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RESEARCH ARTICLE OPEN ACCESS

Impact of Menstrual Cycle and Oral Contraceptives on Haematological and Inflammatory Biomarkers in Highly Trained Female Athletes

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Keywords: contraception | elite female athletes | hormonal status | reticulocytes | transferrin

ABSTRACT

Haematological and inflammatory biomarkers play an important role in athlete performance and health, with some of them used in the fight against doping. However, little is known about how they are modulated by sex hormone fluctuations in highly trained female athletes. We therefore measured the haematological parameters monitored in the athlete biological passport (ABP) as well as erythropoietin, serum markers of iron and inflammatory statuses (iron, ferritin, transferrin, transferrin saturation, albumin, creatinine, total protein, interleukin-6 and TNF- α) in 20 highly trained female athletes: 10 with normal menstrual cycle (NMC) during the early follicular and mid-luteal phases and 10 using a combined oral contraceptive (COC, i.e., ethinylloestradiol and levonorgestrel) during active and inactive hormone intake. Body composition, leptin and lipid profile (total cholesterol, HDL, LDL and triglycerides) were determined in parallel. No changes were observed throughout NMC phases. Irrespective of active/inactive pill intake, COC use increased transferrin, triglycerides as well as reticulocyte count ($p < 0.05$) and decreased interleukin-6 ($p < 0.05$), with no significant changes in the other parameters studied. In conclusion, given our results across NMC phases in highly trained athletes, it seems warranted to investigate whether intense physical training would mitigate the impact of endogenous sex hormones on body composition and haematological and inflammatory parameters. In addition, further studies are needed to determine the extent of the changes induced by COCs on these blood biomarkers in elite female athletes when subjected to extreme environments such as intensive training or competition in humid heat, cold and/or hypoxia or when using other medications in parallel.

1 | Introduction

Haematological and inflammatory biomarkers as well as body composition play an important role in athlete performance and health as they mediate many adaptations to exercise [1]. Some of them are also used in the fight against doping. However, little is

known about how they are modulated by sex hormone fluctuations in highly trained female athletes.

Female specificity is partly linked to the menstrual cycle, consisting of the follicular and the luteal in a normal menstrual cycle (NMC). Oestradiol (E2) increases in both follicular and luteal

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phases, whereas progesterone (PG) increases only in the luteal phase, with E2 and PG levels at their lowest during the early follicular phase [2]. Between 20% and 70% of female athletes use hormonal contraception [3–5], with a large majority taking combined oral contraceptive (COC), containing most of the time, ethinylloestradiol and levonorgestrel [2]. COCs are taken for 21 days followed by 7 days off to reproduce NMC, but without the E2 and PG peaks, thanks to the inhibition of the hypothalamic–pituitary–gonadal axis.

Curiously, there is still no consensus regarding the effect of NMC phases on body composition in female athletes, with studies reporting or not [6–11] change in body mass across NMC or COC phases, though the impact of hormonal status on lipid profile and leptin levels has been little investigated either with or without COC intake [1, 12–14]. A recent review [1] about the effects of NMC phases on immune function and inflammation revealed contradictory results. In healthy active females, a study on haematological athlete biological passport (ABP) in NMC volunteers [15] showed lower reticulocyte % in the follicular vs. the luteal phase, without any change in the other parameters, whereas a remarkable stability of all ABP markers was observed for women taking COC [16]. In addition, few changes were obtained throughout NMC on serum markers of iron status [17], but higher serum iron, transferrin and transferrin saturation were found in COC vs. NMC during active hormone intake. Finally, although erythropoietin (EPO) levels do not appear to change during the menstrual cycle in healthy women [18–20], there is no information on the potential effect of COCs on EPO secretion or on thrombotic risk in the elite athlete population, as has been demonstrated in women subjected to extreme environments [21].

Given the scarcity of studies carried out on female athletes, we proposed to examine in both COC and NMC female athletes, haematological, iron and inflammatory biomarkers, as well as body composition, across two phases: active and inactive hormone intake for COC and early follicular (low E2 and low PG) and mid-luteal phases (high E2 and high PG) for NMC.

2 | Material and Methods

2.1 | Participants

Twenty highly trained judokas (12) and horse riders (8) volunteered to participate in the study and signed a written consent form after being informed of the objectives and risks of the study.

These two sporting disciplines, which involve multiple intermittent high-intensity efforts, require highly developed technical skills and levels of physical fitness, not only anaerobic but also aerobic, with a significant increase in energy expenditure, as an average of over 75% of maximum oxygen consumption possibly being reached during training [22–25]. All procedures were approved by the ethics committee (ID-RCB: 2020-A02965-34) and were in accordance with the Declaration of Helsinki. Prior to the study, subjects were screened with a medical history and physical examination. Inclusion criteria required that participants be highly physically active training (6–11 times per week, 3–6 h per day, for at least 3 years).

TABLE 1 | Initial characteristics of subjects (mean ± SEM) in the NMC and COC groups.

Subjects' characteristics	NMC (10)	COC (10)
Age (years)	19.8 ± 0.4	20.2 ± 0.6
Height (cm)	164.0 ± 2.6	166.7 ± 2.9
Weight (kg)	61.1 ± 2.6	60.2 ± 2.7
BMI (kg/m ²)	22.7 ± 0.5	21.6 ± 0.6

Exclusion criteria were the presence of cardiovascular, liver, biliary or renal disease; hyperlipidaemia; high blood pressure; endocrinological disorder; oligomenorrhoea or amenorrhoea; pregnancy; history of thromboembolic disorder. Subjects were separated into two groups: Group 1 (10 subjects) with NMC, without hormonal contraception for at least 1 year; and Group 2 (10 subjects) with monophasic COC use: 20 µg/100 µg (8); 30 µg/150 µg (2) of ethinylloestradiol and levonorgestrel per pill, respectively. Age, height and body mass were similar in the both groups at the start of the study (Table 1).

2.2 | Study Design

Each subject visited the laboratory two times after the inclusion visit, at 10-week intervals, in order to shift their cycle phase and reach the two target statuses. NMC participants came to the laboratory during the early follicular (Days 1–7, i.e., NMP_{EF}) and mid-luteal (Days 18–24, i.e., NMP_{ML}) phases. Ovulation was detected with PG measurement in all NMC participants and menstrual phases were retrospectively confirmed by the analysis of serum sex female hormones. COC participants attended the laboratory during active (Days 8–18, i.e., COC_{AC}) and inactive hormone intake (Days 23–28, i.e., COC_{IN}).

The protocol for each trial was held at the same time of day to prevent diurnal variation. Participants were asked to maintain their normal food dietary intake during the experiment and to refrain from ingesting either caffeine or alcohol and strenuous efforts for at least 24 h prior to the trial sessions.

2.3 | Body Composition Determination

On trial days, subjects reported in a fasting state to the laboratory between 6:45 am and 7:45 am. After a 15-min rest, body composition was assessed by bioelectrical impedance analysis using a Tanita MC-780 multifrequency segmental body composition analyser [26] which included body mass, fat mass, muscle mass and body water (total and intra- and extracellular).

2.4 | Blood Analysis

Venous samples (10 mL) were then collected from an antecubital vein using standard procedures and analysed within 24 h for the markers registered in the haematological module of ABP (4 mL on EDTA tube). Automatic haematology analyser Sysmex XN-1000 (Norderstedt, Germany) was used for the analyses of haemoglobin

TABLE 2 | Oestradiol and progesterone concentrations (mean \pm SEM) across NMC and COC phases.

Sex hormones	NMC (10)	COC (10)
Estradiol (pg/mL)	NMP _{EF} : 60.1 \pm 10.8 NMP _{ML} : 110.8 \pm 19.7 ⁺	COC _{IN} : 68.2 \pm 8.4 COC _{AC} : 28.2 \pm 6.7 [§]
Progesterone (ng/mL)	NMP _{EF} : 0.75 \pm 0.09 NMP _{ML} : 11.61 \pm 1.63 ⁺⁺	COC _{IN} : 0.63 \pm 0.07 COC _{AC} : 0.60 \pm 0.05

Abbreviations: COC_{AC}, active intake; COC_{IN}, inactive intake; NMP_{EF}, early follicular; NMP_{ML}, mid-luteal.

⁺ $p < 0.05$.

⁺⁺ $p < 0.01$, difference between NMP_{ML} and the other conditions.

[§] $p < 0.05$, difference between COC_{AC} and COC_{IN}.

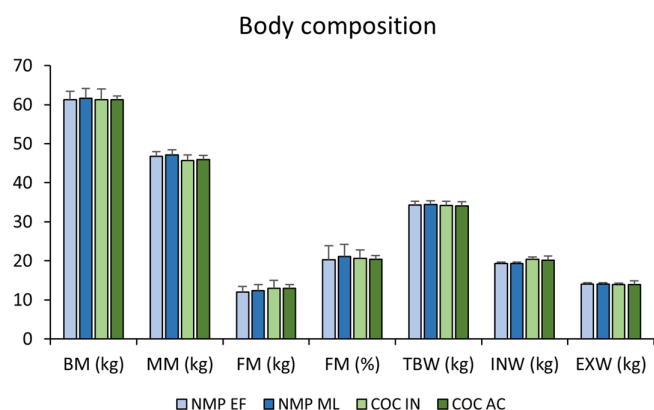


FIGURE 1 | Body composition (mean \pm SEM) across NMC and COC phases: body mass (BM), muscle mass (MM), fat mass (FM), total body water (TBW) and intra- (INW) and extra- (EXW) cellular water. COC_{AC}, active intake; COC_{IN}, inactive intake; NMP_{EF}, early follicular; NMP_{ML}, mid-luteal.

(HGB), haematocrit (HCT), red blood cell count, mean corpuscular volume, mean corpuscular haemoglobin, mean corpuscular haemoglobin concentration, reticulocyte percentage (RET%), absolute reticulocytes count (RET#), immature reticulocyte fraction, white blood cell (total and eosinophils, monocytes, lymphocytes, basophiles) and platelets, according to the procedure in WADA document TD2021BAR. The remaining blood (6 mL) was immediately transferred into serum, EDTA and heparin tubes and centrifuged at 3500rpm for 10min before serum and plasma being stored in a freezer at -20°C for later analysis. Serum iron, ferritin, transferrin and transferrin saturation (TSAT), total proteins, albumin, creatinine, total cholesterol, HDL, LDL and triglycerides were determined by classical immunoturbometry or colorimetry (1mL heparin plasma). In addition, E2, PG, EPO, leptin, interleukin-6 (IL-6) and TNF-alpha were determined by ELISA (EPO kit from R&D Systems; other kits from DRG Diagnostic), with assays made in duplicate and coefficients of variation for all parameters were always $< 10\%$.

2.5 | Statistical Analyses

Results are presented as mean values \pm standard error of the mean (SEM). After evaluating the normality of the samples, differences between the trials were investigated with a two-way (group and period) analysis of variance (ANOVA) with repeated measurements (Statistica 12) and group/period interaction factor indicated,

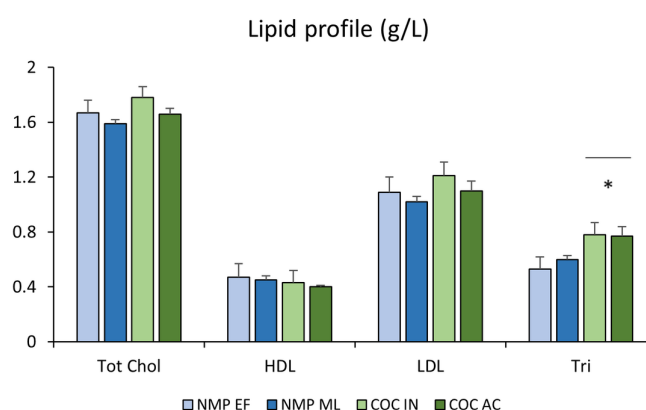


FIGURE 2 | Lipid profile (mean \pm SEM) across NMC and COC phases: total cholesterol (Tot Chol), HDL, LDL and triglycerides (Tri). COC_{AC}, active intake; COC_{IN}, inactive intake; NMP_{EF}, early follicular; NMP_{ML}, mid-luteal. * $p < 0.05$, difference between COC and NMC.

if any. A post hoc Newman–Keuls test was performed to determine which parameters showed significant differences, in the event of an ANOVA revealing a significant main effect. Correlations were calculated using Pearson's product moment correlation test. The null hypothesis was rejected at p value < 0.05 .

3 | Results

3.1 | Sex Hormones (Table 2)

There was a group and condition effect with lower E2 and PG levels in COC vs. NMC ($p < 0.01$). In NMC subjects, higher E2 and PG were obtained in NMP_{ML} vs. the other phases ($p < 0.05$ and $p < 0.01$, respectively). E2 were lower in COC_{AC} vs. COC_{IN} ($p < 0.05$). A significant group/period interaction factor was found for PG ($p < 0.01$).

3.2 | Body Composition, Lipid Profile and Cytokines (Figures 1–3)

No effects linked to NMC and COC phases on body mass and composition and leptin levels were evident. For all subjects, there was a high correlation between leptin and fat mass, expressed in either % or kg ($r = 0.72$, $p < 0.05$). Triglycerides increased ($p < 0.05$) in COC

vs. NMC with no change in other lipid profile parameters. In parallel, IL-6 levels were lower ($p < 0.05$), whereas TNF- α levels were unchanged in COC vs. NMC, with no phase effects.

3.3 | EPO, Serum Iron and Protein Concentrations (Table 3)

Irrespective of the period of active/inactive pill intake, COC vs. NMC increased transferrin ($p < 0.01$), with no significant change in EPO, TSAT, ferritin, serum iron, total protein, albumin or creatinine levels.

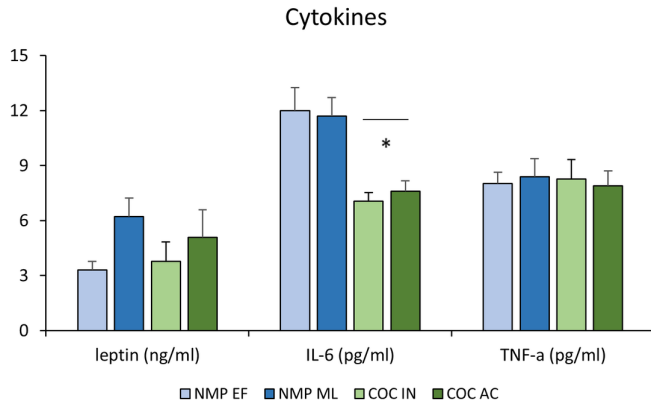


FIGURE 3 | Cytokines (mean \pm SEM) across NMC and COC phases: leptin, IL-6 and TNF- α . COC_{AC}, active intake; COC_{IN}, inactive intake; NMP_{EF}, early follicular; NMP_{ML}, mid-luteal. * $p < 0.05$, difference between COC and NMC.

3.4 | Haematological Parameters (Table 4)

HGB and HCT did not differ in the two groups across the different phases as well as the other red blood cell indices. However, RET# was significantly higher in COC vs. NMC subjects, whatever the phase ($p < 0.05$).

4 | Discussion

In highly trained female athletes, body composition was not influenced by either NMC or COC phases. Irrespective of active/inactive intake, COC use increased transferrin, RET# and triglyceride levels and decreased IL-6, whereas no parameter was modified throughout NMC phases.

The NMC and COC female athletes had similar body mass and composition at the start of the study, and no changes were observed when their sexual hormone status fluctuated over the course of the experiment. Previous studies have provided conflicting results. Indeed, some studies have reported a difference in body mass due to a change in body water caused by fluctuations in sex hormones. Kanellakis et al. [9] observed a higher body weight the first day of menstruation compared to the mid-follicular phase, which could be attributed to an increase in extracellular water. This finding was also revealed by other authors whereas increased body weight was also noted during the 3 days before the beginning of menstruation [7]. However, most of the recent studies performed on female athletes, using either bioimpedance meter or skinfold methodology [6, 8, 10, 11], did not describe any significant change in body composition across NMC and COC phases. According to Stachenfeld et al. [27, 28]

TABLE 3 | Erythropoietin (EPO), serum iron, ferritin, transferrin, transferrin saturation (TSAT), albumin, creatinine and total protein concentrations (mean \pm SEM) across NMC and COC phases.

Parameters	NMC (10)	COC (10)
EPO (mIU/mL)	NMP _{EF} : 6.9 \pm 1.1 NMP _{ML} : 6.5 \pm 0.9	COC _{IN} : 6.9 \pm 0.9 COC _{AC} : 7.7 \pm 1.0
Serum iron (mg/L)	NMP _{EF} : 0.68 \pm 0.09 NMP _{ML} : 0.77 \pm 0.08	COC _{IN} : 0.72 \pm 0.08 COC _{AC} : 0.74 \pm 0.09
Ferritin (ng/mL)	NMP _{EF} : 35.9 \pm 5.8 NMP _{ML} : 39.8 \pm 6.6	COC _{IN} : 30.6 \pm 5.6 COC _{AC} : 28.3 \pm 8.5
Transferrin (g/L)	NMP _{EF} : 2.47 \pm 0.13 NMP _{ML} : 2.49 \pm 0.12	COC _{IN} : 3.19 \pm 0.10** COC _{AC} : 3.09 \pm 0.09**
TSAT (%)	NMP _{EF} : 0.19 \pm 0.02 NMP _{ML} : 0.23 \pm 0.03	COC _{IN} : 0.17 \pm 0.02 COC _{AC} : 0.17 \pm 0.02
Albumin (g/L)	NMP _{EF} : 43.7 \pm 1.9 NMP _{ML} : 44.2 \pm 1.6	COC _{IN} : 43.1 \pm 0.8 COC _{AC} : 42.8 \pm 2.2
Creatinine (mg/L)	NMP _{EF} : 8.1 \pm 0.5 NMP _{ML} : 8.3 \pm 0.3	COC _{IN} : 9.0 \pm 0.5 COC _{AC} : 8.9 \pm 0.8
Total proteins (g/L)	NMP _{EF} : 69.0 \pm 2.7 NMP _{ML} : 68.8 \pm 2.1	COC _{IN} : 71.9 \pm 1.0 COC _{AC} : 70.9 \pm 2.5

Abbreviations: COC_{AC}, active intake; COC_{IN}, inactive intake; NMP_{EF}, early follicular; NMP_{ML}, mid-luteal.

* $p < 0.05$.

** $p < 0.01$, difference between COC and NMC.

TABLE 4 | Haematological parameters (mean \pm SEM) across NMC and COC phases.

Parameters	NMC		COC	
	NMP _{EF}	NMP _{ML}	COC _{IN}	COC _{AC}
RBC count ($10^6/\mu\text{L}$)	4.48 \pm 0.09	4.42 \pm 0.08	4.60 \pm 0.07	4.53 \pm 0.08
HGB (g/dL)	13.0 \pm 0.3	12.9 \pm 0.2	13.6 \pm 0.2	13.5 \pm 0.2
HCT (%)	40.1 \pm 0.6	39.8 \pm 0.4	41.3 \pm 0.4	40.8 \pm 0.4
MCV (fL)	89.7 \pm 1.2	90.1 \pm 1.3	89.8 \pm 0.8	90.1 \pm 1.2
MCH (pg)	29.0 \pm 0.5	29.2 \pm 0.6	29.5 \pm 0.4	29.8 \pm 0.5
MCHC (g/dL)	32.3 \pm 0.3	32.0 \pm 0.5	32.8 \pm 0.2	33.1 \pm 0.3
RET (%)	1.26 \pm 0.07	1.23 \pm 0.08	1.56 \pm 0.13	1.52 \pm 0.09
RET# ($10^6/\mu\text{L}$)	55.0 \pm 3.7	54.3 \pm 3.7	71.1 \pm 5.3*	68.5 \pm 3.8*
IRF (%)	8.8 \pm 1.1	9.3 \pm 1.0	7.8 \pm 1.0	9.8 \pm 1.5
WBC count ($10^3/\mu\text{L}$)	5.9 \pm 0.4	5.7 \pm 0.5	5.4 \pm 0.4	6.2 \pm 0.4
Eosinophils (%)	2.2 \pm 0.2	2.5 \pm 0.4	3.4 \pm 0.5	3.8 \pm 0.6
Monocytes (%)	10.0 \pm 0.8	10.0 \pm 0.8	9.1 \pm 0.6	8.1 \pm 0.6
Lymphocytes (%)	38.3 \pm 2.2	40.6 \pm 2.5	41.1 \pm 2.6	38.7 \pm 2.4
Basophiles (%)	0.7 \pm 0.1	0.7 \pm 0.1	1.0 \pm 0.2	0.9 \pm 0.1
Platelets ($10^3/\mu\text{L}$)	268.5 \pm 12.9	255.9 \pm 17.9	286.0 \pm 21.0	278.2 \pm 20.4

Abbreviations: COC_{AC}, active intake; COC_{IN}, inactive intake; HCT, haematocrit; HGB, haemoglobin; IRF, immature reticulocyte fraction; MCH, mean corpuscular haemoglobin; MCHC, mean corpuscular haemoglobin concentration; MCV, mean corpuscular volume; NMP_{EF}, early follicular; NMP_{ML}, mid-luteal; RBC, red blood cell count; RET#, reticulocytes count; RET%, % of reticulocytes; WBC, white blood cell count (eosinophils, monocytes, lymphocytes, basophiles and platelets).

* $p < 0.05$, difference between COC and NMC.

and Thompson et al. [10], it may therefore be concluded that, despite the alterations induced by sex hormone levels on body water distribution within the extracellular space, overall body water and total extracellular fluid volume remained largely unaffected by either NMC or COC phases [16]. Alongside the lack of change in fat mass, we detected no significant variation in leptin levels throughout NMC and COC phases, with a high positive correlation between leptin and fat mass, both when expressed in percentage and kg of body mass in all subjects. Increased leptin during luteal phases was reported in about half of the previous studies conducted on active women [1] and possibly linked to E2 fluctuations or the corpus luteum [14, 29]. In view of our data, it may be hypothesised that intense physical training may mitigate the natural and synthetic sex hormone effects on both fluid volume and leptin secretion.

In terms of lipid profile, no change across NMC or COC phases was obtained in total, HDL and LDL cholesterol although triglyceride levels were significantly higher in COC subjects. In parallel, higher transferrin levels were observed in this group, without any change in total protein and albumin. These data seemed to partially correlate with the literature. Indeed, old studies reported alteration in the lipid profile with COC intake through modification of the LDL and HDL cholesterol level, increasing triglyceride serum level, reducing glucose tolerance, raising blood pressure and promoting clotting mechanisms [30, 31] although these effects appear much more limited with the new formulations used today [30]. Elevated transferrin with COCs in the present study was not due to altered renal function

as creatinine concentrations were similar in both groups of subjects and more likely reflected COC stimulation of hepatic production of binding proteins [32]. Similarly, we did not notice any change in albumin concentrations. Moreover, if no change was found in IL-6 concentrations during the menstrual cycle as previously reported [33], an anti-inflammatory climate seems to prevail under COC. Indeed, higher cortisol level was described [34], and we detected lower pro-inflammatory IL-6 levels with COC vs. NMC. As above, our data are partially in accordance with previous works performed on non-highly trained subjects. Decreased IL-6 concentrations with COC were previously noted by Eagan et al. [12] vs. the low-hormone NMC phase. The authors therefore suggested a decreased inflammation status with COC compared to the menses phase in NMC women, which could be specifically linked to the pharmacological role of the synthetic estrogen itself in disrupting IL-6 production and development. To explain the lower IL-6 levels maintained during the washout period, they proposed that the administered dose of exogenous hormones may not be fully cleared through the system during this period [12]. Similarly, Zwart et al. [21] reported that female astronauts who used COCs exhibited higher concentrations of transferrin, a protein involved in the clotting cascade but lower concentrations of serum albumin than male and female astronauts not taking COCs, combined with higher serum cortisol concentrations and whole blood viscosity. The authors concluded that lower circulating albumin concentrations, higher transferrin levels and elevated inflammation markers may contribute to an increased risk of venous thromboembolism (VTE) during spaceflight. In view of our data, it seems doubtful that

the risk of VTE is intensified by COCs for female athletes, at least under the current experimental setting, although further studies are needed to assess health risks of COCs in extreme environments such as intensive training or competitions performed in hot, cold and/or hypoxic conditions or in combination with other medications. Indeed, prospective and epidemiological studies on VTE incidence among female high-altitude travellers using COCs are lacking, with only some case reports [35] and none, to our knowledge, having studied the combined effect of COC administration and intense physical activity in hypoxic conditions. Similarly, the potential adverse effects of COCs in conditions of humid heat or extreme cold, which increase thermoregulatory stress, on endurance/ultra-endurance exercise hyponatraemia, dehydration, pulmonary insufficiency, oedema or cardiovascular disease [36–38] need to be clarified. Last, recent studies highlighted that non-steroidal anti-inflammatory drugs, commonly used by athletes, significantly increased the number of VTE with concomitant COC intake [39].

At present, scarce literature exists about the physiological and pharmacological effects of natural or synthetic oestrogens and progestins on the ABP haematological module, iron metabolism and immune function. In concordance with studies by Mullen et al. [15] and Moreillon et al. [16], haemoglobin, haematocrit and red blood cell count remained stable here across NMC and COC. However, unlike the study conducted by Mullen et al. [15] performed on healthy young active women, we did not observe a significant change in reticulocyte percentage throughout NMC among our highly trained female athletes. Similarly, we detected no evidence of change for absolute leucocyte and granulocyte counts across NMC, which contrasts with findings from around half of the studies [1]. Irrespective of active/inactive intake, COC induced a significant increase in reticulocyte counts but without any parallel change in EPO concentrations. As previously proposed [16, 40], we may thus hypothesise that this reticulocytosis was caused by reduced erythrocyte filterability, indicating poor microcirculation, due to synthetic progestin. Finally, Alfaro-Magallanes et al. [17] previously suggested that the use of COCs in female athletes could lead to improved iron status, with an increase in serum iron availability but not in iron stores. However, we were unable to determine any change in these parameters according to the hormonal status of our NMC or COC subjects in the present study. Once again, it can be intimated that this discrepancy may be related to the very high physical level of activity experienced by our subjects.

5 | Conclusion

The parameters studied were not altered by NMC phases in highly trained female athletes unlike untrained or less physically trained subjects. It therefore seems warranted to investigate whether intense physical training would minimise the impact of endogenous sex hormones on body composition and haematological and inflammatory markers as previously demonstrated for the perception of menstrual cycle symptoms [41]. In addition, further studies are needed to determine the extent of COC-induced changes in these blood biomarkers in elite female athletes when subjected to extreme environments such as intensive training or competition in humid heat, cold and/or hypoxia or when using other medications in parallel.

Author Contributions

K.C. and A.O.: study conception, data analyses, interpretation and manuscript writing. **C.T., C.C.:** study conception and interpretation. **J.B., A.M., C.B. and N.C.:** data analyses. **E.F., M.Z., V.A. and N.C.:** participation in the experiment. **M.E. and E.D.:** critical revision of the manuscript for critical intellectual content. All authors read and approved the final version of the manuscript.

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Conflicts of Interest

The authors declare no conflicts of interest.

Data Availability Statement

The data that support the findings of this study are available from the corresponding author upon reasonable request.

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