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

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

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43 **ABSTRACT:**
44 This study sought to determine the effects of a women's collegiate soccer season on
45 psychological markers, biomarkers, sleep, and performance. Athletes participated in maximal
46 countermovement vertical jump height (CMJ) assessments and biomarker monitoring in
47 conjunction with subjective measures of psychological wellness and sleep questionnaires prior to
48 preseason (S1) and every four weeks following (S2, S3, S4). Training was monitored during
49 practices and games using global positioning satellite systems and heart rate technology. Total
50 training load was highest from S1-S2, decreased from S2-S3 (effect size [ES]=-2.5; $p<0.001$),
51 and remained stable S3-S4. CMJ declined at S2 ($ES_{1-2}=-0.51$; $p=0.001$) and returned to baseline
52 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
53 baseline by S3 and S4 (free cortisol). Total cortisol was elevated throughout the season before
54 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
55 returned to baseline values at S4, whereas growth hormone declined at S2 ($ES_{1-2}=-0.50$; $p=0.01$)
56 and remained depressed. Interleukin-6 increased at S4 ($ES_{1-4}=0.71$; $p=0.02$). Total training
57 distress decreased from S1-S2 ($ES_{1-2}=-0.38$; $p=0.02$), returned to baseline by S3 and increased by
58 S4 ($ES_{1-4}=0.57$; $p=0.01$). No changes were observed in markers of sleep ($p>0.05$). Biomarkers
59 showed notable changes following the highest workload period (S1-S2), which coincided with
60 CMJ decrements. Biomarker perturbations preceded declines in subjective psychological
61 wellness (S4) which occurred in the latter half of the season, indicating an accumulation of
62 fatigue as the season progressed.

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

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4 **67 INTRODUCTION:**

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6 **68** Adequate recovery from stress is an essential aspect of an athlete's training program.
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9 **69** Strenuous training and match demands may increase an athlete's psychological and
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11 **70** physiological stress (36); while collegiate soccer players, in particular, are further burdened with
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13 **71** additional stressors that include a short preseason (<3 weeks), multiple games per week, frequent
14
15 **72** travel, and academic requirements (14, 41). These demands may have cumulative effects
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17 **73** throughout the competitive season which can exacerbate recovery needs. Insufficient recovery
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19 **74** can present in the form of psychological and physiological changes that may progress to
20
21 **75** performance decrements (16, 27, 31). Accordingly, methods of assessing and profiling recovery
22
23 **76** status and readiness to train/compete are valuable when seeking to optimize training and prevent
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25 **77** chronic declines in performance. Research is warranted to assess the utility of multiple athlete-
26
27 **78** monitoring tools, particularly in women's team sports, to discover how athletes respond to the
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29 **79** stress of the competitive season. Little has been done to examine pertinent physiological and
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31 **80** psychological challenges and changes that occur in this population, particularly for those
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33 **81** competing at a high level.
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43 **82**
44 **83** Early detection and prevention of excessive fatigue in athletes should be the goal of
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46 **84** monitoring techniques in order to avoid non-functional overreaching and overtraining syndrome
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48 **85** from developing (30). Athlete monitoring methods have emerged as a tool to prevent declines in
49
50 **86** performance stemming from inappropriate training loads and inadequate recovery (16). While no
51
52 **87** single reliable monitoring technique has been established (19), various methods of tracking an
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54 **88** athlete's internal load (i.e. physiological response) and external load (i.e. work completed by the
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56 **89** athlete) in conjunction with psychological assessments may prove beneficial (16, 42). Load
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4 90 monitoring systems include technologies, such as global positioning satellite systems (GPS),
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7 91 accelerometry, and continuous heart rate (HR) measurements, which provide the ability to track
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9 92 both on-field external and internal loads throughout a competitive season (16). While tracking
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11 93 on-field training load is an important step to gauge the athlete's training demands, other factors
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13 94 may also play a role in determining readiness to perform. The use of blood-based biomarkers has
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15
16 95 the capacity to provide an objective evaluation of the athlete's physiological response to training
17
18 96 as well as other aspects of *overall* health, recovery, metabolic and nutritional status (23).
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21 97 Monitoring relative changes in biomarkers related to stress, inflammation, anabolism, muscle
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23 98 breakdown/turnover, metabolism, nutrition, and reproductive health, are important means of
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26 99 evaluating training status and fatigue.
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31 101 In addition to monitoring physiological responses, changes in psychological states have been
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33 102 established as an important indicator of training distress and may be linked to decrements in
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36 103 performance (15). The study by Morgan and colleagues monitoring mood states in competitive
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38 104 swimmers showed mood disturbances increase during times of high training volumes in a dose-
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41 105 response manner (31). Questionnaires and surveys designed to assess athlete's psychological
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43 106 wellness have the ability to provide subjective information as to how the athlete is adapting to
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46 107 the imposed training load (16). Further, surveys that track athletes' sleep habits may help provide
47
48 108 important information to coaches and support staff. One of the most commonly reported methods
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51 109 for managing fatigue and enhancing recovery is related to sleep (20, 37). Adequate sleep may act
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53 110 to buffer the negative effects of increased training demands, while inadequate sleep may lead to a
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55 111 worsened state of fatigue with significant negative effects on performance, perceived effort, and
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4 112 cognition as well as other biological functions (17). As a result, evaluating sleep quality and
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7 113 duration may help to better understand the athlete's overall readiness to perform.

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11 Previous research has shown an imbalance between training demands and recovery can result
12 115
13 in physiological, biochemical, and psychological/behavioral alterations that may occur before or
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15 in conjunction with deteriorations in performance (18, 39). Tracking changes in these metrics
16 117
17 may help to elucidate the effects of the competitive season in women's team sport athletes as
18 118
19 well as help to determine the point at which training may become maladaptive. The purpose of
20 119
21 this study was to monitor the physiological and psychological profile of National Collegiate
22 120
23 Athletic Association's (NCAA) Division I women's soccer players to determine the time course
24 121
25 of change in various markers of fatigue in conjunction with fluctuating training demands
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27 throughout the course of a season. It was hypothesized that an increase in training load metrics
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29 would be accompanied by perturbations in biomarkers, psychological wellness, and sleep, as
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31 well as declines in performance.
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41 127 **METHODS:**

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43 128 *Experimental Approach to the Problem:* This observational study sought to determine the effects
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45 of a competitive collegiate soccer season on biomarkers, psychological athlete wellness, sleep,
46 129
47 and performance outcomes in high-level women collegiate athletes. Maximal aerobic capacity
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49 tests in conjunction with body composition assessments were performed prior to the start of the
50 131
51 season to accurately program the on-field athlete monitoring system for each player. Training
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53 variables (i.e. training load [TL], exercise energy expenditure [EEE], time spent at a percentage
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55 of HR maximum [HR_{max}], total distance covered [DIS], and distance covered in high speed
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4 135 running) were monitored during all practices and games. Biomarkers, maximal counter-
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6 136 movement vertical jump height (CMJ), subjective psychological wellness, and sleep were
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9 137 assessed before the start of preseason and every 4-weeks following to evaluate the effects of the
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12 138 accumulating demands during the competitive season.

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16 140 *Subjects:* Twenty-five NCAA DI women's soccer players (Age = 19 ± 1 yrs; Height = 167 ± 6
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18 141 cm; Weight = 65.1 ± 1.6 kg) participated in monitoring and assessments during a competitive
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21 142 season. All participants performed testing as part of the regular team activity associated with
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24 143 their sports science program. All subjects received clearance by the University sports medicine
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26 144 staff prior to testing. This research was approved, and written consent waived, by the
27
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29 145 University's Institutional Review Board (#16-050) for the Protection of Human Subjects. All
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31 146 procedures performed were in accordance with the 1964 Declaration of Helsinki and its later
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34 147 amendments.

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38 149 *Performance Testing:* Athletes reported to the laboratory prior to the start of the collegiate fall
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41 150 preseason in late July to complete performance and body composition testing. Subjects were
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43 151 instructed to arrive euhydrated, 2 hours fasted, and having abstained from strenuous exercise 24
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46 152 hours prior to testing. All athletes with prior participation on this team had previous experience
47
48 153 in testing procedures. All incoming athletes were familiarized with the testing procedures
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51 154 through instruction and demonstration by the researchers prior to testing. Body composition was
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53 155 assessed by air displacement plethysmography (11) (BOD POD, COSMED, Concord, CA) to
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55 156 determine percent body fat (%BF), and fat free mass (FFM), using the Brozek formula (4). The
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58 157 error of body volume reading is roughly 0.02%, which allows for the calculation of %BF with
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4 158 only 0.01% error (11). Following a self-selected warm-up, a maximal graded treadmill exercise
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6 159 test was used to measure aerobic capacity ($\dot{V} O_{2max}$) and ventilatory threshold via direct gas
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9 160 exchange measured by a COSMED Quark CPET (COSMED, Concord, CA). The error of the
10
11 161 Quark CPET has been shown to be in the 4-12% range (2, 8). A speed-based protocol was used
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14 162 with stages that were metabolically equivalent (MET) to the Bruce protocol. This protocol
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16 163 included two-minute stages at a constant 2% incline. The speeds were as follows: 6.4, 7.9, 10.0,
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19 164 11.7, 13.7, 15.6, 17.1, 18.2, 19.8, 21.1 km·h⁻¹ (29). Subjects continued the test with
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21 165 encouragement from the laboratory staff until volitional fatigue. $\dot{V} O_{2max}$ was determined using
22
23 166 breath-by-breath analysis consisting of 30 second rolling time averages. At least three of the
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25 167 following criteria were met verifying the attainment of $\dot{V} O_{2max}$: a leveling off or plateauing of
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27
28 168 $\dot{V} O_2$ with an increase in exercise intensity, attainment of age predicted maximal heart rate
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31 169 (HR_{max}), a respiratory exchange ratio greater than 1.10, and/or an RPE \geq 18. Heart rate was
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33 170 continuously monitored to obtain HR_{max} (Polar H7; Polar Electro Co., Woodbury, NY, USA).
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36 171 Subject's ventilatory threshold was calculated after the completion of each test as the point
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38 172 where ventilation increased nonlinearly with $\dot{V} O_2$, which is expressed as a percentage of $\dot{V} O_{2max}$.
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41 173 Body composition and performance data are presented in *Table 1*.

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43 174 INSERT TABLE 1 ABOUT HERE

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46 175 *Biomarker Collection and Analysis:* Athletes were instructed to report for blood draws following
47
48 176 an overnight fast in a normally hydrated state. Athletes were instructed to drink water *ad libitum*
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50 177 before arrival. Blood draws occurred prior to the start of pre-season (S1), and every four weeks
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53 178 following, (S2) (S3) & (S4). S4 occurred 25 days prior to the last competitive game of the season
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55 179 as logistics prevented biomarker assessments post season. All blood draws were taken between
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58 180 0700-0900 hours. Blood draws at S2, S3, and S4 occurred ~18-36 hours following a game.
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4 181 Blood samples were obtained (antecubital fossa, 21G, BD Vacutainer, Safety-Lok) from the
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7 182 antecubital vein in the arm by an experienced phlebotomist while subjects were seated. Whole
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9 183 blood was collected and left to clot at room temperature. Samples were drawn into collection
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11 184 tubes consisting of anticoagulant (EDTA), or clot activator (gel free and Serum Separator
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13
14 185 Tubes). Following collection, blood was centrifuged for 10 minutes at 4,750 revolutions·min⁻¹
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16 186 (Allegra x-15R, Beckman Coulter, Brea, CA, USA). Plasma and serum samples were shipped (in
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19 187 containers designed to maintain approximately 20°C, 4°C, or -20°C depending on the analyte) to
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21 188 a Clinical Laboratory Improvements Amendment (CLIA) certified processing facility (Quest
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24 189 Diagnostics, Inc., San Juan Capistrano, CA, USA) for analysis. Samples were run in duplicate
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26 190 and the coefficient of variation for all biomarkers were between 0.5 – 10.0 %. Biomarkers in the
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29 191 analysis included markers of stress (free cortisol [FCORT], total cortisol [TCORT]) muscle
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31 192 breakdown/turnover (creatine kinase [CK]), inflammatory markers (interleukin-6 [IL-6]),
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34 193 markers of anabolism (growth hormone [GH], insulin-like growth factor-1 [IGF-1], total
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36 194 testosterone [TTEST], free testosterone [FTEST]), markers of reproductive health (total
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38 195 estrogens [E], prolactin [PRL], sex hormone binding globulin [SHBG]), markers of metabolism
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41 196 (tri-iodothyronine [T₃], thyroxine [T₄]) and nutritional markers (iron [Fe], tryptophan [TRP],
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43 197 glutamine [GLN], phenylalanine [PHE], and taurine [TAU]) (23).

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48 199 *Season Training and Monitoring:* All practices and games were monitored using the Polar
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51 200 TeamPro system (Polar Electro Co., Woodbury, NY, USA) which utilizes HR, GPS, and
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53 201 accelerometry technology (21). Physiological attributes of the player obtained from laboratory
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55 202 testing (i.e., player age, height, weight, sex, $\dot{V}O_{2max}$, HR_{max}, and HR at ventilatory threshold)
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58 203 were used to program each player's monitor through the Polar TeamPro online platform.
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4 204 Training metrics were assessed during all practices and games and included total DIS, EEE (9,
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6 205 13), time spent in HR zones expressed as a percentage of HR_{max} , (HR_{Z1} : 50-59%; HR_{Z2} : 60-69%;
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9 206 HR_{Z3} : 70-79%; HR_{Z4} :80-89%; HR_{Z5} : 90-100%) and distance covered in speed zones (DIS_{Z1} =3.0-
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11 207 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} = \geq
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13 208 19 km/h) based on similar speed zones used by female athletes (3). A total TL score was also
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16 209 determined for each practice and game which was calculated via an algorithm developed by
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18 210 PolarTM based on the quantification of an individual player's output. **GPS signal acquisition is**
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21 211 **confirmed for each practice and game from a mobile tablet containing the Polar TeamPro**
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23 212 **software. Confirmation required a minimum of four satellites for the athlete's unit number to**
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26 213 **appear on the tablet.**

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31 215 *Subjective Psychological Wellness and Sleep:* Prior to the start of preseason (S1), and every four
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33 216 weeks following (S2, S3, S4) paper-based surveys were administered and completed by the
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36 217 athletes before the start of practice. These surveys included the Multi-Component Training
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38 218 Distress Scale (MTDS) to monitor subjective athlete wellness (26) and the Pittsburgh Sleep
39
40 219 Quality Index to determine sleep quality (SQ) and duration (SD) (6). The MTDS included the
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43 220 composite score: total training distress (TTD), and the six subscales: depressed moods, vigor,
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46 221 physical signs and symptoms, sleep disturbances, perceived stress, and general fatigue. The
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48 222 MTDS consisted of 22-item questionnaire in which responses were formed from a Likert scale
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51 223 with 0 corresponding to "Not at all" and 5 corresponding to "extremely". For all subscales,
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53 224 scores were calculated based on the sum of the questions (Q) for each category (e.g. depressed
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55 225 moods: 5Q, vigor: 4Q, physical signs and symptoms: 3Q, sleep disturbances: 3Q, perceived
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58 226 stress: 4Q, and general fatigue: 3Q). All questions regarding vigor were positively weighted in

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4 227 the questionnaire and therefore responses were reverse scored when included in the composite
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6 228 (TTD) score and when reporting (i.e. higher scores represent greater vigor). Higher scores on the
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9 229 Pittsburgh Sleep Quality Index were representative of adverse sleep.

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14 231 *Countermovement Vertical Jump Height:* Survey completion was followed by a 10-15 min
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16 232 generalized dynamic warmup and subsequent maximal CMJ height assessments. Subjects were
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18 233 given two attempts at CMJ assessed using a digital contact mat (Just Jump system, Probotics,
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20 234 Huntsville, AL, USA) with the highest jump height recorded. Athletes maintained their hands on
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22 235 their hips for all jumps. Athletes were given 30-45 seconds between jump attempts. If form was
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24 236 incorrect, athletes were instructed to repeat attempt after guidance from the researcher. The error
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26 237 of vertical jump height for females has been reported with a standard error of the mean of 1.7 cm
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29 238 and a coefficient of variation of 4.4% (33). Following S1, this protocol was continued every 4
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31 239 weeks during the first practice of the week, consistent with the same schedule as blood draws
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34 240 (S2, S3 & S4).

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41 242 *Statistical Analysis:* Repeated measures (RM)-MANOVAs were performed with univariate
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43 243 follow-ups to assess changes in biomarkers, CMJ performance, and training metrics (TL, DIS,
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45 244 EEE) throughout the season. For each univariate analysis, the Huynh-Feldt epsilon was
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48 245 examined for the general model to evaluate sphericity. If the Huynh-Feldt epsilon exceeded 0.75,
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50 246 sphericity was considered to have been met, and the unadjusted statistic was used. If epsilon was
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52 247 less than 0.75, the adjusted Huynh-Feldt statistic was used to test significance. Planned simple
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55 248 contrasts were conducted using the baseline values as the comparison term. Pairwise contrasts
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58 249 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

273 periods are represented in Figures 1-3. Time spent in HR zones (1-5) and distances covered in
274 high-speed running zones (1-5) during the four-week time periods are presented in Figures 4 &
275 5.

276 INSERT FIGURES 1-5 ABOUT HERE

277 **Biomarker Response:** All biomarker data are presented in *Table 2*. FCORT increased from S1-
278 S2 (ES= 0.88; p=0.03) and returned to baseline by S4. Compared to S1, there was a decline in
279 TCORT at S4 (ES= -0.41; p=0.03), but no significant differences at S2 or S3 (p>0.05). CK
280 increased from S1 to S2 (ES= 1.74; p=0.03), then returned to baseline by S3 and remained steady
281 through S4. IL-6 significantly increased above baseline by S4 (ES= 0.71; p=0.02). FTEST
282 increased from S1 to S2 (ES=1.27; p<0.001). Although not significant, FTEST remained
283 moderately elevated above baseline at S3 (ES=0.51; p=0.057) before returning to baseline by S4.
284 TTEST followed a similar pattern with an increase from S1 to S2 (ES=3.5; p=0.02) and a return
285 to baseline by S3. No statistically significant changes were seen in SHBG; however, modest
286 declines were observed from S1 to S4 (ES= -0.13; p=0.07). There were no differences from
287 baseline values throughout the season for E, PRL, or T₃ (p>0.05). T₄ increased from S1 to S2
288 (ES=0.81; p=0.003), declined below baseline at S3 (ES=-0.65; p=0.001) and then returned to
289 baseline at S4. Compared to baseline, GH significantly decreased at S2 (ES= -0.50; p=0.01) and
290 remained depressed for the remainder of the season (ES_{S3}=-0.56; p=0.001; and ES_{S4}=-0.60;
291 p=0.002). IGF-1 significantly declined from S1 to S3 (ES=-0.54; p=0.02), before returning to
292 baseline at S4. Fe declined from S1 to S2 (ES= -0.73; p=0.001). Although not significant, Fe
293 remained moderately suppressed at S3 (ES=-0.42; p=0.09) before returning towards baseline
294 values at S4. GLN increased at S2 (ES= 1.07; p=0.002) and remained elevated at S3 (ES= 0.63;
295 p=0.03) before returning to baseline at S4. The moderate increase in TRP from S1 to S2

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4 296 approached significance (ES=0.62; p=0.08), followed by a steady decline which approached
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7 297 significance at S4 (ES=-0.39; p=0.09). There were no significant changes in PHE and TAU from
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9 298 S1 throughout the season (p>0.05).

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12 299 INSERT TABLE 2 ABOUT HERE
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17 301 ***Psychological Response:*** All changes in scores represent the increases or decreases in
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19 302 subscales construct. TTD declined from S1 to S2 (ES=-0.38; p=0.02) and increased from
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21 303 baseline by S4 (ES=0.57; p=0.01). Compared to baseline values, depressed mood became worse
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23 304 at S4 (ES=0.62; p=0.03). Vigor decreased from S1 to S2 (ES= 0.48; p=0.03) and continued to
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26 305 decline below baseline values (ES_{S3}=1.28, ES_{S4}=1.47; p<0.001). No changes in physical signs
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29 306 and symptoms or as sleep disturbances were observed. Perceived stress decreased from S1 to S2
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31 307 (ES=-0.89; p<0.001) before returning to baseline at S3 (p>0.05). Although not significant,
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33
34 308 reported general fatigue moderately increased at S3 (ES=0.52; p=0.056) compared to baseline
35
36 309 before increasing above baseline at S4 (ES=0.61; p=0.01). See *Table 3* for the full change in
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38
39 310 MTDS subscales.

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42 311 INSERT TABLE 3 ABOUT HERE
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45 312 ***Sleep Quality and Duration Response:*** No changes were seen in SQ or SD from baseline
46
47 313 values (P>0.05).
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50 314
51 315 ***Performance Response:*** CMJ declined from S1 to S2, (ES=-0.51; p=0.001) before
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53
54 316 returning to baseline values at S3. Although not significant, there was a small decline in CMJ
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56 317 from baseline at S4 (ES=-0.22, p=0.079). See Figure 6 for full changes in CMJ throughout the
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59 318 season.
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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.

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4 341 Comprehensive biomarker monitoring can provide insight into the physiological changes
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6 342 athletes experience throughout a season (40). Chronic elevations in resting cortisol can indicate a
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9 343 maladaptation of the HPA-axis (30) and an impaired ability to recover from training (23).
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11 344 Significant elevations in the metabolically active FCORT were apparent at S2, immediately
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14 345 following the preseason time block, and persisted into S3. It is important to note that although
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16 346 TCORT levels were maintained followed by a decline at S4, TCORT values were above the
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19 347 normal clinical reference ranges at all time points (127-569 nmol/L when assessed 0800-1000h).
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21 348 The above range values may indicate a persistent catabolic state in these athletes throughout the
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24 349 season. CK, an indicator of muscle damage (23), increased nearly 56% from S1 values after the
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26 350 initial training block. Although within athlete-specific reference ranges, the increased CK seen at
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29 351 S2 in conjunction with increases in FCORT, indicates the need for enhanced recovery strategies
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31 352 during this time block to combat the increase in training related stressors. Interestingly elevations
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33 353 in IL-6, a pro- and anti-inflammatory cytokine, occurred at S4, in conjunction with increases in
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36 354 TTD, fatigue, depressed moods, reduced vigor, and modest declines in CMJ height. Although IL-
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38 355 6 has been shown to increase in response to decreased muscle glycogen, muscle contraction, and
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41 356 muscle damage in order to activate an immune response (34), it has also been implicated in the
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43 357 production and enhancement of negative mood states, sleep disturbances, and fatigue (24, 27).
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48 359 Testosterone, often used in conjunction with cortisol to provide a relative indication of an
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50 360 anabolic status in male athletes (22, 23), may play a role in helping to maintain performance by
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53 361 reducing protein breakdown and promoting protein synthesis (23). FTEST and TTEST
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55 362 significantly increased following the preseason training block. We speculate that this
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58 363 upregulation in testosterone may have been a physiological response to combat the stress of the
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4 364 first training block, although more research is warranted regarding testosterone's response to
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7 365 overload training, specifically in women. Other biomarkers that changed in response to the stress
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9 366 of the season include the anabolic hormones, IGF-1 and GH, both of which are involved in
10
11 367 muscle protein synthesis and the regulation of muscle mass (23) and may play a significant role
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14 368 in the anabolic response in women. Previous research evaluating acute GH responses to exercise
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16 369 have found blunted effects in overreached compared to healthy subjects (7). Relative declines in
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19 370 IGF-1 and chronic reductions in GH seen throughout the season may impact muscular
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21 371 adaptations to training (23). In addition, IGF-1 has been shown to be associated with energy
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24 372 status, as inadequate energy intakes to match high volumes of training have been shown to
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26 373 reduce IGF-1 (12).

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31 375 Additionally, thyroid hormones respond to energy demands and may act to maintain
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33 376 metabolic function during training in order to accommodate the increased EEE. Conversely,
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36 377 thyroid hormones may act by reducing metabolic function in times of high EEE to provide an
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38 378 "energy sparing" effect contingent on training demands and energy intake (40). Free T₄ increased
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41 379 following S2 before declining below baseline at S3, which may be reflective of the high caloric
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43 380 expenditures experienced in these athletes. Alterations in free T₄, which is converted to the
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46 381 biologically active form, T₃ may be reflective of the variations in workload. Previous research
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48 382 has shown that chronic elevations in cortisol suppress the conversion of T₄ to the active T₃ (28),
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51 383 which may have contributed to the lack of changes observed in T₃ throughout the season.
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53 384 Examining the overlap between the HPA- and hypothalamic-pituitary-thyroid (HPT)-axes during
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55 385 prolonged periods of high workloads and energy expenditures may aid in understanding changes
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58 386 in metabolic markers throughout the season.
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388 Other nutritional markers may play a role in evaluating athlete recovery and fueling
389 strategies. Total Fe declined significantly after the first training block before returning to
390 baseline values. Although not clinically classified as “iron-deficient”, this decrease represents a
391 ~32% decline from baseline. Monitoring relative changes in Fe stores throughout the season may
392 provide an opportunity to intervene before deficiencies can occur and impact performance. The
393 amino acid GLN has been studied in association with overreaching for its role in immune system
394 function (32), with decreased concentrations of GLN reported during prolonged chronic exercise
395 and heavy training (19, 35). Interestingly, GLN increased throughout the middle of the season
396 which may suggest immune system compensation. Tracking GLN in conjunction with rates of
397 infection, illness, and select immune markers throughout the season may help clarify the utility
398 of this marker as an indicator of recovery status. Of additional importance is the amino acid
399 TRP, the precursor for serotonin, which in conjunction with IL-6 may provide a mechanism for
400 understanding changes in mood typically reported with overreaching (39). Increased brain levels
401 of serotonin are believed to result in mood and behavioral changes such as inducing sleep and
402 reducing appetite, both behaviors evident in overreaching and overtraining (39). TRP displayed a
403 moderate effect for an increase at S2 when TTD, perceived stress, depressed moods, physical
404 signs and symptoms and fatigue were the lowest. Meanwhile, the small decline in TRP at S4
405 corresponded to elevations in general fatigue, depressed mood, and TTD. Although PHE and
406 TAU may provide an indication of protein turnover in athletes (23), no significant changes were
407 seen throughout the season despite elevations in CK. Monitoring nutritional markers is important
408 to assess overall athlete health or to provide justification for the implementation of nutrition
409 education programs, particularly for athletes that experience high energetic demands.

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6 411 In addition to tracking physiological response, monitoring athletes' psychological state
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9 412 and perceived wellness can identify athletes who are negatively responding to training (30), with
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11 413 low vigor and high ratings of fatigue and depression indicative of a maladaptive training
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13 414 response (31). In the current study, the greatest perturbation in athlete wellness occurred at S4,
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15 415 which included significant increases in TTD, fatigue, depressed moods and declines in vigor.
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17 416 Similar results have been found using the Profile of Mood States throughout professional,
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19 417 university and recreational soccer seasons (25). Researchers found the greatest change in
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21 418 negative mood states occurred for professional players as the seasons progressed, suggesting
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23 419 psychological changes may be associated with the total demands of a highly-competitive soccer
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25 420 season (25). Interestingly in the current study, compared to baseline values, the lowest scores for
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27 421 TTD, perceived stress, physical signs and symptoms, fatigue, and depressed moods occurred
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29 422 after the period of highest workload. These positive psychological changes occurred in
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31 423 conjunction with declines in CMJ performance, indicating factors other than training load alone
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33 424 may be driving psychological responses.
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43 426 Subjective measures, while helpful in determining athletes' perceptions of wellness, may
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45 427 also be reflective of environmental stressors. In these athletes, the greatest perceived stress (at
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47 428 S1) may have been in anticipation of the upcoming season, and therefore may have allowed for
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49 429 improvements as the season progressed and team dynamics became established. For example, the
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51 430 high perceived stress could be a result of the upcoming physical demands of the condensed
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53 431 preseason, the need to perform for a starting position, or related to new experiences inherent to a
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55 432 collegiate environment. Although speculative, this could aid in explaining the improvements
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4 433 observed in several measures of athlete wellness (TTD, perceived stress) that occurred after the
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7 434 first training block, despite physiological perturbations and downturns in CMJ performance.
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9 435 Alternatively, certain subscales may not have been sensitive enough to detect changes along with
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11 436 workload fluctuations. It is important to note, psychological questionnaires rely on self-report
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13 437 data that can be manipulated either intentionally or unintentionally. Although confidentiality of
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16 438 survey responses was emphasized to the athletes, survey recording occurred in the team
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19 439 environment. It may be players fear their honest responses will negatively reflect playing time or
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21 440 make them look “weak” in the eyes of coaches or teammates. Further, the frequency of
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24 441 administration and length of the survey also affects reporting accuracy (38). For these reasons, it
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26 442 may be beneficial to use subjective measures of athlete readiness alongside objective measures
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29 443 of physiological responses to training, especially in collegiate team sports.
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33 445 In addition to subjective psychological surveys, questionnaires designed to evaluate sleep
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36 446 are also an important means of assessing recovery (37). Although no reported changes in sleep
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38 447 disturbances, quality, or duration occurred throughout the season, this could be influenced by the
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41 448 sensitivity of subjective measures to detect SQ changes in a real-world setting using actively
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43 449 training athletes. Future research should consider alternative, objective methods of tracking
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46 450 sleep, as these methods may be advantageous to determining SQ in response to fluctuating
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48 451 workloads in collegiate athletes.
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53 453 Previous research has shown CMJ height, an effective measure of lower limb muscular
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55 454 power ability, to be related to soccer performance across a season (1, 43). Following the highest
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58 455 training load block, CMJ height declined, but then returned to baseline before beginning to
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4 456 modestly decline at S4 (which approached significance). This final decline occurred when the
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7 457 athletes were entering the most critical time of the competitive season: tournament play, which
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9 458 consisted of post-season appearances in a division tournament final and the NCAA tournament.
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11 459 It appears CMJ may have been sensitive enough to detect changes in athlete readiness throughout
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14 460 the season. Therefore, evaluating changes in performance outcomes, such as vertical jump, that
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16 461 can be rapidly tested without adding additional fatigue to the athletes may help coaches to better
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19 462 prepare training sessions and recovery strategies, particularly upon entering tournament play.
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24 464 This study sought to evaluate the effects of a competitive collegiate season on various
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26 465 markers of fatigue in an applied setting within a team environment; however, there are certain
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29 466 limitations that are inherent to the study. First, a post-season blood draw in conjunction with
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31 467 maximal performance testing would have been ideal to shed light on the physiological state of
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33 468 these athletes following tournament play. Unfortunately, this was not possible due to the
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36 469 tournament structure and travel logistics. In addition, diet and nutritional supplementation, which
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38 470 may influence biomarker responses as well as overall recovery status, was not assessed during
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41 471 the season. While a limitation, the feasibility and accuracy of self-reported dietary intakes make
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43 472 assessments difficult to achieve in a team setting (5). Further considerations specific to female
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46 473 athletes such as hormonal contraceptive use and menstrual status may further influence several
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48 474 performance and biomarker measures over the course of the season. Given the nature of the
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51 475 applied setting, researchers sought to evaluate typical conditions affecting a women's
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53 476 competitive soccer season in an effort to increase the generalizability of the study. As such,
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55 477 although no changes were seen in E, SHBG, or PRL throughout the season, these reproductive
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58 478 hormones may be affected by menstrual status and hormonal contraceptive use and thereby
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4 479 impact the physiological adaptations to training seen throughout the study. Further research is
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6
7 480 warranted to determine the influence of hormonal contraceptives and menstrual status on these
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9 481 markers.

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12 482 Despite these limitations, this study sheds a novel light on the effect of a collegiate soccer
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14 483 season on various physiological and psychological responses. The time course of change in
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16 484 markers of athlete readiness revealed the greatest physiological disruptions and performance
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19 485 decrements occurred following the highest workload period. Yet, the findings of this study also
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21 486 point to an accumulation of fatigue as the season progressed and stress the need to determine
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24 487 optimal loading and appropriate periodization by coaches and training staff during the collegiate
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26 488 preseason time block. While no one measure was shown in isolation to indicate fatigue, a
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29 489 combination of monitoring techniques may better aid in the evaluation of athlete health and
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31 490 readiness throughout a women's competitive soccer season.

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36 492 **PRACTICAL APPLICATION:**

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38 493 Appropriately structured training is essential to athlete recovery and performance
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41 494 throughout a competitive season. Tracking workload is an important first step to determine the
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43 495 on-field training stress imposed on the athlete. However, the cumulative training stress is not
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46 496 always reflected in a ~2-hour training session. Therefore, the use of multiple monitoring
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48 497 techniques may provide better clarity regarding individual player's response to the season
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51 498 demands. When selecting a monitoring technique, it is important to consider the efficacy and
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53 499 sensitivity of that method to detect fatigue and insufficient recovery, while also minimizing the
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55 500 burden on the athlete. While subjective measures may be helpful in determining how athletes
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58 501 respond to training and environmental stressors, tracking changes in a multitude of biomarkers

502 may give a more comprehensive and objective assessment of the athlete's physiological load as
503 well as insight into recovery status and overall athlete health. Therefore, a combination of
504 subjective and objective monitoring techniques may be most useful to combat maladaptive
505 training responses from the accumulated stress of a competitive season and may afford an
506 opportunity for intervention before declines in performance can occur.

507

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669 Figure Legends:

670

671 **Figure 1:** Changes in Training Load throughout the Season.

672 ▲u=arbitrary units

673 ■ Black bars represent total training load accumulated from games during the four-week time blocks

674 □ Gray bars represent total training load accumulated from practices during the four-week time blocks

675 (*) Denotes significant difference from (S1-S2) $p < 0.05$

676 Values represent Means

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680 **Figure 2:** Changes in Exercise Energy Expenditure throughout the Season.

681 Kcals=calories expended

682 ■ Black bars represent total exercise energy expenditure accumulated from games during the four-week time blocks

683 □ Gray bars represent total exercise energy expenditure accumulated from practices during the four-week time blocks

684 (*) Denotes significant difference from (S1-S2) $p < 0.05$

685 Values represent Means

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689 **Figure 3:** Changes in Distance Covered Throughout the Season.

690 KM=kilometers

691 ■ Black bars represent total distances accumulated from games during the four-week time blocks

692 □ Gray bars represent total distances accumulated from practices during the four-week time blocks

693 (*) Denotes significant difference from (S1-S2) $p < 0.05$

694 Values represent Means

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698 **Figure 4:** Time Spent in Heart Rate Zones as a Percentage of HR_{max}

699 Values represent Means

700 HRZ=Heart Rate Zone

701 □ bars represent time spent in HR_{Z1} :50-59% of HR_{max} accumulated during the four-week time period

702 □ bars represent time spent in HR_{Z2} :60-69% of HR_{max} accumulated during the four-week time period

703 □ bars represent time spent in HR_{Z3} :70-79% of HR_{max} accumulated during the four-week time period

704 □ bars represent time spent in HR_{Z4} :80-89% of HR_{max} accumulated during the four-week time period

705 □ bars represent time spent in HR_{Z5} : 90-100% of HR_{max} accumulated during the four-week time period

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55
56
57
58
59
60
61
62
63
64
65

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

Performance and Body Composition Metrics	
Body Fat (%)	20.8 ± 3.1
Fat Free Mass (kg)	51.6 ± 5.0
VO_{2max} (ml·kg⁻¹·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·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
T₃	nmol/L	1.76 ± 0.5	1.80± 0.5	1.76 ± 0.4	1.85 ± 0.4
T₄	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=creatin kinase, IL-6=interleukin-6, FTEST= free testosterone, TTEST=total testosterone, E=total estrogen, SHBG=sex-hormone binding globulin, T₃=free tri-iodothyronine, T₄= Total thyroxine, GH=growth hormone, IGF-1=insulin-like growth factor-1, Fe=iron, PRL=prolactin, GLN=glutamine, TAU=taurine, TRP=tryptophan, PHE=phenylalanine.

S₁₋₄= Timepoints taken every four weeks throughout the season.

Values represent Means \pm Standard Deviations.

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

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

S₁₋₄= 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

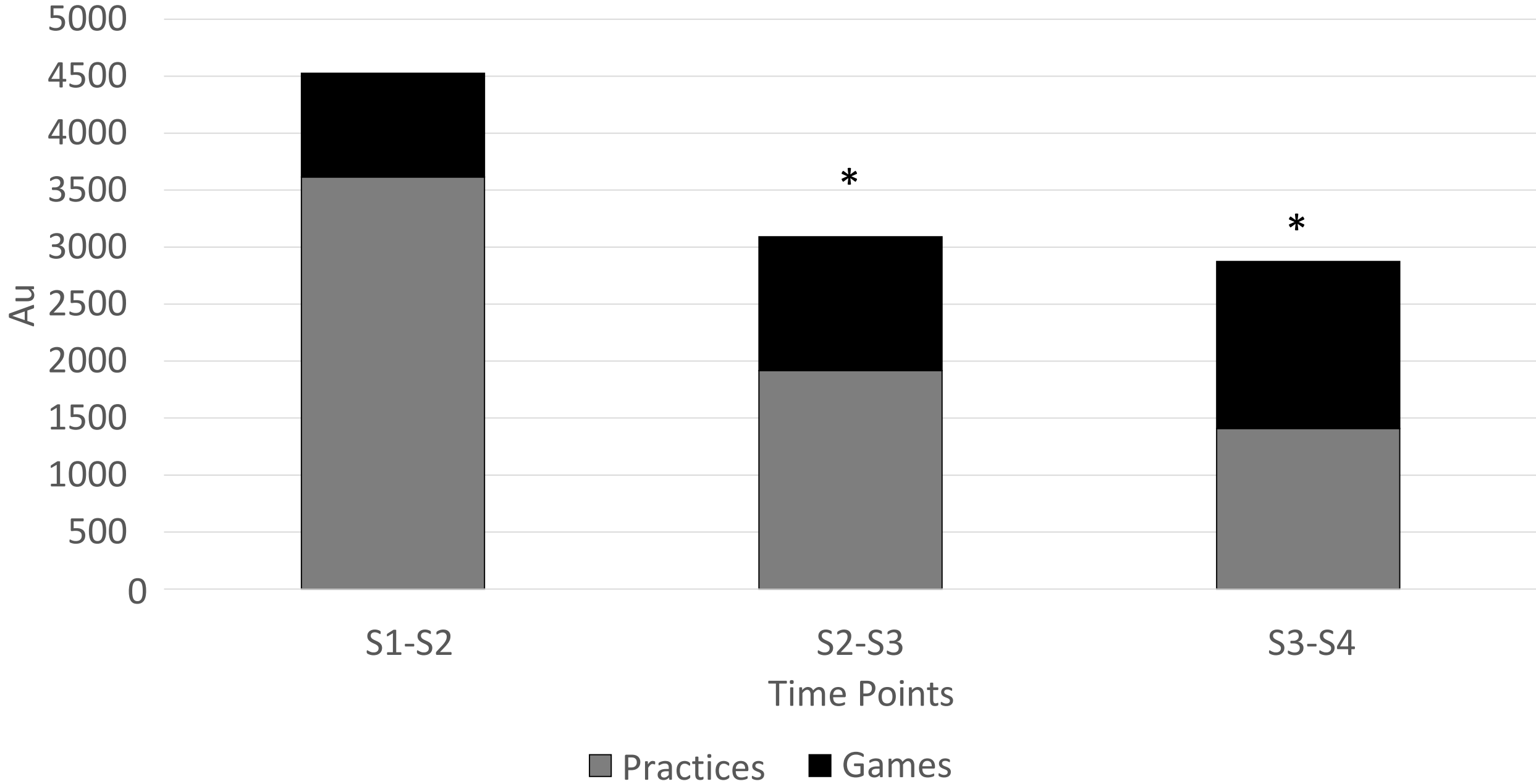


Figure 2

Exercise Energy Expenditure

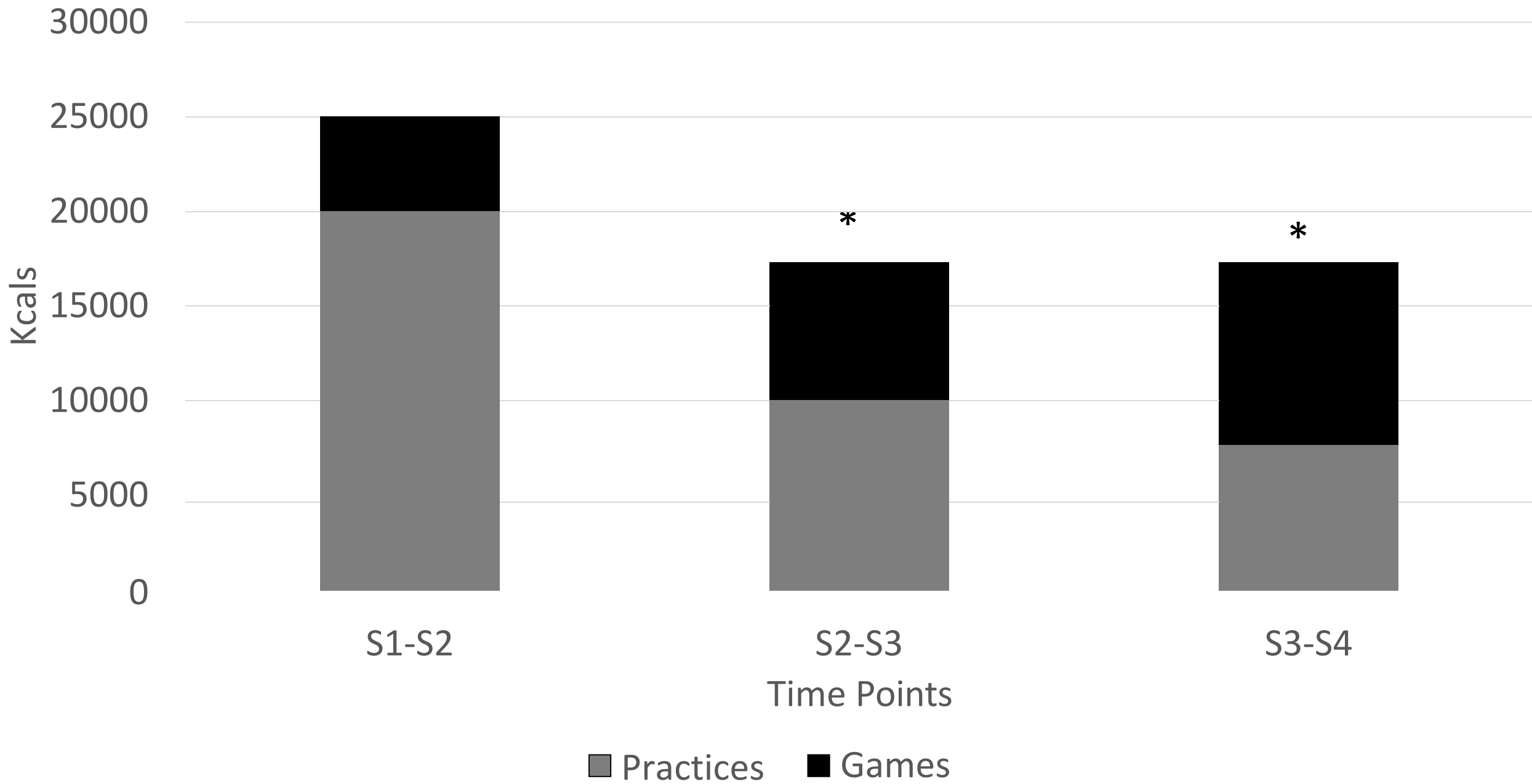


Figure 3

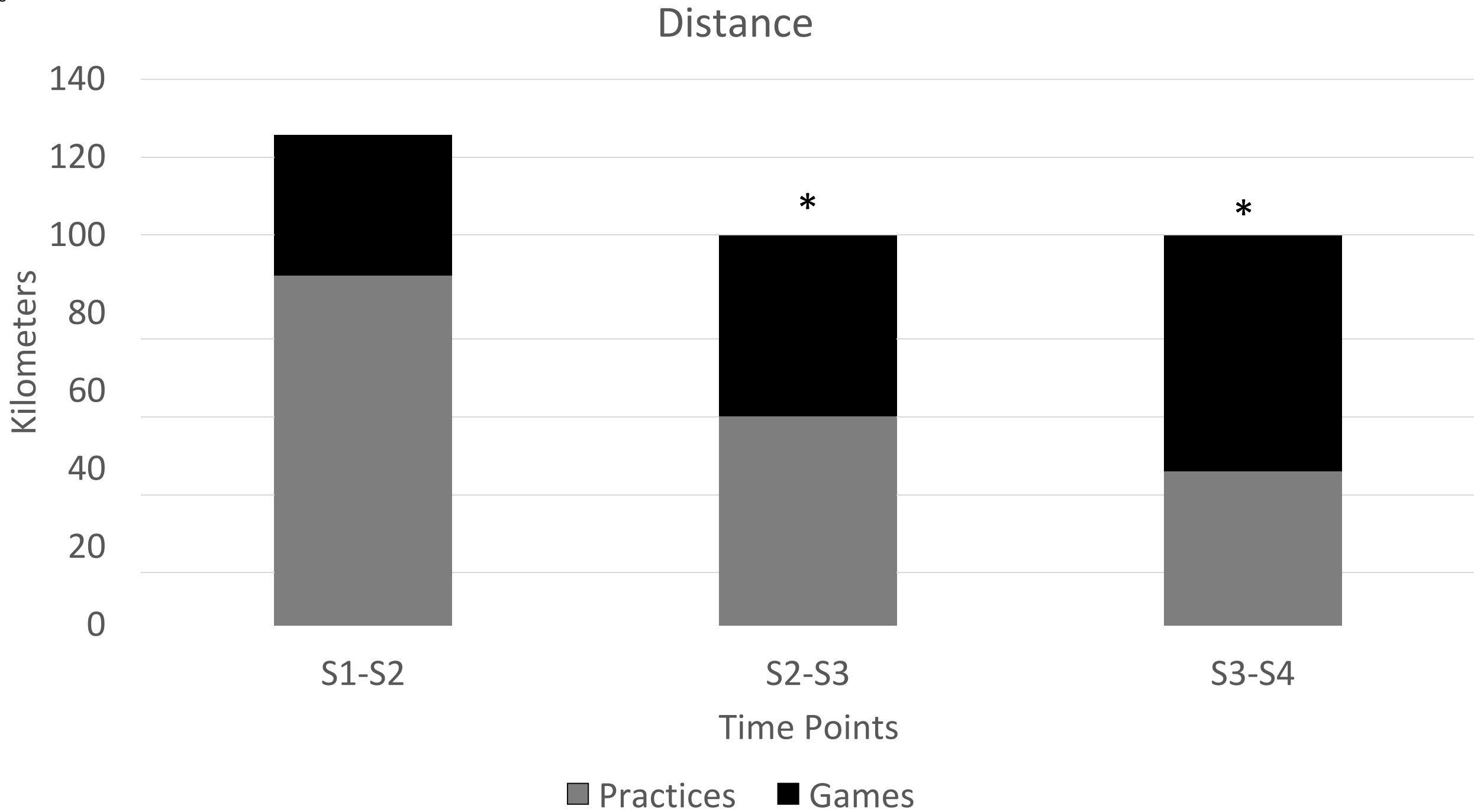


Figure 4

Time Spent in Heart Rate Zones (1-5)

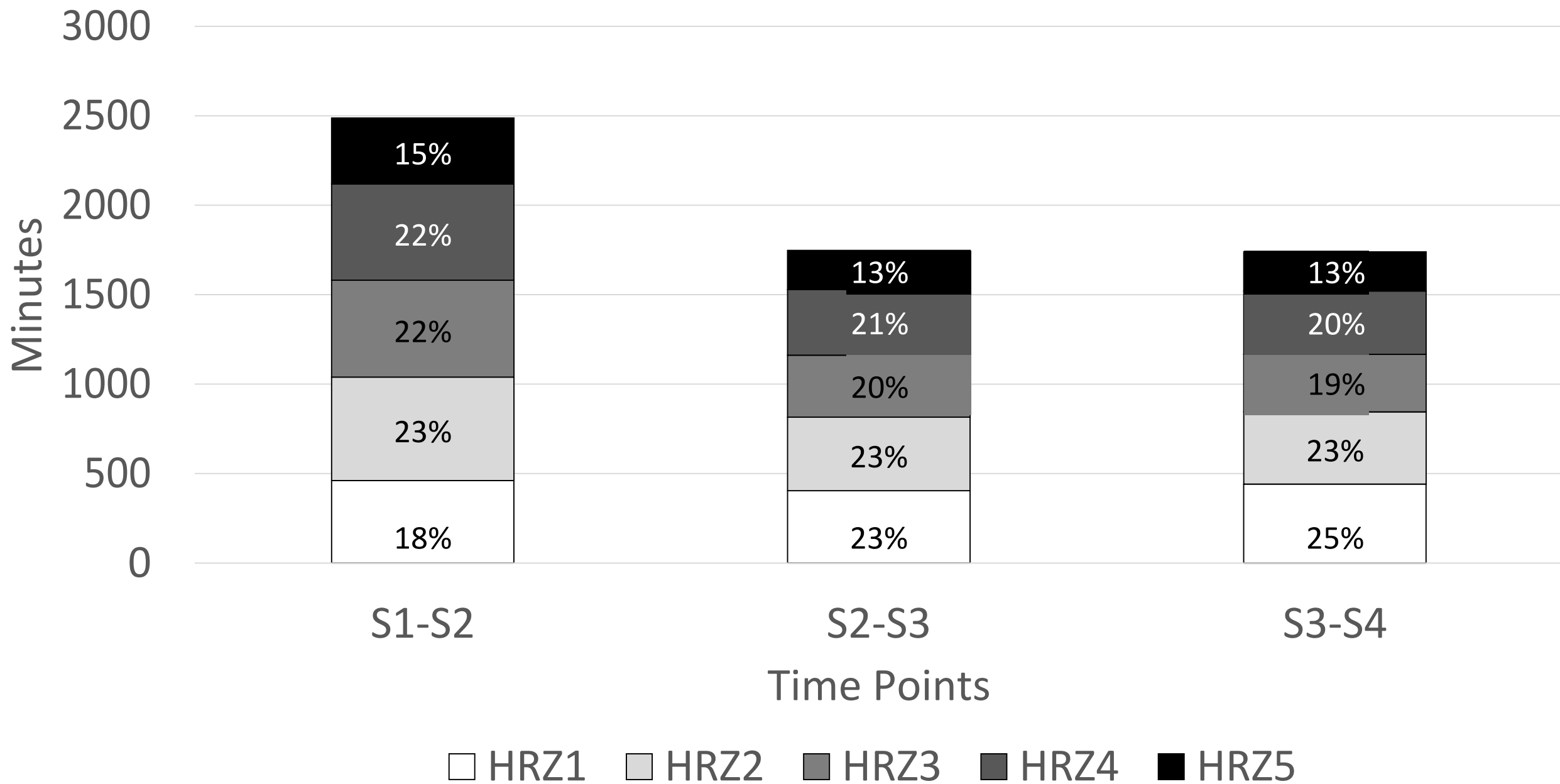


Figure 5

Distance Covered in Speed Zones

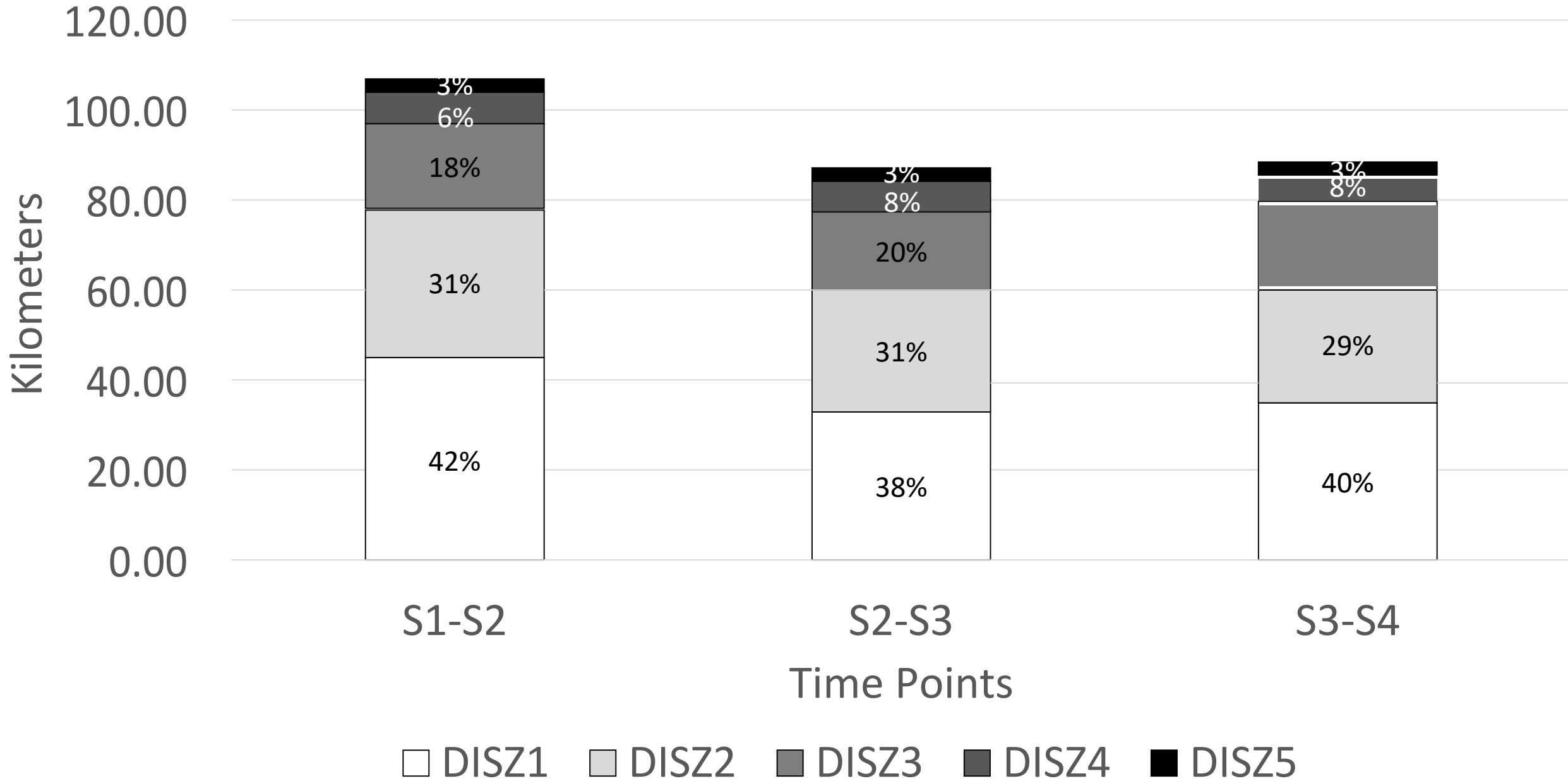


Figure 6

Counter-Movement Vertical Jump

