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

4
5 **ABSTRACT**

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

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

26
27 **KEY WORDS:** female athletes, hormonal contraceptives, training loads, performance

28
29 **NEW & NOTEWORTHY:** This study highlights the influence of OC use on physiological
30 changes that occur over a four-month intense, competitive season and the differential systemic
31 exposure to biomarkers, specifically those of inflammation, stress, anabolism, and energy
32 balance, between OC-using and non-using soccer players. Additionally, this study provides
33 insight into changes in body composition with prolonged training between female athletes with
34 and without OC use.

35 36 **1. INTRODUCTION**

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

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

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

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

91 **2. METHODS**

92 *2.1 Experimental Design*

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

104 *2.2 Participants*

105 Female collegiate soccer players (N=30) were monitored throughout the course of the
106 competitive season. Players were stratified into two groups: oral contraceptive (OC: n=6; Mean
107 \pm SD: age=19 \pm 1yr; weight= 67.6 \pm 3.0 kg; height= 168.4 \pm 4.4 cm) and control (CON: n=17;
108 age=19 \pm 1yr; weight= 66.0 \pm 8.0 kg; height= 168.2 \pm 6.5 cm) based on their reported OC use. OC
109 usage was determined by a Menstrual Status Questionnaire completed prior to the start of pre-
110 season, and was also repeated post-season for confirmation of OC status. At baseline, all OC
111 players reported at least one-year of OC use and all CON players reported menstrual cycle
112 lengths of 25-35 days. Self-reported age of menarche was 13 \pm 1 years for the CON group and
113 14 \pm 2 years for the OC group. Players were excluded from analysis if they were using intrauterine
114 contraception (n=4), altered contraception method mid-season (n=1), did not participate in team
115 training (n=1), or had a known metabolic disorder (n=1). Written, informed consent was

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

121 122 *2.3 Performance Testing*

123 Prior to the start of pre-season and upon completion of the competitive season, players
124 underwent a battery of performance tests and body composition assessments. All pre- and post-
125 season testing sessions, as well as blood draws, occurred within a one-week period. Prior to the
126 start of season, players reported to the lab ≥ 2 hours fasted and having refrained from exercise in
127 the preceding 12-hours. Body composition was assessed using air displacement plethysmography
128 via the BodPod (BODPOD, COSMED, Concord, CA, USA) with a predicted lung volume via
129 the Brozek formula (7, 16) to determine percent body fat (BF%) and fat free mass (FFM). After a
130 ~10-minute standardized dynamic warm-up, players performed maximal countermovement
131 vertical jumps with hands-on-hips (CMJ_{HOH}) on a contact mat (Probotics Inc., Huntsville, AL,
132 USA) (36). Players were allowed two attempts with highest jump height recorded.

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

139 used in collegiate soccer players and consisted of two-minute stages at a constant 2% incline,
140 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).
141 Players continued the test with encouragement from research assistants until volitional fatigue.
142 At least two of the following criteria were met for attainment of VO_{2max} : $RER \geq 1.1$, observation
143 of a plateau in O_2 consumption (increase ≤ 150 ml/min with increasing workload), and $HR > 85\%$
144 age-predicted HR_{max} ($208 - 0.7 \times \text{age}$). For athletes who did not meet the above criteria, VO_{2peak}
145 was used ($n=3$). Player's VT was analyzed after the completion of each test as the inflection
146 point where VCO_2 increased nonlinearly with VO_2 , expressed as a percentage of VO_{2max} (5).

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

151 152 *2.4 Blood Draws*

153 Blood draws were performed prior to pre-season, on weeks 2 (end of pre-season), 4, 8, &
154 12 of the season, and post-season. Athletes reported to the lab between 0700 and 0900h and were
155 instructed to arrive in an euhydrated state following an overnight fast. All draws during the
156 season were performed between 18-24 hours following a game (T2-T5), with the exception of
157 pre-season (T1: 'baseline') and post-season draws (T6: ~58h post-game). The T2 blood draw
158 was performed in order to assess changes in biomarkers following pre-season in which
159 workloads are the highest for the athletes, while the T6 blood draw offered a snapshot of
160 recovery post-season. For all draws, blood samples were drawn from participants while seated
161 via the antecubital fossa (21G, BD Vacutainer, Safety-Lok) by three experienced phlebotomists

162 into clot activator collection tubes (SST and gel-free tubes). Blood samples were centrifuged for
163 10-minutes at 4,750 rpm (Allegra x-15R; Beckman Coulter, Brea, CA, USA), serum/plasma
164 were aliquoted from centrifuged tubes and immediately shipped, in containers designed to
165 maintain 4°, 20°, or -20°C depending on the analyte, to a Clinical Laboratory Improvements
166 Amendment (CLIA)-certified processing facility for analysis (Quest Diagnostics, Secaucus, NJ,
167 USA). Samples were run in duplicate and the coefficient of variation (CV) was between 0.5-
168 10.0% for all biomarkers. Results were provided to the researchers via the Quest Diagnostics
169 Care360 online portal. Biomarkers analyzed included total cortisol (TCORT), free cortisol
170 (FCORT), creatine kinase (CK), CRP, IL-6, tumor necrosis factor- α (TNF- α), estradiol (E₂),
171 growth hormone (GH), insulin-like growth factor-1 (IGF-1), ferritin (Fer), iron (Fe), total iron
172 binding capacity (TIBC), percent transferrin saturation (%SAT), transferrin, leptin, total
173 triiodothyronine (TT₃), free triiodothyronine (FT₃), total thyroxine (TT₄), free thyroxine (FT₄),
174 thyroid-stimulating hormone (TSH), prolactin, sex-hormone binding globulin (SHBG), follicle-
175 stimulating hormone (FSH), progesterone (P₄), total testosterone (TTEST), and free testosterone
176 (FTEST).

177 178 *2.5 In-season athlete-monitoring*

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

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

192 *2.6 Statistical Analysis*

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

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

226 3. RESULTS

227 3.1 Reproductive markers: E_2 , P_4 , FSH, SHBG, TTEST, FTEST, Prolactin

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

231 (Table 1) in both OC (-0.38: FSH; to 0.14: TTEST) and CON (-0.27: SHBG; to 0.43: TTEST)
232 groups. The area under the curve ratios indicated the OC users were exposed to substantively
233 higher levels of SHBG (AUC ratio: 1.4 [95%CrI: 1.3 – 1.5]; $p>0.99$), but substantively lower
234 levels of E₂ (AUC ratio: 0.36 [95%CrI: 0.11 – 0.61]; $p<0.001$), P₄ (AUC ratio: 0.48 [0.13 –
235 0.89]; $p=0.008$), FTEST (AUC ratio: 0.58 [95%CrI: 0.47 – 0.70]; $p<0.001$), and FSH (AUC
236 ratio: 0.67 [95%CrI: 0.51 – 0.85]; $p<0.001$) compared with the CON group across the season.
237 Graphical outputs of Bayesian hierarchical linear models for the reproductive biomarkers are
238 presented in *Figure S2*; DOI: <http://doi.org/10.6084/m9.figshare.12996794>.

239
240 **Insert Table 1 here**

241 242 **3.2 Stress and inflammatory markers: TCORT, FCORT, CRP, IL-6, TNF- α**

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

254 under the curve ratios indicated the OC group was exposed to a substantively greater amount of
255 TCORT (AUC ratio: 1.7 [95%CrI: 1.6 – 1.8]; $p>0.99$) and CRP (AUC ratio: 5.2 [95%CrI: 3.7 –
256 8.3]; $p>0.99$) compared with the CON group across the season. Graphical outputs of Bayesian
257 hierarchical linear models for stress and inflammatory biomarkers are presented in *Figure S3*;
258 DOI: <http://doi.org/10.6084/m9.figshare.12996794>.

260 **Insert Table 2 here**

262 **3.3 Markers of muscular growth and breakdown: GH, IGF-1, CK**

263 Linear responses were identified for all biomarkers indicative of growth and muscular
264 breakdown across the season. The OC group experienced a large increase in GH ($d=1.5$), but a
265 moderate decrease in IGF-1 ($d=-0.52$) across the season (*Table 3*). In contrast, median point
266 estimates were below a medium threshold ($|d|<0.5$) for all muscular anabolic and catabolic
267 biomarkers in the CON group (-0.14: IGF-1; to -0.07: CK) (*Table 3*). The area under the curve
268 ratios indicated OC users were exposed to substantively higher levels of GH (AUC ratio: 1.5
269 [95%CrI: 0.97– 2.2]; $p=0.97$), but substantively lower levels of CK (AUC ratio: 0.33 [95%CrI:
270 0.16 – 0.50]; $p<0.001$) compared with the CON group across the season. Graphical outputs of
271 Bayesian hierarchical linear models for biomarkers of muscular growth and breakdown are
272 presented in *Figure S4*; DOI: <http://doi.org/10.6084/m9.figshare.12996794>.

274 **Insert Table 3 here**

276 **3.4 Markers of iron status: Fe, Fer, %Sat, TIBC, Transferrin**

277 Linear responses were identified for the majority of biomarkers indicative of iron status
278 in the athletes across the season. Both OC and CON groups were found to experience a moderate
279 decrease in Fe ($d=-0.51$, $d=-0.56$), with the CON group also demonstrating a moderate increase
280 in TIBC ($d=0.63$) (*Table 4*). Similar non-linear responses were identified for %SAT with OC and
281 CON groups experiencing a decrease between T1-T5 (combined $d = -0.42$; [50%CrI: -0.60 – -
282 0.23]), followed by a subsequent increase between T5-T6 (combined $d= 0.34$; [50%CrI: 0.17 –
283 0.51]). Graphical outputs of Bayesian hierarchical linear models for biomarkers of iron status are
284 presented in *Figure S5*; DOI: <http://doi.org/10.6084/m9.figshare.12996794>.

285
286 **Insert Table 4 here**

287 288 **3.5 Markers of metabolism: TSH, TT₄, FT₄, TT₃, FT₃, Leptin**

289 Linear responses were identified for all biomarkers indicative of metabolism and energy
290 balance across the season. OC users were found to experience increases in the majority of
291 biomarkers with large effects for TT₄ ($d=0.91$) and leptin ($d=1.2$), and moderate effects for TT₃
292 ($d=0.71$) and FT₃ ($d=0.78$), but a moderate effect for a decrease in FT₄ ($d=-0.52$) (*Table 5*).
293 Similarly, the CON group experienced moderate effects for increases in TT₄ ($d=0.53$) and leptin
294 ($d=0.51$), and moderate effects for decreases in both TSH ($d=-0.61$) and FT₄ ($d=-0.70$) (*Table 5*).
295 The area under the curve ratios indicated the OC group were exposed to substantially greater
296 amounts of TSH (AUC ratio: 1.4 [95%CrI: 1.3– 1.6]; $p>0.99$), TT₄ (AUC ratio: 1.3 [95%CrI:
297 1.2– 1.4]; $p>0.99$), TT₃ (AUC ratio: 1.3 [95%CrI: 1.2– 1.3]; $p>0.99$), and leptin (AUC ratio: 1.4
298 [95%CrI: 1.3– 1.6]; $p>0.99$) compared with the CON group across the season. Graphical outputs

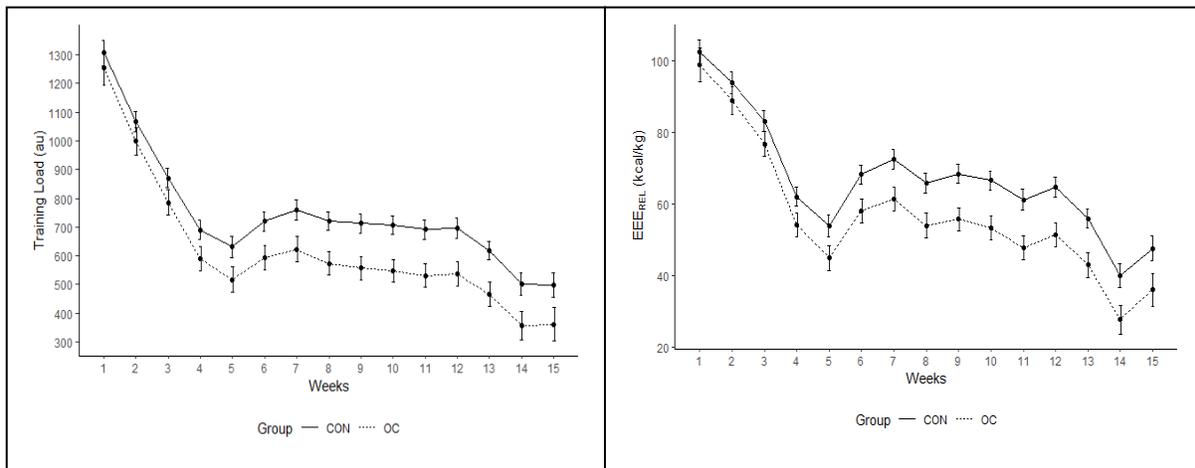
of Bayesian hierarchical linear models for metabolic biomarkers are presented in *Figure S6* DOI: <http://doi.org/10.6084/m9.figshare.12996794>.

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



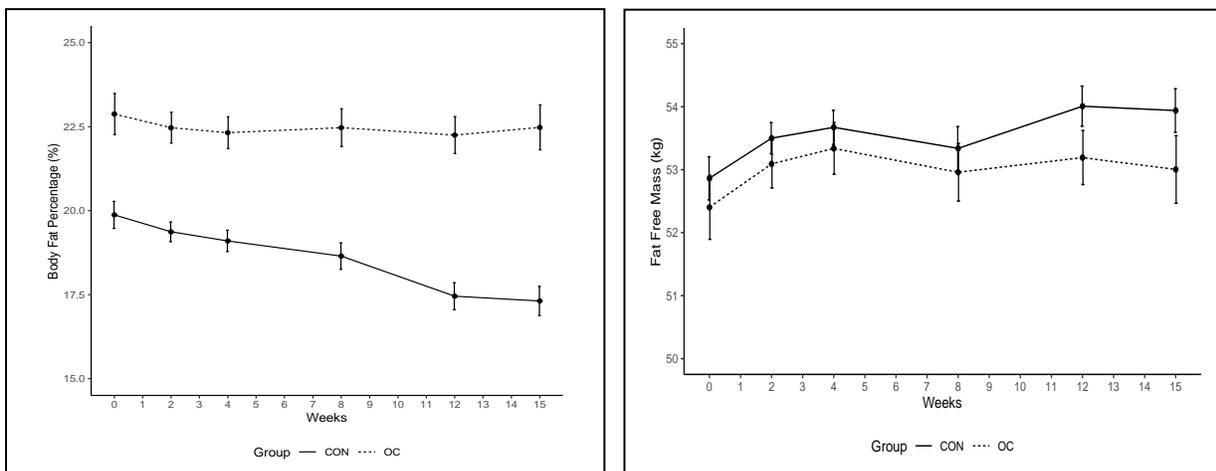
EEE_{REL} : relative exercise energy expenditure; Plots illustrate smoothed data obtained from Bayesian hierarchical generalized linear models. Circles represent averages and error bars represent \pm standard deviations.

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



326

Plots illustrate smoothed data obtained from Bayesian hierarchical generalized linear models. Circles represent averages and error bars represent \pm 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 $EEES_{REL}$ 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.

357 Over the season, effect sizes revealed concentrations of sex hormones E₂ and P₄ were
358 relatively stable; however, the CON group experienced a ~3x greater exposure to E₂ and ~2x
359 greater exposure to P₄ compared to OC users over the season. This is expected as OCs act by
360 suppressing endogenous production of E₂ and P₄ through the inhibition of the HPG-axis (43).
361 Oral contraceptive-mediated suppression of ovarian hormone production is coupled with a
362 decreased production and secretion of FSH and luteinizing hormone (LH) (43). This is supported
363 by the finding that the CON group exhibited larger concentrations of FSH (~2x greater exposure)
364 over the season than the OC group. Although LH concentrations and exogenous hormone doses
365 were not quantified in this study, the differences in female reproductive hormones between OC
366 and CON groups illustrate the typical reproductive hormonal profiles associated with OC use.
367 Unlike the CON group, OC users experienced a small effect for a decreased FSH concentrations
368 over the season. This increased suppression of FSH levels may in part be mediated by HPA-axis
369 interactions and inhibition on the HPG-axis as TCORT was elevated in OC versus CON groups.
370 Previous research has shown decreased FTEST and increased SHBG levels with OC use (52).
371 This mirrored the findings in this study as the OC group had about ~2x less FTEST and ~1.5x
372 greater SHBG exposure over the season compared with the CON group. This builds upon acute
373 findings in elite athletes where salivary testosterone levels remained lower in OC users after
374 exercise regardless of training session intensity (15). Finally, no differences in prolactin AUC
375 were observed between groups. Prolactin levels can be influenced by IL-6 production (32),
376 potentially explaining the similar prolactin levels across the season as both groups experienced
377 similar increases in IL-6. Additionally, although the timing of blood draws in this study may
378 have influenced the observed reproductive hormone concentrations in the CON group due to
379 cyclic nature of fluctuations in sex hormones during a typical menstrual cycle, overall the

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

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

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

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

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

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

439 Overall, CK levels in the CON group started and remained elevated above OC users,
440 yielding about a ~3x greater exposure in the CON group throughout the season. Previous
441 research has shown E₂ to potentially play a protective role against muscle damage through
442 mechanisms such as increased membrane stabilization (46). Findings on acute elevations in CK
443 post-exercise with OC use remain equivocal (47); however, greater reductions in CK values 72-
444 hours post-exercise have been observed in OC users (11). The greater CK levels observed in the
445 CON group may be indicative of greater skeletal muscle turnover in these athletes (3), especially
446 when taken into context with the FFM gains over the competitive season.

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

449 through week 12 for %SAT followed by a small increase during the remainder of the season.
450 These changes may indicate a trend towards a training-induced Fe deficiency particularly over
451 the first 12-weeks of the season before the final decline in TL/EEE_{REL} as observed in previous
452 research (51). Fe deficiency, defined as Fer concentrations <12 µg/L and percent saturation
453 <16%, has been reported in endurance and team sport athletes, with females experiencing a
454 greater risk for reduced Fe status (30). The similar responses between groups in iron status over
455 the collegiate season reflect previous findings that Fer and Fe concentrations are not affected
456 with OC use (47).

457 For all athletes, FT₃ levels increased from baseline through week 12 before declining
458 through week 15, demonstrating a similar response to that previously described in female
459 collegiate soccer players (51). Decreased or no change in FT₃ levels have often been shown over
460 training periods in athletes, potentially as an effort to promote energy conservation during high
461 EEE (4, 48). Perhaps the FT₃ decline observed indicates decreased muscular metabolism “needs”
462 as FT₃ regulates skeletal muscle metabolism (45) and these declines corresponded to further
463 decreases in TL/EEE_{REL} in weeks 12-15. Future research examining the relationship between
464 changes in TL, EEE, and energy intake along with thyroid hormone responses in female athletes
465 is warranted due to the conflicted findings in these hormones over periods of intense training.
466 Between groups, OC athletes had considerably greater TSH, TT₄, and TT₃ levels, yet no
467 differences were observed for FT₃ exposure compared to CON players. It appears that OCs
468 potentially influence thyroid hormone levels; however, this does not necessarily correspond to
469 increased levels of the biologically active FT₃ above non-OC users. This lends support to
470 previous findings that OCs may increase TSH as well as TT₄ and TT₃ levels due to increased

471 binding capacity of thyroxine-binding globulin, without significant changes in FT₄ and FT₃
472 levels (53).

473 For both groups, moderate to large increases were observed in leptin, an adipose-derived
474 hormone whose levels are reflective of changes in energy balance (1), over the season.
475 Previously in collegiate rowers, changes in FT₃ levels were related to leptin changes, with rowers
476 experiencing either a decrease in both FT₃ and leptin or no change in the hormones over 20-
477 weeks of training (4). Conversely, in this study increases in FT₃ and leptin were observed. It
478 appears a relationship exists between thyroid hormones and leptin production that may be
479 reflective of energy balance in athletes. Throughout the season OC athletes exhibited an almost
480 ~1.5x greater exposure to leptin compared to CON. The elevated leptin levels correspond with
481 the divergent results in BF% identified, with OC athletes maintaining values and the evidence
482 obtained that CON progressively decreased values throughout the season. Leptin expression has
483 been shown to correlate with adipose stores (1), supporting the disparity in leptin levels observed
484 at baseline and throughout the season between the groups. Previous research examining the
485 effects of OC use on body composition is inconsistent in its findings, with some studies reporting
486 no change (40, 41), while others reporting increases in body weight (9, 12, 41). It appears
487 however, that changes in leptin across a training block may occur independent of body
488 composition changes, as previously evidenced in collegiate rowers (4). The authors speculate
489 that while leptin may indicate fat storage, changes may be primarily influenced by fluctuations in
490 energy balance (1) with training.

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

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

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

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

524

525 **6. CONCLUSION**

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

535

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541 **Conflicts of Interest:** The authors have no conflicts of interest to report.

542

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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 \pm standard deviations.

Fig 2:

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