Evaluating the effects of oral contraceptive use on biomarkers and body composition during a competitive season in collegiate female soccer players.

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Title: **EVALUATING THE EFFECTS OF ORAL CONTRACEPTIVE USE ON BIOMARKERS AND BODY COMPOSITION DURING A COMPETITIVE SEASON IN COLLEGIATE FEMALE SOCCER PLAYERS**

Running Heading: EFFECTS OF ORAL CONTRACEPTIVE USE IN FEMALE ATHLETES

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ABSTRACT

High training demands throughout the competitive season in female collegiate soccer players have been shown to induce changes in biomarkers indicative of stress, inflammation, and reproduction, which may be exacerbated in athletes using oral contraceptives (OCs). **Purpose:** To compare biomarkers and body composition between OC-using and non-using (CON) female soccer players throughout a competitive season. **Methods:** Female collegiate soccer players were stratified into two groups based on their reported OC use at the start of pre-season (OC: n=6; CON: n=17). Prior to the start of pre-season and immediately post-season, athletes underwent a battery of performance tests. Blood draws and body composition assessments were performed prior to pre-season, on weeks 2, 4, 8, and 12 of the season, and post-season. **Results:** Area-under-the-curve ratios (OC_{AUC}:CON_{AUC}) indicated the OC group were exposed to substantially higher levels of sex-hormone binding globulin (AUC_{ratio}=1.4, probability=p>0.999), total cortisol (1.7; p>0.999), c-reactive protein (5.2; p>0.999), leptin (1.4; p=0.990), growth hormone (1.5; p=0.97), but substantively lower amounts of estradiol (0.36; p<0.001), progesterone (0.48; p=0.008), free testosterone (0.58; p<0.001), follicle-stimulating hormone (0.67; p<0.001) and creatine kinase (0.33, p<0.001) compared with the CON across the season. Both groups increased fat free mass over the season, but CON experienced a greater magnitude of increase along with decreased body fat percentage. **Conclusion:** Although similar training loads were observed between groups over the season, the elevated exposure to stress, inflammatory, and

metabolic biomarkers over the competitive season in OC users may have implications on body composition, training adaptations, and recovery in female athletes.

KEY WORDS: female athletes, hormonal contraceptives, training loads, performance

NEW & NOTEWORTHY: This study highlights the influence of OC use on physiological changes that occur over a four-month intense, competitive season and the differential systemic exposure to biomarkers, specifically those of inflammation, stress, anabolism, and energy balance, between OC-using and non-using soccer players. Additionally, this study provides insight into changes in body composition with prolonged training between female athletes with and without OC use.

1. INTRODUCTION

Due to its power-endurance nature, soccer is a physically demanding sport, which is compounded by the stress of academics, frequent travel, and environmental stressors in collegiate players (19). Athlete-monitoring methods, such as heart rate (HR) and global positioning systems (GPS), allow for the assessment of internal and external workloads and recovery during training and competition; however tracking changes in blood biomarkers may offer a more comprehensive picture of the cumulative demands of a collegiate season outside of just on-field training sessions (2). In National Collegiate Athletic Association (NCAA) Division I (DI) soccer, the high training demands throughout the competitive season have been shown to induce changes in biomarkers of stress and reproduction in male (24, 26) and female players (51). Chronic elevations in stress and inflammatory biomarkers such as cortisol and interleukin-6

(IL-6) and decreases in reproductive markers (e.g. testosterone, estrogen) amongst other biomarker changes can be indicative of inadequate recovery (29), and thus have implications on performance (26) and health (17).

Current research shows that the majority of elite female athletes have at some point in their career taken hormonal contraceptives (HC), with almost half (49.5%) reporting current HC usage (31). Of the various HC methods reported, oral contraceptives (OC) were the most widely used (78.4%) amongst female athletes (31). As such, it is important to understand any implications HCs, especially OCs, have on training adaptations, recovery, and performance. HC use is a potential confounding factor in the stress response from training in female athletes due to the overlap between hypothalamic-pituitary-adrenal (HPA) and hypothalamic-pituitary-gonadal (HPG) axes (32, 35). In females, HCs modify normal hormonal fluctuations, suppressing endogenous production of estrogen and progesterone (43). HPA-axis activation inhibits the HPG-axis, through the influence of corticotropin-releasing hormone (CRH) on gonadotropin-releasing hormone (GnRH) either directly or indirectly through β-endorphin or cortisol (32). Cortisol, whose production can also be stimulated by vasopressin (AVP) during stress, acts to inhibit all levels of the HPG-axis beyond just GnRH (32). A recent study investigating the effects of OC use on the HPA-axis demonstrated that OCs alter the activation of the HPA-axis by increasing circulating levels of cortisol, thereby inducing metabolic alterations such as increasing circulating levels of triglycerides (23). This finding demonstrates that OC use may have an analogous impact on the HPA-axis as training, with both activating this stress response. Therefore, OC use in conjunction with training, particularly during times of high training demands, such as during the competitive soccer season (51), may produce an augmented stress response in female athletes.

OC use has also been linked to increased c-reactive protein (CRP) levels at rest in female athletes, but not other acute phase proteins (13). Moreover, this finding has been shown in active females who underwent 10-weeks of high intensity training as HC users (7 out of 8 subjects on OCs) displayed increased CRP levels as well as reduced lean mass gains post-intervention than the non-HC users (25). In elite female athletes, increased resting cortisol concentrations (6) and blunted cortisol responses to high intensity training sessions have been reported with OC use (15). In addition to the blunted cortisol responses, elite female hockey players on OCs also had decreased resting testosterone levels and a reduced testosterone response to training over 15 days compared to their non-user teammates (15). This mirrors previous findings in which OC use had been shown to decrease free testosterone and increase sex hormone-binding globulin (SHBG) levels in healthy women (52). As such, changes in biomarkers may be exacerbated or altered in athletes using OCs in response to prolonged periods of intense training. This possible enhanced activation of stress and inflammatory responses in female athletes using OCs may indicate a greater recovery need. Furthermore, side effects such as increased in body weight or fat mass have been reported in female endurance athletes and active females on OCs (12, 41), which may impact performance outcomes; however, these findings have not been consistent (40, 41). The purpose of this study was to compare biomarker and body composition responses in female soccer players with and without OC use during a NCAA DI competitive soccer season. It was hypothesized that the players using OCs would have altered physiological responses compared to their non-user counterparts over the competitive season.

2. METHODS

2.1 Experimental Design

Female collegiate soccer players were monitored throughout a competitive fall season to determine the effects of OC use on body composition and biomarkers indicative of stress, inflammation, reproduction, anabolism, metabolism, and hematological status. Prior to the start of pre-season, players underwent maximal performance testing that was used to determine their endurance and power characteristics as well as to individualize each athlete's Polar TeamPro monitor. The Polar TeamPro system utilized GPS, accelerometry, and HR monitoring technology to determine training load (TL) and exercise energy expenditure (EEE) for all team training sessions, practices, and games. Additionally, body composition and biomarkers assessments were performed prior to pre-season as well as on weeks 2, 4, 8, 12, and immediately post-season (*Figure S1;* DOI: http://doi.org/10.6084/m9.figshare.12996794).

2.2 Participants

Female collegiate soccer players (N=30) were monitored throughout the course of the competitive season. Players were stratified into two groups: oral contraceptive (OC: n=6; Mean ±SD: age=19±1yr; weight= 67.6±3.0 kg; height= 168.4±4.4 cm) and control (CON: n=17; age=19±1yr; weight= 66.0±8.0 kg; height= 168.2±6.5 cm) based on their reported OC use. OC usage was determined by a Menstrual Status Questionnaire completed prior to the start of pre-season, and was also repeated post-season for confirmation of OC status. At baseline, all OC players reported at least one-year of OC use and all CON players reported menstrual cycle lengths of 25-35 days. Self-reported age of menarche was 13±1 years for the CON group and 14±2 years for the OC group. Players were excluded from analysis if they were using intrauterine contraception (n=4), altered contraception method mid-season (n=1), did not participate in team training (n=1), or had a known metabolic disorder (n=1). Written, informed consent was

obtained from all subjects prior to participation and all subjects received clearance by the university Sports Medicine staff prior to testing. All players competed on the same NCAA DI women's soccer team in the Big Ten Conference. Research was approved by the University's Institutional Review Board for the Protection of Human Subjects and conducted in accordance with the Declaration of Helsinki.

2.3 Performance Testing

Prior to the start of pre-season and upon completion of the competitive season, players underwent a battery of performance tests and body composition assessments. All pre- and post-season testing sessions, as well as blood draws, occurred within a one-week period. Prior to the start of season, players reported to the lab ≥2 hours fasted and having refrained from exercise in the preceding 12-hours. Body composition was assessed using air displacement plethysmography via the BodPod (BODPOD, COSMED, Concord, CA, USA) with a predicted lung volume via the Brozek formula (7, 16) to determine percent body fat (BF%) and fat free mass (FFM). After a ~10-minute standardized dynamic warm-up, players performed maximal countermovement vertical jumps with hands-on-hips (CMJ_{HOH}) on a contact mat (Probotics Inc., Huntsville, AL, USA) (36). Players were allowed two attempts with highest jump height recorded.

Afterwards, a maximal graded exercise test (GXT) on a treadmill was used to measure maximal aerobic capacity (VO_{2max}) and ventilatory threshold (VT) via direct gas exchange by a COSMED Quark CPET (COSMED, Concord, CA, USA). HR was continuously monitored throughout the test using a Polar S610 HR monitor to obtain maximal heart rate (HR_{max}) (Polar Electro Co., Woodbury, NY, USA). A speed-based protocol was used with stages that were metabolic equivalents (MET) to the standard Bruce protocol. This protocol has previously been

used in collegiate soccer players and consisted of two-minute stages at a constant 2% incline, with increasing speeds of 6.4, 7.9, 10.0, 11.7, 13.7, 15.6, 17.1, 18.2, 19.8, 21.1 km/h (34). Players continued the test with encouragement from research assistants until volitional fatigue. At least two of the following criteria were met for attainment of VO_{2max}: RER ≥1.1, observation of a plateau in O_2 consumption (increase ≤150 ml/min with increasing workload), and HR >85% age-predicted HR_{max} (208 – 0.7 x age). For athletes who did not meet the above criteria, VO_{2peak} was used (n=3). Player's VT was analyzed after the completion of each test as the inflection point where VCO_2 increased nonlinearly with VO_2, expressed as a percentage of VO_{2max} (5).

All performance tests were repeated post-season and body composition assessments were repeated during all blood draw timepoints in addition to post-season. One athlete at baseline (n=1) and four athletes at post-testing (n=4) were limited in participation for maximal testing by the team physician and did not participate in all testing sessions (see *Table 8*).

2.4 Blood Draws

Blood draws were performed prior to pre-season, on weeks 2 (end of pre-season), 4, 8, & 12 of the season, and post-season. Athletes reported to the lab between 0700 and 0900h and were instructed to arrive in an euhydrated state following an overnight fast. All draws during the season were performed between 18-24 hours following a game (T2-T5), with the exception of pre-season (T1: 'baseline') and post-season draws (T6: ~58h post-game). The T2 blood draw was performed in order to assess changes in biomarkers following pre-season in which workloads are the highest for the athletes, while the T6 blood draw offered a snapshot of recovery post-season. For all draws, blood samples were drawn from participants while seated via the antecubital fossa (21G, BD Vacutainer, Safety-Lok) by three experienced phlebotomists

into clot activator collection tubes (SST and gel-free tubes). Blood samples were centrifuged for 10-minutes at 4,750 rpm (Allegra x-15R; Beckman Coulter, Brea, CA, USA), serum/plasma were aliquoted from centrifuged tubes and immediately shipped, in containers designed to maintain 4º, 20º, or -20ºC depending on the analyte, to a Clinical Laboratory Improvements Amendment (CLIA)-certified processing facility for analysis (Quest Diagnostics, Secaucus, NJ, USA). Samples were run in duplicate and the coefficient of variation (CV) was between 0.5-10.0% for all biomarkers. Results were provided to the researchers via the Quest Diagnostics Care360 online portal. Biomarkers analyzed included total cortisol (TCORT), free cortisol (FCORT), creatine kinase (CK), CRP, IL-6, tumor necrosis factor-α (TNF-α), estradiol (E_2), growth hormone (GH), insulin-like growth factor-1 (IGF-1), ferritin (Fer), iron (Fe), total iron binding capacity (TIBC), percent transferrin saturation (%SAT), transferrin, leptin, total triiodothyronine (TT_3), free triiodothyronine (FT_3), total thyroxine (TT_4), free thyroxine (FT_4), thyroid-stimulating hormone (TSH), prolactin, sex-hormone binding globulin (SHBG), follicle-stimulating hormone (FSH), progesterone (P_4), total testosterone (TTEST), and free testosterone (FTEST).

2.5 In-season athlete-monitoring

Players were evaluated during all team training sessions using the Polar TeamPro system during the fall competitive season. The Polar TeamPro system utilized GPS, accelerometry, and HR technology to determine TL and EEE (14, 18, 21) for all lifts, practices, and games. The Polar TeamPro system was individualized to each athlete using their pre-season testing results of height, weight, age, VO_{2max}, HR_{max}, and HR_{VT}. During the season, daily TL and EEE data were downloaded from the athletes' monitors by the researchers and then averaged weekly throughout

the season. TL, expressed as arbitrary units (au), was calculated via an algorithm developed by Polar™ based on the training impulse concept and factors in an athlete's HR responses, caloric expenditure, and mechanical impact incurred during a training session as well as the duration of the session. EEE was normalized for body weight (EEE_{REL}, expressed as kcal/kg), which was obtained from body composition assessments, in order to account for relative size differences between players.

2.6 Statistical Analysis

The purpose of the statistical analysis was to model the time series nature of biomarker and body composition data and assess the extent to which values changed across the season for both OC and CON groups. To conduct the analyses, hierarchical generalized linear models (HGLMs) were fitted within a Bayesian framework. HGLMs accounted for structure in the data and were fitted to smooth the time series data, identifying the underlying shape of the physiological signal (38). With a Bayesian framework, dichotomous interpretations of results (*e.g.* with null hypothesis significance testing) can be avoided and greater emphasis placed on describing the most likely results and their practical consequences (27). Analogous to mixed-effect models with varying slopes, the HGLMs were fitted with a single common smoother plus group-level smoothers with the same "wiggliness" (38). The HGLMs also accounted for the repeated measures nature of the data by including random intercepts for each player. All models were fitted within the brms package (8) that interfaced with the Bayesian software Stan (10). Models were fitted with 5 chains each comprising 10,000 sets of posterior estimates. These model estimates with smoothers were then used to generate 50,000 new data sets to account for uncertainty in coefficients and variance parameters. Means were then calculated in each data set

across time intervals for both OC and CON groups. Visual inspection of the distribution of means revealed that most outcomes exhibited linear behavior (e.g. constant throughout the season or consistent increase/decrease). The proportion of gradients with for example a positive slope was interpreted as the probability of an increase in the outcome across the season. To quantify the magnitude of any change, effect sizes (Cohen's *d*) were calculated for each data set by dividing the change in value across the season by the pre-season standard deviation. Effect sizes (*d*) of 0.20, 0.50, and 0.80 were considered indicative of small, medium, and large effects, respectively. To quantify differences in biomarker levels across the season between OC and CON groups, the ratio of the area under the curve (AUC) was calculated. The distribution of all calculations across the generated data sets were used to derive percentage credible intervals (%CrIs). Descriptive statistics (Mean ± SD) were used to quantify team, OC, and CON performance characteristics pre- and post-season. Frequency counts for OC and CON groups were used to present changes in performance from baseline values (increase, maintain, decrease) due to changes in sample size for each performance variable from pre- to post-season. Changes were considered an increase or decrease based on the sensitivity of the equipment to detect significant changes (VO_{2max}: ± 2.3 $ml \cdot kg^{-1}min^{-1}$; VT: ± 2.0%; CMJ_{HOH}: ± 1.7 cm) (22, 36), otherwise no change (maintenance) was indicated.

3. RESULTS

3.1 Reproductive markers: E_2, P_4, FSH, SHBG, TTEST, FTEST, Prolactin

Inspection of modelled time series indicated linear (constant or increasing/decreasing) responses for all reproductive biomarkers across the season. However, median point estimates describing linear changes were below a medium threshold ($|d|<0.5$) for all reproductive markers

(*Table 1*) in both OC (-0.38: FSH; to 0.14: TTEST) and CON (-0.27: SHBG; to 0.43: TTEST) groups. The area under the curve ratios indicated the OC users were exposed to substantively higher levels of SHBG (AUC ratio: 1.4 [95%CrI: 1.3 – 1.5]; p>0.99), but substantively lower levels of E_2 (AUC ratio: 0.36 [95%CrI: 0.11 – 0.61]; p<0.001), P_4 (AUC ratio: 0.48 [0.13 – 0.89]; p =0.008), FTEST (AUC ratio: 0.58 [95%CrI: 0.47 – 0.70]; p<0.001), and FSH (AUC ratio: 0.67 [95%CrI: 0.51 – 0.85]; p<0.001) compared with the CON group across the season. Graphical outputs of Bayesian hierarchical linear models for the reproductive biomarkers are presented in *Figure S2*; DOI: http://doi.org/10.6084/m9.figshare.12996794.

Insert Table 1 here

3.2 Stress and inflammatory markers: *TCORT, FCORT, CRP, IL-6, TNF-α*

Inspection of modelled time series indicated linear responses for the majority of stress and inflammatory biomarkers across the season. Results indicated that OC users experienced a large increase for CRP (d=0.85) and a moderate increase for IL-6 (d=0.66) (*Table 2*). In contrast, median point estimates describing linear changes were below a medium threshold ($|d|$<0.5) for all stress and inflammatory biomarkers in the CON group (0.10: CRP; to 0.46: IL-6) (*Table 2*). A non-linear response was identified for FCORT in both OC and CON groups, with values increasing between T1-T4 (combined d =0.40; [50%CrI: 0.21 – 0.59]) followed by a return towards original values between T4-T6 (combined d=-0.23; [50%CrI: -0.42 – 0.05]). During the season, both OC and CON groups also experienced a similar non-linear trend with decreasing TNF-α values between T1-T5 (combined d=-0.89; [50%CrI: -1.1 – 0.57]), followed by a subsequent large increase between T5-T6 (combined d=1.2; [50%CrI: 1.0 – 1.4]). The area

under the curve ratios indicated the OC group was exposed to a substantively greater amount of TCORT (AUC ratio: 1.7 [95%CrI: 1.6 – 1.8]; p>0.99) and CRP (AUC ratio: 5.2 [95%CrI: 3.7 – 8.3]; p>0.99) compared with the CON group across the season. Graphical outputs of Bayesian hierarchical linear models for stress and inflammatory biomarkers are presented in *Figure S3*; DOI: http://doi.org/10.6084/m9.figshare.12996794.

Insert Table 2 here

3.3 Markers of muscular growth and breakdown: *GH, IGF-1, CK*

Linear responses were identified for all biomarkers indicative of growth and muscular breakdown across the season. The OC group experienced a large increase in GH (d=1.5), but a moderate decrease in IGF-1 (d=-0.52) across the season (*Table 3*). In contrast, median point estimates were below a medium threshold ($|d|$<0.5) for all muscular anabolic and catabolic biomarkers in the CON group (-0.14: IGF-1; to -0.07: CK) (*Table 3*). The area under the curve ratios indicated OC users were exposed to substantively higher levels of GH (AUC ratio: 1.5 [95%CrI: 0.97– 2.2]; p=0.97), but substantively lower levels of CK (AUC ratio: 0.33 [95%CrI: 0.16 – 0.50]; p<0.001) compared with the CON group across the season. Graphical outputs of Bayesian hierarchical linear models for biomarkers of muscular growth and breakdown are presented in *Figure S4*; DOI: http://doi.org/10.6084/m9.figshare.12996794.

Insert Table 3 here

3.4 Markers of iron status: *Fe, Fer, %Sat, TIBC, Transferrin*

Linear responses were identified for the majority of biomarkers indicative of iron status in the athletes across the season. Both OC and CON groups were found to experience a moderate decrease in Fe (*d*=-0.51, *d*=-0.56), with the CON group also demonstrating a moderate increase in TIBC (*d*=0.63) (*Table 4*). Similar non-linear responses were identified for %SAT with OC and CON groups experiencing a decrease between T1-T5 (combined *d* = -0.42; [50%CrI: -0.60 – -0.23]), followed by a subsequent increase between T5-T6 (combined *d*= 0.34; [50%CrI: 0.17 – 0.51]). Graphical outputs of Bayesian hierarchical linear models for biomarkers of iron status are presented in *Figure S5*; DOI: http://doi.org/10.6084/m9.figshare.12996794.

Insert Table 4 here

3.5 Markers of metabolism: *TSH, TT_4, FT_4, TT_3, FT_3, Leptin*

Linear responses were identified for all biomarkers indicative of metabolism and energy balance across the season. OC users were found to experience increases in the majority of biomarkers with large effects for TT_4 (*d*=0.91) and leptin (*d*=1.2), and moderate effects for TT_3 (*d*=0.71) and FT_3 (*d*=0.78), but a moderate effect for a decrease in FT_4 (*d*=-0.52) (*Table 5*). Similarly, the CON group experienced moderate effects for increases in TT_4 (*d*=0.53) and leptin (*d*=0.51), and moderate effects for decreases in both TSH (*d*=-0.61) and FT_4 (*d*=-0.70) (*Table 5*). The area under the curve ratios indicated the OC group were exposed to substantially greater amounts of TSH (AUC ratio: 1.4 [95%CrI: 1.3– 1.6]; p>0.99), TT_4 (AUC ratio: 1.3 [95%CrI: 1.2– 1.4]; p>0.99), TT_3 (AUC ratio: 1.3 [95%CrI: 1.2– 1.3]; p>0.99), and leptin (AUC ratio: 1.4 [95%CrI: 1.3– 1.6]; p>0.99) compared with the CON group across the season. Graphical outputs

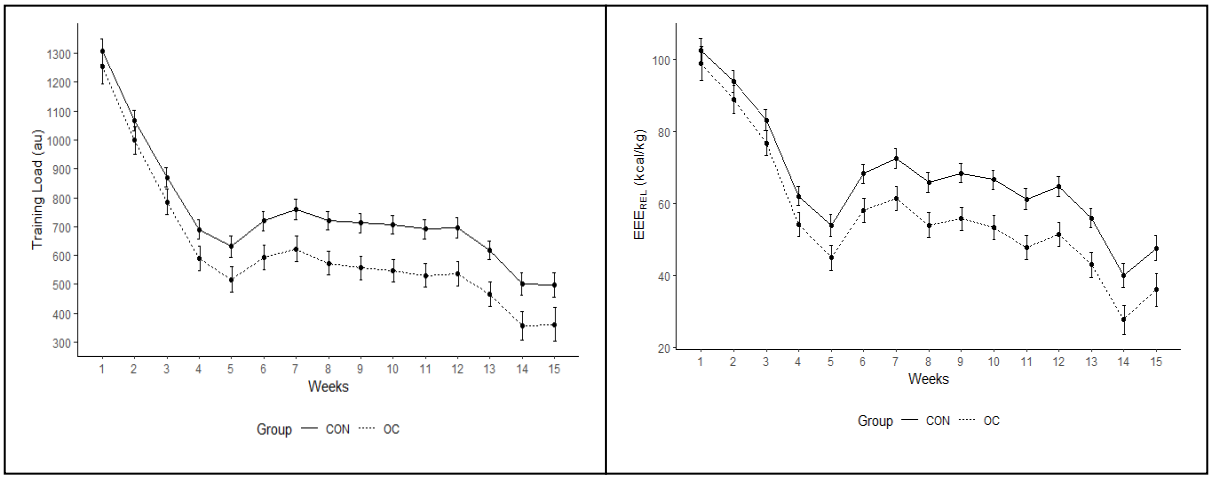
of Bayesian hierarchical linear models for metabolic biomarkers are presented in *Figure S6* DOI: http://doi.org/10.6084/m9.figshare.12996794.

Insert Table 5 here

3.6 Training Load / Exercise Energy Expenditure

Large linear decreases were found for TL and EEE_{REL} across the season (TL: combined *d*=-2.3; [50%CrI: -2.5 – -2.1]; EEE_{REL}: combined *d*=-2.2; [50%CrI: -2.4 – -2.0]); however, OC users were identified to exhibit a lower TL (AUC ratio: 0.83 [95%CrI: 0.76 – 0.89]; p<0.001) and EEE_{REL} (AUC ratio: 0.85 [95%CrI: 0.79 – 0.90]; p<0.001) across the season than the CON group.

Fig 1: Changes in Training Load and Exercise Energy Expenditure Over Time



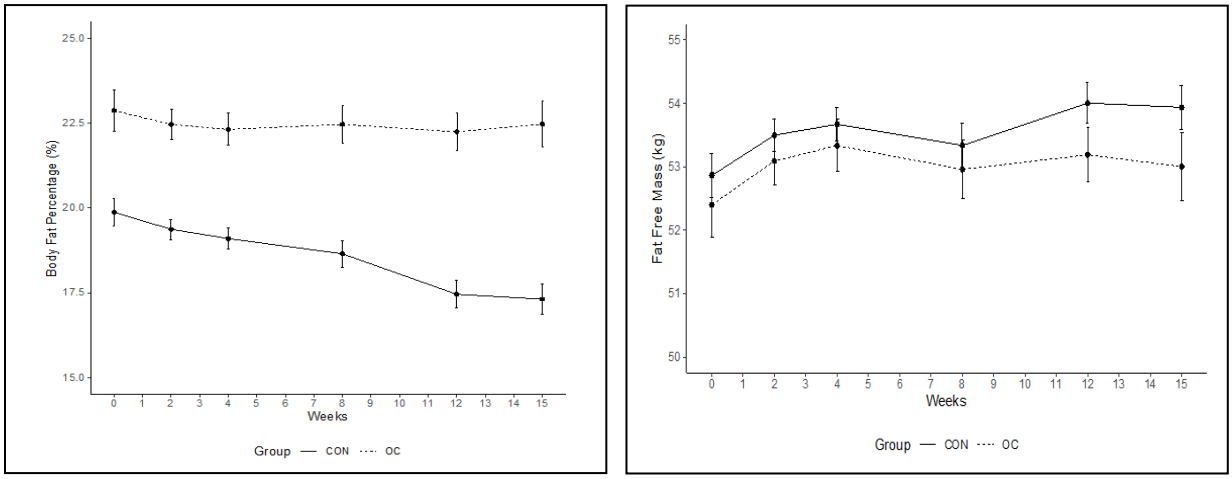
EEE_{REL}: relative exercise energy expenditure; Plots illustrate smoothed data obtained from Bayesian hierarchical generalized linear models. Circles represent averages and error bars represent ± standard deviations.

Insert Table 6 here

3.7 Body Composition

Investigation of body composition data indicated that both OC and CON groups maintained body mass across the season (d_{OC} = 0.04 [50%CrI: -0.06 – 0.14]; d_{CON} = -0.03 [50%CrI: -0.09 – 0.04]; *Table 7*), with limited evidence that both groups increased FFM slightly (d_{OC} = 0.11 [50%CrI: 0.02 – 0.20]; d_{CON} = 0.20 [50%CrI: 0.14 – 0.26]). The CON group also experienced moderate decreases in BF% (d_{CON} = -0.50 [50%CrI: -0.58 – -0.43]), with no such changes identified for OC users (d_{OC} = -0.08 [50%CrI: -0.19 – 0.04]; *Figure 2*).

Fig 2: Changes in Body Fat Percentage and Fat Free Mass Over the Season



Plots illustrate smoothed data obtained from Bayesian hierarchical generalized linear models. Circles represent averages and error bars represent ± standard deviations.

Insert Table 7 here

3.8 Performance Variables

Team and group performance characteristics from pre- and post-season testing are presented in *Table 8*.

Insert Table 8 here

4. DISCUSSION

The TLs and EEEs experienced by female collegiate soccer players throughout the competitive season corresponded with various perturbations in blood biomarkers and changes in body composition. TL and EEE_{REL} were highest for both groups during the first two-weeks of pre-season, with players experiencing reductions in workload as the season progressed. Between OC and CON groups; however, there were substantially different exposures to biomarkers of reproduction, stress, inflammation, metabolism, and muscular anabolism/catabolism throughout the competitive season. These differences were observed despite similar training loads, although OC users exhibited an accumulative 15% lower training load across the season. Yet, the OC group experienced substantially greater exposure to inflammatory and stress biomarkers than the CON group even with the reduced total workloads. Additionally, neither group exhibited changes in BM across the season; however, findings indicated that CON players experienced greater increases in FFM and substantially greater decreases in BF% compared with OC users. These findings indicate that although both groups displayed similar temporal biomarker responses overall, the relative magnitude of these responses to training were exacerbated in OC users, particularly for CRP, GH, and leptin. This study highlights the influence of OC use on physiological changes that occur over a four-month intense competitive season and the differential systemic exposure to biomarkers, specifically those of inflammation, stress, anabolism, and energy balance. These differences observed as a result of OC use may have implications on body composition, training adaptations, and recovery during the competitive season in female athletes.

Over the season, effect sizes revealed concentrations of sex hormones E_2 and P_4 were relatively stable; however, the CON group experienced a ~3x greater exposure to E_2 and ~2x greater exposure to P_4 compared to OC users over the season. This is expected as OCs act by suppressing endogenous production of E_2 and P_4 through the inhibition of the HPG-axis (43). Oral contraceptive-mediated suppression of ovarian hormone production is coupled with a decreased production and secretion of FSH and luteinizing hormone (LH) (43). This is supported by the finding that the CON group exhibited larger concentrations of FSH (~2x greater exposure) over the season than the OC group. Although LH concentrations and exogenous hormone doses were not quantified in this study, the differences in female reproductive hormones between OC and CON groups illustrate the typical reproductive hormonal profiles associated with OC use. Unlike the CON group, OC users experienced a small effect for a decreased FSH concentrations over the season. This increased suppression of FSH levels may in part be mediated by HPA-axis interactions and inhibition on the HPG-axis as TCORT was elevated in OC versus CON groups. Previous research has shown decreased FTEST and increased SHBG levels with OC use (52). This mirrored the findings in this study as the OC group had about ~2x less FTEST and ~1.5x greater SHBG exposure over the season compared with the CON group. This builds upon acute findings in elite athletes where salivary testosterone levels remained lower in OC users after exercise regardless of training session intensity (15). Finally, no differences in prolactin AUC were observed between groups. Prolactin levels can be influenced by IL-6 production (32), potentially explaining the similar prolactin levels across the season as both groups experienced similar increases in IL-6. Additionally, although the timing of blood draws in this study may have influenced the observed reproductive hormone concentrations in the CON group due to cyclic nature of fluctuations in sex hormones during a typical menstrual cycle, overall the

findings of this study underscore the consistent differences over time in circulating sex hormones in female athletes with and without OC use.

Across the season, athletes exhibited an initial increase in FCORT followed by a small decrease during the second-half of the season. This continued increase in FCORT in the first two-months of the season occurred despite dramatic decreases in weekly TL and EEE_{REL} following pre-season. This increased catabolic environment observed in the first-half of the season may be a result of the high TL and EEE_{REL} that occurred during pre-season, where workloads were nearly double those observed from weeks 4 to 15 of the season. Previous research in collegiate fall-sport athletes has characterized the deleterious effects of a condensed pre-season (50, 51), with similar effect sizes observed for increased FCORT in female field-hockey players (50). The observed perturbations in FCORT described herein occurred earlier and to a smaller magnitude than those previously reported in female soccer players (51), which may point to differences in player management between studies. Interestingly, OC players were exposed to nearly ~2x greater TCORT throughout the season compared to CON players, with no differences in FCORT between groups. OC use has been shown to enhance corticosteroid-binding globulin binding capacity, which may influence circulating FCORT levels (53). In female athletes on OCs, increased resting cortisol concentrations have been reported (6) along with blunted acute cortisol responses to exercise (6, 15). This study adds further support to the notion that OCs alter the activation of the HPA-axis by increasing circulating levels of cortisol (23). Research regarding cortisol and OC use in athletes has, however, been equivocal. For example, Larsen and colleagues showed no differences in cortisol concentrations between elite female athletes on OCs (28); however, exercise participation prior to blood draws and time of day varied between subjects, potentially washing out any between group differences as both

factors have been shown to impact cortisol levels. The elevated TCORT levels across the season in the OC group may indicate an increased catabolic environment in these athletes and thus, a reduced capacity for protein synthesis (29), especially when taken in conjunction with the smaller FFM gains observed in OC users. The sustained elevated TCORT levels, along with the exacerbated inflammatory responses observed in OC athletes, may also have implications on recovery and immune function (29), through the inhibition of muscle protein synthesis (20) and immunosuppression (20, 44).

For inflammatory biomarkers, the athletes TNF-α levels decreased through week-12 of the season followed by an increase from weeks 12 to 15. Interestingly, this contrasting response in TNF-α is opposite that of FCORT over the season, and may be due to an interaction and feedback between FCORT, IL-6, and TNF-α responses (37). Compared with pre-season baseline values, OC users experienced large increases in CRP and moderate increases in IL-6 and TNF-α concentrations, whereas the CON group had a small overall increase in IL-6. Thus, there appears to be greater inflammatory responses to training with OC use, despite the increased resting TCORT levels. This may lead to augmented systemic inflammation in these athletes as OC users exposure to CRP was over 5x greater than CON players over the season. This aligns with previous findings that have shown increased CRP at rest and in response to intense training with OC use (13, 25, 28). The heightened systemic inflammation seen with OCs may have long-term implications on athlete health as elevated CRP levels have been associated with an increased cardiovascular disease risk (39). Additionally, chronic inflammation may influence training adaptations, as reduced FFM gains and FM loss alongside elevated CRP levels have been shown over a 10-week training block (25) and similar changes in body composition measures were observed in this present study. It appears OCs may exacerbate inflammatory responses to

training, with the enhanced systemic inflammation contributing to a hindered ability to adapt to a training stimulus.

While the CON group experienced no changes in biomarkers indicative of muscular anabolism, OC users displayed a large increase in GH accompanied by a concomitant moderate decrease in IGF-1 from pre- to post-season. Moreover, AUC comparisons revealed ~1.5x greater exposure to GH in the OC group than the CON group throughout the season. This is in agreement with previous findings in female endurance athletes, in which increased GH levels without changes in IGF-1 were observed following OC treatment (42). Similar declines in IGF-1 have been observed in ovarian suppressed female athletes with intense training, with declines becoming more pronounced over the 12-weeks of training, indicating a potentially increased catabolic environment in these athletes (48). The decreased IGF-1 levels observed over the season in OC users may indicate an impaired ability to induce muscular adaptations in these athletes (29).

Overall, CK levels in the CON group started and remained elevated above OC users, yielding about a ~3x greater exposure in the CON group throughout the season. Previous research has shown E_2 to potentially play a protective role against muscle damage through mechanisms such as increased membrane stabilization (46). Findings on acute elevations in CK post-exercise with OC use remain equivocal (47); however, greater reductions in CK values 72-hours post-exercise have been observed in OC users (11). The greater CK levels observed in the CON group may be indicative of greater skeletal muscle turnover in these athletes (3), especially when taken into context with the FFM gains over the competitive season.

Overall, linear trends for decreases in Fer and Fe and increases in TIBC and transferrin were shown in the players over the soccer season. Additionally, a small decrease occurred

through week 12 for %SAT followed by a small increase during the remainder of the season. These changes may indicate a trend towards a training-induced Fe deficiency particularly over the first 12-weeks of the season before the final decline in TL/EEE_{REL} as observed in previous research (51). Fe deficiency, defined as Fer concentrations <12 μg/L and percent saturation <16%, has been reported in endurance and team sport athletes, with females experiencing a greater risk for reduced Fe status (30). The similar responses between groups in iron status over the collegiate season reflect previous findings that Fer and Fe concentrations are not affected with OC use (47).

For all athletes, FT_3 levels increased from baseline through week 12 before declining through week 15, demonstrating a similar response to that previously described in female collegiate soccer players (51). Decreased or no change in FT_3 levels have often been shown over training periods in athletes, potentially as an effort to promote energy conservation during high EEE (4, 48). Perhaps the FT_3 decline observed indicates decreased muscular metabolism "needs" as FT_3 regulates skeletal muscle metabolism (45) and these declines corresponded to further decreases in TL/EEE_{REL} in weeks 12-15. Future research examining the relationship between changes in TL, EEE, and energy intake along with thyroid hormone responses in female athletes is warranted due to the conflicted findings in these hormones over periods of intense training. Between groups, OC athletes had considerably greater TSH, TT_4, and TT_3 levels, yet no differences were observed for FT_3 exposure compared to CON players. It appears that OCs potentially influence thyroid hormone levels; however, this does not necessarily correspond to increased levels of the biologically active FT_3 above non-OC users. This lends support to previous findings that OCs may increase TSH as well as TT_4 and TT_3 levels due to increased

binding capacity of thyroxine-binding globulin, without significant changes in FT_4 and FT_3 levels (53).

For both groups, moderate to large increases were observed in leptin, an adipose-derived hormone whose levels are reflective of changes in energy balance (1), over the season. Previously in collegiate rowers, changes in FT_3 levels were related to leptin changes, with rowers experiencing either a decrease in both FT_3 and leptin or no change in the hormones over 20-weeks of training (4). Conversely, in this study increases in FT_3 and leptin were observed. It appears a relationship exists between thyroid hormones and leptin production that may be reflective of energy balance in athletes. Throughout the season OC athletes exhibited an almost ~1.5x greater exposure to leptin compared to CON. The elevated leptin levels correspond with the divergent results in BF% identified, with OC athletes maintaining values and the evidence obtained that CON progressively decreased values throughout the season. Leptin expression has been shown to correlate with adipose stores (1), supporting the disparity in leptin levels observed at baseline and throughout the season between the groups. Previous research examining the effects of OC use on body composition is inconsistent in its findings, with some studies reporting no change (40, 41), while others reporting increases in body weight (9, 12, 41). It appears however, that changes in leptin across a training block may occur independent of body composition changes, as previously evidenced in collegiate rowers (4). The authors speculate that while leptin may indicate fat storage, changes may be primarily influenced by fluctuations in energy balance (1) with training.

Team performance characteristics demonstrated the power-endurance nature of the sport with similar average team aerobic capacity and greater CMJ_{HOH} ability as those previously reported in DI female soccer players (49). Additionally in female collegiate soccer athletes, body

composition changes and biomarker perturbations across a competitive season have been shown to occur alongside performance changes pre- to post-season (51). Specifically, changes in IL-6, IGF-1, GH, and TCORT have been shown to correlate to changes in body composition and performance metrics across a collegiate season (33). Although statistical comparison of performance changes between groups was not possible in this study due to reduced sample size at post-season testing; visual inspection of the data appears to show no discernable differences in aerobic performance metrics between groups pre- to post-season. In terms of power, it seems players in the CON group tended to experience increases in CMJ_{HOH} across the season (n=8), while the OC group tended to maintain baseline values (n=4). Future research investigating the effects of OC use on long-term changes in athletic performance in a larger sample size is warranted in light of the increased catabolic and inflammatory environment that exists in OC athletes.

As previously noted, this study is not without its limitations. Although only one team was examined in this study yielding a small sample size, this also allowed for OC and CON athletes to partake in the same prescribed training throughout the entire 15-weeks of the season, in the same environment, with the same training system, and with the same coaching and monitoring strategies. The substantially different exposure to stress, inflammatory, and metabolic biomarkers between OC and CON groups across the season indicate a difference in physiological response, despite the sample size. Future research investigating the long-term effects of OC use on biomarker responses, body composition, and performance metrics across multiple teams and sports is warranted to corroborate these findings. Additionally, dose and type of OC was not controlled for in this study; however, the concentrations of reproductive hormones observed in the OC group reflected the typical reproductive hormonal profile associated with OC use (43).

As a variety of OC prescriptions currently exist, further research examining the effect of different OC formulations on female athletes is potentially needed. Finally, the timing of blood draws in this study may have influenced the observed reproductive hormone concentrations in the CON group due to cyclic nature of fluctuations in sex hormones during a typical menstrual cycle. Although the timing of blood draws was established in relation to seasonal demands, consistent differences were observed in circulating sex hormones through the season between players with and without OC use.

6. CONCLUSION

Overall, the TL and EEE_{REL} incurred during a NCAA DI soccer season corresponded to perturbations in biomarkers of stress, inflammation, hematologic status, metabolism, anabolism, and reproduction as well as changes in body composition. The majority of biomarker response patterns were similar between groups; however, large differences in biomarker exposures existed over the season. Specifically, OC use was related to exacerbated stress, inflammatory, and metabolic disruptions that corresponded to a potentially reduced capacity for training adaptations and recovery. This study highlights the need for further research examining the impact of OCs on changes in performance with training as well as to investigate the effect of other hormonal contraceptive methods on biomarkers and body composition changes.

DISCLOSURES

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Figure Legends

Fig 1:

EEE_{REL}: relative exercise energy expenditure; Plots illustrate smoothed data obtained from Bayesian hierarchical generalized linear models. Circles represent averages and error bars represent ± standard deviations.

Fig 2:

Plots illustrate smoothed data obtained from Bayesian hierarchical generalized linear models. Circles represent averages and error bars represent ± standard deviations.

Table 1: Changes in Reproductive Biomarkers Over Time and Differences in Exposure Between Groups

	Effect size [50% CrI] *Probability (p) of increase ↑ or decrease ↓ across the season*		**Area Under Curve Ratio [95% CrI];** *Probability (p) OC exposure > CON*
	OC	**CON**	**OC_{AUC} : CON_{AUC}**
E_2 *(pmol/L)*	-0.03 [-0.64 – 0.55] ↓p=0.53	0.14 [-0.28 – 0.56] ↑p=0.75	0.36 [0.11 – 0.61]; p <0.001
P_4 *(nmol/L)*	-0.00 [-0.19 - 0.19] ↓p=0.50	0.06 [-0.20 – 0.12] ↓p=0.62	0.48 [0.13 – 0.89]; p =0.008
FSH *(IU/L)*	-0.38 [-1.2 – 0.45] ↓p=0.83	0.14 [-0.68 – 0.40] ↓p=0.70	0.67 [0.51 – 0.85]; p <0.001
SHBG *(nmol/L)*	-0.08 [-0.53 – 0.39] ↓p=0.64	0.27 [-0.58 – 0.02] ↓p=0.97	1.4 [1.3 – 1.5]; p >0.99
FTEST *(nmol/L)*	-0.06 [-0.22 – 0.10] ↓p=0.61	-0.05 [-0.14 – 0.05] ↓p=0.62	0.58 [0.47 – 0.70]; p =0.150
TTEST *(nmol/L)*	0.14 [-0.10 – 0.37] ↑p=0.65	0.43 [0.28 – 0.58] ↑p=0.98	0.94 [0.85 – 1.0]; p <0.001
Prolactin *(nmol/L)*	0.02 [-0.22 – 0.28] ↑p=0.53	0.24 [0.08 – 0.40] ↑p=0.85	0.92 [0.76 – 1.09]; p =0.178

CrI: Credible Interval; E_2: estradiol, P_4: progesterone, FSH: follicle-stimulating hormone, SHBG: sex-hormone binding globulin, FTEST: free testosterone, TTEST: total testosterone; Effect sizes (*d*) indicate the magnitude of change from baseline across the season; OC_{AUC} : CON_{AUC} ratio > 1 indicative of greater exposure in OC group, OC_{AUC} : CON_{AUC} ratio <1 indicative of greater exposure in CON.

Table 2: Changes in Stress & Inflammatory Biomarkers Over Time and Differences In Exposure Between Groups

	Effect size [50% CrI] *Probability (p) of increase ↑ or decrease ↓ across the season*		**Area Under Curve Ratio [95% CrI];** *Probability (p) OC exposure > CON*
	OC	**CON**	**OC_{AUC} : CON_{AUC}**
FCORT *(nmol/L)*	0.15 [-0.08 – 0.37] ↑p=0.68	0.18 [0.01 – 0.34] ↑p=0.77	0.99 [0.89 – 1.1]; p =0.420
TCORT *(nmol/L)*	0.11 [-0.02 – 0.25] ↑p=0.72	0.12 [0.03 – 0.22] ↑p=0.81	1.7 [1.6 – 1.8]; p >0.99
CRP *(IU/L)*	0.85 [0.64 – 1.1] ↑p=0.99	0.10 [-0.03 – 0.05] ↑p=0.70	5.2 [3.7 – 8.3]; p >0.99
IL-6 *(pg/mL)*	0.66 [-0.73 – 2.1] ↑p=0.84	0.46 (-0.49 – 1.3) ↑p=0.84	1.0 [0.80 – 1.2]; p =0.491
TNF-α *(pg/mL)*	0.67 [0.42 – 0.89] ↑p=0.96	0.07 [-0.08 – 0.22] ↓p=0.63	1.02 [0.95 – 1.1]; p =0.724

CrI: Credible Interval; FCORT: free cortisol, TTCORT: total cortisol, CRP: c-reactive protein, IL-6: interleukin-6, TNF-α: tumor necrosis factor-alpha; Effect sizes (*d*) indicate the magnitude of change from baseline across the season; OC_{AUC} : CON_{AUC} ratio > 1 indicative of greater exposure in OC group, OC_{AUC} : CON_{AUC} ratio <1 indicative of greater exposure in CON.

Table 3: Changes in Biomarkers of Muscular Growth and Breakdown Over Time and Differences in Exposure Between Groups

	Effect size [50% CrI] *Probability (p) of increase ↑ or decrease ↓ across the season*		**Area Under Curve Ratio [95% CrI];** *Probability (p) OC exposure > CON*
	OC	**CON**	**OC_{AUC} : CON_{AUC}**
GH *(µg/L)*	1.5 [1.0 – 1.9] ↑p=0.99	-0.12 [-0.42 – 0.15] ↓p=0.62	1.5 [0.97 – 2.2); p =0.97
IGF-1 *(µg/L)*	-0.52 [-0.68 – -0.35] ↓p=0.99	-0.14 [-0.23 – -0.03] ↓p=0.81	0.88 [0.81 – 0.96]; p =0.002
CK *(U/L)*	-0.11 [-0.35 – 0.12] ↓p=0.63	-0.07 [-0.24 – 0.11] ↓p=0.60	0.33 [0.16 – 0.50]; p <0.001

CrI: Credible Interval; GH: growth hormone, IGF-1: insulin-like growth factor-1, CK: creatine kinase; Effect sizes (*d*) indicate the magnitude of change from baseline across the season; OC_{AUC} : CON_{AUC} ratio > 1 indicative of greater exposure in OC group, OC_{AUC} : CON_{AUC} ratio <1 indicative of greater exposure in CON.

Table 4: Changes in Iron Status Over Time and Differences in Exposure Between Groups

	Effect size [50% CrI] *Probability (p) of increase ↑ or decrease ↓ across the season*		**Area Under Curve Ratio [95% CrI];** *Probability (p) OC exposure > CON*
	OC	**CON**	**OC_{AUC} : CON_{AUC}**
Fe *(µmol/L)*	-0.51 [-0.75 – -0.28] ↓p=0.98	-0.56 [-0.73 – -0.39] ↓p=0.99	1.2 [1.0 – 1.4]; p =0.974
Fer *(pmol/L)*	-0.25 [-0.42 – -0.05] ↓p=0.80	-0.36 [-0.49 – -0.22] ↓p=0.96	1.1 [0.98 – 1.2]; p =0.95
%SAT *(%)*	0.07 [-0.13 – 0.29] ↑p=0.60	-0.20 [-0.33 – 0.06] ↓p=0.83	0.99 [0.82 – 1.2]; p =0.487
TIBC *(µmol/L)*	0.22 [0.04 – 0.38] ↑p=0.80	0.42 [0.30 – 0.54] ↑p=0.99	1.10 [1.07 – 1.13]; p >0.99
Transferrin *(g/L)*	0.25 [0.07 – 0.42] ↑p=0.83	0.63 [0.52 – 0.75] ↑p=0.99	1.09 [1.05 – 1.13]; p >0.99

CrI: Credible Interval; Fe: iron, Fer: ferritin, %Sat: percent transferrin saturation, TIBC: total iron binding capacity; Effect sizes (*d*) indicate the magnitude of change from baseline across the season; OC_{AUC} : CON_{AUC} ratio > 1 indicative of greater exposure in OC group, OC_{AUC} : CON_{AUC} ratio <1 indicative of greater exposure in CON.

Table 5: Changes in Metabolic Biomarkers Over Time and Differences in Exposure Between Groups

	Effect size [50% CrI] *Probability (p) of increase ↑ or decrease ↓ across the season*		**Area Under Curve Ratio [95% CrI];** *Probability (p) OC exposure > CON*
	OC	**CON**	**OC_{AUC} : CON_{AUC}**
TSH *(mIU/L)*	-0.41 [-0.56 – 0.24] ↓p=0.95	-0.61 [-0.71 – 0.50] ↓p>0.99	1.4 [1.3 – 1.6]; p >0.99
TT_4 *(nmol/L)*	0.91 [0.72 – 1.1] ↑p>0.99	0.53 [0.41 – 0.64] ↑p>0.99	1.3 [1.2 – 1.4]; p >0.99
FT_4 *(pmol/L)*	-0.52 [-0.72 – 0.30] ↓p=0.95	-0.70 [-0.83 – 0.57] ↓p>0.99	0.97 (0.94 – 1.0); p =0.045
TT_3 *(nmol/L)*	0.71 [0.50 – 0.92] ↑p=0.99	-0.32 [-0.45 – 0.18] ↓p=0.94	1.3 [1.2 – 1.3]; p >0.99
FT_3 *(pmol/L)*	0.78 [0.55 – 1.0] ↑p=0.99	0.18 [0.03 – 0.33] ↑p=0.79	0.98 (0.95 – 1.0]; p =0.141
Leptin *(µg/L)*	1.2 [0.48 – 1.9]; ↑p>0.99	0.51 [0.08 – 0.95] ↑p>0.99	1.4 [1.3 – 1.6]; p >0.99

CrI: Credible Interval; TSH: thyroid stimulating hormone, TT_4: total thyroxine, FT_4: free thyroxine, TT_3: total triiodothyronine, FT_3: free triiodothyronine; Effect sizes (*d*) indicate the magnitude of change from baseline across the season; OC_{AUC} : CON_{AUC} ratio > 1 indicative of greater exposure in OC group, OC_{AUC} : CON_{AUC} ratio <1 indicative of greater exposure in CON.

Table 6: Changes in Training Load and Exercise Energy Expenditure Over Time and Differences Between Groups

	Effect size [50% CrI] *Probability (p) of increase ↑ or decrease ↓ across the season*		**Area Under Curve Ratio [95% CrI];** *Probability (p) OC exposure > CON*
	OC	**CON**	**OC_{AUC} : CON_{AUC}**
TL *(au)*	-2.5 [-2.6 – -2.3] ↓p>0.99	-2.2 [-2.3 – -2.1] ↓p>0.99	0.83 [0.76 – 0.89]; p <0.001
EEE_{REL} *(kcal/kg)*	-2.5 [-2.6 – -2.3] ↓p>0.99	-2.1 [-2.3 – -2.0] ↓p>0.99	0.85 [0.79 – 0.90]; p <0.001

CrI: Credible Interval; TL: Training Load, EEE_{REL}: relative exercise energy expenditure; Effect sizes (*d*) indicate the magnitude of change from baseline across the season; OC_{AUC} : CON_{AUC} ratio > 1 indicative of greater exposure in OC group, OC_{AUC} : CON_{AUC} ratio <1 indicative of greater exposure in CON.

Table 7: Changes in Body Composition Over Time

	Effect size [50% CrI] *Probability (p) of increase ↑ or decrease ↓ across the season*	
	OC	**CON**
Body Mass *(kg)*	0.04 [-0.06– 0.14] ↑p=0.61	-0.03 [-0.09 – 0.04] ↓p=0.61
BF% *(%)*	-0.08 [-0.19 – 0.04] ↓p=0.67	-0.50 [-0.58 – 0.43] ↓p>0.99
FFM *(kg)*	0.11 [0.02 – 0.20] ↑p=0.81	0.20 [0.14 – 0.26] ↑p=0.99

CrI: Credible Interval; BF%: body fat percentage, FFM: fat free mass; Effect sizes (*d*) indicate the magnitude of change from baseline across the season

Table 8: Team and Group Performance Characteristics Pre- and Post-season

	TEAM *(baseline)*	**TEAM** *(post-season)*	**CON** *(baseline)*	**CON** *(post-season)*	**OC** *(baseline)*	**OC** *(post-season)*
VO_{2max} *($ml \cdot kg^{-1} min^{-1}$)*	49.03 ± 4.1	47.67 ± 4.3	49.21 ± 4.6	Increase(n=3) Maintain(n=5) Decrease(n=6)	48.53 ± 2.9	Increase(n=1) Maintain(n=1) Decrease(n=3)
VT *(%VO_{2max})*	79.41 ± 4.6	80.74 ± 4.0	79.81 ± 4.2	Increase(n=5) Maintain(n=7) Decrease(n=2)	78.33 ± 5.9	Increase(n=2) Maintain(n=1) Decrease(n=2)
CMJ_{HOH} *(cm)*	46.82 ± 4.8	48.01 ± 5.0	46.59 ± 4.6	Increase(n=8) Maintain(n=5) Decrease(n=1)	47.41 ± 5.9	Increase(n=1) Maintain(n=4) Decrease(n=0)

Values are expressed as Mean ± SD; VO_{2max}: aerobic capacity, VT: ventilatory threshold, CMJ_{HOH}: hands on hip countermovement jump; Frequency counts for individual changes in performance variables (increase, maintenance, decrease) are presented for post-season testing values for OC and CON.