the Season.
Au=arbitrary units
Black bars represent total training load accumulated from games during the four-week time blocks
Gray bars represent total training load accumulated from practices during the four-week time blocks
(*) Denotes significant difference from (S1-S2) $p<0.05$
Values represent Means

Figure 2: Changes in Exercise Energy Expenditure throughout the Season.
Kcals=calories expended
Black bars represent exercise energy expenditure accumulated from games during the four-week time blocks
Gray bars represent total exercise energy expenditure accumulated from practices during the four-week time blocks
(*) Denotes significant difference from (S1-S2) $p<0.05$
Values represent Means

Figure 3: Changes in Distance Covered Throughout the Season.
KM=kilometers
Black bars represent total distances accumulated from games during the four-week time blocks
Gray bars represent total distances accumulated from practices during the four-week time blocks
(*) Denotes significant difference from (S1-S2) $p<0.05$
Values represent Means

Figure 4: Time Spent in Heart Rate Zones as a Percentage of HR_{max}
Values represent Means
HRZ=Heart Rate Zone
bars represent time spent in HR_{Z1}:50-59% of HR_{max} accumulated during the four-week time period
bars represent time spent in HR_{Z2}:60-69% of HR_{max} accumulated during the four-week time period
bars represent time spent in HR_{Z3}:70-79% of HR_{max} accumulated during the four-week time period
bars represent time spent in HR_{Z4}:80-89% of HR_{max} accumulated during the four-week time period
bars represent time spent in HR_{Z5}: 90-100% of HR_{max} accumulated during the four-week time period

Figure 5: Distance Covered in Speed Zones
Values represent Means
DISZ=Distance Zone
bars represent distance covered in DIS_{Z1}=3.0-6.99 kilometers/hour accumulated during the four-week time period
bars represent distance covered in DIS_{Z2}= 7.0-10.99 kilometers/hour accumulated during the four-week time period
bars represent distance covered in DIS_{Z3}=11.0-14.99 kilometers/hour accumulated during the four-week time period
bars represent distance covered in DIS_{Z4}=15.0-18.99 kilometers/hour accumulated during the four-week time period
bars represent distance covered in DIS_{Z5}= ≥ 19 kilometers/hour accumulated during the four-week time period

Figure 6: Counter Movement Vertical Jump.
Values represent Means ± Standard Deviations.
(*) Denotes significant difference from S1. P<0.05

Table 1

Performance and Body Composition Metrics	
Body Fat (%)	20.8 ± 3.1
Fat Free Mass (kg)	51.6 ± 5.0
VO_{2max} ($ml \cdot kg^{-1} \cdot min^{-1}$)	51.2 ± 2.8
Ventilatory Threshold (% of $\dot{V}O_{2max}$)	84.2 ± 3.4
Time to exhaustion (min)	11.38 ± 1.1
Average final speed ($km \cdot h^{-1}$)	15.6

Table 1: Preseason Performance Testing.
Values represent Means ± Standard Deviations

Table 2

Biomarkers	Units	S1	S2	S3	S4
FCORT	nmol/L	29.45 ± 10.0	38.33± 14.6*	35.12± 9.8*	29.80± 09.7
TCORT	nmol/L	720.91 ± 333.8	675.32± 203.2	704.46± 281.7	585.34± 280.7*
CK	U/L	160.96 ± 118.0	365.84 ± 461.3*	200.88 ± 133.5	213.16 ± 143.4
IL-6	pg/ml	1.77 ± 1.3	1.84 ± 1.1	2.66 ± 2.5	2.69 ± 1.35*
FTEST	nmol/L	9.00 ± 6.3	17.01±8.0*	12.21± 6.6	11.31±8.4
TTEST	nmol/L	1.10 ± 0.4	2.50±2.9*	1.42±1.0	1.08±0.5
E	ng/L	196.56 ± 88.1	198.91± 113.3	173.59± 78.3	204.82± 109.2
SHBG	nmol/L	81.48 ± 61.2	78.52 ± 61.5	76.12 ± 59.1	73.32 ± 55.6
T_3	nmol/L	1.76 ± 0.5	1.80± 0.5	1.76 ± 0.4	1.85 ± 0.4
T_4	nmol/L	14.05 ± 1.4	15.18 ± 1.7*	13.13 ± 1.6*	14.38 ± 2.3
GH	ng/mL	4.30 ± 4.2	2.23 ± 2.1*	1.94 ± 3.4*	1.78 ± 2.5*
IGF-1	ng/mL	299.04 ± 95.1	280.84 ± 95.7	248.00 ± 66.1*	281.60 ± 77.9
Fe	Umol/L	16.15 ± 7.3	10.85 ±5.8*	13.07 ±6.2	16.22 ±6.0
PRL	ng/mL	17.02 ± 8.7	20.50 ± 12.5	19.31 ± 8.3	19.31 ± 8.6
GLN	Umol/L	530.32 ± 76.9	612.44 ± 105.6*	579.00 ± 86.6*	540.24 ± 93.1
TAU	Umol/L	40.44 ± 9.6	44.04 ± 11.8	38.44 ± 12.6	38.40 ± 12.9
TRP	Umol/L	64.80 ± 13.9	73.12 ± 22.3	62.24 ± 12.9	59.32 ± 13.6
PHE	Umol/L	70.56 ± 9.3	66.40 ± 10.9	72.08 ± 11.03	70.68 ± 9.2

Table 2: Biomarker Values Throughout the Season. FCORT=free cortisol, TCORT=total cortisol, CK=creatine kinase, IL-6=interleukin-6, FTEST= free testosterone, TTEST=total testosterone, E=total estrogen, SHBG=sex-hormone binding globulin, T_3=free tri-iodothyronine, T_4= Total thyroxine, GH=growth hormone, IGF-1=insulin-like growth factor-1, Fe=iron, PRL=prolactin, GLN=glutamine, TAU=taurine, TRP=tryptophan, PHE=phenylalanine.

$S_{1\text{-}4}$= Timepoints taken every four weeks throughout the season.

Values represent Means ± Standard Deviations.

(*) Denotes significant differences from baseline (S1).

Table 3

MTDS Subscales (AU)	Highest Possible Score	S1	S2	S3	S4
Total Training Distress↓	88	23.40 ± 10.5	19.00 ± 8.3*	27.04 ± 8.3	29.12 ± 11.7*
Depressed Moods ↓	20	1.08 ± 2.5	0.72 ± 1.9	1.84 ± 3.2	2.64 ± 3.5*
Vigor ↑	16	10.84 ± 2.9	9.44± 3.8*	7.12 ± 3.5*	6.56 ± 3.9*
Physical Signs & Symptoms ↓	12	6.12 ± 3.0	4.48 ± 3.3	4.80 ± 3.3	4.88 ± 3.9
Sleep Disturbances ↓	12	1.84 ± 2.8	2.12 ± 2.9	2.16 ± 2.0	1.52 ± 2.0
Perceived Stress ↓	16	5.00 ± 3.7	1.72 ± 1.9*	3.48 ±2.4	4.12 ± 3.2
General Fatigue ↓	12	4.16 ± 3.1	3.28 ±2.4	5.76 ± 3.1	6.04 ± 3.6*

Table 3: Changes in the Multi-Component Training Distress Scale (MTDS).

AU=Arbitrary Units

S_{1-4}= Timepoints taken every four weeks throughout the season.

Values represent Means ± Standard Deviations.

(*) Denotes significant differences from baseline (S1).

(↑) Indicates higher scores are better

(↓) Indicates lower scores are better

Figure 1

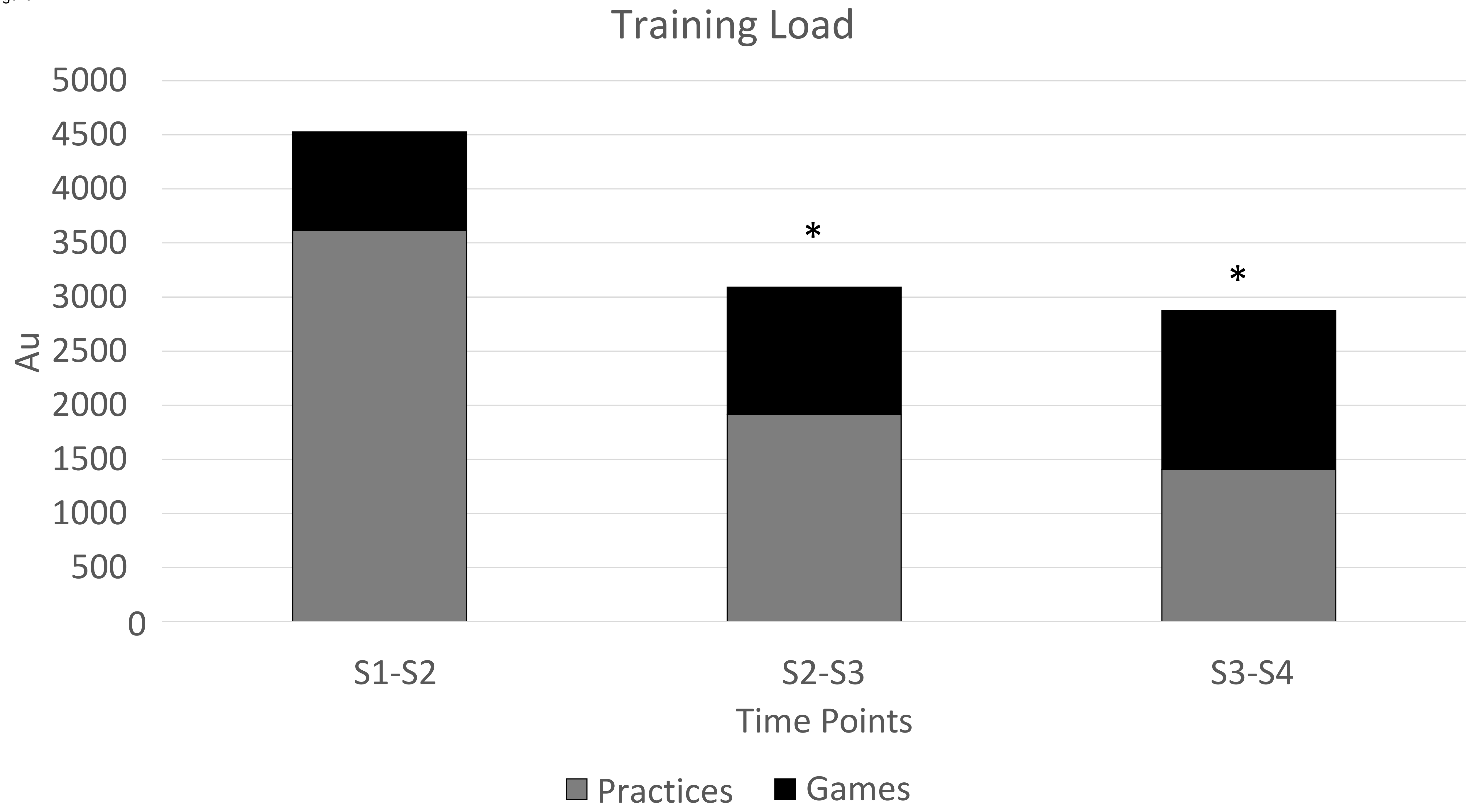
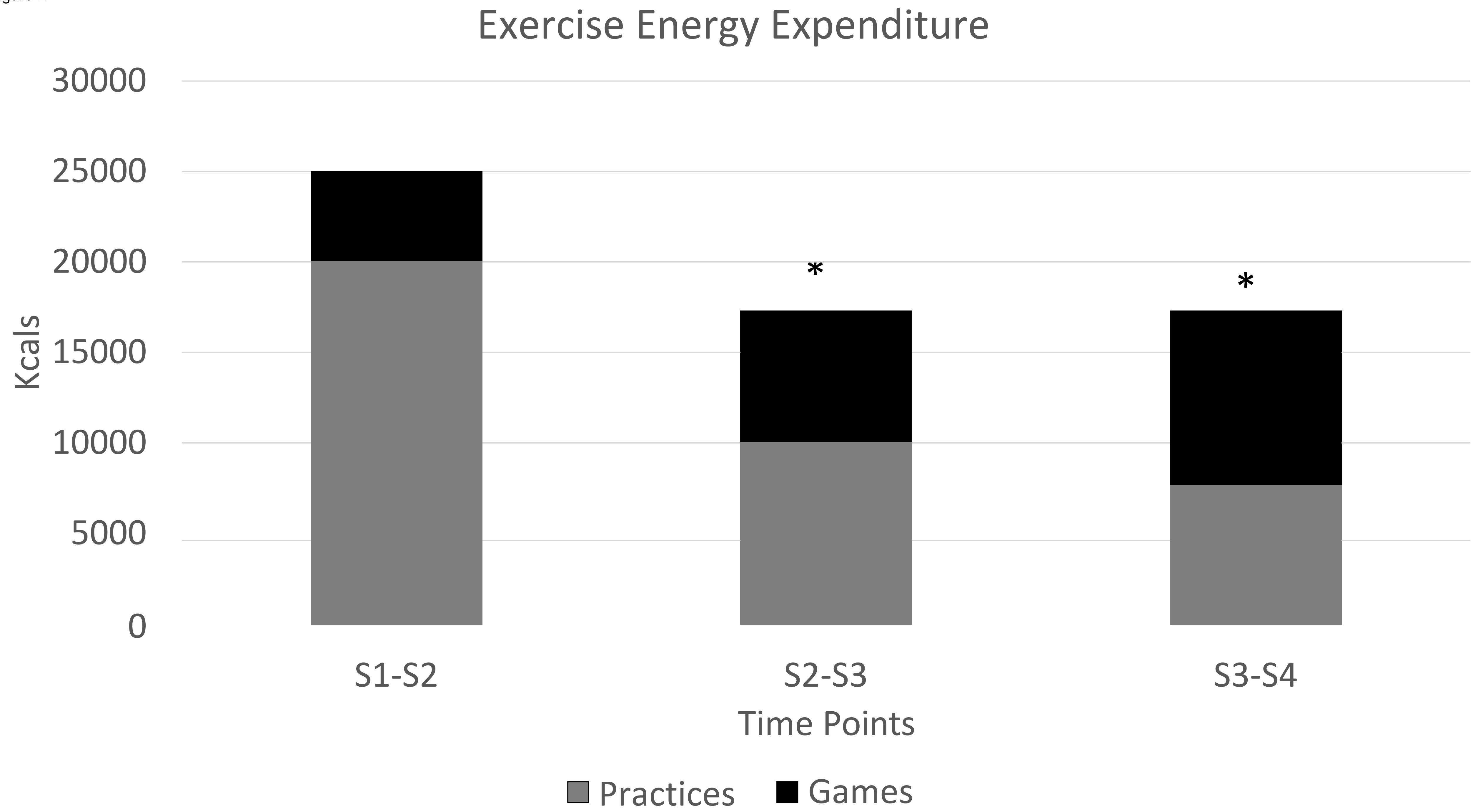

Training Load
5000
4500
4000
3500
3000
2500
2000
1500
1000
500
0
Au
*
*
S1-S2
S2-S3
S3-S4
Time Points
Practices
Games

Figure 2



Exercise Energy Expenditure
30000
25000
20000
15000
10000
5000
0
Kcals
*
*
S1-S2
S2-S3
S3-S4
Time Points
Practices
Games

Figure 3

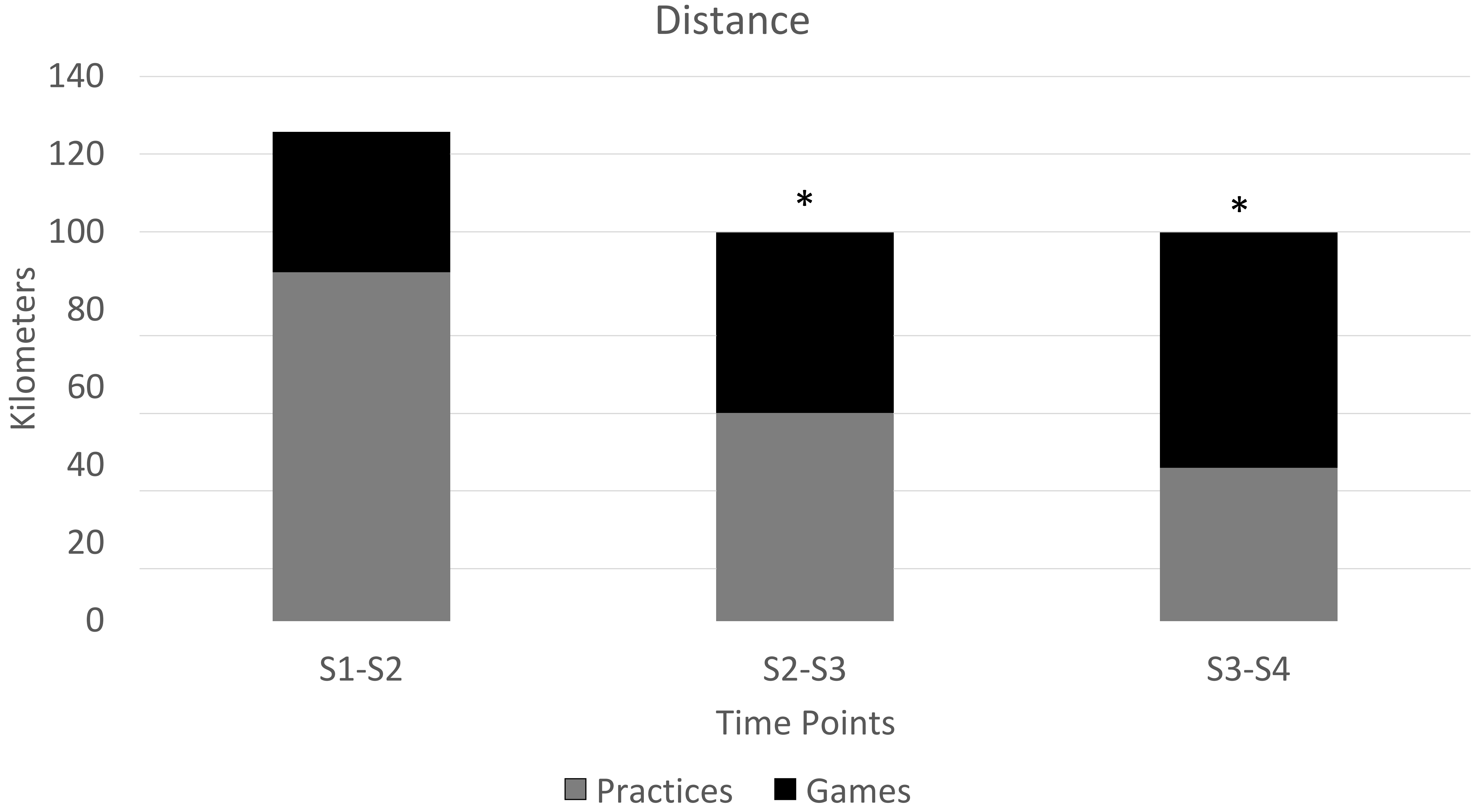
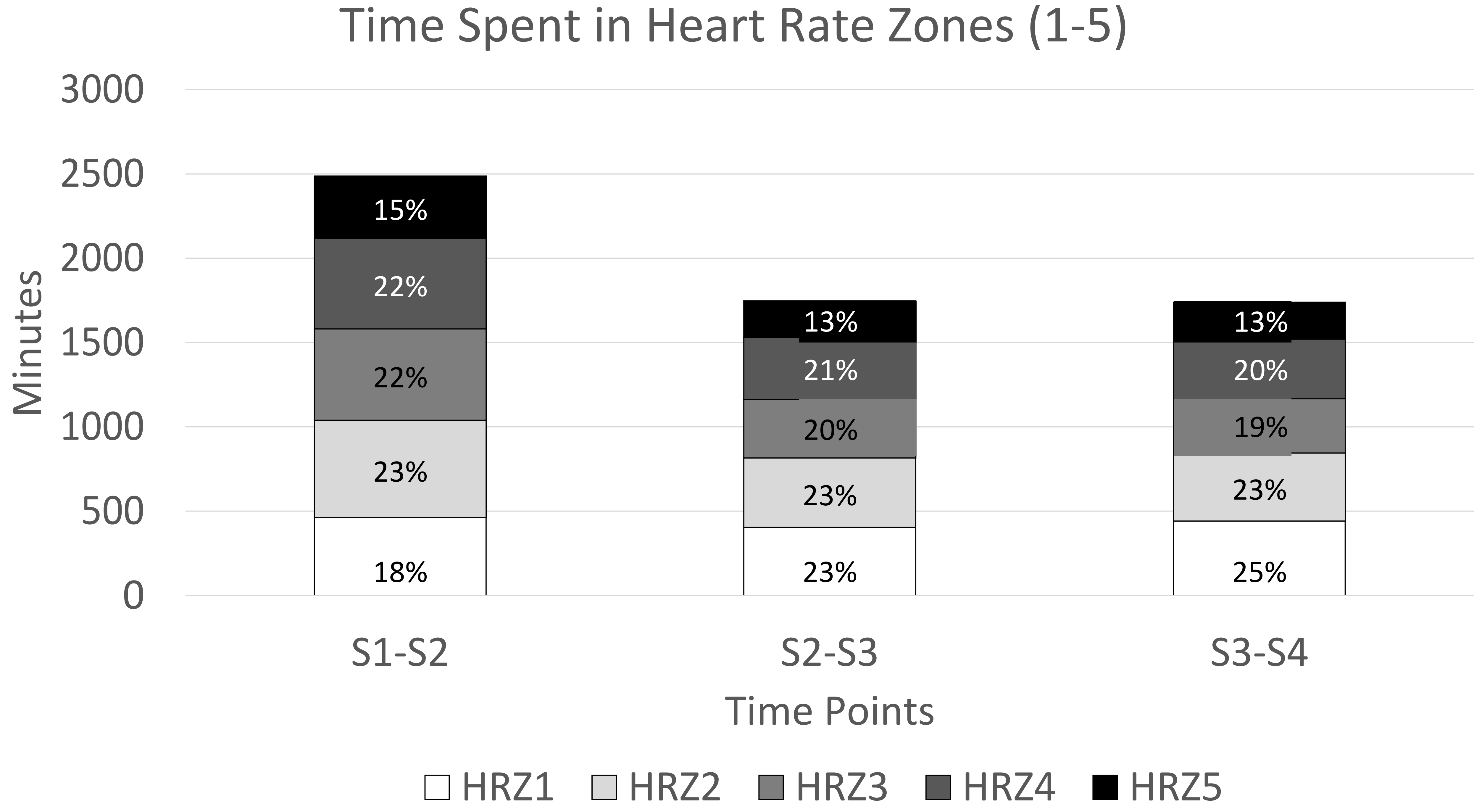

Distance
140
120
100
80
60
40
20
0
Kilometers
*
*
S1-S2
S2-S3
S3-S4
Time Points
Practices
Games

Figure 4



Time Spent in Heart Rate Zones (1-5)
3000
2500
2000
1500
1000
500
0
Minutes
15%
22%
22%
23%
18%
13%
21%
20%
23%
23%
13%
20%
19%
23%
25%
S1-S2
S2-S3
S3-S4
Time Points
HRZ1
HRZ2
HRZ3
HRZ4
HRZ5

Figure 5

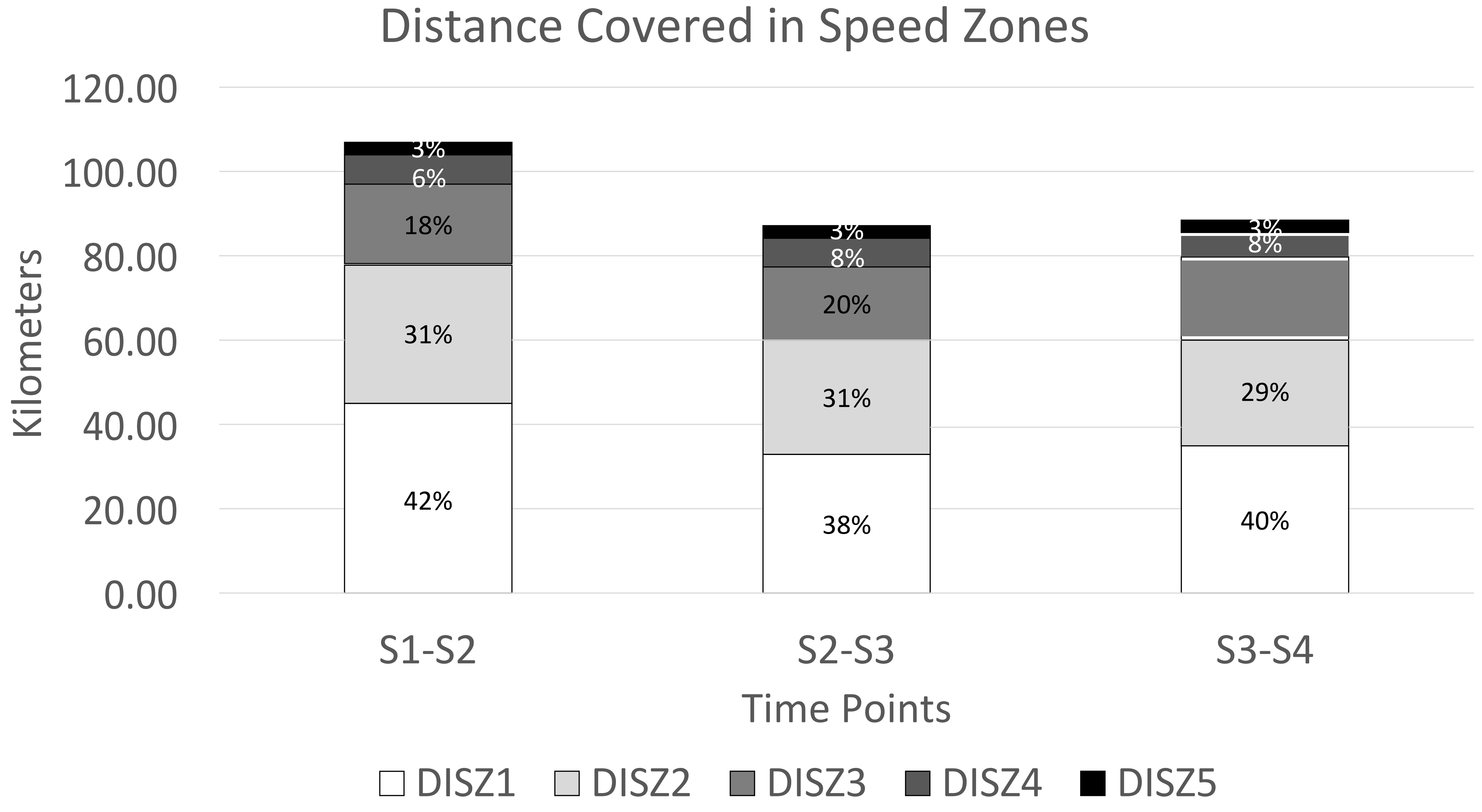
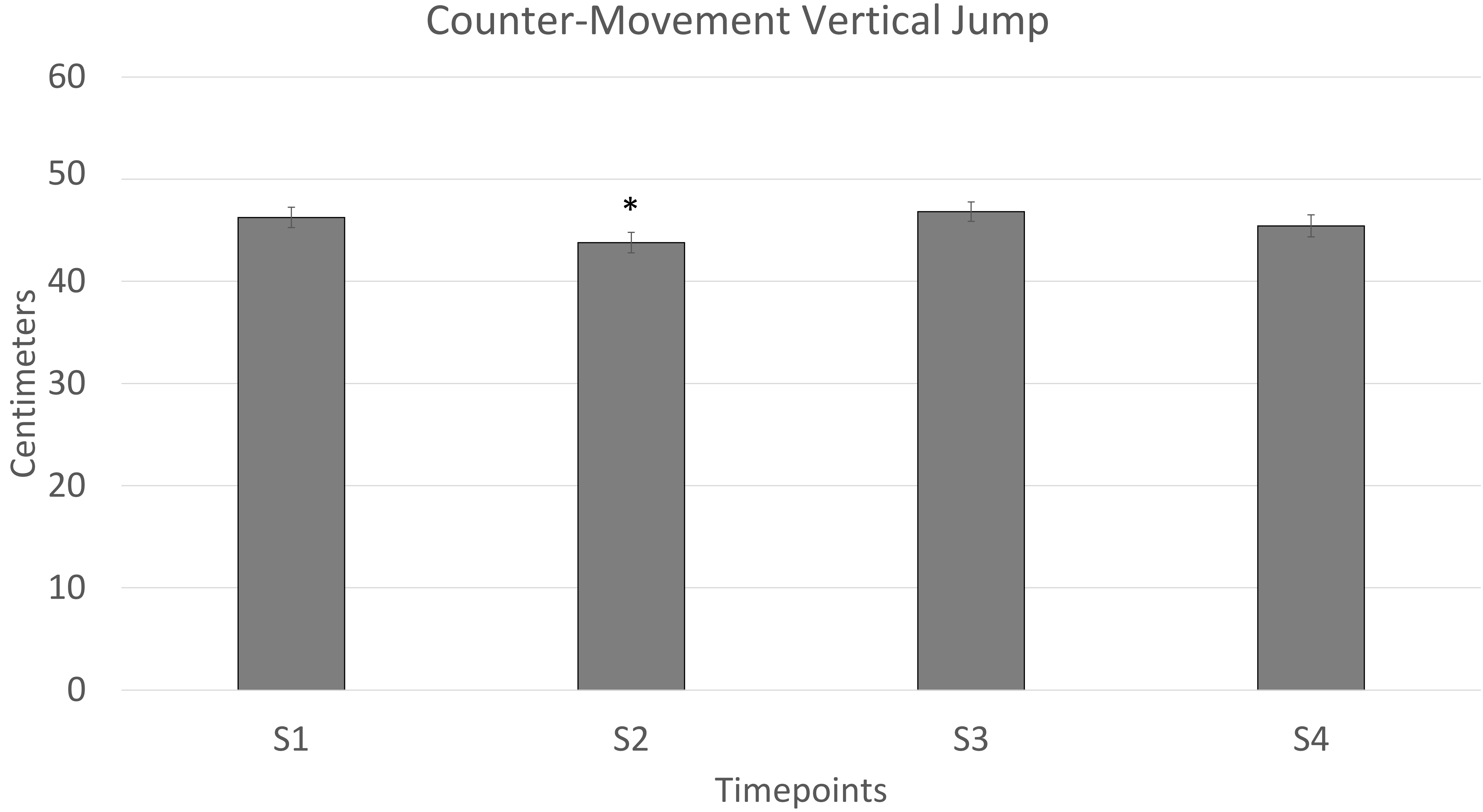

Distance Covered in Speed Zones
120.00
100.00
80.00
60.00
40.00
20.00
0.00
Kilometers
3%
6%
18%
31%
42%
3%
8%
20%
31%
38%
3%
8%
29%
40%
S1-S2
S2-S3
S3-S4
Time Points
DISZ1
DISZ2
DISZ3
DISZ4
DISZ5

Figure 6


Counter-Movement Vertical Jump
60
50
40
30
20
10
0
Centimeters
*
S1
S2
S3
S4
Timepoints