CORTISOL, HIGH SENSITIVITY C-REACTIVE PROTEIN, AND FERRITIN ADAPTATION TO ENDURANCE TRAINING IN NATURALLY MENSTRUATING FEMALES VS. FEMALES USING HORMONAL CONTRACEPTIVES

Sarah Kordrostami

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Faculty of Sport and Health Science
University of Jyväskylä
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Abstract

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Introduction: Ferritin, hs-CRP and cortisol are markers of iron metabolism, inflammation and stress, respectively. The current longitudinal study investigated the effect of eight weeks of moderate-intensity aerobic exercise on cortisol, high-sensitivity C-reactive protein (hs-CRP), and ferritin among naturally menstruating females and hormonal contraceptive users.

Methods: Thirty-two healthy young female volunteers were assigned to two experimental groups: the control group (n=13) (CON) (naturally menstruating females) and the hormonal contraceptive users (HC) group (n=19). Each group engaged in 1-3 workouts (running or brisk walking) per week for eight weeks, with each session lasting between 30-90 minutes. The training program involved maintaining a heart rate within the range of 60-75% of the maximum heart rate. Blood samples were collected in the early follicular phase and hormonal contraceptive active pill phase before and after eight weeks. Both groups follow the same training program with equal intensity and volume. The menstrual cycle (MC) phases were determined from the first day of bleeding. Clearblue advanced digital ovulation (CB-OT) kits were used for ovulation detection.

Results: No significant changes in cortisol and ferritin concentrations were reported after 8 weeks in either group, and the relative changes in cortisol and ferritin were not significant between groups ($p>0.05$). Cortisol showed a statistically significant difference between groups at both baseline and post-MIET, with the HC group demonstrating higher concentrations. A significant within-group difference was observed in hs-CRP for both groups. The CON group experienced a reduction, while the HC group saw an increase post-MIET, and there was a significant difference in the relative change in hs-CRP between the groups ($p < 0.05$).No significant relationship was reported between cortisol, hs-CRP, or between cortisol and ferritin ($p>0.05$)

Conclusion: The results suggest that an 8-week moderate-intensity endurance training programme may not lead to distinct adaptations in serum ferritin and cortisol between the CON and HC groups. However, the impact of MIET training on hs-CRP varies significantly depending on the use of hormonal contraceptives, with the concentration increasing in the HC group and decreasing in the CON group. No significant relationship was reported between cortisol and hs-CRP as well cortisol and ferritin.

Key words: ferritin, hs-CRP, cortisol, hormonal contraceptive, naturally menstruating females, moderate intensity endurance training

ABBREVIATIONS

ATP	adenosine triphosphate
APR	acute phase reactants
ACTH	adrenocorticotropic hormone
BMI	body mass index
CBG	corticosteroid-binding globulin
CRH	corticotropin- releasing hormone
CRP	C-reactive protein
E2	estradiol
FP	follicular phase
FSH	follicle stimulating hormone
GC	glucocorticoid
GnRH	gonadotropin- releasing hormone
Hb	hemoglobin
HPA	hypothalamic- pituitary adrenal axis
HPO	hypothalamic-pituitary ovarian
hs-CRP	high sensitivity C-reactive protein
IL-6	interléukin-6
LH	luteinizing hormone
LP	luteal phase
LT	lactate threshold
MC	menstrual cycle
MD	mean difference
O_2	oxygen
P4	progesterone
PMS	premenstrual symptoms
Q	cardiac output
SV	stroke volume
TNF-a	Tumor necrosis factor-alfa
VO_2max	maximal oxygen consumption
VO_2	oxygen uptake
VT	ventilatory threshold

CONTENTS

1 INTRODUCTION

A key challenge in studying females lies in the inherent hormonal fluctuations experienced throughout the menstrual cycle (MC) (Bruinvels et al., 2017). The use of hormonal contraceptives adds additional challenges as they alter endogenous sex hormones, impacting various physiological functions in females (Fleischman et al., 2010). This alteration may include modifications in stress, inflammation, and iron stores.

Cortisol, high-sensitivity C-reactive protein (hs-CRP), and ferritin are indicators of stress (Thau et al., 2019), inflammation (Bassuk et al., 2004), and iron storage (Knovich et al., 2009) respectively. Earlier studies demonstrated crosstalk between the immune and neuroendocrine systems (Liu et al., 2017; Quan & Banks., 2007). Hence, chronic alterations in one biomarker can potentially disrupt the balance of other biomarkers. Briefly, chronic stress triggers the hypothalamic-pituitary-adrenal axis (HPA), leading to the release of glucocorticoids (GC), such as cortisol. Dysfunction in the HPA axis may increase proinflammatory cytokines, ultimately releasing inflammatory biomarkers such as hs-CRP (Liu et al., 2017). The presence of hs-CRP has the potential to disturb iron metabolism, leading to an increase in ferritin levels, regardless of iron status. (Ueda & Takasawa, 2018).

Indeed, endurance training is recognized as a stressor that can potentially impact the HPA axis and influence the levels of these biomarkers. However, conflicting findings exist regarding the impact of exercise training on the concentration of cortisol and ferritin (Martínez-Díaz & Carrasco., 2021; Bijeh et al., 2018), while other studies reported no change in the concentration of cortisol and ferritin following endurance training (Häkkinen et al., 2005; Ishibashi et al., 2022). Regarding hs-CRP, there is a consistent trend in the results, suggesting that concentrations of hs-CRP decrease in naturally menstruating females with regular exercise training (Mogharnasi et al., 2019). This indicates an anti-inflammatory effect of regular exercise training. Among hormonal contraceptive users, research findings indicate elevated levels of cortisol (Wiegratz et al., 2003), high-sensitivity C-reactive protein (hs-CRP) (Park, 2022), and ferritin (Gellert & Hahn, 2017) in comparison to females with natural menstruation. It is proposed that synthetic sex hormones may potentially disrupt the HPA axis, consequently impacting the inflammatory status in females using hormonal contraceptives (Wiegratz et al., 2003; Cerqueira et al., 2020; Michigan et al., 2011; Margen & King, 1975).

The effect of high-intensity endurance training on cortisol, hs-CRP, and ferritin levels is well studied, even though not all those studies control for MC and the hormonal contraceptive phases. However, the impact of moderate-intensity endurance training on these specific biomarkers in naturally menstruating females and hormonal contraceptive users is less explored. Therefore, to broaden our understanding of how different intensity of endurance training influences these biomarkers, the present study aimed to explore the potential influence of moderate-intensity endurance training on the adaptation of serum cortisol, hs-CRP, and ferritin in both naturally menstruating females and users of monophasic hormonal contraceptive pills. The unique aspect of this research is the comparison of two distinct groups, which gives us better insights into female physiology. These insights, in turn, will serve as a guide for making informed decisions when designing training program, ensuring that the level of these essential biomarkers is not compromised. The selection of moderate-intensity endurance training is based on the understanding that such training is believed to elicit various positive effects on the immune system and overall health while imposing less stress on the immune system than high-intensity endurance training (da Luz Scheffer & Latini., 2020).

2 LITERATURE REVIEW

2.1 Adaptation to endurance training

Endurance exercise involves the repeated contraction of a large group of skeletal muscles and is required to sustain a given power output or velocity for a prolonged duration (Jones & Carter., 2000). This type of training generates considerable physiological changes over time, which cause enhanced exercise performance and capacity (Jones & Carter., 2000; Hughes et al., 2018). Endurance training induces cardiovascular and musculoskeletal adaptations, promoting an overall enhancement in exercise capacity and performance. Musculoskeletal adaptations include enhancing the capacity of skeletal muscles to store glycogen and triglycerides (Hughes et al., 2018) and changing the substrate use by increasing lipid oxidation and decreasing carbohydrate utilization, which consequently enhances substrate availability (Henriksson., 1977; Holloszy & Coyle., 1984). The improved mitochondrial biogenesis and increased capillary density, on the other hand, contribute to the body's capacity to transport and utilize oxygen for energy production (Joyner & Coyle., 2008). Cardiovascular adaptation to endurance training significantly boosts aerobic power, improving endurance performance by enhancing maximal cardiac output (Q) due to an enlargement in cardiac dimensions. This adaptation also increases muscle perfusion capacity, allowing for greater oxygen delivery (Hellsten & Nyberg., 2015). These adaptations enable individuals to engage in prolonged exercise or sustain a higher intensity for a specified duration (Jones & Carter., 2000).

Maximal oxygen consumption (VO_2max) is an aspect that collectively defines aerobic fitness (Whipp et al., 1982). This aspect is related to cardiovascular and musculoskeletal adaptation to endurance training. VO_2max can be defined as the ability of the skeletal muscle to use and deliver oxygen during exercise (Santisteban et al., 2022). It reflects a person's maximal rate of aerobic metabolism (Jones & Carter., 2000). Greater Q with elevated oxygen (O2) extraction via skeletal muscle tissue during maximal exercise leads to better VO_2max (Hellsten & Nyberg., 2015).

Ventilatory threshold (VT) and lactate threshold (LT) are other aspects that define aerobic fitness (Whipp et al., 1982). They are valid indicators of endurance performance and exercise intensity (Goodwin et al., 2007). LT and lactate curves are essential in interpreting endurance

performance (Faude et al., 2009). VT is defined as changes in gas exchange during exercise in which ventilation rises faster than VO_2. LT, by turn, is defined as the gradual increase in blood lactate concentration at the onset of exercise, which then accelerates as the exercise intensity rises. This threshold is a key marker in understanding the transition between aerobic and anaerobic metabolism during physical activity (Goodwin et al., 2007). The characteristics of a successful endurance training program involve shifting the VT and LT curves toward the right. These adaptations allow the individual to work at higher absolute and relative exercise intensity without accumulating blood lactate (Jones & Carter.,2000). Nonetheless, various physiological and methodological factors can impact the lactate curve. These include depleted glycogen stores resulting from a low-carbohydrate diet (McLellan & Gass, 1989; Yoshida, 1984), muscle fiber composition, the activity of glycolytic and lipolytic enzymes, as well as capillary or mitochondrial density (Midgley et al., 2007).

In summary, endurance-type exercise training results in several physiological adaptations in the body. The magnitude of training adaptation is affected by several factors, including the intensity, frequency, and duration of exercise sessions, age, gender (Wenger & Bell, 1986), and the recovery period after exercise (Neufer., 1989).

2.2 Cortisol and endurance training

Cortisol is the primary stress hormone with different roles and effects on the human body. The cortisol level in the blood circulation constantly changes due to physiological, physical, and psychological stressors (Hill et al., 2008). Cortisol is a GC hormone released by the adrenal cortex (Wittert et al., 1996) plays a crucial role in regulating metabolism, mediating the stress, and inflammatory responses, and immune function. (Thau et al., 2019). However, chronic high levels of cortisol promote adverse physiological effects (Kraemer & Ratamess, 2005).

The sympathetic nervous system stimulates the hypothalamus, leading to the secretion of corticotropin-releasing hormone (CRH). Then, CRH stimulates the anterior pituitary gland (Hill et al., 2008) to release adrenocorticotropic hormone (ACTH), resulting in the secretion of cortisol (Viru, 2004). After the secretion of cortisol from the adrenal glands, various tissues such as the liver, skeletal muscle, and adipose tissue take it up from the blood. The availability of cortisol in these tissues mediates various physiological functions in recovery and exercise

capacity (Hill et al., 2008). A prolonged increase in cortisol levels within skeletal muscle initiates catabolic responses, suppresses anabolism, and notably affects type 2 muscle fibers (Kraemer & Ratamess, 2005). Consequently, cortisol diminishes protein synthesis and promotes protein degradation in muscle cells, reducing lean muscle mass (Torres et al., 2021). In adipose tissue, cortisol enhances lipolysis (Thau et al., 2019). Moreover, heightened cortisol levels in the liver activate the gluconeogenesis pathway while inhibiting glycogen synthesis (Kuo et al., 2015). Essentially, increased cortisol availability prompts muscle cells to produce glucose from amino acids, lactate, and other sources, resulting in an elevation of protein degradation and a reduction in protein synthesis (Thau et al., 2019).

Physical exercise is a stressor that stimulates the HPA axis, resulting in cortisol secretion (Hill et al., 2008). Studies have shown that cortisol concentrations positively correlate with the intensity and duration of exercise (Torres et al., 2021). Thus, with high-intensity and prolonged exercise, the cortisol level is greater. Several other factors, such as types of exercise, training status, gender, and rest interval, influence cortisol levels (Torres et al., 2021). The hydration state is another factor influencing cortisol concentration, irrespective of exercise intensity, as reported by Maresh et al. (2005).

During endurance exercises, the HPA axis is stimulated by a combination of adequate duration and intensity in training (Duclos & Tabarin, 2016). In a singular session of endurance exercise, the minimum intensity required to trigger cortisol release is approximately 60% of VO_2max (Viru et al., 2008; Hill et al., 2008). Beyond this threshold, cortisol concentration increases proportionally with exercise intensity (Duclos et al., 1997). Conversely, exercising below 60% VO_2max only prompts the HPA axis to release cortisol after extending the duration of the exercise (Duclos et al., 1997; Athanasiou et al., 2022). However, regular exercise training induces adaptations in the neuroendocrine system, reducing basal stress hormone levels and decreasing stress hormone concentration in response to both maximal and submaximal exercise (Hackney, 2006). As a result, the HPA axis exhibits reduced sensitivity to such stressors.

Studies examining the effects of moderate-intensity training on cortisol levels in females are mainly focused on evaluating the acute response to exercise, with limited attention given to assessing cortisol concentration longitudinally. A review by Torres et al. (2021) studied the effect of different types and intensities of exercise on cortisol production. The result indicated

that cortisol production is significantly greater in endurance-type training when compared with resistance training. They concluded that duration and intensity were the primary factors in increasing cortisol production. Other investigations have also confirmed the latter point (Martínez-Díaz & Carrasco., 2021; Vega et al., 2006), although it is worth noting that these studies focused on the acute responses.

Kraemer et al. (1989) showed the effect of different types of exercise, including sprint intervals, endurance, and a mix of both, on plasma cortisol concentration over ten weeks. Their study revealed that sprint interval training and combined training, including endurance training and sprint intervals, significantly increased resting cortisol concentration. In contrast, endurance training showed a negligible effect. They also reported that anaerobic exercises induces different cortisol responses compared to aerobic training (Kraemer et al., 1989). Taipale et al. (2020) similarly found no significant alteration in the absolute cortisol concentrations during 10 weeks of combined high-intensity endurance and strength training in both of their study groups, which comprised men and females. They observed a slight decrease in cortisol levels, though it did not reach statistical significance.

To sum up, previous studies indicate that the key factors significantly triggering cortisol secretion are sufficient exercise intensity and duration. There seems to be a threshold of approximately 60% VO_2max required to elicit a cortisol response. This underscores the importance of considering both the intensity and duration of exercise when examining cortisol dynamics.

2.3 Inflammation and its roles

Inflammation is a state induced by stressors, and it plays a key role in maintaining body homeostasis, by beginning to destroy and excreting harmful compounds and damaged tissues (Cerqueira et al., 2020). Acute phase reactants (APR) are inflammation biomarkers and essential mediators built in the liver during acute and chronic inflammatory states (Gulhar et al., 2018). APRs are classified into positive and negative APRs. The markers of positive APR are C-reactive protein (CRP), ferritin, and hepcidin, the concentrations of which rise with inflammation. On the other hand, the markers of negative APR, such as albumin, transferrin, and retinol-binding protein, drop in inflammation conditions (Gruys et al., 2005).

CRP is a protein that increases in response to tissue injury, infection, and inflammation and is believed to be a risk marker of chronic systemic inflammation, cardiovascular risk, and hs-CRP (Ün et al., 2016; Fedewa et al., 2017). Hs-CRP has a higher sensitivity than CRP and allows a detection level of <3mg/L (Ridker, 2009). Hs-CRP is recognized as a marker indicating a low-grade systemic inflammation condition (Li et al., 2017) and is presumed to be a risk factor for various conditions, including atherosclerosis, myocardial infarction, and stroke (Galkina & Ley, 2009; Pradhan et al., 2001; Calabro et al., 2012). Evidence suggests that age, adiposity (Gentile et al., 2010; Kawamoto et al., 2013), sex (with higher hs-CRP in females compared to men (Lu et al., 2017; Garcia et al., 2016), race, and ethnicity (Khera et al., 2005; Majka et al., 2009) can influence the production of hs-CRP.

2.4 Endurance training and hs-CRP

Exercise is one of the stressors that leads to significant physiological changes in the body and immune system, resulting in the secretion of inflammation biomarkers (Silveira et al., 2016; Docherty et al., 2022). The release of proinflammatory cytokines post-exercise is crucial in long-term adaptive responses to exercise training. This process is essential for facilitating repair procedures, particularly those resulting from training demands (Oishi & Manabe, 2018; Cerqueira et al., 2020).

The level of hs-CRP, produced by hepatocytes, increases in response to proinflammatory cytokines, including interleukin-6 (IL-6), tumor necrosis factor (TNF-a), after exercise with adequate intensity (Cerqueira et al., 2020; Michigan et al., 2011). However, studies have suggested an inverse relationship between regular physical activity and serum concentration of hs-CRP (Kasapis & Thompson, 2005). Previous studies suggested a 19-35 % lower CRP among individuals who are more physically active compared with less active counterparts (Fedewa et al., 2017). Indeed, there is a reported trend indicating that regular physical training has the potential to reduce inflammatory cytokines and enhance basal anti-inflammatory cytokines (Petersen & Pedersen, 2005; Wilund., 2007).

It is worth noting that factors such as mode, intensity, and duration of exercise are associated with changes in the serum concentration of hs-CRP (Strachan et al., 1984; Nosaka & Clarkson.,

1996; King et al., 2003). Factors such as muscle injury and muscle mass involvement have been reported to influence the serum concentration of inflammatory biomarkers. (Kasapis & Thompson., 2005). Generally, high-intensity exercise shows a higher concentration of inflammatory biomarkers, mainly when conducted with fewer resting periods, while moderate to vigorous exercise with proper recovery periods showed less or no increase in serum concentration of inflammatory biomarkers (Cerqueira et al., 2020).

The majority of the investigations have indicated a significant decline in serum hs-CRP concentration by regular exercise and physical activity (Campbell et al., 2009; Daray et al., 2011; King et al., 2003; Mogharnasi et al., 2019; Rosenbaum et al., 2007; Sponder et al., 2017). Several mechanisms have been proposed to explain how exercise contributes to the reduction of CRP. One key aspect is the suggested importance of fat loss in lowering CRP levels (Campbell et al., 2009). As adipose tissue decreases, proinflammatory cytokines such as IL-6 and TNF-α secretion diminish. This, in turn, leads to a decline in hs-CRP concentration since these cytokines play a role in stimulating hs-CRP release (Campbell et al., 2009). Changes in fat metabolism play a role in the anti-inflammatory benefits of exercise, with an inverse relationship observed between abdominal lipolysis and inflammatory cytokines (You et al., 2004). Furthermore, an increase in the rate of muscle protein synthesis is believed to elicit an anti-inflammatory response (Donges et al., 2010).

Numerous studies have explored the impact of different types of exercise on the levels of inflammatory biomarkers. Mogharnasi et al. (2019) examined the effect of the different types of exercise (endurance and resistance) on hs-CRP in obese females. They reported that the serum level of hs-CRP decreased significantly in response to both types of exercise, but a greater decrease was seen in the endurance group. The possible mechanism of lower hs-CRP level in endurance training was reduced fat mass. On the other hand, a study by Daray et al. (2011) illustrated that resistance training combined with endurance training might produce a better response to decreasing the plasma CRP concentration in females regardless of changes in aerobic capacity or body composition.

A study by King et al. (2003) indicated that after adjusting for potential confounding variables, such as age, race, sex, body mass index (BMI), smoking, and health status in the logistic regression model, only participants who were regularly jogging and participating in aerobic

dancing were considered unlikely to have elevated inflammatory biomarkers compare with other forms of exercise including cycling and swimming and weightlifting. Their findings suggest that certain types of physical activity are linked to a reduced likelihood of increased inflammatory markers. However, it is essential to note that variations observed could be influenced by factors such as exercise intensity or duration. Further, Dufaux et al. (1984) conducted a comparative analysis involving athletes from various disciplines, including rowers, swimmers, middle and long-distance runners, football players, and racing cyclists. They were compared with an untrained control group. The results indicated that hs-CRP concentration was lower in the athlete groups, both men and females, compared to the untrained group, with swimmers showing notably lower concentrations.

In summary, regular exercise training offers an anti-inflammatory treatment. However, factors such as different exercise intensity and duration play a defining role in the anti-inflammatory effects of regular training. This is apparent in specific investigations where certain sports seem to induce a more substantial decrease in hs-CRP levels, while others showed less pronounced changes.

2.5 Iron and its importance

Iron is a micronutrient that influences multiple bodily functions, such as oxidative metabolism, and is essential to exercise performance and endurance exercise training (DellaValle., 2013). Iron is an essential component of hemoglobin (Hb), hematocrit (Alaunyte et al., 2015), and myoglobin (Hinton., 2014) , which is crucial for supplying oxygen to tissues throughout the body (Rubeor et al., 2018). Hence, the low Hb level affects the oxygen transportation to an active muscle, which then influences performance and consequently, VO_2max and aerobic power decrease (DellaValle., 2013).

The body is not able to build iron; therefore, sufficient dietary iron is crucial in preserving iron stores because the low level of iron stores disturbs the production of adenosine triphosphate (ATP), which can influence the aerobic capacity and push the body to anaerobic metabolism of glucose to create ATP (Hinton., 2014). According to the Food & Nutrition Board (2001), the daily iron recommendation for females is 18 mg.

Numerous biomarkers are available to identify iron status, such as hepcidin (primary iron regulatory hormone), serum iron, transferrin, transferrin saturation, and soluble transferrin receptor (Aimone-Gastin., 2006). However, serum ferritin is the most clinically applicable for keeping track of changes in iron status (Daru et al., 2017; Mei et al., 2005). Ferritin, a blood protein containing iron, is considered a total body iron storage (Rubeor et al., 2018). However, it is worth mentioning that serum ferritin is an APR; thus, it is not a reliable predictor of iron status in individuals with liver disease, obesity, and inflammation (World Health Organization., 2020), as it shows a considerable difference in serum concentration during these conditions (Gulhar et al., 2018; Dignass et al., 2018). Consequently, the result from the measurement of serum ferritin is only reliable in the absence of any disease.

Studies have shown that exercise training acutely and chronically changes the concentration of ferritin (Roecker et al., 2005; Sinclair & Hinton., 2005; Mcclung et al., 2009; Peeling et al., 2009; Newlin et al., 2012). During exercise, several mechanisms such as hematuria, hemolysis (foot strike, impact), gastrointestinal bleeding, sweating, inflammation, and poor dietary iron intake contribute to iron loss (Auersperger et al., 2013; DellaValle & Haas., 2011; Akabas & Dolins., 2005). Menstruation is another essential factor contributing to iron loss among premenopausal females. Furthermore, research indicates significant day-to-day variability in ferritin levels regardless of the training load (Malczewska et al., 2000; Cooper & Zlotkin, 1996). This underscores that ferritin alone is not a reliable indicator of iron metabolism (Malczewska-Lenczowska et al., 2010).

2.6 Endurance training and ferritin

Studies examining the impact of endurance training on ferritin levels mostly have found a strong association between endurance training and low ferritin, particularly in long-distance running and triathlons (Beard & Tobin, 2000; Röcker et al., 2002). In athletes and physically active populations, ferritin levels are generally lower compared to the sedentary population (Roecker et al., 2005). This phenomenon can be explained by the body's demand for iron, which increases during exercise to improve the production of red blood cells (erythropoiesis) (Montero & Lundby, 2011).

Several investigations regarding the ferritin adaptation to exercise training demonstrated an exercise-induced change in ferritin concentration (Newlin et al., 2012; Terink et al., 2018; Mcclung et al., 2009; Blum et al., 1986; Bijeh et al., 2018), while others demonstrated no changes in the level of ferritin following an exercise training (Ishibashi et al., 2022; Malczewska-Lenczowska et al., 2010). Studies indicated that exercise-induced changes in ferritin levels correlate with training intensity (Malczewska et al., 2000) and ferritin baseline level (Peeling et al., 2014). Therefore, this could explain the variation in the results. Earlier studies demonstrated that long-term and more intense exercise imposes more stress on the immune system than moderate-intensity exercise (Suzuki & Hayashida., 2021). Thus, when exercise triggers a rise in serum ferritin, it could be linked to the immune response to exercise training and inflammation. These factors stimulate the APR, leading to consistently elevated ferritin levels (Schumacher et al., 2002) . On the other hand, the decline in ferritin levels after a training period reported in some investigations might be associated with inadequate iron intake and hemolysis following exercise training (Badenhorst et al., 2019).

Malczewska et al. (2000) reported a day-to-day variability of ferritin ranging from 13 to 77% and found a significant positive correlation between training intensity and ferritin concentration in female elite judokas. In a study conducted by Bourque et al. (1997), no significant group-by-time interaction in ferritin serum concentration was observed among their walking/running group, cycling group, and nonexercised control group over 12 weeks of training consisting of three to four times per week at 80% VO_2max. They reported a notable reduction in ferritin concentration across all study groups over time, including a nonexercised control group; they concluded that 12 weeks of exercise training did not result in a significant decrease in serum ferritin concentration in previously untrained females. This conclusion contrasts with the findings of Blum et al. (1986), who reported a significant decrease in serum ferritin concentration among females participating in aerobic dance four times per week for 35 minutes per session over 13 weeks compared to their control group. Factors such as methodological and statistical approaches, research design, and different populations may influence the outcomes.

2.7 Physiology of menstrual cycle and hormonal contraceptive

MC is regulated by the monthly cyclic physiological changes resulting from hormones secreted by the hypothalamus, pituitary, and ovaries, which lead to the removal of the uterine lining (Reed & Carr., 2015). The hypothalamic–pituitary–ovarian (HPO) axis is called the female reproductive axis, as it is the primary regulator of estradiol (E2) and progesterone (P4) (Davis & Hackney., 2017). Hormones throughout the MC are secreted in the form of negative and positive feedback. The hypothalamus begins to release gonadotropin-releasing hormone (GnRH) at the onset of puberty. GnRH travels down to the anterior pituitary gland and binds to its receptors on the gland. This signals the anterior pituitary to promote the release of luteinizing hormone (LH) and follicle-stimulating hormone (FSH). LH and FSH travel through the bloodstream to the ovaries and bind to their receptors in the ovaries. This stimulates the production of E2 and P4. Once the level of E2 and P4 is increased in the luteal phase (LP), negative feedback is provided and sends the signal to the anterior pituitary to decrease FSH and LH. On the other hand, during ovulation, positive feedback is provided when the required amount of E2 is secreted, resulting in increased LH and FSH production (Thiyagarajan et al., 2021). FIGURE 1 illustrates the HPO axis pathways.

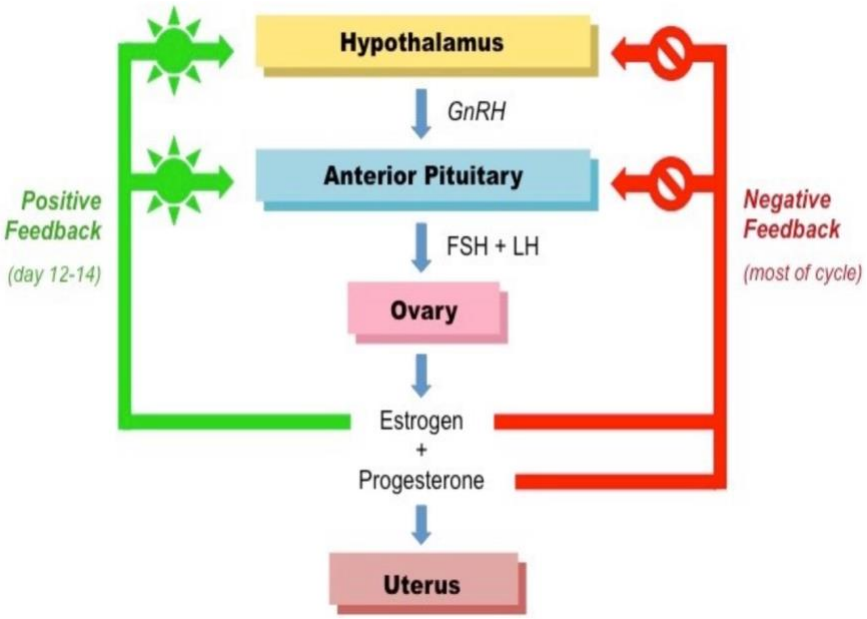

FIGURE 1. Hypothalamic–pituitary–ovarian (HPO) axis pathway. (Holtzman & Ackerman., 2019). FSH= follicle-stimulating hormone; LH = luteinizing hormone; GnRH= gonadotropin-releasing hormone;

The cyclic fluctuations of E2, P4, LH, and FSH in healthy, naturally menstruating females typically divided the MC into two main phases: follicular phase (FP) and LP. These phases are

separated by ovulation, which may result in fertilization and/or menstruation (Davis & Hackney., 2017). The average length of a menstrual cycle is typically 28 days, with most cycle lengths falling between 25 to 30 days (Bull et al., 2019). The duration is calculated from the initial day of bleeding in one cycle to the initial day of bleeding in the next cycle. (Reed & Carr., 2015). FIGURE 2 illustrates the hormonal fluctuation throughout MC.

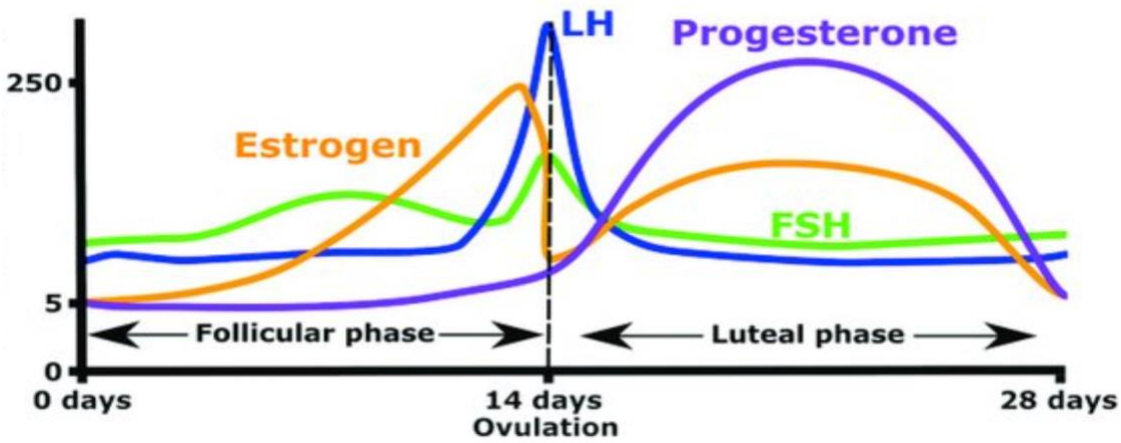

FIGURE 2. Hormonal fluctuation over the mensuration. (Chidi-Ogbolu & Baar., 2019)

The FP marks the initial stage of the MC, spanning from the first day of bleeding through day 14 in an average 28-day cycle (Farage et al., 2009). Early FP is marked as a low E2, P4, LH, and FSH concentration. However, the concentration of E2 increases towards late FP, followed by a rise in LH and FSH just before ovulation (Rael et al., 2021). During the FP, the primary goal is the maturation of ovarian follicles, preparing one of them for eventual release during ovulation (Monis & Tetrokalashvili., 2022). Ovulation typically occurs on day 14 of an average 28-day cycle (Thiyagarajan et al., 2021), marked by a subsequent sharp decline in concentrations of E2, LH, and FSH right after this pivotal event (Rael et al., 2021). LP, the second phase of the MC, is typically observed from day 14 to day 28 in an average 28-day cycle. During this period, P4 takes centre stage as the primary hormone. P4 concentrations start to rise and peak in mid-LP. In the late LP, all hormones decline to prepare the uterus for the next menstrual cycle (Thiyagarajan et al., 2021; Rael et al., 2021; Farage et al., 2009).

It is essential to consider the influence of hormonal fluctuations when investigating females, because the variations in E2 and P4 during different phases of the menstrual cycle may result in diverse responses and adaptations to exercise, influencing females' performance and

recovery (McNulty et al., 2020; Hackney et al., 2019). Understanding these dynamics can pave the way for more tailored approaches to training and healthcare for females.

Hormonal contraceptives. The use of synthetic sex hormones in hormonal contraceptives adds an additional layer of complexity that might result in different exercise adaptations compared to females not using hormonal contraceptives. Below, we will delve into the indications for use, types, and the mechanism of how hormonal contraceptives work.

Generally, the hormonal contraceptive pill is widely utilized for birth control among both athletic and non-athletic females. It decreases variation in cycle length, ensuring a consistent 28-day cycle (Burrows & Peters., 2007). Hormonal contraceptive serve various purposes beyond birth control, including promoting bone health, managing premenstrual symptoms (PMS), manipulating or regulating the menstrual cycle, and addressing conditions like amenorrhea and painful menstruation (Rickenlund et al., 2004; Davis & Westhoff, 2001; Bennell et al., 1999). They come in diverse forms, including oral pills, intrauterine devices, vaginal rings, transdermal patches, injections, and subcutaneous implantation (Martin & Elliot-Sale, 2016).

Hormonal contraceptive pills consist of ethinyl estradiol (EE) and progestin, available in two main forms: "combined hormonal contraceptive pills" containing both EE and progestin and "progestin-only" or mini-pills, which exclusively contain progestin (Cooper et al., 2017). Among females, combined hormonal contraceptive pills are predominantly prescribed. Pills containing EE and progestin come in monophasic and triphasic forms, with less common biphasic pills (Bennell et al., 1999).

Combined hormonal contraceptive pills are offered in 21-day or 28-day packages. The 28-day pack comprises 21 pills containing EE and progestin (active pill phase) and an additional 7 placebo pills (inactive pill phase). In contrast, a 21-day package consists entirely of active pills with no inactive ones. A pill is taken daily for three weeks, followed by a seven-day break during which no pills are consumed. The only distinction between the 28-day and 21-day boxes is the presence of the 7 placebo or inactive pills. (Findlay., 1986).

Monophasic pills deliver a consistent amount of EE and progestin throughout the pill cycle, typically spanning three weeks (FIGURE 3a). Various brands of monophasic pills may contain differing quantities of EE and progestin. On the other hand, biphasic pills provide a fixed dose of EE, with two distinct dosages of progestin administered between days 7-10 and days 11-14, followed by 7 days of placebo (FIGURE 3b). Triphasic pills undergo three hormonal changes over the pill cycle, involving varied amounts of EE and progestin (FIGURE 3c). The specific dosage of EE and progestin can vary depending on the pill brand (Burrows & Peters., 2007).

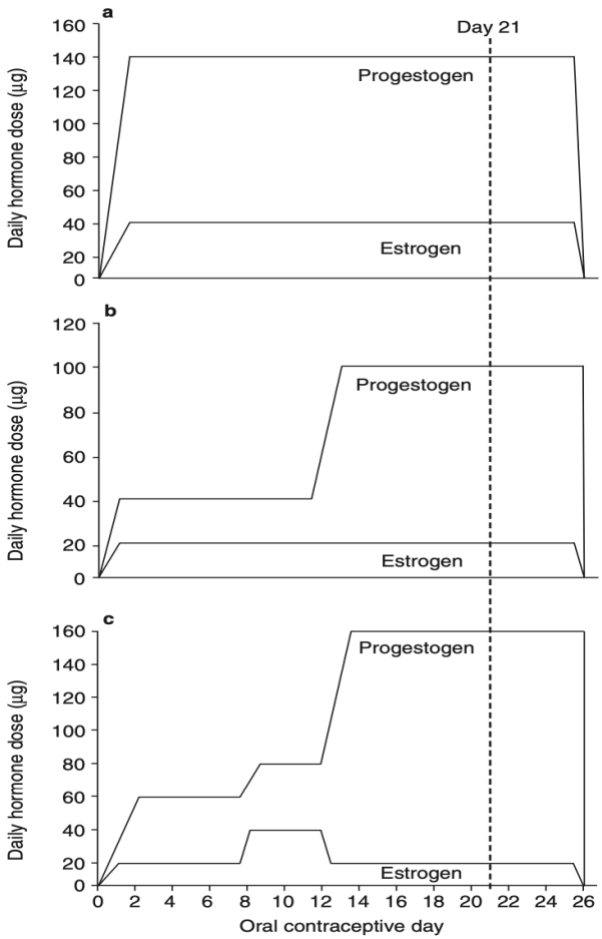

FIGURE 3. Hormonal pattern in (a) monophasic, (b) biphasic, and (c) triphasic hormonal contraceptive pills (Burrows and Peters., 2007).

The primary mechanism of hormonal contraceptive pills is to prevent ovulation by inhibiting the pituitary secretion of gonadotropins, which regulate the production of endogenous E2 and P4 (reduce the production of E2 and P4) (Cooper et al., 2017). Hormonal contraceptives function by exerting negative feedback on the hypothalamus and pituitary gland, leading to suppression of LH, FSH, and GnRH. Consequently, follicular development and ovulation are

prevented by low concentrations of LH and FSH (Elliott-Sale et al., 2013; Cooper et al., 2017), which then causes consistent down-regulation of endogenous E2 and P4 (Elliott-Sale et al., 2013). As a result, ovulation is prevented when there is no follicle development and no LH surge to trigger the release of the follicle (Cooper et al., 2017). FIGURE 4 illustrates the mechanism of hormonal contraceptive pills.

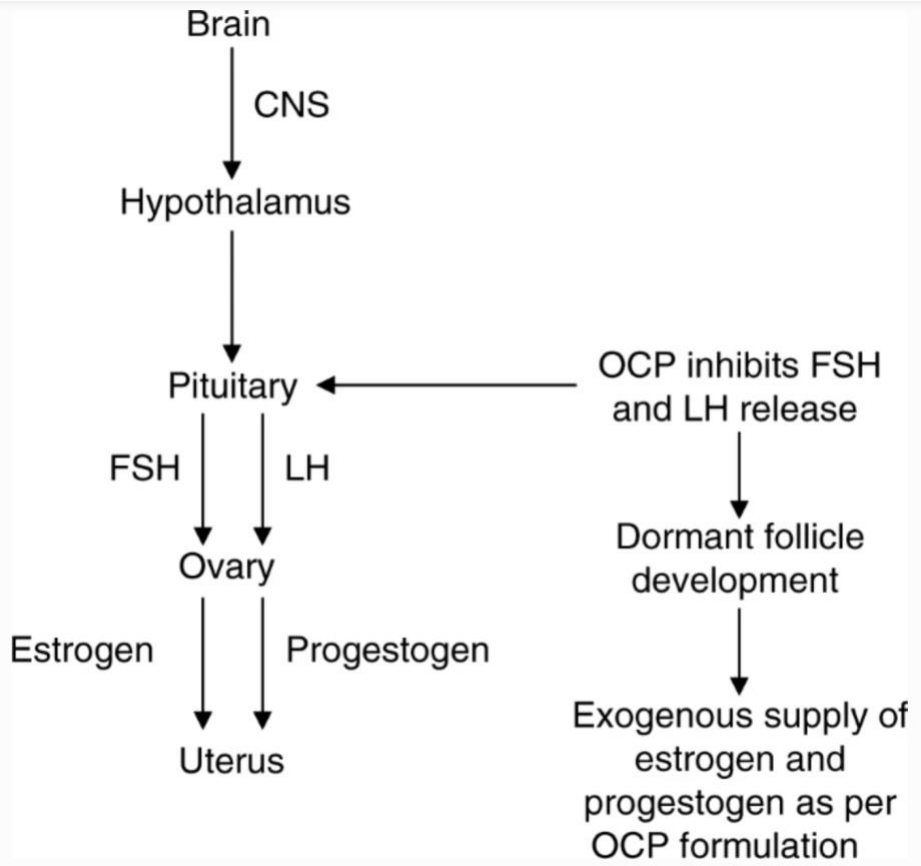

FIGURE 4. The mechanism of hormonal contraceptive pills. LH= Luteinizing hormone. FSH= follicle-stimulating hormone. OCP= oral contraceptive pill (Burrows & Peters., 2007).

In summary, hormonal contraceptives are available in different formulations and chemical compositions, which affect the body differently. Thus, it is essential to comprehend the mechanisms of each and group different forms separately (Elliott-Sale et al., 2013) as some types of hormonal contraceptive supply the body with more EE (3-5 folds) and progestin (1-3-fold) than endogenous level (Myllyaho et al., 2021; Elliott et al., 2005) compared to other. This might impact the adaptation to exercise training, recovery, and performance (Elliott-Sale et al., 2020; Dalgaard et al., 2019). In the studies investigating hormonal contraceptives and exercise

performance, hormonal contraceptive users are mainly included as a control group; this allows researchers to compare the effect of MC (Elliott-Sale et al., 2013).

2.7.1 Influence of menstrual cycle and hormonal contraceptive on cortisol

As mentioned, MC is involved in considerable hormonal changes, leading to physical and physiological changes. Generally, E2 is recognized as a potential regulator of the cortisol response to stressors in females. (Boisseau et al., 2013).

In a meta-analysis conducted by Hamidovic et al. (2020), it was reported that the concentration of cortisol changes continuously throughout MC phases. This suggests that cortisol availability is particularly crucial during the FP to regulate adaptive physiological procedures in response to environmental stressors when the levels of E2 and P4 are low (Hamidovic et al., 2020). However, the studies in this area reported mixed results, with some indicating no changes (Timon et al., 2013; Stewart et al., 1993; Boisseau et al., 2013) and some suggesting higher concentrations in FP (Hamidovic et al., 2020; Villada et al., 2014), and some reported higher concentrations in LP (Genazzani et al., 1975) in naturally menstruating females.

The stress response varies between females using hormonal contraceptives and naturally menstruating females. Previous investigations reported that hormonal contraceptives increased serum cortisol compared to non-users (Kirschbaum et al., 1996; Timmons et al., 2005; Kuhl et al., 1993; Wiegratz et al., 1995). Users of hormonal contraceptives may potentially experience elevated cortisol levels due to the alteration of the HPA axis (Kirschbaum et al., 1996). Hormonal contraceptives have been suggested to contribute to the elevation in serum cortisol by inhibiting hepatic metabolism of corticosteroids (Wiegratz et al., 2003).

There are a couple of mechanisms through which hormonal contraceptives may influence cortisol levels. Firstly, hormonal contraceptives may enhance the synthesis of corticosteroid-binding globulin (CBG). CBG is a protein responsible for transporting GC like cortisol in the bloodstream, consequently influencing the tissue availability of these hormones This, in turn, increased serum cortisol binding by CBG. Second, hormonal contraceptive has been proposed to contribute to the elevation of serum cortisol by potentially inhibiting hepatic corticosteroid metabolism (Scott et al., 1990; Wiegratz et al., 2003).

A study by Wiegratz et al. (2003) examined the effect of different hormonal contraceptives on cortisol. They indicated that the elevation in cortisol concentration is linked to the dosage of EE present in the pills. This highlights the complex interplay between hormonal contraceptives and cortisol, suggesting that cortisol dynamics are influenced by factors such as the dosage and composition of hormonal contraceptives.

2.7.2 Influence of menstrual cycle and hormonal contraceptives on hs-CRP

Female's physiological reproductive systems, such as ovulation and menstruation, exhibit signs of inflammation (Chaireti et al., 2016). As previously mentioned, the lining of the uterus experiences cyclic inflammation, leading to tissue destruction and menstruation (Maybin & Critchley., 2011).

Research has shown that hs-CRP production varies throughout the menstrual cycle, irrespective of body weight (Puder et al., 2006). Additionally, both endogenous and exogenous female sex hormones appear to have distinct impacts on hs-CRP levels. E2 shows a negative association with hs-CRP, while endogenous P4 demonstrates a positive association. In contrast, synthetic hormones_ EE and progestin_ exhibit the opposite effect. (Wander et al., 2008; Chaireti et al., 2016). The majority of studies demonstrated a significant rise in hs-CRP concentration in FP compared to LP in regularly menstruating females (Vashishta et al., 2017; Puder et al., 2006; Wander et al., 2008; Gursoy et al., 2015). In contrast, Wunder et al. (2006) and Saxena et al. (2012) found no significant difference in hs-CRP concentration over MC. The inconsistency in the result might be because of the different methodologies used to determine hs-CRP (Vashishta et al., 2017).

Generally, hs-CRP concentration is higher in hormonal contraceptive users than non-users (Fedewa et al., 2018). Studies on the association between hormonal contraceptives and inflammatory biomarkers have demonstrated that hormonal contraceptives increase chronic low-grade inflammation in the lack of any disease process (Park, 2022; Fedewa et al., 2018). However, the underlying mechanism is not clear (Park., 2022). The data from different cohorts, including healthy young females, have illustrated that hormonal contraceptive users possibly have a six to nine times higher elevation of hs-CRP than non-users (Fedewa et al., 2018; Cauci et al., 2008; Krintus et al., 2010). Van Rooijen et al. (2006) reported a significant increase in

the level of hs-CRP after two months of using various types of hormonal contraceptives, including both third generation and second-generation formulations.

2.7.3 Influence of menstrual cycle and hormonal contraceptives on ferritin

Previous investigations have indicated that the mean values of serum ferritin tend to be low during the bleeding phase of the menstrual cycle and reach their peak during the LP in a normal cycle (Kim et al., 1993). Additionally, serum ferritin has shown an inverse relationship with the duration of bleeding (Milman et al., 1992). Therefore, the fluctuation of both endogenous and exogenous reproductive hormones, mainly E2 and P4, throughout the menstrual cycle and hormonal contraceptive phases could notably impact how ferritin adapts to exercise. Considering that females of reproductive age lose 10–40 mg of iron through menstrual bleeding in each cycle, it is possible that this blood loss might play a crucial role in influencing overall iron storage (Higham et al., 1990).

On the other hand, hormonal contraceptives have the potential to positively impact iron status by reducing menstrual blood loss (Fischer et al., 2021). In earlier studies, Bathija et al. (1998) noted higher ferritin levels in individuals using hormonal contraceptives compared to non-users. This trend continues in recent research by Gellert & Hahn (2017), where females, especially those using the fourth progestin generation, exhibit higher ferritin concentrations than naturally menstruating females. The specific type of hormonal contraceptive appears to play a role in determining iron status, as Gellert and Hahn (2017) indicated. However, conflicting results arise from investigations like Larsson et al. (1992) who reported no difference in the level of ferritin among naturally menstruating females and the users of hormonal contraceptives. A study by Alfaro-Magallanes et al. (2023), supported these findings by reporting no distinction.

Despite this, Yang et al. (2012) suggested that the improved iron status in hormonal contraceptive users is likely linked to reduced menstrual blood loss and/or suppressed hepcidin transcription by E2 treatment, which is a protection mechanism to enhance iron uptake during bleeding. Further, enhanced gut absorption of iron in hormonal contraceptive users is another mechanism associated with better serum ferritin status (Margen & King., 1975

When interpreting the results related to hormonal contraceptives, it is crucial to consider that the higher ferritin concentration observed may be associated with increased inflammation. However, uncertainty arises when determining whether elevated serum ferritin reflects existing inflammation, a contributor to the inflammatory process, or part of a cyclical relationship, as discussed by Kell and Pretorius (2014).

On the other hand, the explorations into ferritin concentration throughout the MC have demonstrated that different phases of the MC influence the concentration of ferritin, as is evident in Kim et al. (1993) and Alfaro-Magallanes et al. (2022) study, which proposed decreased iron parameters, including ferritin, during the early FP compared to the LP. However, contradictory findings are also available, which suggested no significant difference in mean serum ferritin across menstrual cycle phases (Belza et al., 2005; Zheng et al., 2021).

3 PURPOSE OF THE STUDY AND RESEARCH QUESTIONS

Exercise is one of the stressors that activates the neuroendocrine and immune systems, influencing hormonal responses (Hackney., 2006; Scheffer & Latini, 2020) . In females, hormonal fluctuations occur naturally throughout the MC or with the use of hormonal contraceptives, potentially triggering distinct biological responses.

Investigating the adaptation of cortisol, hs-CRP, and ferritin following endurance training in naturally menstruating females and hormonal contraceptive users is crucial for several reasons. Firstly, understanding how these biomarkers respond to endurance training in naturally menstruating female and hormonal contraceptive users provides valuable insights into the physiological adaptations specific to hormonal status. Although relying on a single biomarker for different conditions may not be sufficient to draw definitive conclusions, this understanding contributes to the development of informed decisions when designing training program for naturally menstruating females and the users of hormonal contraceptives. Secondly, monitoring changes in these biomarkers helps assess the impact of endurance training on overall health and well-being in females. Previous research has indicated diverse responses to high-intensity exercise training, with limited attention given to the impact of hormonal contraceptives on these biomarkers in the context of exercise training. Additionally, the impact of moderate-intensity endurance training on these biomarkers remains relatively understudied. Understanding how different training intensities and modes affect markers of stress, inflammation, and iron status can offer useful insights for designing training programs for these specific populations. This ensures that the levels of these particular biomarkers are not compromised during the training process. Therefore, the current study aims to explore the impact of an eight-week moderate-intensity training program on serum cortisol, hs-CRP, and ferritin levels in naturally menstruating females and hormonal contraceptive users. The investigation seeks to answer the following questions:

Question 1: Does the influence of moderate-intensity training on serum cortisol, hs-CRP, and ferritin differ between naturally menstruating females and hormonal contraceptive users?

Hypothesis 1: Yes. Hormonal contraceptive users are anticipated to exhibit a distinct response pattern compared to naturally menstruating females in terms of cortisol, hs-CRP, and ferritin. Regarding cortisol, the administration of oral contraceptives containing E2 has been proposed

to contribute to the elevation of serum cortisol by potentially inhibiting hepatic corticosteroid metabolism. Therefore, this leads to increase in the level of cortisol among hormonal contraceptive users (Wiegratz et al., 2003). Synthetic sex hormones in hormonal contraceptives also have the potential to disrupt the body's inflammatory status by interfering with endogenous reproductive hormones. This disruption can increase the levels of proinflammatory markers, such as IL-6 and TNF-α, which, in turn, may stimulate the liver to produce hs-CRP (Cerqueira et al., 2020; Michigan et al., 2011). In terms of ferritin, the synthetic E2 present in hormonal contraceptives may suppress hepcidin transcription, which serves as a protective mechanism to enhance iron uptake during bleeding. This leads to higher levels of ferritin among hormonal contraceptive users (Margen & King., 1975).

Question 2: What is the relationship between cortisol and hs-CRP after the training period?
Hypothesis 2: It is hypothesized that if the cortisol concentration exceeds the reference value, a positive relationship between cortisol and hs-CRP will be observed. Chronically elevated cortisol is linked to increased adiposity. Increased adiposity subsequently increases the inflammatory response in the body (Van der Valk et al., 2018) . Elevated cortisol level has the potential to disturb the HPA axis. As a result, proinflammatory cytokines are downregulated while anti-inflammatory cytokines are upregulated, ultimately resulting in the release of inflammation markers such as hs-CRP
(Liu et al., 2017).

Question 3: What is the relationship between cortisol and ferritin after the training period?
Hypothesis 3: A positive correlation is anticipated after the training period if the ferritin concentration is elevated. To date, no authors have demonstrated the relationship between cortisol and ferritin concentration over the training period among naturally menstruating female and the users of hormonal contraceptive. Nevertheless, earlier studies on unhealthy populations have indicated that elevated ferritin levels can impact adrenal gland function, potentially resulting in an increase in cortisol secretion.

4 METHODS

4.1 Participants and ethics

The current study is part of a bigger multidisciplinary study (NaisQ study) where the primary aim was to compare menstrual cycle-mediated endurance training with traditional endurance training in females. The study was accomplished at the Faculty of Sport and Health Sciences, University of Jyväskylä, and included volunteers who signed up through the advertisement (social media, public places, sports halls, and gyms).

A group of thirty-three healthy young female volunteers were included in this thesis. After obtaining informed consent and delivering written and oral information regarding the study's protocols and objectives, the participants were categorized into two experimental groups: a control group (CON), comprising naturally menstruating females without the use of hormonal contraceptives, and a hormonal contraceptive users (HC) group. They were assigned based on their use of hormonal contraceptive users and non-users. The initial sample size in this study was 13 in the CON group and 19 in the HC group. However, 4 participants in the CON group and 6 in the HC group did not complete the baseline or post-MIET measurements. Various factors such as personal reasons, sickness, and changes in hormonal contraceptive type contributed to these incomplete measurements. These participants were excluded all these participants from all analyses. Therefore, the final number of participants in the CON-group was 9 and, in the HC,-group was 13.

The inclusion criteria were as follows: healthy eumenorrheic females of reproductive age between 18-35 (with a cycle length of 26-35 days) without the use of hormonal contraceptives (no hormonal contraceptives for the last 12 months), and BMI of 19.5-35 kg/m2. Participants in the HC group were required to be using hormonal contraceptives for at least one year before participating in the study. The exclusion criteria were chronic diseases, musculoskeletal and/or cardiac problems, medications that would affect a volunteer's ability to perform training and testing and might influence exercise responses, sedentary behavior, pregnancy and lactating, competing in sports, smoking, injury and disability, absence of any acute or chronic inflammatory/infective, and anemia. All participants filled out the health questionnaire and were checked by a physician before participating in this study to ensure that participants met

the inclusion criteria. Hormonal contraceptives used in this study were packed as 21-day (with 7 pill-free days) and 28-day (with placebo pills). One participant used 24- a 24-day monophasic pill. Hormonal contraceptive types are shown in TABLE 1.

TABLE 1. List of hormonal contraceptives used in this study.

Product	**n**	**Type**	**Content**
Gestinyl (21+7 free pill)	3	Monophasic	EE 20 µg / gestoden75µg
Gestinyl (21+7 free pill)	2	Monophasic	EE 30 µg / gestoden75µg
Daisynelle (21+7 free pill)	1	Monophasic	EE 20 µg / desogestrel 150µg
Tasminetta (21+7 free pill)	1	Monophasic	EE 0.03 mg / drospirenone 3mg
Dienorette (21+7 placebo)	6	Monophasic	EE 0.03 mg / dienogest 2 mg
Levesia (21+7 free pill)	1	Monophasic	EE 20 µg / Levonorgestrel 100 µg
Yasmin (21+7 free pill)	1	Monophasic	EE 0.03 mg / drospirenone 3 mg
Yasminille (21+7 free pill)	1	Monophasic	EE 0.02 mg / drospirenone 3 mg
Dizminelle (21+7 free pill)	1	Monophasic	EE 0.02 mg / drospirenone 3 mg
Mercilon (21+7 free pill)	1	Monophasic	EE 20 µg / desogestrel 150 µg
Stefaminelle (24+4 free pill)	1	Monophasic	EE 0.02 mg / drospirenone 3 mg

EE= Ethinyl Estradiol

The current study followed the approaches of the Finnish National Board on Research Integrity (TENK). All the methodologies in this study received approval from the JYU Ethical Committee in accordance with the regulations of TENK. The study was carried out in accordance with the latest Declaration of Helsinki provisions.

4.2 Study design and training

A longitudinal study was used to examine the research questions. The study included a control period of one MC or hormonal contraceptive cycle. Participants filled in menstrual and exercise diaries and measured their ovulation, followed by 8 weeks of moderate-intensity endurance training (MIET) (two MC/ hormonal contraceptive cycles). FIGURE 5 illustrates the study design.

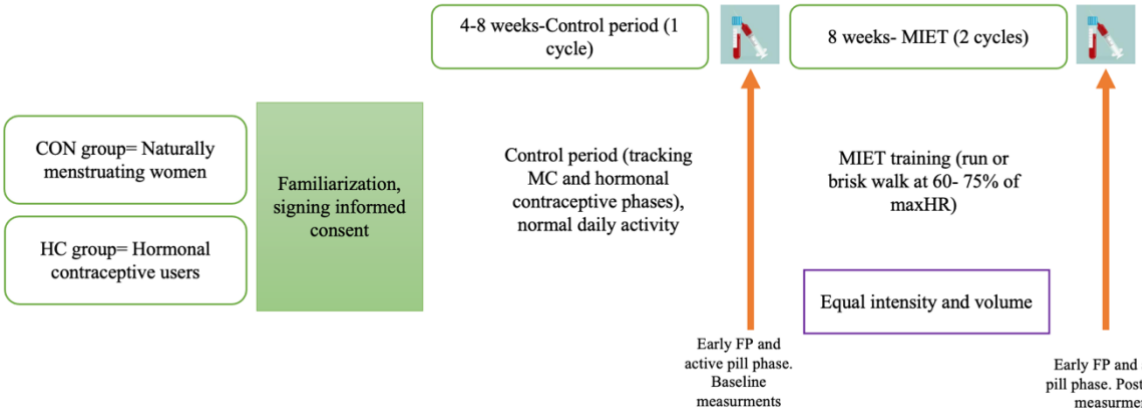

FIGURE 5. Study design. CON= control group. HC= hormonal contraceptive group. MC= menstrual cycle. MIET= moderate intensity endurance training. FP= follicular phase

Both groups adhered to an identical training program, with the same volume and intensity maintained consistently throughout the study. Each group performed 1-3 workouts per week, and the training program consisted of running or brisk walking with 60- 75% of maximum heart rate (maxHR) for 30-90 min per session (TABLE 2). Throughout the training sessions, HR was monitored constantly with the watch (Garmin® Venu 2 Series, Garmin Ltd., Taiwan) and heart rate monitor belt (Garmin® HRM-Dual, Garmin Ltd., Taiwan) to ensure that exercise remained at the desired intensity. All training sessions were compeleted independently and unsupervised. Training intensity for each participant was prescribed based on maxHR calculated from the incremental treadmill running test results.

Baseline measurements were carried out at the start of the training program. This took place during the early FP (days 1-5 in the CON group) and days 2-7 in the HC group during the active pill phase. Post-MIET measurements were conducted in identical phases. Despite the difference in the hormonal environment in the early FP and early active pill phase, the natural levels of E2 and P4 are generally lower in hormonal contraceptive users, as the EE and progestin component contained by the pill suppress the natural E2 and P4 (Elliott-Sale et al., 2013) (Elliott-Sale et al., 2018). Therefore, E2 and P4 levels are low in the active pill phase, miming the early FP. All the measurements were done in a fasted state (10 hours) between 6:00 and 10:00 AM to reduce circadian rhythms' potential confounding effects. Each participant visited the laboratory three times during the study period. The first session was for familiarization, the second for baseline measurements, and the third after completing the 8-week MIET training. Participants

were instructed to refrain from strenuous exercise, caffeine, and alcohol 24 hours before withdrawing blood. By following these guidelines, we aimed to obtain blood samples that reflect the individual's baseline physiological state without the influence of recent activities or substances that could temporarily alter blood parameters. This allows for a more accurate interpretation of the test results.

TABLE 2. Moderate intensity exercise training program. Week 1 through week 8.

Week	HC and CON
1	2 training session= 90 min
2	3 training sessions= 150 min
3	3 training sessions = 180 min
4	3 training sessions = 195 min
5	3 training sessions= 165 min
6	3 training sessions= 195 min
7	3 training sessions= 210 min
8	1 training session=60 min

HC= hormonal contraceptive group

CON= control group

4.3 Anthropometric measurements

Before and after 8 weeks of MIET training, measurements of weight, body composition, and circumferences (waist, hip, thigh, upper arm) were taken in a fasted state under consistent conditions for all participants. Height was measured only once with bare feet at the beginning of the study with a wall-mounted stadiometer. Weight was measured at each measurement in a fasted state with a digital scale, and the participants were wearing underwear. Body composition was measured with a bioimpedance device (Inbody 770, Biospace Co. Ltd, Seoul, South Korea). Circumferences were measured during each fasting assessment and recorded three times at each visit. In cases where the repeated measurements were not within 0.5 cm, an additional measurement was taken to ensure accuracy and precision. The result of anthropometrics remained hidden from participants until the end of their intervention in order to avoid changes in eating manners.

4.4 Blood markers and hormonal analysis

Blood samples were collected at each fasting measurement. A qualified lab technician withdrew venous blood samples using sterile needles into serum tubes (Vacuette® TUBE, Greiner Bio-One GmBH, Austria) and K2 EDTA tubes (Vacuette® TUBE, Greiner Bio-One, USA) in the morning. The blood was centrifuged at 2245 g (Megafuge 1.0R, Heraeus, Germany) for 15 minutes, and then the serum was extracted and was kept at -80 °C until further analysis of the following biomarkers: cortisol, hs-CRP, ferritin, E2, and P4. These hormones were analyzed using chemical luminescence techniques (Immulite 2000XPi, Siemens, New York, USA). The inter and intra-assay coefficient of variation (CV%) and sensitivity of blood serum are shown in TABLE 3. At the beginning of measurements, a basic blood count was employed as the primary test to detect any abnormalities (e.g., infection). Throughout the study period, blood was collected 2 times for each participant (at the beginning of the study to determine the baseline and after 8 weeks of MIET training.

TABLE 3. The sensitivity and the inter-assay coefficients of variations (CV) for serum hormones, ferritin, hs-CRP, and cortisol.

Variables	Sensitivity	CV%
Cortisol	73.4 nmol/L	7.43%
Hs-CRP	1.9 mg/L	8.34%
Ferritin	71.0 ug/L	6.19%
E2	55 pmol/L	6.6%
P4	0.3 nmol/L	9.7%

Hs-CRP=high sensitivity CRP. E2= estrogen. P4 progesterone.

4.5 Menstrual cycle assessment

The menstrual cycle phases were determined from the first day of bleeding. Participants were instructed to track their menstrual cycles using diaries. Ovulation was detected by monitoring the LH surge using a Clearblue two-hormone fertility monitor (Clearblue® Advanced Digital Ovulation, SDP Swiss Precision Diagnostics GmbH (SDP), Geneva, Switzerland), which is capable of detecting LH and E2 hormone levels. Volunteers received written instructions on

using and interpreting the results from the ovulation kits according to the manufacturer's guidelines.

4.6 Statistical analysis

All statistics were conducted using IBM- SPSS Statistics (version 28). Microsoft Excel was used when needed. The statistician assisted as required. All data are expressed as mean values ±SD. The statistical significance level was set at the level of $p \leq 0.05$. Nonparametric tests were used due to the study's small sample size (Dwivedi et al., 2017). The Mann–Whitney U test was used to assess group differences. Friedman tests were conducted to assess the influence of 8 weeks of MIET training. Spearman's rank-order correlation was conducted to specify the association between cortisol, hs-CRP, and ferritin over the training period. A sample correlation coefficient (r) of $\leq \pm 0.2$ was considered weak, $r \leq \pm 0.6$ was deemed medium, $r \leq \pm 0.9$ was classified as strong, and an r of ±1 was considered perfect (Akoglu, 2018). Effect sizes (ES) for the Mann–Whitney U test was expressed as the standardized test statistic divided by the square root of the number of observations for the two groups demonstrating a difference. For Friedman tests, results from the Kendall W test were reported (Tomczak & Tomczak., 2014). In this context, ES of ≤ 0.2 were categorized as small, those between 0.2 and 0.5 were considered medium, and those $0.5 \leq$ were deemed large (Sullivan & Feinn, 2012)

5 RESULTS

5.1 Participants characteristics and anthropometric measures

The results of characteristic and anthropometric measures of participants in the CON and HC groups are shown in TABLE 4. The comparison between groups revealed no significant differences in anthropometric characteristics at both the baseline and after 8 weeks of MIET training (post-MIET). The only significant within-group change in characteristic and anthropometric measures among participants was observed in the upper arm circumferences, only in the CON group ($\chi 2 = 6.0$, $p = 0.01$).

TABLE 4. Mean (±SD) age, anthropometric characteristics of subject in control (CON), and hormonal contraceptives (HC) groups at baseline and after 8 weeks of moderate intensity endurance training (post-MIET).

Variables	CON (n=9)	HC (n=13)
Age (yrs)	28.7±5.5	27.1±4.1
Height (cm)	169±5	169 ±6
Weight (kg)	70,1.0±12.6	70.1±5.6
Body fat (%)	28.9±7.9	28.5±5.2
Upper arm (cm)		
Baseline	31.4±3.9	31.1±1.9
Post- MIET	**29.9±3.9***	30.6±2.1
Waist (cm)		
Baseline	83.9±9.7	82.2±5.5
Post- MIET	79.6±10.8	82.5±7.5
Hip (cm)		
Baseline	102.7±7.9	101.6±4.2
Post- MIET	98.7±10.3	100.6±7.7
Thigh (cm)		
Baseline	54.9±5.2	54.7±3.7
Post- MIET	54.9±5.2	54.1±2.9
BMI (kg/m^2)		
Baseline	24.8±4.1	24.3±1.8
Post- MIET	23.6±3.5	24.2±1.8

BMI= Body mass index

*= Significantly different from the baseline

5.2 Between groups comparison

Changes in mean serum cortisol, hs-CRP, ferritin, and endogenous sex hormones at the baseline and after 8 weeks of the MIET training are summarized in Table 5. The between-group analysis at the baseline indicated no statistically significant difference in the average serum ferritin and hs-CRP levels between the CON and HC groups ($p \geq 0.05$). However, the results showed a significant difference between groups at the baseline for cortisol with the medium effect size

(ES) (ES=0.57). Specifically, the HC group had a higher mean cortisol concentration than the CON group. The between groups mean difference (MD) for cortisol at the baseline was reported as 279.2 nmol/L. E2 and P4 also showed no significant difference between the groups at the baseline.

Further, post-MIET between-group analysis showed no statistically significant difference in serum ferritin concentration. However, the results indicated a statistically significant difference between groups in serum hs-CRP and cortisol concentrations. In particular, the HC group indicated a higher concentration of both hs-CRP and cortisol compared to the CON group. The ES was small for hs-CRP (ES=0.25) and medium for cortisol (ES=0.47). The MD between group for serum hs-CRP and cortisol was 1 mg/L and 217.8 nmol/L, respectively. Moreover, there was a significant difference in endogenous sex hormones between groups, with higher concentrations reported in the CON group with large ES for E2 (ES=0.72) and P4 (ES= 0.66).

The relative changes (Δ%) in cortisol, hs-CRP, and ferritin concentrations in the CON and HC groups were reported in TABLE 6. The between-group comparison revealed that the relative change was statistically significant only for hs-CRP with the large ES of -0.75 but not for ferritin and cortisol.

5.3 Within groups comparison

TABLE 5 summarize the changes in mean serum cortisol, hs-CRP, ferritin, and endogenous sex hormones at the baseline and after 8 weeks of the MIET training.The result of the within-group comparison indicated no significant change in the concentration of ferritin and cortisol in the CON and HC groups ($p>0.05$). However, there was a statistically significant change in hs-CRP in the CON group **(**$\chi2= 5.4$, $p=0.02$) (ES=0.60) and the HC group ($\chi2 = 5.3$, $p= 0.02$) (ES=0.41). In particular, the concentration decreased by about 22% in the CON group, while it increased by approximately 33% in the HC group following 8 weeks of the MIET training. E2 indicated a significant change only in the CON **(**$\chi2= 9.0$, $p=0.003$) (ES=1.0) but not in the HC groups **(**$p>0.05$). Specifically, the concentration of E2 increased following MIET training. P4 also showed a significant change in the CON **(**$\chi2= 9.0$, $p=0.003$) (ES=1.0). But the change in P4 was reported close to significant **(**$\chi2= 3.6$, $p=0.052$).

TABLE 5. Mean (±SD**)** change in serum ferritin, hs-CRP, cortisol, and endogenous sex hormones.

	Pre		**Post**		Between group	P value	Within group	P value
Variables	CON(n=9)	HC(n=13)	CON(n=9)	HC(n=13)	**Pre**	**Post**	CON(n=9)	HC(n=13)
Cortisol(nmol/L)	300.2±114.2	579.4±135.6	348.4±76.6	566.2±124.2	**<0.001***	**0.002***	0.48	0.78
Hs-CRP(mg/L)	2.2±1.4	2.7±1.8	2.1±2.8	3.1±1.6	0.66	**0.02***	**0.02** ■	**0.02** ■
Serum ferritin(ug/L)	35.1±17.1	35.6±18.2	36.4±31.3	33.1±12.7	0.92	0.57	0.31	0.78
E2 (pmol/L)	232.5 ±237.8	143.5±109.3	529.8±224.7	96.5±50.6	0.13	**<0.001***	**0.003**■	0.78
P4 (nmol/L)	6.4 ±10.8	1.1±0.8	26.9±17.8	0.9±0.8	0.36	**<0.001***	**0.003** ■	0.52

*= Between group differences

■ = Within group differences

Hs-CRP= high-sensitivity C-reactive protein. E2= estrogen. P4 progesterone.

TABLE 6. Relative change (Δ%) in the concentration of serum ferritin, high-sensitivity C-reactive protein (hs-CRP), and cortisol.

Variables	CON (Δ%)	HC (Δ%)	Between group p value (Δ%)
Cortisol (nmol/L)	85.6.1 %	-0.07 %	0.28
Hs-CRP (mg/L)	-22.2 %	33.2 %	**0.002***
Serum ferritin (ug/L)	-2.4 %	16.1 %	0.32

*= Between group differences

5.4 Relationship between cortisol, hs-CRP, and ferritin

The result from the relationship between cortisol and hs-CRP at baseline indicated a non-significant correlation both in the CON group (r=0.18, p=0.63) and in the HC group (r=-0.04, p=0.88). The post-MIET relationship between the same variables also showed a non-significant correlation between the CON group (r=-0.63, p=0.06) and the HC group (r=-0.18, p=0.54). Additionally, the relationship between Δ% in cortisol and hs-CRP also indicated no significant correlation in the CON (FIGURE 6a) and HC groups (FIGURE 6b).

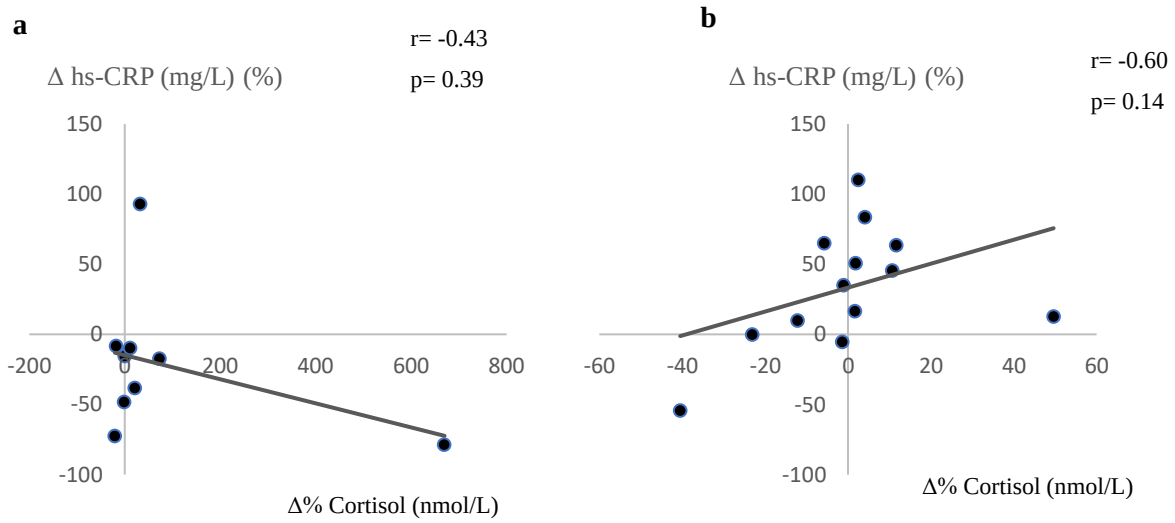

FIGURE 6. Scatterplot showing relationships between relative changes (Δ%) in cortisol and hs-CRP in the CON group (a) and the HC group (b) Hs-CRP= high-sensitivity C-reactive protein

Regarding the relationship between cortisol and ferritin at baseline, both the CON and the HC groups exhibited a non-significant correlation (CON group: r=-0.13, p=0.73; HC group: r=-0.003, p=0.99). No significant association between ferritin and hs-CRP was observed following the MIET in the CON group (r= -0.20, p=0.60) and in the HC group (r=0.22, p=0.45). Furthermore, the correlation analysis between Δ% in cortisol and ferritin also revealed a non-significant correlation in the CON group (FIGURE 7a) and in the HC group (FIGURE 7b).

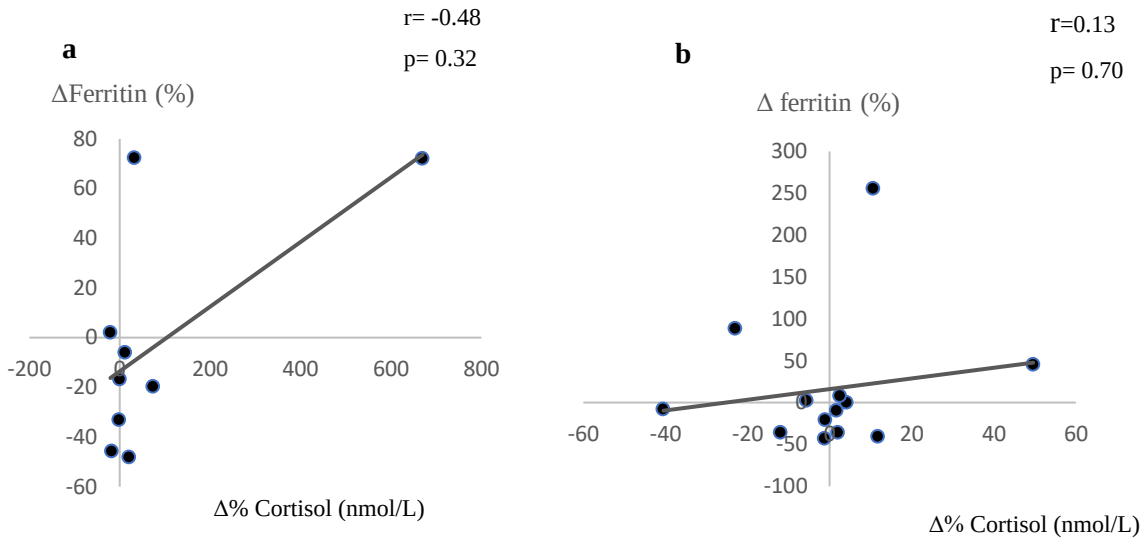

FIGURE 8. Scatterplot showing relationships between relative changes (Δ%) in cortisol and ferritin in the CON group (a) and the HC group (b).

6 DISCUSSION

The primary objective of the present study was to evaluate the impact of 8 weeks of the MIET training on serum ferritin, hs-CRP, and cortisol levels in naturally menstruating females (CON group) and users of combined hormonal contraceptive pills (HC group). Additionally, the study examined the relationship between cortisol and hs-CRP, as well as cortisol and ferritin.

The primary outcome of the current study indicates that 8 weeks of the MIET training does not induce any changes in serum ferritin and cortisol levels in either the CON or HC groups. Furthermore, no distinct adaptations were observed between the two groups. On the other hand, hs-CRP showed a statistically significant decrease in the CON group and increase in the HC group after 8 weeks of MIET. Additionally, the Δ% in hs-CRP between the two groups was significantly different. Further, no significant relationship was identified between cortisol, hs-CRP, and ferritin in either the HC or CON groups.

6.1 The effect of 8-week MIET on cortisol

Despite significant between-group differences at the baseline and post-MIET, the current study's findings indicated no influence of the 8 weeks of MIET training on the concentration of cortisol in the CON and HC groups. Even though the Δ% did not indicate a significant difference between group, the CON group experienced an increase, and the HC group experienced a reduction following 8-weeks of MIET training in the mean cortisol concentration after 8 weeks of MIET training. However, these changes did not reach statistical significance. This phenomenon may be attributed to the simultaneous increase in blood CBG, resulting in increased cortisol binding to CBG when the HPA axis is triggered among hormonal contraceptive users which might affect the cortisol concentration (Crewther et al., 2015; Kirschbaum et al., 1995)

Previous studies reported a decline in basal cortisol concentration following regular exercise training, as Grandys et al. (2016) indicated. The concept of the body adapting to the stressor and subsequently experiencing a diminished sensitivity of the HPA axis, as Hackney. (2006) suggested, adds an intriguing layer to understanding how the body responds to regular physical

activity. However, this occurrence was not observed in the current study, and the cortisol level remained unchanged in both groups.

The lack of significant change in the cortisol concentration in the current study could be attributed to the fact that the total intensity and duration of the training program were not sufficient for the exercise-induced change in cortisol in our study groups (Häkkinen et al., 2005). Indeed, the change in cortisol concentration can be influenced by various factors, including the duration of recovery between training sessions and the type of training (Häkkinen et al., 2005; Corazza et al., 2014). Further, the participant's initial fitness level could be another factor contributing to the absence of significant changes in cortisol levels, as indicated by Wood et al. (2018). Although their study focused on psychological stress, they suggested that individuals with higher fitness levels experience reduced cortisol secretion during psychosocial stress. This indicates a potential role of physical activity in mitigating the impact of stress on health. Therefore, stratifying participants based on their fitness level and hormonal status in future studies could enhance our understanding of the variability in cortisol responses.

The results of this study indicated no change in the level of cortisol following 8 weeks of MIET training, which agrees with the report of Häkkinen et al. (2005). They assessed the effect of two types of training (combined strength and endurance training) on serum cortisol in females with stable rheumatoid arthritis and healthy females as a control group. Häkkinen et al. (2005) reported no effect of training on the concentration of cortisol after 21 weeks of simultaneous strength and endurance training period. However, the training intensity in their study was high, and they did not report the state of MC. Other investigations also indicated no statistically significant change in serum cortisol concentration after chronic training (Häkkinen et al., 2002; de Souza Vale et al., 2009; Kraemer et al., 1989; Taipale et al., 2020). Nonetheless, the training mode (e.g., strength training), population (e.g., unhealthy, athletic), fitness levels, and training duration differed from the current study. Conflicting findings are also present, suggesting an increase (Hill et al., 2008; Rojas Vega et al., 2006) and decrease (Arazi et al., 2013) in cortisol concentration following exercise training. Notably, studies reported increase, focused on the acute effects of exercise on cortisol levels.

There is limited supporting data available in the context of exercise training regarding the effect of hormonal contraceptives on cortisol responses. There are few investigations that reported

attenuated cortisol responses to psychological, pharmacological, and physical stressors among hormonal contraceptive users, which is different from the findings of the current studies (Roche et al., 2013; Kirschbaum et al., 1996; Crewther et al., 2015). Crewther et al. (2015) reported lower reactivity of cortisol among hormonal contraceptive users by pooling all the activities (gym training, light training, heavy training, and competition) and groups (hormonal contraceptives and non-users) in elite females' athletes. While the within-activity analysis indicated a rise in the level of cortisol in both groups during the competition but not the training session.

The significantly higher concentration of cortisol (at baseline and post-MIET) in the HC group suggests a potential differential effect of exogenous hormones on cortisol metabolism in females using hormonal contraceptives compared to naturally menstruating females. The higher cortisol concentration in the HC group compared with the CON in the current study confirms other investigations (Kirschbaum et al., 1996; Timmons et al., 2005; Boisseau et al., 2013). The higher cortisol levels in hormonal contraceptive users can be attributed to the impact of synthetic hormones on the HPA axis (Kirschbaum et al., 1996). The magnitude of this change also depends on the dosage of EE within the pill, with a higher dose of EE correlating to a higher cortisol concentration (Wiegratz et al., 2003).

In summary, the significant difference in cortisol concentration between groups suggests potential variations in cortisol regulation linked to hormonal status. The lack of a statistically significant change in cortisol levels following the 8-week MIET training, along with the absence of distinct adaptation between the CON and HC groups, might be due to the relatively small sample sizes in the CON and HC groups, which could limit the statistical power to detect significant differences. To confirm these findings, future studies with larger sample sizes are essential.

6.2 The effect of 8-week MIET on hs-CRP.

The findings of the present study indicate that the impact of MIET training on hs-CRP may vary depending on the use of hormonal contraceptives. The concentration of hs-CRP increased significantly in the HC group, while it decreased in the CON group. This distinct physiological response observed here suggests a potential influence of hormonal contraceptives on this

particular biomarker. Despite the between-group difference at post-MIET (but the magnitude of the difference was small), there was no significant difference between the groups reported at the baseline in the concentration of hs-CRP.

The observed significant decrease in the hs-CRP levels among naturally menstruating females is in line with previous studies. For instance, Mogharnasi et al. (2019) reported a decrease in the concentration of hs-CRP, in their exercise groups, but the reduction was more effective in the endurance exercise group than in resistance training. It is worth noting that their study exclusively involved resistance and endurance training. The exercise programs in their investigation included 8 weeks of endurance and resistance training at intensities ranging from 65% to 80% of maxHR and one-repetition maximum. Daray et al. (2011) also observed a decrease in hs-CRP concentration in their exercise groups, comprising an endurance group (E) involved in marathon training and a combined endurance and resistance group (ER) participating in marathon training with additional resistance training, when compared to their inactive group after a 15-week training period. They noted that the reduction was particularly pronounced in the ER group. These results are consistent with the conclusions drawn by Donges et al. (2010) and Stewart et al. (2007), indicating that incorporating resistance training may be more effective than relying only on aerobic training in reducing CRP levels. Despite the variation in the study's populations and the type of training, the consistent finding across these studies is a reduction in hs-CRP concentration in the exercise intervention groups compared to the inactive groups. Other investigations also confirm the results of the current study demonstrating a reduced serum concentration of hs-CRP following regular training (Dufaux et al., 1984; Campbell et al., 2009; King et al., 2003; Rosenbaum et al., 2007).

Various potential explanations could underlie the observed effects. The decrease in hs-CRP concentration after 8 weeks of MIET in the CON group implies that MIET might provide anti-inflammatory benefits for non-hormonal contraceptive users (Cerqueira et al., 2020). Earlier research has indicated that regular physical activity can elicit anti-inflammatory effects by influencing the release of different cytokines and adipokines. Changes in body composition resulting from exercise, such as decreased adipose tissue and modifications in adipokine secretion, could contribute to the observed decline in hs-CRP levels (Campbell et al., 2009; You et al., 2004). However, this study did not find significant changes in anthropometric measurements following the MIET training, with only the upper arm showing significant

changes. Additionally, enhancements in cardiovascular fitness and endothelial function due to regular exercise training may play a role in reducing hs-CRP levels (Sae et al., 2009).

Investigation into the impact of hormonal contraceptives on the concentration of hs-CRP in athletes and physically active females indicated a progressive increase in hs-CRP in hormonal contraceptive users compared to non-users. Cauci et al. (2017) found that non-obese, healthy, white Italian females using hormonal contraceptives and exercising regularly for more than 5 hours per week had elevated hs-CRP compared to their non-hormonal contraceptive counterparts. They also hypothesize that individuals with elevated baseline levels of hs-CRP will likely undergo a more pronounced inflammatory response to stressors. Aligning with the findings of the current study, the study by Ihalainen et al. (2019) also observed an increase in the concentration of hs-CRP among hormonal contraceptive pill users, while it decreased in non-users after 10 weeks of high-intensity combined strength and endurance training.

The rise in hs-CRP levels following regular endurance training among hormonal contraceptives could be attributed to the impact of synthetic sex hormones on HPO axis, potentially disrupting endogenous reproductive hormones (Elliott-Sale et al., 2013). This disturbance may lead to changes in inflammatory status by increasing levels of proinflammatory markers such as IL-6 and TNF-α. These proinflammatory markers can, in turn, stimulate the liver to produce hs-CRP (Cerqueira et al., 2020; Michigan et al., 2011). Hence, the rise in hs-CRP observed in the hormonal contraceptive group could be attributed to an intensified inflammatory response induced by hormonal effects that, in terms, blunt the anti-inflammatory effect of regular exercise training.

Kasapis and Thompson (2005) also demonstrated that regular exercise training is a lasting anti-inflammatory treatment. The anti-inflammatory effect of regular training extends to aging, as indicated by Nicklas et al. (2008), and persists in conditions characterized by chronic inflammation, such as metabolic diseases, as highlighted by Lancaster and Febbraio (2014). Generally, physically active individuals exhibit hs-CRP levels that are 19–35% lower compared to their inactive counterparts, and there is an observed inverse dose–response relationship among adults engaging in light, moderate, or vigorous physical activity (Fedewa et al., 2017). The current investigation also observed about a 22% decrease in hs-CRP levels among naturally menstruating females, post-MIET. Interestingly, this anti-inflammatory effect was not evident

in hormonal contraceptive users, where the MIET training resulted in an approximately 33% increase in hs-CRP levels. The complexity of monophasic hormonal contraceptives and their potential influence on hs-CRP levels during exercise training suggests that more investigations are needed to understand the underlying mechanisms comprehensively.

To sum up, the opposite effects of MIET training on hs-CRP levels in the CON and HC groups highlight the significance of individual characteristics in designing exercise interventions. To validate and extend our findings, future studies should involve larger populations and include a broader panel of inflammatory markers or cytokines in future research, which would contribute to a more thorough understanding of the effects of MIET on inflammation. Our focus on hs-CRP alone leaves room for a more comprehensive exploration of the underlying mechanisms.

6.3 The effect of 8-week MIET on ferritin

The findings of the present study suggested that MIET training has no effect on ferritin concentrations in the CON and HC groups. Additionally, no group difference was reported. Contrary to these findings, earlier investigation has indicated that endurance training can impact iron metabolism, resulting in a decrease in ferritin levels (Beard & Tobin, 2000). Longitudinal studies indicated a reduction in ferritin concentration in physically active females, which can be attributed to the increased demand for iron during regular exercise to enhance the production of red blood cells (Montero & Lundby., 2011). In other words, the increased demand for iron during training has been suggested to potentially deplete iron stores.

In contrast with our findings, Bijeh et al. (2018) explored the impact of eight weeks of aerobic exercise on iron parameters, including serum ferritin, in non-athlete females. The training programs in their study involved endurance training, three sessions/ week, each lasting one hour and conducted at 60-70% of maxHR. The results revealed a significant decline in the concentration of ferritin. Similarly, Blum et al. (1986) reported a decline in serum ferritin levels after 13 weeks in females engaged 4 days per week of 35 minutes of aerobic exercise. Mcclung et al. (2009) confirmed these findings, but their study consisted of 9 weeks of aerobic and resistance training in female soldiers 4-6 times/week for 1-1.5h.

Few previous studies indicated no change in ferritin concentration, which is in line with the findings of this study. For instance, Di Santolo et al. (2008) examined the effects of regular physical exercise on iron status parameters, including ferritin, in young non-professional female athletes, primarily volleyball players, who trained for more than 9 hours per week. They compared this group with a control group engaged in physical exercise for less than 3 hours/week. Interestingly, they reported no significant difference between the two groups. Nevertheless, it is essential to note that their study design differed from the current one, as they opted for a cross-sectional design. A study by Ishibashi et al. (2022) reported a not significant change in ferritin concentration following twice-a-day (one in the morning and one in the afternoon) endurance exercise in female long-distance runners. Regardless, it is worth noting that they also assessed the acute response, and blood samples were collected up to 24 hours after the intervention (at baseline, 8, 12, and 24 hours). However, none of these studies took into account the MC or hormonal contraceptive status, and some lacked a control group.

Indeed, there are several potential explanations for the differences observed. Factors including dietary intake of iron could also play crucial roles in influencing the study outcomes (Alaunyte et al., 2015). Additionally, as implemented in the current study, the 8-week training duration, with 30-90 minutes each session, may have been insufficient to induce substantial changes in ferritin levels. Additional investigations are required to explore the potential impacts of more extended training periods and enhance our comprehension of how hormonal contraceptives affect iron metabolism. Indeed, other reasons could be attributed to the lack of change in ferritin concentration. Previous studies have indicated that physical exertion (aerobic and anaerobic), and even the specific type of muscle contractions (concentric and eccentric), can play a significant role in serum ferritin concentration. As an example, Malczewska-Lenczowska et al. (2010) reported no training effect on serum ferritin levels in cross-country skiers over 8 consecutive days. In their study, the training program was predominantly aerobic, with the anaerobic threshold rarely surpassed. Besides, concentric contraction (if dominant in endurance-type exercise) yielded a smaller degree of damage and inflammation compared with eccentric contraction (Córdova-Martínez et al., 2022). As a result, exercise training that emphasizes eccentric muscle actions and incorporates multiple short anaerobic sessions might induce more significant muscle fiber damage, activating the inflammation signalling pathway. This, in turn, could lead to the accumulation of ferritin as an acute phase reactant (APR) protein (Malczewska-Lenczowska et al., 2010). Hence, the training loads used in this study could

potentially be below the threshold required to induce significant changes in serum ferritin concentration.

The prevailing trend in existing studies examining the impact of exercise training on ferritin among hormonal contraceptive users and naturally menstruating females (both athletes and non-athletes) has predominantly focused on assessing acute responses, sometimes in the lack of exercise intervention (Alfaro-Magallanes et al., 2023; Badenhorst et al., 2023; Belza et al., 2005; Zheng et al., 2021; Larsson et al., 1992). However, most of these studies identified no differences in ferritin concentration across MC and hormonal contraceptive phases, and Larsson et al. (1992) also confirmed these findings and suggested that hormonal contraceptives may enhance ferritin concentration only for those with initially low levels at the study's commencement. Notably, Alfaro-Magallanes et al. (2023) and Badenhorst et al. (2023) took it a step further by reporting no distinctions between hormonal contraceptive users and naturally menstruating females among physically active females. This agrees with the findings of the current study.

Indeed, 8 weeks of MIET training with 60-75% of maxHR may not influence the concentration of ferritin based on the use of hormonal contraceptives. However, when interpreting the results, it is crucial to acknowledge that this study solely assesses the levels of ferritin, which might not provide a comprehensive understanding of the overall iron status following the MIET training programs. Additional markers and assessments could offer a more thorough insight into the intricate dynamics of iron metabolism in response to the training program.

6.4 Relationship between cortisol and hs-CRP

There was no notable correlation found between the Δ% in cortisol and hs-CRP ($p > 0.05$) in both study groups. To the best of our knowledge, there have been no studies conducted to assess the correlation between cortisol and hs-CRP during the course of exercise training among the naturally menstruating female and hormonal contraceptive users. Research examining the interaction between cortisol and hs-CRP throughout the training period is limited, with the majority of studies predominantly including men in their study populations (Costello et al., 2018; Rohnejad & Monazzami, 2023). Moreover, researchers are currently investigating the interplay between cortisol and hs-CRP in diverse populations, ranging from depressed adults

(Suarez et al., 2017) to individuals with early acute pancreatitis (Mohammed et al., 2022) rather than healthy and physically active females.

The relationship between cortisol and hs-CRP is a topic exploring the complex relationships between stress and the immune system. As per findings from Suarez et al. (2017), the dysregulation of the neuro-immune relationship, indicated by an insufficient release of cortisol along with elevated hs-CRP levels, plays a role in stress sensitivity, particularly in individuals displaying elevated symptoms of depression. In the current study, participants maintained cortisol and hs-CRP levels within the normal range both at baseline and post-MIET. Nevertheless, it is crucial to emphasize that it is inappropriate to compare these two studies as the nature of Suarez and Colleague's study varies from the current investigation.

Several studies propose that stress can initiate a proinflammatory and anti-inflammatory response both centrally_in the brain_ and peripherally by disturbing HPA axis (Rohleder, 2014; Calcia et al., 2016). However, the proinflammatory and anti-inflammatory functions of cortisol depend upon the nature and intensity of stressors (Liu et al., 2017). It is important to note that while an acute increase in cortisol concentration following a single bout of exercise acts as an anti-inflammatory biomarker (Hannibal & Bishop, 2014), chronically elevated cortisol levels contribute to a proinflammatory state (Sorrells et al., 2009).

Additionally, factors such as hydration status, can influence the response of cortisol and hs-CRP to exercise, as indicated by Costello et al. (2018). In their study, Costello and colleagues examined the impact of acute and chronic heat exposure, exercise, and dehydration on cortisol levels and inflammation biomarkers, including hs-CRP, in trained males. They found that acute exercise in the heat led to increased cortisol and inflammation biomarkers, mainly when fluid intake was restricted. Further, no interaction between these biomarkers were reported which is in line with the result of current study. However, they assess the acute response. Furthermore, ethnicity is another factor that may indicate an interaction between cortisol and hs-CRP, as reported by Tolmay et al. (2012). However, it is essential to note that none of these investigations are directly comparable to the current study, as this study did not consider the hydration state. In addition, some investigations did not incorporate any exercise training, and the population of their study differed from this study.

To sum up, the relationship between cortisol and hs-CRP is influenced significantly by individual variations, the characteristics of the study's population, and the study's specific context, as with any physiological interaction. In the current study, the concentration of both cortisol and hs-CRP fell within the reference range for most participants, which could contribute to the absence of a relationship between these two biomarkers.

6.5 Relationship between cortisol and ferritin

Both study groups showed no significant relationships between Δ% in cortisol and ferritin ($p > 0.05$). To the best of our knowledge, no studies have explored the relationship between cortisol and ferritin, specifically in the context of exercise training among healthy, physically active females and users of hormonal contraceptives. Existing studies examining the relationship between cortisol and ferritin primarily focus on populations apart from the healthy and physically active population, such as gestational diabetes mellitus (Feng et al., 2020) and patients with iron deficiency (Saad et al., 1991). These findings suggest that chronic alterations in either cortisol or ferritin levels can impact each other.

Numerous studies have explored the impact of iron supplements on fatigue and mood states, incorporating assessments of iron metabolism biomarkers like ferritin and stress biomarkers such as cortisol in athletic populations. The findings indicate that endurance athletes may benefit from iron supplementation, improving stress levels, mood states, and subjective fatigue compared to those who do not take iron supplements (Córdova et al., 2019; Kapoor et al., 2023). These findings suggest that the iron supplementation regimen was effective in maintaining hematological levels such as ferritin and reduced accumulated stress in response to exercise intensity, as evidenced by lower cortisol levels in both men and females. The findings in the current study differ from those of previous research, as no relationship was reported between the two biomarkers. However, a direct comparison with these studies might not be suitable for several reasons. Firstly, those studies did not provide information on the MC status and whether participants were using hormonal contraceptives. Additionally, in the present study, participants adhered to their regular dietary habits without using any iron supplementation, which is critical when interpreting the results. Moreover, the population in this study consisted of healthy non-athletic females, whereas the populations in the other studies comprised athletes.

The relationship between cortisol and ferritin might depend on the study's context and population. The participants in the present study were healthy, naturally menstruating females and users of hormonal contraceptives. We observed no alterations in serum ferritin and cortisol concentrations following 8 weeks of MIET training, and most participants exhibited values within the reference range.

To sum up, cortisol and ferritin are distinct biomarkers representing different physiological processes in the body. While there is no direct relationship between cortisol and ferritin, it is essential to consider that various factors, including overall health, nutritional status, and the type, intensity, and duration of exercise, can influence both cortisol and ferritin levels independently.

6.6 Strengths, limitations, and future research

The strengths of this study are worth considering. Firstly, we had a well-designed experiment with two groups, which allowed us to compare the effect of moderate-intensity endurance training on cortisol, hs-CRP, and ferritin in naturally menstruating females and those using combined monophasic hormonal contraceptive pill. Furthermore, our focus on a specific population enhances the applicability of our findings to individuals with similar characteristics. Additionally, we employed reliable and validated analytical methods to measure biomarkers, ensuring the precision and credibility of our results.

While our findings provide valuable insights into the effects of moderate-intensity endurance training on specific biomarkers, there are limitations to consider:

1. The sample size is a common limitation, and expanding it in future studies would strengthen the findings' reliability and generalizability. It is a step toward building a more comprehensive understanding of the impact of moderate-intensity endurance training on these biomarkers.
2. Stress, inflammation, and iron metabolism are complex physiological processes, and each biomarker (cortisol, hs-CRP, and ferritin) provides only a partial picture of the overall status. Selecting only one biomarker from each category does not allow us to draw comprehensive conclusions about an individual's health or physiological condition related to stress, inflammation, and iron metabolism.

3. Additionally, the duration and frequency of our study's training sessions were standardized. This may differ from real-world scenarios where individuals engage in various training schedules. Thus, further research should consider incorporating more diverse training protocols to understand better the effects of moderate-intensity endurance training on these biomarkers.
4. The exclusive focus in this thesis on the follicular phase of the menstrual cycle and the active pill phase of hormonal contraceptive pills restricts our results' generalizability to ovulation, the LP of the MC, and the inactive phase of hormonal contraceptive pills. We recommend that future studies incorporate additional phases, such as the inactive phase of hormonal contraceptive pills and the LP of the natural menstrual cycle while employing the same training program.
5. Nutritional aspects were not incorporated into this thesis, and this omission is crucial for comprehensively interpreting the alterations in the status of cortisol, hs-CRP, and ferritin.

Further investigation is essential to address these limitations. Understanding the role of fluctuating levels of E2 and P4 during natural menstruation and hormonal contraceptive pills is crucial, given their pivotal impact on various physiological pathways, including the regulation of the immune system. Further, existing research on the effects of exercise training on ferritin, hs-CRP, and cortisol levels frequently fails to consider age-related variations. Exploring the influence of training on biomarker levels in diverse age groups of menstruating females and hormonal contraceptive users may enhance our overall comprehension of the subject. Additionally, expanding the scope to include a broader range of biomarkers associated with stress, inflammation, and iron metabolism adds depth to the study. This approach enhances the overall comprehension of how moderate-intensity endurance training impacts the physiological responses in both naturally menstruating females and hormonal contraceptive users.

6.7 Conclusions

In conclusion, the findings from the present study indicate that MIET does not significantly impact cortisol and ferritin levels in both the CON and HC groups. Interestingly, hs-CRP exhibited a decrease in the CON group following MIET, whereas it showed an increase in the

HC group. The current study suggests that the use of hormonal contraceptives disrupts the HPA axis, irrespective of engagement in exercise training, as the concentration of cortisol was higher in the HC group before and after the training programs. Further, despite the well-documented research demonstrating the anti-inflammatory effects of regular exercise training, the physiological benefits of exercise would seem to be diminished by long-term hormonal contraceptive use in females. Therefore, the impact of moderate-intensity endurance training on hs-CRP among hormonal contraceptive users remains uncertain. Nonetheless, further research is warranted, considering their potential implications for public health, as they respond differently in some conditions, specifically inflammation.

REFERENCES

Aimone-Gastin, I. (2006). Biochemical markers of iron status. Nephrologie & Therapeutique, 2, S321-6.

Akabas, S. R., & Dolins, K. R. (2005). Micronutrient requirements of physically active women: what can we learn from iron? The American journal of clinical nutrition, 81(5), 1246S-1251S.

Akoglu, H. (2018). User's guide to correlation coefficients. Turkish journal of emergency medicine, 18(3), 91-93.

Alaunyte, I., Stojceska, V., & Plunkett, A. (2015). Iron and the female athlete: a review of dietary treatment methods for improving iron status and exercise performance. Journal of the international society of sports nutrition, 12(1), 38.

Alfaro-Magallanes, V. M., Barba-Moreno, L., Romero-Parra, N., Rael, B., Benito, P. J., Swinkels, D. W., ... & IronFEMME Study Group. (2022). Menstrual cycle affects iron homeostasis and hepcidin following interval running exercise in endurance-trained women. European Journal of Applied Physiology, *122*(12), 2683-2694.

Alfaro-Magallanes, V. M., Romero-Parra, N., Barba-Moreno, L., Rael, B., Benito, P. J., Díaz, Á. E., ... & IronFEMME Study Group. (2023). Serum iron availability, but not iron stores, is lower in naturally menstruating than in oral contraceptive athletes. European Journal of Sport Science, 23(2), 231-240.

Arazi, H., Damirchi, A., & Asadi, A. (2013, February). Age-related hormonal adaptations, muscle circumference and strength development with 8 weeks moderate intensity resistance training. In *Annales d'endocrinologie* (Vol. 74, No. 1, pp. 30-35). Elsevier Masson.

Athanasiou, N., Bogdanis, G. C., & Mastorakos, G. (2022). Endocrine responses of the stress system to different types of exercise. Reviews in Endocrine and Metabolic Disorders, 1-16.

Auersperger, I., Škof, B., Leskošek, B., Knap, B., Jerin, A., & Lainscak, M. (2013). Exercise-induced changes in iron status and hepcidin response in female runners. PLoS One, 8(3), e58090.

Badenhorst, C. E., Black, K. E., & O'Brien, W. J. (2019). Hepcidin as a prospective individualized biomarker for individuals at risk of low energy availability. International journal of sport nutrition and exercise metabolism, *29*(6), 671-681.

Badenhorst, C. E., Govus, A. D., & Mündel, T. (2023). Does chronic oral contraceptive use detrimentally affect C-reactive protein or iron status for endurance-trained women? Physiological Reports, *11*(14), e15777.

Banfi, G., Marinelli, M., Roi, G. S., & Agape, V. (1993). Usefulness of free testosterone/cortisol ratio during a season of elite speed skating athletes. International journal of sports medicine, 14(07), 373-379.

Barba-Moreno, L., Alfaro-Magallanes, V. M., de Jonge, X. A. J., Díaz, A. E., Cupeiro, R., & Peinado, A. B. (2022). Hepcidin and interleukin-6 responses to endurance exercise over the menstrual cycle. European Journal of Sport Science, 22(2), 218-226.

Bassuk, S. S., Rifai, N., & Ridker, P. M. (2004). High-sensitivity C-reactive protein: clinical importance. Current problems in cardiology, *29*(8), 439-493.

Bathija, H., Lei, Z. W., Cheng, X. Q., Xie, L., Wang, Y., Rugpao, S., ... & Boukhris, R. (1998). Effects of contraceptives on hemoglobin and ferritin. Contraception, 58(5), 261-273.

Beard, J., & Tobin, B. (2000). Iron status and exercise. The American journal of clinical nutrition, 72(2), 594S-597S.

Belza, A., Henriksen, M., Ersbøll, A., Thilsted, S., & Tetens, I. (2005). Day-to-day variation in iron-status measures in young iron-deplete women. British Journal of Nutrition, 94(4), 551-556.

Bennell, K., White, S., & Crossley, K. (1999). The oral contraceptive pill: a revolution for sportswomen? British journal of sports medicine, 33(4), 231-238.

Bijeh, N., Askari, A., Ramezani, S., & Askari, B. (2018). Effect of Eight Weeks of Aerobic Exercise on Iron Status and Coagulation and Immune Indices in Non-Athlete Females. Medical Laboratory Journal, 12(1), 37-43.

Blum, S. M., Sherman, A. R., & Boileau, R. A. (1986). The effects of fitness-type exercise on iron status in adult women. The American journal of clinical nutrition, 43(3), 456-463.

Boisseau, N., Enea, C., Diaz, V., Dugué, B., Corcuff, J. B., & Duclos, M. (2013). Oral contraception but not menstrual cycle phase is associated with increased free cortisol levels and low hypothalamo-pituitary-adrenal axis reactivity. Journal of Endocrinological Investigation, 36, 955-964.

Bourque, S. P., Pate, R. R., & Branch, J. D. (1997). Twelve weeks of endurance exercise training does not affect iron status measures in women. Journal of the American Dietetic Association, 97(10), 1116-1121.

Bruinvels, G., Burden, R. J., McGregor, A. J., Ackerman, K. E., Dooley, M., Richards, T., & Pedlar, C. (2017). Sport, exercise and the menstrual cycle: where is the research? British Journal of Sports Medicine, 51(6), 487-488.

Bull, J. R., Rowland, S. P., Scherwitzl, E. B., Scherwitzl, R., Danielsson, K. G., & Harper, J. (2019). Real-world menstrual cycle characteristics of more than 600,000 menstrual cycles. NPJ digital medicine, 2(1), 83.

Burrows, M., & Peters, C. E. (2007). The influence of oral contraceptives on athletic performance in female athletes. Sports medicine, 37(7), 557–574.

Calabro, P., Golia, E., & TH Yeh, E. (2012). Role of C-reactive protein in acute myocardial infarction and stroke: possible therapeutic approaches. Current pharmaceutical biotechnology, 13(1), 4-16.

Calcia, M. A., Bonsall, D. R., Bloomfield, P. S., Selvaraj, S., Barichello, T., & Howes, O. D. (2016). Stress and neuroinflammation: a systematic review of the effects of stress on microglia and the implications for mental illness. Psychopharmacology, *233*(9), 1637–1650.

Campbell, P. T., Campbell, K. L., Wener, M. H., Wood, B., Potter, J. D., McTIERNAN, A. N. N. E., & Ulrich, C. M. (2009). A yearlong exercise intervention decreases CRP among obese postmenopausal women. Medicine and science in sports and exercise, *41*(8), 1533–1539.

Cauci, S., Di Santolo, M., Culhane, J. F., Stel, G., Gonano, F., & Guaschino, S. (2008). Effects of third-generation oral contraceptives on high-sensitivity C-reactive protein and homocysteine in young women. Obstetrics & Gynecology, 111(4), 857-864.

Cauci, S., Francescato, M. P., & Curcio, F. (2017). Combined oral contraceptives increase high-sensitivity C-reactive protein but not haptoglobin in female athletes. Sports Medicine, 47, 175-185.

Cerqueira, É., Marinho, D. A., Neiva, H. P., & Lourenço, O. (2020). Inflammatory effects of high and moderate intensity exercise—A systematic review. Frontiers in physiology, 10, 1550.

Chaireti, R., Lindahl, T. L., Byström, B., Bremme, K., & Larsson, A. (2016). Inflammatory and endothelial markers during the menstrual cycle. Scandinavian Journal of Clinical and Laboratory Investigation, 76(3), 190-194.

Chen, L., Deng, H., Cui, H., Fang, J., Zuo, Z., Deng, J., ... & Zhao, L. (2018). Inflammatory responses and inflammation-associated diseases in organs. Oncotarget, 9(6), 7204.

Chidi-Ogbolu, N., & Baar, K. (2019). Effect of Estrogen on Musculoskeletal Performance and Injury Risk. Frontiers in physiology, *9*, 1834.

Cooper, D. B., Patel, P., & Mahdy, H. (2017). Oral Contraceptive Pills. In StatPearls. StatPearls Publishing.

Cooper, M. J., & Zlotkin, S. H. (1996). Day-to-day variation of transferrin receptor and ferritin in healthy men and women. The American journal of clinical nutrition, 64(5), 738-742.

Corazza, D. I., Sebastião, É., Pedroso, R. V., Andreatto, C. A. A., de Melo Coelho, F. G., Gobbi, S., ... & Santos-Galduróz, R. F. (2014). Influence of chronic exercise on serum cortisol levels in older adults. European Review of Aging and Physical Activity, 11(1), 25-34.

Costello, J. T., Rendell, R. A., Furber, M., Massey, H. C., Tipton, M. J., Young, J. S., & Corbett, J. (2018). Effects of acute or chronic heat exposure, exercise and dehydration on plasma cortisol, IL-6 and CRP levels in trained males. *Cytokine*, *110*, 277-283.

Crewther, B. T., Hamilton, D., Casto, K., Kilduff, L. P., & Cook, C. J. (2015). Effects of oral contraceptive use on the salivary testosterone and cortisol responses to training sessions and competitions in elite women athletes. *Physiology & behavior*, *147*, 84-90.

Córdova, A., Mielgo-Ayuso, J., Fernandez-Lazaro, C. I., Caballero-García, A., Roche, E., & Fernández-Lázaro, D. (2019). Effect of iron supplementation on the modulation of iron metabolism, muscle damage biomarkers and cortisol in professional cyclists. *Nutrients*, *11*(3), 500.

Córdova-Martínez, A., Caballero-García, A., Bello, H. J., Perez-Valdecantos, D., & Roche, E. (2022). Effects of Eccentric vs. Concentric Sports on Blood Muscular Damage Markers in Male Professional Players. Biology, *11*(3), 343.

Dalgaard, L. B., Dalgas, U., Andersen, J. L., Rossen, N. B., Møller, A. B., Stødkilde-Jørgensen, H., ... & Hansen, M. (2019). Influence of oral contraceptive use on adaptations to resistance training. Frontiers in Physiology, 10, 824.

Da Luz Scheffer, D., & Latini, A. (2020). Exercise-induced immune system response: Anti-inflammatory status on peripheral and central organs. Biochimica et Biophysica Acta (BBA)-Molecular Basis of Disease, 1866(10), 165823.

Daray, L. A., Henagan, T. M., Zanovec, M., Earnest, C. P., Johnson, L. G., Winchester, J., ... & Stewart, L. K. (2011). Endurance and resistance training lowers C-reactive protein in young, healthy females. Applied Physiology, Nutrition, and Metabolism, 36(5), 660-670.

Daru, J., Colman, K., Stanworth, S. J., De La Salle, B., Wood, E. M., & Pasricha, S. R. (2017). Serum ferritin as an indicator of iron status: what do we need to know? The American journal of clinical nutrition, 106(suppl_6), 1634S-1639S.

Davis, A. R., & Westhoff, C. L. (2001). Primary dysmenorrhea in adolescent girls and treatment with oral contraceptives. Journal of pediatric and adolescent gynecology, 14(1), 3-8.

Davis, H. C., & Hackney, A. C. (2017). The hypothalamic–pituitary–ovarian axis and oral contraceptives: regulation and function. Sex hormones, exercise and women: scientific and clinical aspects, 1-17.

DellaValle, D. M. (2013). Iron supplementation for female athletes: effects on iron status and performance outcomes. Current sports medicine reports, 12(4), 234-239.

DellaValle, D. M., & Haas, J. D. (2011). Impact of iron depletion without anemia on performance in trained endurance athletes at the beginning of a training season: a study of female collegiate rowers. International journal of sport nutrition and exercise metabolism, 21(6), 501-506.

De Souza Vale, R. G., de Oliveira, R. D., Pernambuco, C. S., da Silva Novaes, J., & de Andrade, A. D. F. D. (2009). Effects of muscle strength and aerobic training on basal serum levels of IGF-1 and cortisol in elderly women. Archives of gerontology and geriatrics, 49(3), 343-347.

Dignass, A., Farrag, K., & Stein, J. (2018). Limitations of Serum Ferritin in Diagnosing Iron Deficiency in Inflammatory Conditions. *International journal of chronic diseases*, *2018*, 9394060.

Di Santolo, M., Stel, G., Banfi, G., Gonano, F., & Cauci, S. (2008). Anemia and iron status in young fertile non-professional female athletes. European journal of applied physiology, *102*, 703-709.

Docherty, S., Harley, R., McAuley, J. J., Crowe, L. A., Pedret, C., Kirwan, P. D., ... & Millar, N. L. (2022). The effect of exercise on cytokines: implications for musculoskeletal health: a narrative review. BMC Sports Science, Medicine and Rehabilitation, 14(1), 1-14.

Donges, C., Duffield, R., & Drinkwater, E. (2010). Effect of resistance or aerobic exercise training on interleukin-6, C-reactive protein, and body. Med Sci Sports Exerc, 42(2), 304-13.

Duclos, M., Corcuff, J. B., Rashedi, M., Fougère, V., & Manier, G. (1997). Trained versus untrained men: different immediate post-exercise responses of pituitary adrenal axis. A

preliminary study. European journal of applied physiology and occupational physiology, 75(4), 343–350.

Duclos, M., & Tabarin, A. (2016). Exercise and the Hypothalamo-Pituitary-Adrenal Axis. Frontiers of hormone research, *47*, 12–26.

Dufaux, B., Order, U., Geyer, H., & Hollmann, W. (1984). C-reactive protein serum concentrations in well-trained athletes. International Journal of Sports Medicine, 5(02), 102-106.

Dwivedi, A. K., Mallawaarachchi, I., & Alvarado, L. A. (2017). Analysis of small sample size studies using nonparametric bootstrap test with pooled resampling method. *Statistics in medicine*, *36*(14), 2187-2205.

Elliott, K. J., Cable, N. T., & Reilly, T. (2005). Does oral contraceptive use affect maximum force production in women? British journal of sports medicine, 39(1), 15-19.

Elliott-Sale, K. J., & Hicks, K. M. (2018). Hormonal-based contraception and the exercising female. In The Exercising Female (pp. 30-43). Routledge.

Elliott-Sale, K. J., McNulty, K. L., Ansdell, P., Goodall, S., Hicks, K. M., Thomas, K., ... & Dolan, E. (2020). The effects of oral contraceptives on exercise performance in women: a systematic review and meta-analysis. *Sports medicine*, *50*(10), 1785-1812.

Elliott-Sale, K. J., Smith, S., Bacon, J., Clayton, D., McPhilimey, M., Goutianos, G., ... & Sale, C. (2013). Examining the role of oral contraceptive users as an experimental and/or control group in athletic performance studies. Contraception, 88(3), 408-412.

Farage, M. A., Neill, S., & MacLean, A. B. (2009). Physiological changes associated with the menstrual cycle: a review. Obstetrical & gynecological survey, *64*(1), 58-72.

Faude, O., Kindermann, W., & Meyer, T. (2009). Lactate threshold concepts: how valid are they? Sports medicine,*39*(6), 469–490.

Fedewa, M. V., Hathaway, E. D., Higgins, S., Das, B. M., Forehand, R. L., Schmidt, M. D., & Evans, E. M. (2018). Interactive associations of physical activity, adiposity, and oral contraceptive use on C-reactive protein levels in young women. Women & health, 58(2), 129-144.

Fedewa, M. V., Hathaway, E. D., & Ward-Ritacco, C. L. (2017). Effect of exercise training on C reactive protein: a systematic review and meta-analysis of randomised and non-randomised controlled trials. British journal of sports medicine, 51(8), 670-676.

Feng, Y., Feng, Q., Lv, Y., Song, X., Qu, H., & Chen, Y. (2020). The relationship between iron metabolism, stress hormones, and insulin resistance in gestational diabetes mellitus. Nutrition & diabetes, *10*(1), 17.

Findlay D. (1986). The pill. Australian family physician, *15*(12), 1587.

Fischer, J. A., Sasai, C. S., & Karakochuk, C. D. (2021). Iron-containing oral contraceptives and their effect on hemoglobin and biomarkers of iron status: a narrative review. Nutrients, *13*(7), 2340.

Fleischman, D. S., Navarrete, C. D., & Fessler, D. M. (2010). Oral contraceptives suppress ovarian hormone production. Psychological science, *21*(5), 750-752.

Institute of Medicine (US) Panel on Micronutrients. (2001). Dietary Reference Intakes for Vitamin A, Vitamin K, Arsenic, Boron, Chromium, Copper, Iodine, Iron, Manganese, Molybdenum, Nickel, Silicon, Vanadium, and Zinc. National Academies Press (US).

Frassinelli-Gunderson, E. P., Margen, S., & Brown, J. R. (1985). Iron stores in users of oral contraceptive agents. The American journal of clinical nutrition, 41(4), 703-712.

Galkina, E., & Ley, K. (2009). Immune and inflammatory mechanisms of atherosclerosis. Annual review of immunology, *27*, 165-197.

Garcia, V. P., Rocha, H. N., Sales, A. R., Rocha, N. G., & da Nóbrega, A. C. (2016). Sex Differences in High Sensitivity C-Reactive Protein in Subjects with Risk Factors of Metabolic Syndrome. Arquivos brasileiros de cardiologia, *106*(3), 182–187.

Gellert, S., & Hahn, A. (2017). Iron status in relation to oral contraceptive use in women of reproductive age. Austin J Women Health, *4*, 1025.

Genazzani, A. R., Lemarchand-Beraud, T. H., Aubert, M. L., Felber, J. P., Muller, A., Lavanchy, M., & Gomez, J. (1975). Pattern of plasma ACTH, hGH, and cortisol during menstrual cycle. The Journal of Clinical Endocrinology & Metabolism, 41(3), 431-437.

Gentile, M., Panico, S., Rubba, F., Mattiello, A., Chiodini, P., Jossa, F., ... & Rubba, P. (2010). Obesity, overweight, and weight gain over adult life are main determinants of elevated hs-CRP in a cohort of Mediterranean women. European journal of clinical nutrition, 64(8), 873-878.

Goodwin, M. L., Harris, J. E., Hernández, A., & Gladden, L. B. (2007). Blood lactate measurements and analysis during exercise: a guide for clinicians. Journal of diabetes science and technology, 1(4), 558-569.

Grandys, M., Majerczak, J., Kulpa, J., Duda, K., Rychlik, U., & Zoladz, J. A. (2016). The importance of the training-induced decrease in basal cortisol concentration in the improvement in muscular performance in humans. Physiological Research, *65*(1).

Gruys, E., Toussaint, M. J. M., Niewold, T. A., & Koopmans, S. J. (2005). Acute phase reaction and acute phase proteins. Journal of Zhejiang University. Science. B, 6(11), 1045.

Gulhar, R., Ashraf, M. A., & Jialal, I. (2018). Physiology, Acute Phase Reactants. In StatPearls. StatPearls Publishing

Gursoy, A. Y., Caglar, G. S., Kiseli, M., Pabuccu, E., Candar, T., & Demirtas, S. (2015). CRP at early follicular phase of menstrual cycle can cause misinterpretation for cardiovascular risk assessment. Interventional medicine and applied science, 7(4), 143-146.

Hackney, A. C. (2006). Stress and the neuroendocrine system: the role of exercise as a stressor and modifier of stress. Expert review of endocrinology & metabolism, 1(6), 783-792.

Hackney, A. C., Kallman, A. L., & Ağgön, E. (2019). Female sex hormones and the recovery from exercise: Menstrual cycle phase affects responses. Biomedical human kinetics, 11(1), 87-89.

Hamidovic, A., Karapetyan, K., Serdarevic, F., Choi, S. H., Eisenlohr-Moul, T., & Pinna, G. (2020). Higher circulating cortisol in the follicular vs. luteal phase of the menstrual cycle: A meta-analysis. Frontiers in endocrinology, 11, 311.

Hannibal, K. E., & Bishop, M. D. (2014). Chronic stress, cortisol dysfunction, and pain: a psychoneuroendocrine rationale for stress management in pain rehabilitation. Physical therapy, *94*(12), 1816-1825.

Hellsten, Y., & Nyberg, M. (2011). Cardiovascular adaptations to exercise training. *Comprehensive Physiology*, *6*(1), 1-32.

Henriksson J. (1977). Training induced adaptation of skeletal muscle and metabolism during submaximal exercise. The Journal of physiology, *270*(3), 661–675.

Higham, J. M., O'brien, P. M. S., & Shaw, R. (1990). Assessment of menstrual blood loss using a pictorial chart. BJOG: An International Journal of Obstetrics & Gynaecology, 97(8), 734-739.

Hill, E. E., Zack, E., Battaglini, C., Viru, M., Viru, A., & Hackney, A. C. (2008). Exercise and circulating cortisol levels: the intensity threshold effect. Journal of endocrinological investigation, 31, 587-591.

Hinton, P. S. (2014). Iron and the endurance athlete. Applied Physiology, Nutrition, and Metabolism, 39(9), 1012-1018.

Holloszy, J. O., & Coyle, E. F. (1984). Adaptations of skeletal muscle to endurance exercise and their metabolic consequences. Journal of applied physiology, 56(4), 831-838.

Holtzman, B., & Ackerman, K. E. (2019). Hypothalamic–pituitary–gonadal axis in women's sport: injuries, manipulations, and aberrations. Current Opinion in Endocrine and Metabolic Research, 9, 78-85.

Hughes, D. C., Ellefsen, S., & Baar, K. (2018). Adaptations to endurance and strength training. Cold Spring Harbor perspectives in medicine, 8(6), a029769.

Hakkinen, A., Pakarinen, A., Hannonen, P., Kautiainen, H., Nyman, K., Kraemer, W. J., & Hakkinen, K. (2005). Effects of prolonged combined strength and endurance training on physical fitness, body composition and serum hormones in women with rheumatoid arthritis and in healthy controls. Clinical and experimental rheumatology, *23*(4), 505.

Häkkinen, K., Kraemer, W. J., Pakarinen, A., Tripleltt-Mcbride, T., McBride, J. M., Häkkinen, A., ... & Newton, R. U. (2002). Effects of heavy resistance/power training on maximal strength, muscle morphology, and hormonal response patterns in 60-75-year-old men and women. Canadian Journal of Applied Physiology, *27*(3), 213-231.

Ihalainen, J. K., Hackney, A. C., & Taipale, R. S. (2019). Changes in inflammation markers after a 10-week high-intensity combined strength and endurance training block in women: The effect of hormonal contraceptive use. Journal of science and medicine in sport, 22(9), 1044-1048.

Ishibashi, A., Maeda, N., Kojima, C., & Goto, K. (2022). Iron Metabolism following Twice a Day Endurance Exercise in Female Long-Distance Runners. Nutrients, 14(9), 1907.

Jae, S. Y., Heffernan, K. S., Yoon, E. S., Lee, M. K., Fernhall, B., & Park, W. H. (2009). The inverse association between cardiorespiratory fitness and C-reactive protein is mediated by autonomic function: a possible role of the cholinergic anti-inflammatory pathway. Molecular Medicine, *15*, 291-296.

Jones, A. M., & Carter, H. (2000). The effect of endurance training on parameters of aerobic fitness. Sports medicine, 29(6), 373-386.

Joyner, M. J., & Coyle, E. F. (2008). Endurance exercise performance: the physiology of champions. The Journal of physiology, 586(1), 35-44.

Kapoor, M. P., Sugita, M., Kawaguchi, M., Timm, D., Kawamura, A., Abe, A., & Okubo, T. (2023). Influence of iron supplementation on fatigue, mood states and sweating profiles

of healthy non-anemic athletes during a training exercise: A double-blind, randomized, placebo-controlled, parallel-group study. *Contemporary Clinical Trials Communications*, *32*, 101084.

Kasapis, C., & Thompson, P. D. (2005). The effects of physical activity on serum C-reactive protein and inflammatory markers: a systematic review. Journal of the American College of Cardiology, 45(10), 1563-1569.

Kawamoto, R., Kusunoki, T., Abe, M., Kohara, K., & Miki, T. (2013). An association between body mass index and high-sensitivity C-reactive protein concentrations is influenced by age in community-dwelling persons. Annals of Clinical Biochemistry, 50(5), 457-464.

Kell, D. B., & Pretorius, E. (2014). Serum ferritin is an important inflammatory disease marker, as it is mainly a leakage product from damaged cells. Metallomics, *6*(4), 748-773.

Khera, A., McGuire, D. K., Murphy, S. A., Stanek, H. G., Das, S. R., Vongpatanasin, W., ... & de Lemos, J. A. (2005). Race and gender differences in C-reactive protein levels. Journal of the American college of cardiology, 46(3), 464-469.

Kim, I., Yetley, E. A., & Calvo, M. S. (1993). Variations in iron- status measures during the menstrual cycle. Am. J. Clin. Nutr, 58, 705–709.

King, D. E., Carek, P., MAINOUS III, A. G., & Pearson, W. S. (2003). Inflammatory markers and exercise: differences related to exercise type. Medicine & Science in Sports & Exercise, 35(4), 575-581.

Kirschbaum, C., Pirke, K. M., & Hellhammer, D. H. (1995). Preliminary evidence for reduced cortisol responsivity to psychological stress in women using oral contraceptive medication. Psychoneuroendocrinology, 20(5), 509-514.

Kirschbaum, C., Platte, P., PIRKE, K. M., & Hellhammer, D. I. R. K. (1996). Adrenocortical activation following stressful exercise: further evidence for attenuated free cortisol responses in women using oral contraceptives. Stress Medicine, 12(3), 137-143.

Knovich, M. A., Storey, J. A., Coffman, L. G., Torti, S. V., & Torti, F. M. (2009). Ferritin for the clinician. Blood reviews, *23*(3), 95-104.

Koenig, W. (2013). High-sensitivity C-reactive protein and atherosclerotic disease: from improved risk prediction to risk-guided therapy. International journal of cardiology, 168(6), 5126-5134.

Kraemer, W. J., Fleck, S. J., Callister, R. O. B. I. N., Shealy, M. A. R. I. L. Y. N., Dudley, G. A., Maresh, C. M., ... & Falkel, J. E. (1989). Training responses of plasma beta-

endorphin, adrenocorticotropin, and cortisol. Medicine and science in sports and exercise, 21(2), 146-153.

Kraemer, W. J., & Ratamess, N. A. (2005). Hormonal responses and adaptations to resistance exercise and training. Sports medicine, 35, 339-361.

Krintus, M., Sypniewska, G., & Kuligowska-Prusinska, M. (2010). Effect of second and third generation oral contraceptives on C-reactive protein, lipids and apolipoproteins in young, non-obese, non-smoking apparently healthy women. Clinical biochemistry, 43(6), 626-628.

Kuhl, H., Jung-Hoffmann, C., Weber, J., & Boehm, B. O. (1993). The effect of a biphasic desogestrel-containing oral contraceptive on carbohydrate metabolism and various hormonal parameters. *Contraception*, *47*(1), 55-68.

Kuo, T., McQueen, A., Chen, T. C., & Wang, J. C. (2015). Regulation of glucose homeostasis by glucocorticoids. Glucocorticoid Signaling: From Molecules to Mice to Man, 99-126.

Lampe, J. W., Slavin, J. L., & Apple, F. S. (1986). Poor iron status of women runners training for a marathon. International journal of sports medicine, 7(02), 111-114.

Lancaster, G. I., & Febbraio, M. A. (2014). The immunomodulating role of exercise in metabolic disease. Trends in immunology, *35*(6), 262-269.

Larsson, G., Milsom, L., Lindstedt, G., & Rybo, G. (1992). The influence of a low-dose combined oral contraceptive on menstrual blood loss and iron status. Contraception, 46(4), 327-334.

Li, Y., Zhong, X., Cheng, G., Zhao, C., Zhang, L., Hong, Y., ... & Wang, Z. (2017). Hs-CRP and all-cause, cardiovascular, and cancer mortality risk: a meta-analysis. Atherosclerosis, 259, 75-82.

Liu, Y. Z., Wang, Y. X., & Jiang, C. L. (2017). Inflammation: the common pathway of stress-related diseases. Frontiers in human neuroscience, 11, 316.

Lu, Y., Zhou, S., Dreyer, R. P., Spatz, E. S., Geda, M., Lorenze, N. P., ... & Krumholz, H. M. (2017). Sex differences in inflammatory markers and health status among young adults with acute myocardial infarction: Results from the VIRGO (Variation in Recovery: Role of Gender on Outcomes of Young Acute Myocardial Infarction Patients) study. Circulation: Cardiovascular Quality and Outcomes, 10(2), e003470.

Majka, D. S., Chang, R. W., Vu, T. H. T., Palmas, W., Geffken, D. F., Ouyang, P., ... & Liu, K. (2009). Physical activity and high-sensitivity C-reactive protein: the multi-ethnic study of atherosclerosis. American journal of preventive medicine, 36(1), 56-62.

Malczewska, J., Bach, W., & Stupnicki, R. (2000). The effects of physical exercise on the concentrations of ferritin and transferrin receptor in plasma of female judoists. International journal of sports medicine, 21(03), 175-179.

Malczewska-Lenczowska, J., Stupnicki, R., & Gabryé, T. (2010). Effect of exercise on the markers of iron status in serum of cross-country skiers. Biology of Sport, 27(4).

Maresh, C. M., Whittlesey, M. J., Armstrong, L. E., Yamamoto, L. M., Judelson, D. A., Fish, K. E., ... & Castracane, V. D. (2005). Effect of hydration state on testosterone and cortisol responses to training-intensity exercise in collegiate runners. International journal of sports medicine, 27(10), 765-770.

Margen, S., & King, J. C. (1975). Effect of oral contraceptive agents on the metabolism of some trace minerals. The american journal of clinical nutrition, 28(4), 392-402.

Martin, D., & Elliott-Sale, K. (2016). A perspective on current research investigating the effects of hormonal contraceptives on determinants of female athlete performance. Revista Brasileira de Educação Física e Esporte, 30, 1087-1096.

Martínez-Díaz, I. C., & Carrasco, L. (2021). Neurophysiological stress response and mood changes induced by high-intensity interval training: A pilot study. International Journal of Environmental Research and Public Health, 18(14), 7320.

Maybin, J. A., & Critchley, H. O. (2011). Progesterone: a pivotal hormone at menstruation. Annals of the New York Academy of Sciences, 1221(1), 88-97.

McClung, J. P., Karl, J. P., Cable, S. J., Williams, K. W., Young, A. J., & Lieberman, H. R. (2009). Longitudinal decrements in iron status during military training in female soldiers. British journal of nutrition, 102(4), 605-609.

McLellan, T. M., & Gass, G. C. (1989). The relationship between the ventilation and lactate thresholds following normal, low and high carbohydrate diets. European journal of applied physiology and occupational physiology, 58(6), 568-576.

McNulty, K. L., Elliott-Sale, K. J., Dolan, E., Swinton, P. A., Ansdell, P., Goodall, S., ... & Hicks, K. M. (2020). The effects of menstrual cycle phase on exercise performance in eumenorrheic women: a systematic review and meta-analysis. Sports Medicine, 50, 1813-1827.

Mei, Z., Cogswell, M. E., Parvanta, I., Lynch, S., Beard, J. L., Stoltzfus, R. J., & Grummer-Strawn, L. M. (2005). Haemoglobin and ferritin are currently the most efficient indicators of population response to iron interventions: an analysis of nine randomized controlled trials. The Journal of nutrition, 135(8), 1974-1980.

Michigan, A., Johnson, T. V., & Master, V. A. (2011). Review of the relationship between C-reactive protein and exercise. Molecular diagnosis & therapy, 15, 265-275.

Midgley, A. W., McNaughton, L. R., & Jones, A. M. (2007). Training to enhance the physiological determinants of long-distance running performance. Sports Medicine, 37(10), 857-880.

Milman, N., Kirchhoff, M., & Jørgensen, T. (1992). Iron status markers, serum ferritin and hemoglobin in 1359 Danish women in relation to menstruation, hormonal contraception, parity, and postmenopausal hormone treatment. Annals of hematology, 65, 96-102.

Mogharnasi, M., TaheriChadorneshin, H., & Abbasi-Deloei, N. (2019). Effect of exercise training type on plasma levels of vaspin, nesfatin-1, and high-sensitivity C-reactive protein in overweight and obese women. Obesity medicine, 13, 34-38.

Mohammed, H. S. E. D., Ramadan, H. K. A., El-Mahdy, R. I., Ahmed, E. H., Hosni, A., & Mokhtar, A. A. (2022). The Prognostic Value of Different Levels of Cortisol and High-Sensitivity C-Reactive Protein in Early Acute Pancreatitis. The American Journal of the Medical Sciences, *363*(5), 435-443.

Monis, C. N., & Tetrokalashvili, M. (2022). Menstrual Cycle Proliferative And Follicular Phase. In StatPearls. StatPearls Publishing.

Montero, D., & Lundby, C. (2011). Regulation of red blood cell volume with exercise training. Comprehensive Physiology, 9(1), 149-164.

Myllyaho, M. M., Ihalainen, J. K., Hackney, A. C., Valtonen, M., Nummela, A., Vaara, E., ... & Taipale, R. S. (2021). Hormonal contraceptive use does not affect strength, endurance, or body composition adaptations to combined strength and endurance training in women. The Journal of Strength & Conditioning Research, 35(2), 449-457.

Nascimento Barros, L. A., Ferrari-Piloni, C., Miranda Torres, É., Estrela, C., & Valladares-Neto, J. (2018). Effect size: a statistical basis for clinical practice. Revista Odonto Ciencia, *33*(1).

Neufer, P. D. (1989). The effect of detraining and reduced training on the physiological adaptations to aerobic exercise training. Sports Medicine, 8, 302-320.

Newlin, M. K., Williams, S., McNamara, T., Tjalsma, H., Swinkels, D. W., & Haymes, E. M. (2012). The effects of acute exercise bouts on hepcidin in women. International Journal of Sport Nutrition and Exercise Metabolism, 22(2), 79-88.

Nicklas, B. J., Hsu, F. C., Brinkley, T. J., Church, T., Goodpaster, B. H., Kritchevsky, S. B., & Pahor, M. (2008). Exercise training and plasma C-reactive protein and interleukin-6 in elderly people. Journal of the American Geriatrics Society, *56*(11), 2045-2052.

Nosaka, K., & Clarkson, P. M. (1996). Changes in indicators of inflammation after eccentric exercise of the elbow flexors. Medicine and science in sports and exercise, 28(8), 953-961.

Oishi, Y., & Manabe, I. (2018). Macrophages in inflammation, repair and regeneration. International immunology, 30(11), 511-528.

Park H. (2022). Association between Oral Contraceptive Use and the High-Sensitivity C-Reactive Protein Level in Premenopausal Korean Women. Healthcare (Basel, Switzerland), 10(2), 361.

Peeling, P., Dawson, B., Goodman, C., Landers, G., Wiegerinck, E. T., Swinkels, D. W., & Trinder, D. (2009). Cumulative effects of consecutive running sessions on hemolysis, inflammation and hepcidin activity. European journal of applied physiology, 106(1), 51-59.

Peeling, P., Sim, M., Badenhorst, C. E., Dawson, B., Govus, A. D., Abbiss, C. R., ... & Trinder, D. (2014). Iron status and the acute post-exercise hepcidin response in athletes. PloS one, 9(3), e93002.

Petersen, A. M. W., & Pedersen, B. K. (2005). The anti-inflammatory effect of exercise. Journal of applied physiology, 98(4), 1154-1162.

Pradhan, A. D., Manson, J. E., Rifai, N., Buring, J. E., & Ridker, P. M. (2001). C-reactive protein, interleukin 6, and risk of developing type 2 diabetes mellitus. Jama, 286(3), 327-334.

Prestwood, K. M., Unson, C., Kulldorff, M., & Cushman, M. (2004). The effect of different doses of micronized 17β-estradiol on C-reactive protein, interleukin-6, and lipids in older women. The Journals of Gerontology Series A: Biological Sciences and Medical Sciences, 59(8), M827-M832.

Puder, J. J., Blum, C. A., Mueller, B., De Geyter, C., Dye, L., & Keller, U. (2006). Menstrual cycle symptoms are associated with changes in low-grade inflammation. European journal of clinical investigation, 36(1), 58-64.

Quan, N., & Banks, W. A. (2007). Brain-immune communication pathways. Brain, behavior, and immunity, 21(6), 727-735.

Rael, B., Alfaro-Magallanes, V. M., Romero-Parra, N., Castro, E. A., Cupeiro, R., Janse de Jonge, X. A., ... & IronFEMME Study Group. (2021). Menstrual cycle phases influence on cardiorespiratory response to exercise in endurance-trained females. International Journal of Environmental Research and Public Health, 18(3), 860.

Reed, B. G., & Carr, B. R. (2018). The Normal Menstrual Cycle and the Control of Ovulation. In K. R. Feingold (Eds.) et. al., Endotext. MDText.com, Inc.

Reid, B. M., & Georgieff, M. K. (2023). The Interaction between Psychological Stress and Iron Status on Early-Life Neurodevelopmental Outcomes. Nutrients, *15*(17), 3798.

Rickenlund, A., Carlstrom, K., Ekblom, B., Brismar, T. B., Von Schoultz, B., & Hirschberg, A. L. (2004). Effects of oral contraceptives on body composition and physical performance in female athletes. The Journal of Clinical Endocrinology & Metabolism, 89(9), 4364-4370.

Ridker, P. M. (2009). C-reactive protein: eighty years from discovery to emergence as a major risk marker for cardiovascular disease. Clinical chemistry, 55(2), 209-215.

Roche, D. J., King, A. C., Cohoon, A. J., & Lovallo, W. R. (2013). Hormonal contraceptive use diminishes salivary cortisol response to psychosocial stress and naltrexone in healthy women. Pharmacology Biochemistry and Behavior, 109, 84-90.

Roecker, L., Meier-Buttermilch, R., Brechtel, L., Nemeth, E., & Ganz, T. (2005). Iron-regulatory protein hepcidin is increased in female athletes after a marathon. European journal of applied physiology, 95(5), 569-571.

Rohleder, N. (2014). Stimulation of systemic low-grade inflammation by psychosocial stress. Psychosomatic medicine, *76*(3), 181-189.

Rohnejad, B., & Monazzami, A. (2023). Effects of high-intensity intermittent training on some inflammatory and muscle damage indices in overweight middle-aged men. *Apunts Sports Medicine*, *58*(217), 100404.

Rosenbaum, M., Nonas, C., Weil, R., Horlick, M., Fennoy, I., Vargas, I., ... & El Camino Diabetes Prevention Group. (2007). School-based intervention acutely improves insulin sensitivity and decreases inflammatory markers and body fatness in junior high school students. The Journal of Clinical Endocrinology & Metabolism, 92(2), 504-508.

Rubeor, A., Goojha, C., Manning, J., & White, J. (2018). Does iron supplementation improve performance in iron-deficient nonanemic athletes? Sports Health, 10(5), 400-405.

Röcker, L., Hinz, K., Holland, K., Gunga, H. C., Vogelgesang, J., & Kiesewetter, H. (2002). Influence of endurance exercise (triathlon) on circulating transferrin receptors and other indicators of iron status in female athletes. Clinical Laboratory, 48(5-6), 307-312.

Saad, M. J., Morais, S. L., & Saad, S. T. (1991). Reduced cortisol secretion in patients with iron deficiency. *Annals of nutrition and metabolism*, *35*(2), 111-115.

Santisteban, K. J., Lovering, A. T., Halliwill, J. R., & Minson, C. T. (2022). Sex differences in VO2max and the impact on endurance-exercise performance. International Journal of Environmental Research and Public Health, 19(9), 4946.

Saunders, P. U., Pyne, D. B., Telford, R. D., & Hawley, J. A. (2004). Factors affecting running economy in trained distance runners. Sports medicine, 34, 465-485.

Saxena, A. R., Seely, E. W., & Goldfine, A. B. (2012). Cardiovascular risk factors and menstrual cycle phase in premenopausal women. Journal of endocrinological investigation, 35(8), 715.

Schumacher, Y. O., Schmid, A., König, D., & Berg, A. P. V. D. (2002). Effects of exercise on soluble transferrin receptor and other variables of the iron status. *British journal of sports medicine*, *36*(3), 195-199.

Silveira, L. S., Antunes, B.deM., Minari, A. L., Dos Santos, R. V., Neto, J. C., & Lira, F. S. (2016). Macrophage Polarization: Implications on Metabolic Diseases and the Role of Exercise. Critical reviews in eukaryotic gene expression, *26*(2), 115–132.

Silvestre, O. M., Gonçalves, A., Nadruz Jr, W., Claggett, B., Couper, D., Eckfeldt, J. H., ... & Solomon, S. D. (2017). Ferritin levels and risk of heart failure—the Atherosclerosis Risk in Communities Study. European journal of heart failure, *19*(3), 340-347.

Sim, M., Dawson, B., Landers, G., Swinkels, D. W., Wiegerinck, E., Yeap, B. B., ... & Peeling, P. (2017). Interleukin-6 and hepcidin levels during hormone-deplete and hormone-replete phases of an oral contraceptive cycle: a pilot study. Annals of Nutrition and Metabolism, 70(2), 100-105.

Sim, M., Dawson, B., Landers, G., Swinkels, D. W., Tjalsma, H., Yeap, B. B., ... & Peeling, P. (2015). Oral contraception does not alter typical post-exercise interleukin-6 and hepcidin levels in females. Journal of Science and Medicine in Sport, 18(1), 8-12.

Sinclair, L. M., & Hinton, P. S. (2005). Prevalence of iron deficiency with and without anemia in recreationally active men and women. Journal of the American Dietetic Association, 105(6), 975-978.

Slominski, A. (2009). On the role of the corticotropin-releasing hormone signalling system in the aetiology of inflammatory skin disorders. British Journal of Dermatology, 160(2), 229-232.

Sorrells, S. F., Caso, J. R., Munhoz, C. D., & Sapolsky, R. M. (2009). The stressed CNS: when glucocorticoids aggravate inflammation. Neuron, *64*(1), 33-39.

Sponder, M., Campean, I. A., Emich, M., Fritzer-Szekeres, M., Litschauer, B., Bergler-Klein, J., ... & Strametz-Juranek, J. (2017). Long-term endurance training increases serum cathepsin S and decreases IL-6 and hsCRP levels. Journal of Sports Sciences, 35(21), 2129-2134.

Stewart, L. K., Flynn, M. G., Campbell, W. W., Craig, B. A., Robinson, J. P., Timmerman, K. L., ... & Talbert, E. (2007). The influence of exercise training on inflammatory cytokines and C-reactive protein. Medicine & Science in Sports & Exercise, *39*(10), 1714-1719.

Stewart, P. M., Holder, R., Parton, A., Ratcliffe, J. G., & London, D. R. (1993). The hypothalamo-pituitary-adrenal axis across the normal menstrual cycle and in polycystic ovary syndrome. Clinical endocrinology, 38(4), 387-392.

Strachan, A. F., Noakes, T. D., Kotzenberg, G., Nel, A. E., & de Beer, F. C. (1984). C reactive protein concentrations during long distance running. *British medical journal (Clinical research ed.)*, *289*(6454), 1249–1251.

Suarez, E. C., & Sundy, J. S. (2017). The cortisol: C-reactive protein ratio and negative affect reactivity in depressed adults. Health Psychology, *36*(9), 852.

Sullivan, G. M., & Feinn, R. (2012). Using effect size—or why the P value is not enough. Journal of graduate medical education, *4*(3), 279-282.

Suzuki, K., & Hayashida, H. (2021). Effect of exercise intensity on cell-mediated immunity. Sports, 9(1), 8.

Taipale, R. S., Forssell, J., Ihalainen, J. K., Kyröläinen, H., & Häkkinen, K. (2020). A 10-week block of combined high-intensity endurance and strength training produced similar changes in dynamic strength, body composition, and serum hormones in women and men. *Frontiers in Sports and Active Living*, *2*, 581305.

Terink, R., Ten Haaf, D., Bongers, C. W. G., Balvers, M. G. J., Witkamp, R. F., Mensink, M., ... & Hopman, M. T. E. (2018). Changes in iron metabolism during prolonged repeated walking exercise in middle-aged men and women. European journal of applied physiology, 118(11), 2349-2357.

Thau, L., Gandhi, J., & Sharma, S. (2019). Physiology, cortisol. In StatPearls. StatPearls Publishing.

Thiyagarajan, D. K., Basit, H., & Jeanmonod, R. (2021). Physiology, menstrual cycle. In StatPearls. StatPearls Publishing.

Timon, R., Corvillo, M., Brazo, J., Robles, M. C., & Maynar, M. (2013). Strength training effects on urinary steroid profile across the menstrual cycle in healthy women. European journal of applied physiology, 113, 1469-1475.

Timmons, B. W., Hamadeh, M. J., Devries, M. C., & Tarnopolsky, M. A. (2005). Influence of gender, menstrual phase, and oral contraceptive use on immunological changes in response to prolonged cycling. Journal of Applied Physiology, 99(3), 979-985.

Tolmay, C. M., Malan, L., & Van Rooyen, J. M. (2012). The relationship between cortisol, C-reactive protein and hypertension in African and Causcasian women: the POWIRS study: cardiovascular topics. *Cardiovascular journal of Africa*, *23*(2), 78-84.

Tomczak, M., & Tomczak, E. (2014). The need to report effect size estimates revisited. An overview of some recommended measures of effect size.

Torres, R., Koutakis, P., & Forsse, J. (2021). The Effects of Different Exercise Intensities and Modalities on Cortisol Production in Healthy Individuals: A Review. Journal of Exercise and Nutrition, 4(4).

Ueda, N., & Takasawa, K. (2018). Impact of inflammation on ferritin, hepcidin and the management of iron deficiency anemia in chronic kidney disease. Nutrients, *10*(9), 1173.

Ün, B., Dolapçıoğlu, K. S., Okyay, A. G., Şahin, H., & Beyazıt, A. (2016). Evaluation of hs-CRP and visseral adiposity index in patients with policystic ovary syndrome by clinical and laboratory findings. European Journal of Obstetrics & Gynecology and Reproductive Biology, 204, 16-20.

Van der Valk, E. S., Savas, M., & van Rossum, E. F. (2018). Stress and obesity: are there more susceptible individuals? *Current obesity reports*, *7*, 193-203.

Van Rooijen, M., Hansson, L. O., Frostegård, J., Silveira, A., Hamsten, A., & Bremme, K. (2006). Treatment with combined oral contraceptives induces a rise in serum C-reactive protein in the absence of a general inflammatory response. Journal of Thrombosis and Haemostasis, 4(1), 77-82.

Vashishta, S., Gahlot, S., Singh, A., & Goyal, R. (2017). Impact of menstrual cycle phases on C-reactive protein concentrations. International Journal of Research in Medical Sciences, 5(3), 1090.

Vega, S. R., Strüder, H. K., Wahrmann, B. V., Schmidt, A., Bloch, W., & Hollmann, W. (2006). Acute BDNF and cortisol response to low intensity exercise and following ramp incremental exercise to exhaustion in humans. Brain research, 1121(1), 59-65.

Villada, C., Hidalgo, V., Almela, M., Mastorci, F., Sgoifo, A., & Salvador, A. (2014). Coping with an acute psychosocial challenge: behavioral and physiological responses in young women. PLoS One, 9(12), e114640.

Viru, M., Hackney, A., Janson, T., Karelson, K., & Viru, A. (2008). Characterization of the cortisol response to incremental exercise in physically active young men. Acta Physiologica Hungarica, 95(2), 219-227.

Viru, A., & Viru, M. (2004). Cortisol-essential adaptation hormone in exercise. International journal of sports medicine, 25(06), 461-464.

Wander, K., Brindle, E., & O'Connor, K. A. (2008). C-reactive protein across the menstrual cycle. American Journal of Physical Anthropology: The Official Publication of the American Association of Physical Anthropologists, 136(2), 138-146.

Wenger, H. A., & Bell, G. J. (1986). The interactions of intensity, frequency and duration of exercise training in altering cardiorespiratory fitness. Sports medicine, 3(5), 346-356.

Wessling-Resnick, M. (2010). Iron homeostasis and the inflammatory response. Annual review of nutrition, *30*, 105-122.

Whipp, B. J., Ward, S. A., Lamarra, N., Davis, J. A., & Wasserman, K. (1982). Parameters of ventilatory and gas exchange dynamics during exercise. Journal of applied physiology, 52(6), 1506-1513.

Wiegratz, I., Jung-Hoffmann, C., & Kuhl, H. (1995). Effect of two oral contraceptives containing ethinylestradiol and gestodene or norgestimate upon androgen parameters and serum binding proteins. *Contraception*, *51*(6), 341-346.

Wiegratz, I., Kutschera, E., Lee, J. H., Moore, C., Mellinger, U., Winkler, U. H., & Kuhl, H. (2003). Effect of four oral contraceptives on thyroid hormones, adrenal and blood pressure parameters. Contraception, 67(5), 361-366.

Wilund, K. R. (2007). Is the anti-inflammatory effect of regular exercise responsible for reduced cardiovascular disease? Clinical science, 112(11), 543-555.

Wittert, G. A., Livesey, J. H., Espiner, E. A., & Donald, R. A. (1996). Adaptation of the hypothalamopituitary adrenal axis to chronic exercise stress in humans. Medicine and science in sports and exercise, 28(8), 1015-1019.

Wood, C. J., Clow, A., Hucklebridge, F., Law, R., & Smyth, N. (2018). Physical fitness and prior physical activity are both associated with less cortisol secretion during psychosocial stress. Anxiety, Stress, & Coping, 31(2), 135-145.

World Health Organization. (2020). Serum ferritin concentration for the assessment of iron status in individual and population: Technical brief.

Wunder, D. M., Yared, M., Bersinger, N. A., Widmer, D., Kretschmer, R., & Birkhäuser, M. H. (2006). Serum leptin and C-reactive protein levels in the physiological spontaneous menstrual cycle in reproductive age women. European journal of endocrinology, 155(1), 137-142.

Yang, Q., Jian, J., Katz, S., Abramson, S. B., & Huang, X. (2012). 17β-Estradiol inhibits iron hormone hepcidin through an estrogen responsive element half-site. Endocrinology, 153(7), 3170-3178.

Yoshida, T. (1984). Effect of dietary modifications on lactate threshold and onset of blood lactate accumulation during incremental exercise. European journal of applied physiology and occupational physiology, 53(3), 200-205.

You, T., Berman, D. M., Ryan, A. S., & Nicklas, B. J. (2004). Effects of hypocaloric diet and exercise training on inflammation and adipocyte lipolysis in obese postmenopausal women. The Journal of Clinical Endocrinology & Metabolism, 89(4), 1739-1746.

Zheng, H., Badenhorst, C. E., Lei, T. H., Liao, Y. H., Che Muhamed, A. M., Fujii, N., ... & Mündel, T. (2021). Menstrual phase and ambient temperature do not influence iron regulation in the acute exercise period. American Journal of Physiology-Regulatory, Integrative and Comparative Physiology, 320(6), R780-R790.