STUDY II

Body composition in premenopausal and postmenopausal well-trained females

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ABSTRACT

Purpose: The aim was to analyse the influence of sex hormones on body composition in well-trained females with different hormonal environments.

Methods: Sixty-six eumenorrheic, forty-one low-dose-monophasic oral contraceptive users and sixteen postmenopausal well-trained females participated in this study. Volunteers underwent a Dual-energy X-ray Absorptiometry scan (DXA) and a bioimpedance during the early-follicular and the withdrawal phase, verified with blood samples.

Results: ANCOVA test reported no differences neither in DXA measurements (weight, fat free mass, fat mass, android and gynoid fat mass) nor in bioimpedance variables (weight, fat free mass, fat mass and total body water) among study groups.

Conclusion: Sex hormones seems not to influence body composition in active women. Curiously, premenopausal and postmenopausal active women present the same fat mass distribution. It could be explained by the positive effect exercise has on body composition, and this in turn on preventing cardiovascular and metabolic diseases in this population.

KEY WORDS

Eumenorrheic, oral contraceptive, sex hormones, fat mass, fat free mass, exercise.

INTRODUCTION

Endogenous sex hormones (17 β-estradiol and progesterone) play a key factor in female reproductive system, but they also have an important role influencing lipid metabolism, body fat distribution and skeletal muscle in women. This is because of the presence of sex hormones receptors in non-reproductive tissues such as hypothalamus, cardiovascular system (Heritage et al., 1980), kidney tubules, liver, skeletal muscle and adipose tissue (Faulds et al., 2012).

Sex hormones, specifically estradiol, increase lipoprotein lipase action, stimulating lipid oxidation process and decreasing carbohydrate oxidation (Marchand et al., 2017). However, more than the individual effect of the estradiol is estradiol and progesterone interaction what seems to be crucial. Indeed, the increase of estradiol/progesterone ratio might be important in determining the final effect of these sex hormones on fat metabolism (Isacco & Boisseau, 2017). These hormones are also important for the maintenance of skeletal muscle, promoting not only muscle growth but also its regeneration (Hansen, 2018). In accordance with body fluid regulation, sex hormones play a crucial role influencing water retention in females. Due to the presence of estradiol receptors in the hypothalamus nuclei, where an important hormone involved in the regulation of renal water is produced; arginine vasopressin (AVP), this sex hormone may cause shifts in body fluid regulation (Heritage et al., 1980). Moreover, high levels of progesterone might stimulate water retention as well, because of the increase of aldosterone hormone, hence this sex hormone increases aldosterone production (hormone which acts on kidney tubes stimulating Na+ resorption and K+ excretion) (Stachenfeld & Keefe, 2002).

All these physiological effects caused by endogenous sex hormones might be affected in those females who are using oral contraceptive (OC) pills. In the last decades, the use of OC pills has been widespread among females, inducing a reduction of endogenous hormones production in this population due to the intake of exogenous ones (ethinyl estradiol and progestin) (Di Carlo et al., 2013). In accordance with exogenous sex hormones, there is less knowledge about the effects of its administration on females´ physiology. It has been suggested that ethinyl estradiol has mineralocorticoid actions, which activates renin-angiotensin-aldosterone system encouraging Na^+ and fluid retention, whereas progestin has anti-mineralocorticoid actions which antagonizes the effect of Na^+ and fluid retention (Grandi et al., 2014; Meendering et al., 2009; Torgrimson et al., 2007). On the contrary, other studies reported that ethinyl estradiol and progestin administration increase plasma volume and even the combination of both exogenous sex hormones causes the greatest increase (Stachenfeld & Taylor, 2004).

Furthermore, a recent review concluded that ethinyl estradiol administration could inhibit lipolysis process (Luglio, 2014). Thereby, due to the different hormonal environment presented in OC users regarding eumenorrheic females, it is speculated that differences in females´ physiology may exist between both groups. Postmenopausal females might also have a different behavior due to the loss of the ovarian function and therefore their decrease in sex hormones concentration (Karsenty, 2012). Aging is associated with a decrease in fluid volume (Stachenfeld et al., 1998), a drop in lean body mass and an increase in fatty tissue, specially android fat mass (Chang et al., 2000; Dmitruk et al., 2018; Toth et al., 2000), and this in turn rises metabolic and cardiovascular risks after menopause (Goh & Hart, 2018). However, it seems that these postmenopausal effects could be attenuated by physical activity (Sims et al., 2013).

According to the current literature, it appears that sex hormones concentrations affect substrate metabolism during exercise (increasing lipids oxidation in OC users and reducing it in postmenopausal women) (Isacco & Boisseau, 2017). Nevertheless, it still remains unclear how these changes in females´ metabolism during exercise could affect body composition in these population when training frequently. Although the influence of sex hormones on body fluid regulation and lipid metabolism has been recently studied in sedentary healthy females (Cumberledge et al., 2018; Dmitruk et al., 2018; Hicks et al., 2017), it remains unclear how this influence could modify body water (TBW), fat mass (FM) or fat free mass (FFM) in well-trained females.

OBJECTIVES

The aim of this study is to analyse the influence of sex hormones concentration on body composition variables in well-trained females, comparing three different hormonal profiles: eumenorrheic females, low dose monophasic OC users and postmenopausal women.

METHODS

Subjects

The present work is an observational cross-sectional study performed by sixty-six eumenorrheic females (32.9±10.2 years; 163.7±5.9 cm), forty-one low dose monophasic OC users (26.5±4.7 years; 163.1±5.9 cm) and sixteen postmenopausal women (51.7±3.7 years; 160.9±5.3 cm) participated in this study. Brands and formulation of OC pills used were: Ceciliana® (n=4): ethinyl estradiol 0.03 mg and dienogest 2 mg; Drosure® (n=2): ethinyl estradiol 0.03 mg and drospirenone 3 mg; Yasmin® (n=9): ethinyl estradiol 0.03

mg and drospirenone 3 mg; Loette® (n=5): ethinyl estradiol 0.02 mg and levonorgestrel 0.1 mg; Levobel® (n=3); ethinyl estradiol 0.02 and levonorgestrel 0.1; Diane® (n=5): ethinyl estradiol 0.035 mg and cyproterone 2 mg; Edelsin® (n=2): ethinyl estradiol 0.035 and Norgestimate 0.25 mg; Drosbelalleflex® (n=2): ethinyl estradiol 0.02 mg and Drospirenone 3 mg; Melodene® (n=2): ethinyl estradiol 0.015 mg and gestodene 0.06 mg; Linelle® (n=3): ethinyl estradiol 0.02 mg and levonorgestrel 0.1 mg; Stada® (n=1): ethinyl estradiol 0.02 mg and drospirenone 3 mg; Sibilla® (n=3): ethinyl estradiol 0.03 mg and dienogest 2 mg. All of them were well-trained in endurance and/or in strength training: (i) 1.3±0.4 hours per session, 3.9±1.1 sessions per week with 7.7±5.2 years of experience for eumenorrheic females; (ii) 1.4±2.1 hours per session, 3.7±1.2 sessions per week with 6.6±4.5 years of experience for the OC group; (iii) 1.2±0.3 hours per session, 3.9±1.2 sessions per week with 7.9±3.3 years of experience for postmenopausal women. Running, obstacle races, crossfit and triathlon were the sport activities they trained. Exclusion criteria included smoking, thyroid problems, medication or dietary supplements that alter vascular function (e.g., tricyclic antidepressants, α-blockers, β-blockers, etc.), pregnancy and ovariectomy. At the start of the data collection, all participants conducted a questionnaire gathering information about training experience, health status, the absence of dietary supplements consumption and type of OC pills when appropriate. An informed consent was obtained from each participant with all the information about the procedures and risks involved. The experimental protocol was approved by the ethical Committee of the Universidad Politécnica de Madrid, with DEP2016 code, and it is in accordance with The Code of Ethics of the World Medical Association (Association, 2013).

Experimental protocol

Body composition tests were carried out under similar hormonal environments for all groups (low sex hormonal levels): during the early follicular phase (between the 2nd and 5th day of the menstrual cycle, being the onset of the cycle the first day of menstrual bleeding) for the eumenorrheic females, in the withdrawal phase (between the 3rd and the 7th day of the placebo week) for the OC group and at any time for postmenopausal women. Volunteers performed, in different days, a Dual-energy X-ray Absorptiometry scan (DXA) and a bioimpedance between 8-10 am following the standard recommendations (Khalil et al., 2014). During the first day, DXA test was carried out by all participants. Nonetheless, due to the drop out of 21 volunteers, only fifty-six eumenorrheic females, thirty-five OC users and thirteen postmenopausal females did the bioimpedance test. DXA test was performed to analyse body composition variables such as weight, FM and FFM, in the same way that bioelectrical impedance was conducted to analyse weight, FM, FFM and TBW.

Dual-energy X-ray Absorptiometry scan

A DXA scan (Version 6.10.029GE Encore 2002, GE Lunar Prodigy; GE Healthcare, Madison, WI, USA) was done between 8-10 am in fasting state to obtain body composition variables such as weight, FM and FFM. Volunteers did not perform physical activity 24h previous the test. The scan was calibrated per two days using the phantom supplied by the manufacturer. All volunteers performed the test in underwear, with their body in a supine position and their feet joined by a tape. During the measurements, moving and talking were forbidden. DXA scan was always carried out by the same researcher.

Bioimpedance

A bioimpedance (Biológica Tecnología Médica SL: Tanita BC-418 MA, Tokyo, Japan) was done between 8-10 am to obtain weight, FM, FFM, and TBW. Volunteers either performed physical activity or drank coffee 24h previous the bioimpedance. Firstly, the researcher introduced the age, sex and height of the volunteers, which was previously measured with a stadiometer (SECA-213, Valencia, Spain; range 20-205cm). Then, each subject stood erect with bare feet placed on the contact electrode on the bioimpedance device. Bioimpedance test was always carried out by the same researcher.

Blood samples

All blood samples were obtained by venipuncture into a vacutainer containing clot activator. Following inversion and clotting, the whole blood was centrifuged (Biosan LMC-3000 version V.5AD) for ten minutes at 3000 rpm. After that, serum was transferred into eppendorf tubes and stored frozen at -80ºC until further analysis. Within 1 to 15 days after testing, the serum samples were delivered to the clinical laboratory of the Spanish National Centre of Sport Medicine (Madrid, Spain) to determine sex hormones in order to verify hormonal profiles.

Blood sample analyses were carried out at Agencia Española de Protección de la Salud en el Deporte (AEPSAD) laboratory, in Madrid, Spain. Total 17 β-estradiol, progesterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were measured with a COBAS E411 (Roche Diagnostics, GmbH, Mannheim, Germany), using electrochemiluminescence immunoassay (ECLIA) technology. Inter- and intra-assay coefficients of variation (CV) reported by the laboratory for each variable were, respectively: 11.9% and 8.5% at 93.3 pg/ml and 6.8% and 4.7% at 166 pg/ml for 17β-

Estradiol; 23.1% and 11.8% at 0.7 ng/ml and 5.2% and 2.5% at 9.48 ng/ml for Progesterone; 5.3% and 1.8% at 1.2 mIU/ml for FSH; 5.2% and 1.8% at 0.54 mIU/ml for LH; 5.0% and 4.0% at 300 mIU/l for Prolactin; 4.6% and 1.5% at 3.82 µIU/ml for TSH; 8.5% and 6.0% at 17.3 pg/ml for IL-6.

Statistical analysis

All data are reported as mean ± Standard Deviation (SD). Throughout Kolmogorov-Smirnov test, data showed a normal distribution. Thus, comparisons among study groups (eumenorrheic, OC users and postmenopausal) were performed by one-way ANCOVA and age was used as a covariable. Scheffé test was applied to examine the pairwise comparison. All tests were conducted with a 5% significance level. Statistical analyses were performed using SPSS software for windows, version 20.1 (SPSS Inc, Chicago, IL, USA).

RESULTS

One-way ANCOVA test showed a mean difference among all groups in age ($F_{2,96}$=138.716; p<0.001), while no differences were reported for height ($F_{2,96}$=1.364; p=0.261). In accordance with training status, no significant differences were found for experience ($F_{2,126}$=0.868; p=0.422), sessions per week ($F_{2,126}$=0.906; p=0.407), time per session ($F_{2,126}$=0.178; p=0.837). Finally, hormone results (Figure 17) reported significant differences for FSH and LH (p<0.001 for both sex hormones), presenting postmenopausal females the highest values (p=0.034). Nevertheless, no significant differences were observed either for estradiol (p=0.103), progesterone (p=0.169) or estradiol/progesterone ratio (p=0.287) among study groups.

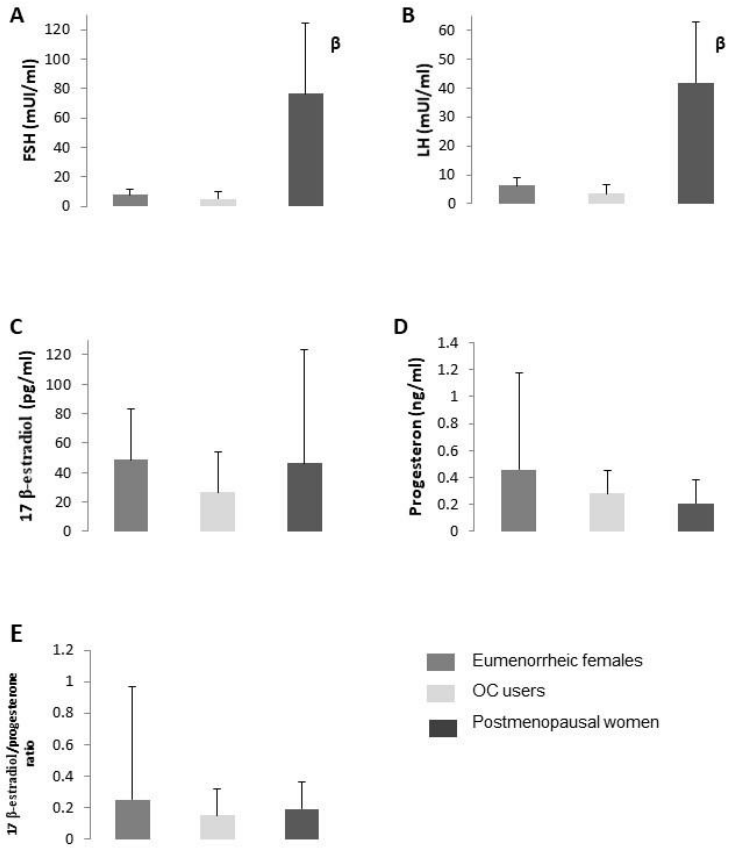

Figure 17: Sex hormones concentrations among females with different hormonal profiles: eumenorrheic females, low-dose monophasic OC users and postmenopausal women. FSH: folliculostimulating hormone; LH: luteinizing hormone; OC: oral contraceptive. β Significant differences in postmenopausal women regarding eumenorrheic females and OC users ($p<0.001$).

With regard to body composition variables (Table 4), DXA measurements did not show significant differences either for weight ($F_{2,119}=1.184$), FFM percentage ($F_{2,119}=0.233$) or FM percentage ($F_{2,119}=0.233$) among well-trained females with different hormonal profiles. Moreover, percentages of android FM and gynoid FM did not vary among study groups ($F_{2,119}=1.037$ and $F_{2,119}=0.515$ respectively). Moving on to bioimpedance results, no significant differences were found for none of the variables measured: weight ($F_{2,100}=0.739$), FFM percentage ($F_{2,101}=1.033$), FM percentage ($F_{2,100}=2.245$) and TBW ($F_{2,100}=0.915$).

Table 4: Body composition variables measured by DXA and bioimpedance in well-trained females with different hormonal profiles.

		Eumenorrheic		OC users		Postmenopausal		*p*	η^2
		Mean±SD	n	Mean±SD	n	Mean±SD	n		
DXA	Weight (Kg)	59.74±8.73	66	57.80±6.02	41	56.69±8.16	16	0.309	0.020
	FFM (%)	75.35±6.73	66	74.59±5.64	41	74.56±7.13	16	0.793	0.004
	FM (%)	24.65±6.73	66	25.41±5.64	41	25.44±7.13	16	0.793	0.004
	Android FM (%)	5.77±1.41	66	6.07±1.08	41	6.31±1.96	16	0.358	0.017
	Gynoid FM (%)	25.73±3.15	66	25.51±2.26	41	24.81±4.10	16	0.599	0.009
BIA	Weight (Kg)	58.28±6.93	56	57.73±5.86	35	54.95±3.71	13	0.423	0.017
	FFM (%)	79.16±5.24	56	79.66±4.09	35	79.21±7.22	13	0.360	0.020
	FM (%)	20.96±5.22	56	20.49±4.14	35	22.54±4.20	13	0.111	0.043
	TBW (kg)	33.84±3.40	56	33.63±2.27	35	31.15±1.57	13	0.404	0.018

OC: Oral contraceptive; BIA: bioimpedance; DXA: Dual-energy x-ray absorptiometry; FFM: fat free mass; FM: fat mass; TBW: total body water.

DISCUSSION

The aim of this study was to analyse the influence of sex hormones concentration on body composition variables in well-trained females, comparing three different hormonal profiles: eumenorrheic females, low dose monophasic OC users and postmenopausal women. The main finding was the lack of differences between postmenopausal women and premenopausal females. Moreover, monophasic OC pills did not impact on body composition.

Retrospective studies strongly suggested that a decrease in FFM occurs after menopause. Muscle mass loss seems to occur in postmenopausal females because of the decrease of sex hormones, specially estradiol, with the ovarian failure (Collins et al., 2019). Evidence is accumulating that estradiol deficiency induces apoptosis in skeletal muscle contributing to loss of muscle mass and, therefore, strength (Collins et al., 2019). Nonetheless, outcomes from this study did not support previous literature. For instance, Distefano, et al. (2018) showed a decrease in muscle mass of 10% in sedentary postmenopausal females (50 years old) (Distefano & Goodpaster, 2018), Baumgartner et al. (1998) reported a drop of 23.6% in sedentary females at the age of 70 years old compared to the premenopausal group (Baumgartner et al., 1998), while our well-trained postmenopausal women did not suffer a reduction in their FFM. Meanwhile, an important reduction in TBW has been observed in sedentary postmenopausal women (Dmitruk et al., 2018). These lower values of TBW could be related to the lower values of FFM in this population, because of the large amount of water stored in the muscle, as the water content in the muscle represents the major component of body weight (about 76%) (Serra-Prat et al., 2019). In addition, aging is associated with a higher plasma osmolality and a reduction of thirst sensation, leading to a decrease in fluid (dehydration), which could be

another explanation for this drop of water in the elderly (Stachenfeld et al., 1998). However, results from this work did not support previous literature, as no differences in TBW has been reported in our postmenopausal women.

The lack of agreement regarding body composition could be explained by differences in physical activity status, since previous studies have been carried out with sedentary women. It is well known that exercise is crucial to prevent the muscle mass decrease in this population, or at least to avoid a pronounced decline (Distefano & Goodpaster, 2018; Marín-Cascales et al., 2018). Therefore, it could be hypothesized that the drop of muscle mass, and consequently TBW, seems to be less pronounced, or even averted, in females who practice exercise regularly. Finally, it is worth mentioning the lack of differences in FM distribution between study groups. Nonetheless, previous research reported an increase in android FM and a decrease in gynoid FM in sedentary postmenopausal women (Chang et al., 2000; Toth et al., 2000), and this in turn rises metabolic and cardiovascular risks in elderly women (Goh & Hart, 2018). Hence, exercise could be a key factor to avoid an increase in android FM in postmenopausal female, preventing them of suffering cardiovascular and metabolic pathologies associated with FM distribution.

In terms of OC users, body composition outcomes obtained in this study are not backed up by previous literature. On the one hand, there is a recent review which concluded a decrease of FFM due to the use of OC pills (Lopez et al., 2016). Nonetheless, there are some aspects we should take into consideration, such as volunteers' characteristics and the type of OC pills. Our participants were well-trained females, using low dose monophasic OC users; whereas the review previously mentioned did not take

into consideration physical activity status and mixed all types of OC pills. It is accepted that exercise stimulates the gain of muscle mass (Schoenfeld et al., 2017), so this factor may be crucial when studying FFM. In any case, if OC had a deleterious effect on FFM, it seems to be counteracted by exercise, specially strength training. On the other hand, when it comes to fluid regulation, controversial results have been reported. Although here is a strongly propose that mineralocorticoid effects of ethinyl estradiol are counterbalanced by the anti-mineralocorticoid actions of progestin (Grandi et al., 2014; Meendering et al., 2009; Torgrimson et al., 2007), some authors reported that ethinyl estradiol and progestin administration increase plasma volume and even the combination of both exogenous sex hormones causes the greatest increase (Stachenfeld & Taylor, 2004). Nevertheless, our data reported no differences in TBW for the OC group. The different dosages of exogenous hormones used in the present study may explain this lack of differences in TBW. Nowadays, sex hormones dosages in OC pills are lower than they used to be (e.g. ethinyl estradiol concentration was 150 mg/day whereas today is 15 mg/day and progestin concentration was 9.85 mg/day whereas today is 0.35 mg/day) (White et al., 2011). Hence, exogenous sex hormones levels from today's monophasic OC pills might not be high enough to promote shifts in TBW in well-trained females. Similar to our findings, other study did not find differences in TBW when comparing eumenorrheic females and OC users in athletes (Hicks et al., 2017).

The current study attempts to address a gap in the research through investigation of important variables of body composition in well-trained females. The strengths of our study included the inclusion of different endogenous and exogenous female hormone profiles and the recruitment of an homogenous group for all of them; eumenorrheic females, OC users and postmenopausal women (active and healthy women). Nonetheless,

it is worth mentioning that the phase in which we have done the measurements could influence the results. OC users were measured during their withdrawal phase, so no exogenous sex hormones were intaking, while eumenorrheic women were measured during their early follicular phase, which is characterized by low dosages of sex hormones and postmenopausal female always have low levels of sex hormones because of their ovarian failure. Therefore, all groups had a similar sex hormones environment the day of measurement. Additionally, longitudinal studies with an intra-subject design should be carried out to explore the influence of the hormonal changes throughout life span. It should be noted that different hormonal stages throughout the menstrual cycle and OC cycle in well-trained females might be also interesting to analyse.

CONCLUSIONS

According to our results, sex hormones from different hormonal profiles (eumenorrheic, low dose monophasic OC users and postmenopausal females) do not influence body composition in physically active women. Therefore, the FFM loss, due to the age and the decrease in sex hormones concentrations, seems to be compensated by exercise. Interestingly, android FM do not vary in active postmenopausal female, which could be explain by the positive effect exercise has on body composition, and this in turn on female´s health. However, more data are needed in order to elucidate the influence of different hormonal profiles in body composition variables when studying physically active women, since most of the previous studies have been carried out without considering physical activity.

STUDY III

Body composition over the menstrual and oral contraceptive cycle in trained females

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ABSTRACT

Purpose: The influence of female sex hormones on body fluid regulation and metabolism homeostasis has been widely studied. However, it remains unclear whether hormone fluctuations throughout the menstrual cycle and with oral contraceptive use affect body composition. Thus, the aim of this study was to investigate body composition over the menstrual cycle and oral contraceptive (OC) cycle in well-trained females.

Methods: Fifty-two eumenorrheic and 33 monophasic OC-taking well-trained females participated in this study. Several body composition variables were measured through bioelectrical impedance analysis three times in the eumenorrheic group (early follicular phase, late follicular phase and mid-luteal phase) and on two occasions in the OC group (withdrawal phase and active pill phase).

Results: Mixed linear model tests reported no significant differences in body composition variables (body weight, body mass index, basal metabolism, fat mass, fat-free mass and total body water) between menstrual cycle phases or between OC phases ($p > 0.05$ for all comparisons). Trivial and small effect sizes were found for all body composition variables when comparing menstrual cycle phases in eumenorrheic females, as well as for OC cycle phases.

Conclusions: According to our results, sex hormone fluctuations throughout the menstrual and OC cycle do not influence body composition variables measured by bioelectrical impedance in well-trained females. Therefore, it seems that bioimpedance analysis can be conducted at any moment of the cycle, both for eumenorrheic women and females using OC.

Keywords: Estrogen, progesterone, fat mass, fat-free mass, total body water

INTRODUCTION

Female endogenous (17 β-estradiol and progesterone) and exogenous (ethinyl estradiol and progestin) hormones play a key role in their reproductive system. Furthermore, there are several non-reproductive tissues, such as the hypothalamus, cardiovascular system, kidney tubules, liver, skeletal muscle and adipose tissue, that have sex hormone receptors (Stachenfeld, 2008). Thus, these sex hormones may also impact on other physiological systems, such as body fluid regulation, sodium content (Stachenfeld, 2008) and metabolic homeostasis (Marchand et al., 2017).

The natural menstrual cycle (MC) is a cyclic process controlled by the hypothalamus-pituitary axis and all hormones involved in it (FSH: follicle-stimulating hormone; LH: luteinizing hormone; estradiol and progesterone). Despite individual variations, it is allocated 28 days. Throughout it, estradiol and progesterone fluctuations take place, giving rise to the different phases of the MC. The first one is the early follicular phase (EFP), coinciding with menstruation, when the lowest concentrations of sex hormones are reached. Then, estradiol starts to rise during the mid-follicular phase, reaching its peak in the late follicular phase (LFP), just prior to ovulation. Estradiol dramatically decreases after ovulation, whereas progesterone starts to increase, achieving its peak in the mid luteal phase (MLP). At this point, estradiol levels are high as well. Finally, during the late luteal phase both estradiol and progesterone drop, starting the cycle again (Janse de Jonge, 2003; Janse de Jonge et al., 2019). Female sex hormones, specifically estradiol, increase lipoprotein lipase action, stimulating the lipid oxidation process and decreasing carbohydrate oxidation (Marchand et al., 2017). Moreover, it seems that estradiol has a slight anabolic effect on muscle mass (Hansen, 2018; Kitajima & Ono, 2016; Sung et al., 2014), promoting not only muscle growth but also its

regeneration (Hansen, 2018; Kitajima & Ono, 2016). With regard to body fluid regulation, high levels of both estradiol and progesterone may encourage water retention (Stachenfeld & Keefe, 2002). All these physiological effects may cause changes in body composition (BC) variables throughout the MC due to fluctuations in sex hormones. However, results of the limited research in this area remain unclear, with some reporting little or no effects of the MC on BC (Cumberledge et al., 2018; Hicks et al., 2017; Teixeira et al., 2013), whereas others found higher weight and fat-free mass during the luteal phase (Meaden et al., 2005; Tomazo-Ravnik & Jakopič, 2006). Some of these conflicting findings may be related to methodological issues. Most studies in the literature have only compared two hormonal environments (Daniusevičiūtė et al., 2010; Gualdi-Russo & Toselli, 2002; Hicks et al., 2017), while the eumenorrheic MC is characterized by three distinctly different hormonal environments (Janse de Jonge et al., 2019). Furthermore, methodologies used to determine MC phases may not have been accurate enough. Specifically, some studies mention MC phases without specifying the testing days (Di Carlo et al., 2013; Janse de Jonge et al., 2019; Stachenfeld & Taylor, 2004), while others estimate MC phase using only self-report information from participants (Stachenfeld & Taylor, 2004; Tomazo-Ravnik & Jakopič, 2006). Due to the large variation in female sex hormones throughout the MC and the common occurrence of menstrual irregularities, it is important to accurately verify the MC phase at the time of testing through the measurement of female sex hormones (Janse de Jonge et al., 2019).

In women taking oral contraceptive (OC) pills, natural endogenous secretion (estradiol and progesterone) is downregulated due to the intake of the exogenous ones (ethinyl estradiol and progestin); thus, they present a different hormonal environment compared to eumenorrheic females (Di Carlo et al., 2013). The OC pattern commonly

consists of 7 days of taking placebo pills without any active ingredients (WP: withdrawal phase), followed by 21 days of taking active pills with exogenous hormones (APP: active pill phase). There are basically two main types of OC pills: the combined one, which has both ethinyl oestradiol and progestin, and the mini pill, which contains just progestin (Burrows & Peters, 2007). It has been shown that the administration of ethinyl estradiol and progestin may increase plasma volume, and that administration of a combination of both exogenous sex hormones causes the greatest increase (Stachenfeld & Taylor, 2004). Furthermore, a recent review concluded that ethinyl oestradiol administration could inhibit the lipolysis process (Luglio, 2014), thereby affecting fat mass and fat-free mass. These findings suggest that there may be differences in BC variables throughout an OC cycle between the active pill phase (APP) and the withdrawal phase (WP). However, research in this population has been limited. Moreover, some research studies in females did not distinguish between OC users and eumenorrheic females (Gleichauf & Roe, 1989; Gualdi-Russo & Toselli, 2002), while others compared the same testing days for both study groups (Hicks et al., 2017). Overall, the influence of the administration of exogenous sex hormones during an OC cycle on BC variables remains unclear.

Body composition plays an important role in not just health, but also in athletic performance. The body composition research on females to date, however, has had limited focus on the training status of participants and has mainly included sedentary participants (Cumberledge et al., 2018; Gualdi-Russo & Toselli, 2002; Hicks et al., 2017). Therefore, the main objective of this study was to determine the effect of sex hormone fluctuations throughout the MC phases (EFP, LFP and MLP) and between the OC phases (WP and APP) on body composition variables (weight, BMI, BM, FM, FFM and TBW) in well-trained females.

MATERIAL AND METHODS

Subjects

Fifty-two eumenorrheic females (31.10±6.25 years; 163.68±6.25 cm; 58.55±6.94 kg) and 33 monophasic OC users (25.67±6.33 years; 162.97±6.06 cm; 57.31±5.81 kg) participated in this study. The first group had regular patterns of menstrual bleeding over the last year and the second one needed to have been taking the monophasic OC for at least the last 6 months. OC pills' brands and formulations are displayed in Table 5. All of the participants were healthy and well trained in endurance and/or in strength training (1.31±0.41 hours per session, 3.9±1.1 sessions per week with 7.65±5.15 years of experience for eumenorrheic females and 1.39±2.08 hours per session, and 3.68±1.15 sessions per week with 6.57±4.48 years of experience for the OC group). Exclusion criteria included smoking, thyroid problems, medication or dietary supplements that alter vascular function (e.g. tricyclic antidepressants, α-blockers, β-blockers, etc.), pregnancy and ovariectomy. At the start of the data collection, all participants conducted a questionnaire gathering information about training experience, health status, dietary supplements and type of OC pills. All participants were informed about the procedures and risks involved and informed consent was provided by each one. The experimental protocol was approved by the institutional Ethics Committee and is in accordance with the Code of Ethics of the World Medical Association (Declaration of Helsinki) (Association, 2001).

Table 5: Brand and formulation of OC pills for the OC group.

Number of users	Brand	Ethinyl estradiol (mg)	Progestin (mg)
3	Ceciliana	0.03	Dienogest (2)
1	Drosure	0.03	Drospirenone (3)
9	Yasmin	0.03	Drospirenone (3)
4	Loette	0.02	Levonorgestrel (0.1)
2	Levobel	0.02	Levonorgestrel (0.1)
4	Diane	0.035	Cyproterone (2)
1	Edelsin	0.035	Norgestimate (0.25)
1	Drosbelalleflex	0.02	Drospirenone (3)
2	Melodene	0.015	Gestodene (0.06)
2	Linelle	0.02	Levonorgestrel (0.1)
1	Stada	0.02	Drospirenone (3)
3	Sibilla	0.03	Dienogest (2)

OC: oral contraceptive

Experimental protocol

The initial screening visit was conducted during the early follicular phase (EFP) (i.e., between the 2nd and 5th day of the MC with day 1 being the onset of menstrual bleeding) and during the withdrawal phase (WP) (i.e., between the 4nd and 7th day of taking the non-active placebo pill). A blood sample was collected in order to ensure that volunteers were healthy and had a normal hormonal profile.

For the eumenorrheic group, BC variables were measured three times over the cycle: EFP (day 3.5±1.2), late follicular phase (LFP) (day 12.3±2.8) and mid luteal phase (MLP) (day 21.9±2.6), with the onset of the cycle being the first day of menstrual bleeding (Figure 18). The OC group was tested on two occasions: WP (day 4.8±1.7) and active pill phase (APP) (day 21.0±4.9), with day 1 being the first day of the placebo pill (Figure 19). Participants underwent the BC testing in a randomized and counterbalanced order.

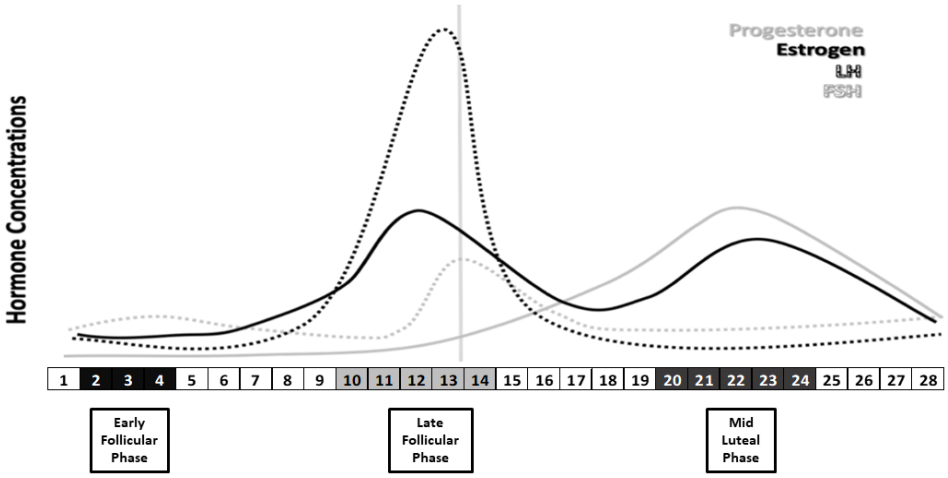

Figure 18: Testing days for the eumenorrheic group. Day 1 is the day of onset of bleeding, and day 14 is ovulation. FSH: follicle-stimulating hormone; LH: luteinizing hormone.

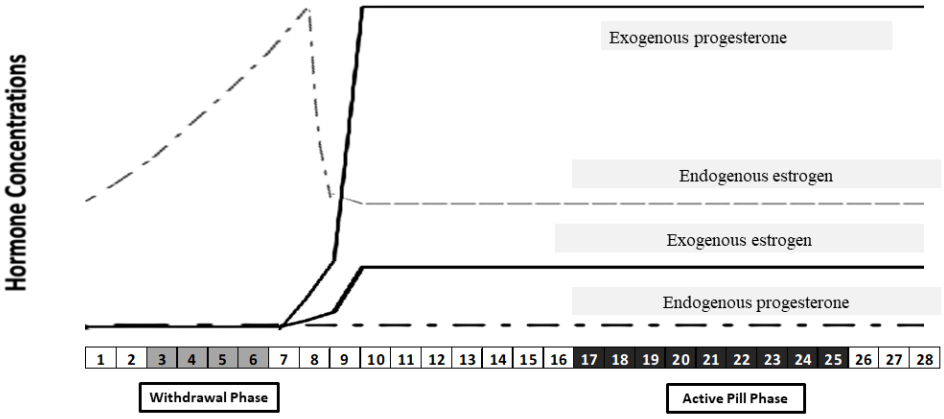

Figure 19: Testing days for the oral contraceptive group. Day 1 is the first day of consumption of the nonactive pill (withdrawal phase), and day 8 is the first day of consumption of the active pill (active pill phase) (adapted from (Rechichi et al., 2009)).

Menstrual cycle determination

In order to determine the average cycle length (number of days from the cycle onset to the next one), all eumenorrheic participants were asked to provide information

about the length of their last six menstrual cycles. These data were provided to a gynaecologist, who calculated the approximate days of EFP, LFP, ovulation and MLP. OC group phases were based on OC ingestion, with day 1 of the OC cycle coinciding with the first placebo pill consumption (7 days of WP) followed by 21 days of active pill consumption (APP). Blood samples were collected at each testing session for determining sex hormone concentrations to confirm that the participants were in the correct phase during testing.

Body composition through bioelectrical impedance analysis

Body composition was assessed through bioelectrical impedance analysis (Tanita BC-418 MA), previously validated (Vasold et al., 2019). Tests were carried out between 8 and 10 am, following standard recommendations (Khalil et al., 2014). Volunteers did not perform physical activity or drink coffee or alcohol for 24 h prior to the bioimpedance. First, the researcher entered the age, sex and height of the participants into the bioelectrical impedance device. Then, each participant stood erect with bare feet placed on the contact electrodes on the bioimpedance device. The bioimpedance tests were always carried out by the same researcher. The BC variables measured through bioimpedance were weight, body mass index (BMI), basal metabolism (BM), fat mass (FM), fat-free mass (FFM) and total body water (TBW).

Blood samples

All blood samples were obtained in a rested state by venipuncture using a Vacutainer containing a clot activator. Following inversion and clotting, the whole blood was centrifuged (Biosan LMC-3000 version V.5AD) for 10 minutes at 3000 rpm. The serum was then transferred into Eppendorf tubes and stored frozen at -80 ºC until further

analysis. Within 1 to 15 days after testing, the serum samples were delivered to the clinical laboratory for determination of sex hormones in order to verify MC phase. Total estradiol 17-Beta (E2), progesterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were measured via ADVIA Centaur ® solid-phase competitive chemiluminescent enzymatic immunoassay (Siemens city, Germany). The coefficients of variation reported by the laboratory were 7.48% for E2, 14.11% for progesterone, 7.74% for FSH and 10.77% for LH.

Statistical analysis

All data are reported as means ± standard deviation (SD). A mixed linear model was used to analyse differences in BC variables over the MC and OC phases. Phase (EFP, LFP and MLP for the eumenorrheic group and WP and APP for the OC group) was included as a fixed effect and individual response as a random effect. LSD's post hoc tests were conducted to examine the pairwise comparisons. All tests were carried out with a 5% significance level. Statistical analyses were performed using SPSS software for Windows, version 20.1 (IBM Corp., Armonk, NY, USA). Cohen effect sizes (ES) were calculated to verify the magnitude of the mean differences between menstrual phases. The ES were interpreted based on the following criteria: < 0.2 = trivial, 0.2 to 0.6 = small effect, 0.6 to 1.2 = moderate effect, 1.2 to 2.0 = large effect, and > 2.0 = very large (Hopkins et al., 2009). The 90% confidence interval (CI) was also calculated.

RESULTS

Hormone levels (mean±SD) presented in each phase of the cycle fulfilled the expected fluctuations for a typical menstrual and OC cycle (Table 6).

In the eumenorrheic group, BC variables showed no differences between the MC phases: body weight ($F_{69,2}$=1.143), BMI ($F_{65,2}$=0.555), BM ($F_{64,2}$=1.702), FM ($F_{78,2}$=0.650), FFM ($F_{63,2}$=1.668) and TBW ($F_{63,2}$=1.582) (Table 7). Trivial and small effect sizes (d < 0.45) for all body composition variables were found when comparing all MC phases in eumenorrheic females.

Similarly, OC group analysis revealed no differences between phases: body weight ($F_{32,1}$=0.214), BMI ($F_{32,1}$=0.105), BM ($F_{32,1}$=0.081), FM ($F_{32,1}$=0.005), FFM ($F_{32,1}$=0.058) and TBW ($F_{32,1}$=0.054) (Table 8). Trivial and small effect sizes (d < 0.46) for all body composition variables were found when comparing hormonal phases in OC users.

Table 6: Sex hormone concentrations on the testing days for the eumenorrheic and OC groups.

	Eumenorrheic females (n=52)			OC users (n=33)	
	EFP	LFP	MLP	WH	APP
LH (mUI/mL)	6.49±3.19	9.95±6.99	8.92±15.35	3.42±3.74	1.61±2.5
FSH (mUI/mL)	8.43±6.50	6.60±2.44	4.37±2.93	5.41±3.94	1.53±1.63
Estrogens (pg/ml)	39.73±26.11	138.60±127.95	125.10±72.41	89.88±150.95	30.44±100.24
Progesterone (ng/ml)	0.56±0.58	0.62±1.32	9.76±5.96	0.37±0.20	0.36±0.16

EFP: early follicular phase; LFP: late follicular phase; MLP: mid-luteal phase; WP: withdrawal phase; APP: active pill phase; OC: oral contraceptive; LH: luteinizing hormone; FSH: folliculostimulating hormone.

Table 7: BC variables (Mean±SD) in eumenorrheic females over the different phases of the menstrual cycle.

	EFP	LFP	MLP	*p*
Weight (kg)	58.608±0.912	58.437±0.912	58.598±0.912	0.325
BMI (kg/m^2)	21.90±0.302	21.835±0.302	21.823±0.302	0.557
BM (Kcal)	1376±17.229	1372±17.229	1379±17.229	0.190
FM (%)	21.021±0.645	21.038±0.645	20.779±0.645	0.525
FFM (kg)	46.088±0.592	45.94±0.592	46.211±0.593	0.197
TBW (kg)	33.737±0.434	33.637±0.434	33.830±0.434	0.214

EFP: early follicular phase; LFP: late follicular phase; MLP: mid-luteal phase; BMI: body mass index; BM: basal metabolism; FM: fat mass; FFM: fat free mass; TBW: total body water.

Table 8: BC variables (Mean±SD) in OC users over the two phases of the hormonal cycle.

	WP	APP	*p*
Weight (kg)	57.339±1.145	57.282±1.145	0.647
BMI (kg/m^2)	21.552±0.339	21.570±0.339	0.778
BM (Kcal)	1376±20.768	1375±20.768	0.748
FM (%)	21.121±0.697	21.103±0.697	0.943
FFM (kg)	45.700±0.725	45.658±0.725	0.811
TBW (kg)	33.455±0.531	33.424±0.531	0.817

WP: withdrawal phase; APP: active pill phase; BMI: body mass index; BM: basal metabolism; FM: fat mass; FFM: fat free mass; TBW: total body water.

Although no statistical differences in BC variables have been reported either across the MC or the OC cycle, some slight individual shifts may occur in well-trained females. Thus, Table 9 presents the percentage of change for each variable during both cycles.

Table 9: Percentage of change throughout different phases of the MC and OC cycle.

	Eumenorrheic females (n=52)						OC users (n=33)	
	EFP-LFP		EFP-MLP		LFP-MLP		WP-APP	
	Mean	Range	Mean	Range	Mean	Range	Mean	Range
Weight (%)	0.31	-6.67-3.66	0.03	-6.27-4.94	-0.31	-3.00-5.13	0.13	-4.07-1.92
BMI (%)	0.32	-6.57-3.88	0.38	-6.11-11.62	0.01	-3.59-11.62	-0.11	-4.43-2.05
BM (%)	0.25	-4.20-4.20	-0.23	-9.30-4.53	-0.50	-7.13-2.95	0.10	-2.53-3.36
FM (%)	-0.89	-36.51-20.63	0.82	-15.00-27.62	1.20	-22.75-32.56	0.01	-14.44-22.40
FFM (%)	0.30	-5.45-5.27	-0.32	-12.24-5.50	-0.66	-9.15-3.66	0.11	-3.33-4.08
TBW (%)	0.28	-5.41-5.11	-0.33	-12.22-5.29	-0.66	-9.06-3.53	0.10	-3.34-4.11

EFP: early follicular phase; LFP: late follicular phase; MLP: mid-luteal phase; OC: oral contraceptive; WP: withdrawal phase; APP: active pill phase; BMI: body mass index; BM: basal metabolism; FM: fat mass; FFM: fat free mass; TBW: total body water.

DISCUSSION

The main objective of this study was to investigate potential differences in BC variables over the different hormonal environments of the MC and OC cycle in well-trained females. Our results showed no differences over the MC phases (EFP, M-LFP and MLP) and between the OC phases (WP and APP) for any of the body composition variables measured through bioelectrical impedance (body weight, BMI, BM, FM, FFM and TBW).

During the MC, sex hormone fluctuations are mainly characterized by a drastic increase in estradiol and LH at the end of the follicular phase (LFP), the rise of progesterone and estradiol during the MLP and the decrease of all female hormones in the EFP. Although sex hormones seem to modify some physiological processes related to BC variables like lipid and carbohydrate oxidation (Marchand et al., 2017), muscle mass metabolism (Hansen, 2018; Kitajima & Ono, 2016; Sung et al., 2014) and body fluid regulation (Stachenfeld & Keefe, 2002), our data did not report variations in BC in well-trained females during the three different hormone phases of the MC. Our outcomes are in line with other studies in females using bioelectrical impedance but none of these tested at the same three points of the MC as the current study. A recent study conducted with physically active women reported no significant differences between the 2nd and the 14th day of the MC for FM, TBW, muscle mass and BMI measured by bioimpedance (Daniusevičiūtė et al., 2010). In the same way, no differences in FM, FFM and TBW were reported by some recent studies carried out with healthy females when comparing EFP and LFP (Hicks et al., 2017); EFP, LFP, early luteal phase and late luteal phase (Cumberledge et al., 2018); and when comparing from the 5th to 8th and from the 9th to 17th day of the cycle (Gualdi-Russo & Toselli, 2002). These last two studies, however,

analysed OC users and eumenorrheic females together, so their results must be considered with caution. Overall, it appears that fluctuations in sex hormones throughout the MC might not be large or long enough to result in changes in BC variables in well-trained females. This is supported by the magnitude-based inference performed in this study, which showed trivial and small effect sizes for all body composition variables when comparing MC phases in eumenorrheic well-trained females.

In contrast, one study including recreational and active sportswomen comparing the EFP (1–5 days), LFP (8–12 days) and MLP (18–23 days) reported a slight increase of body mass, TBW and FFM during the luteal phase (Tomazo-Ravnik & Jakopič, 2006). Self-reported questionnaires were used to verify MC phases in this study. The rise of FFM and body mass may be explained by the increase of TBW, since water is stored in human muscle (Fernández-Elías et al., 2015) and the rise of TBW by the increase of estradiol and progesterone during the luteal phase. Some studies have shown a slight increase of water retention with high estradiol- concentrations (Calzone et al., 2001; Stachenfeld & Keefe, 2002) because of the presence of estradiol receptors in the hypothalamus nuclei, where arginine vasopressin is produced. High levels of progesterone also promote water retention, since this sex hormone increases aldosterone production, which acts on kidney tubes stimulating Na+ resorption and K+ excretion (Stachenfeld & Keefe, 2002). So, estradiol and progesterone might raise plasma volume with the greatest effect when both sex hormones are elevated (Stachenfeld & Taylor, 2004). This is in line with some older studies, which reported weight gain during the luteal phase due to water retention (Cohen, 1973; Dadlani et al., 1981; Janowsky et al., 1973; Reeves et al., 1971). However, the methodology used by earlier studies in this area has several limitations. Some of these studies analysed both study groups (OC users and eumenorrheic females) as a whole

(Cumberledge et al., 2018; Gleichauf & Roe, 1989; Gualdi-Russo & Toselli, 2002; Janowsky et al., 1973; Reeves et al., 1971) and were therefore unable to distinguish between the effects of endogenous and exogenous female hormones. Other studies did not specify or distinguish between the physical activity level of their participants (Gleichauf & Roe, 1989; Janowsky et al., 1973; Reeves et al., 1971). Finally, several studies used very limited verification of MC phases. Some did not specify how the MC phases were determined(Cohen, 1973; Janowsky et al., 1973) or on which day testing was conducted (Gleichauf & Roe, 1989), or only used an MC self-record by volunteers (Reeves et al., 1971). Therefore, differences in results could be explained by inconsistencies in methodology, as well as the often small sample size used ($n < 20$).

For our participants taking OC, no differences in body mass, FM, FFM, BM, BMI and TBW between OC phases (WP and APP) were reported in the present study. These findings are not in line with earlier literature that found an increase of plasma volume (Stachenfeld & Taylor, 2004) and an inhibition of the lipolysis process (Luglio, 2014) with the administration of ethinyl estradiol and progestin. This may be explained by the low dosages of exogenous sex hormones that monophasic OC pills contain nowadays, which may not be high enough to affect physiological processes in well-trained females. Ethinyl estradiol and progestin concentrations in OC pills used to be around 150 mg/day and 9.85 mg/day, respectively, whereas in current OC these concentrations are much lower at approximately 15 mg/day and 0.35 mg/day (White et al., 2011). Our data are in accordance with a recent study performed with sedentary OC users in which FM and TBW measured by bioelectrical impedance remained stable when comparing two time points of the OC cycle (days 1–2 and days 7–14) (Hicks et al., 2017). However, it is important to realize that the hormonal environment in those two phases would have been

very different compared to the testing phases in the current study. At days 1–2 of an OC cycle participants are still likely to be influenced by the exogenous sex hormones taken during the APP just beforehand, while in the current study testing was conducted later in the withdrawal phase to avoid potential effects of the exogenous hormones. The second testing phase in the study by Hicks et al. (2017) was from day 7 to 14, which is in the first week of consumption of the active pill and likely too early to detect a potential effect of the exogenous ethinyl estradiol and progestin on BC variables. The current study tested around day 21 of the OC cycle when exogenous hormone levels are likely to be higher after 2 weeks of active OC consumption. In line with our findings, three further studies reported no changes in body composition variables measured by bioelectrical impedance over four different phases (days 1–7, 8–14, 15–21 and 22–28) (Cumberledge et al., 2018) and two phases of the cycle (Hicks et al., 2017). These three studies all included participants taking OC, but also eumenorrheic participants, and they combined the results of both groups. Therefore, it would have been impossible to distinguish between potential effects of the exogenous hormones in the OC participants and the endogenous hormones in the eumenorrheic participants. Furthermore, there are many different types and dosages of exogenous female hormones in OC and most earlier studies did not provide details on the type of OC their participants were taking. Therefore, to build on the limited research on OC participants, it is important to include specific OC details in future research in this area. In the present study, the OC group used monophasic pills, containing low dosages of ethinyl estradiol (0.03±0.01 mg/day) and progestin (1.8±1.3). Thus, having used different OC brands may have had no impact on the results. Finally, the magnitude-based inference analysis in this study showed trivial and small effect sizes for all body composition variables when comparing hormonal phases of the OC cycle in well-trained

females. Therefore, our findings support the lack of differences found by most previous studies.

PRACTICAL APPLICATIONS

Sex hormone fluctuations throughout the MC and OC cycle appear not to influence BC variables measured by bioelectrical impedance in well-trained females. Therefore, it seems that bioimpedance analysis can be performed at any moment of the cycle, both for eumenorrheic females and for OC users. Although no differences have been found throughout the MC and the OC cycle, it is worth mentioning that BC individual variations that may take place in trained females due to their hormonal fluctuations. Thus, coaches and athletes should be aware of these individual differences, especially regarding FM in eumenorrheic females.

The current study addressed a gap in the research through the investigation of important variables of body composition in well-trained females. The strength of this study is the research design, which included different endogenous and exogenous female hormonal profiles, as well as verification of phases with serum hormone measurement. Furthermore, the recruitment of homogeneous groups of healthy and active females in both the eumenorrheic group and monophasic OC group has ensured practical application for well-trained females. Nonetheless, although equations used by the bioelectrical impedance device have been previously validated, the BM one hasn’t. Thus, the BM variable may not have been accurate enough.

CONCLUSIONS

Different female sex hormone environments throughout the MC and OC cycle do not influence BC variables measured through bioimpedance analysis in well-trained females. Nonetheless, further research is recommended to provide a better understanding of the potential effects of sex hormones on body composition variables in physically active women, with a particular focus on the different types of OC.

STUDY IV

Cardiorespiratory response to exercise in endurance-trained premenopausal and postmenopausal females

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ABSTRACT

Purpose: To assess the influence of different hormonal profiles on the cardiorespiratory response to exercise in endurance-trained females.

Method: Forty-seven eumenorrheic females, 38 low-dose monophasic oral contraceptive (OC) users and 13 postmenopausal women, all of them endurance-trained, participated in this study. A DXA scan, blood sample tests and a maximal aerobic test were performed under similar low sex hormone levels: early follicular phase for the eumenorrheic females; withdrawal phase for the OC group and at any time for postmenopausal women. Cardiorespiratory variables were measured at resting and throughout the maximal aerobic test (ventilatory threshold 1, 2 and peak values). Heart rate (HR) was continuously monitored with a 12-lead ECG. Blood pressure (BP) was measured with an auscultatory method and a calibrated mercury sphygmomanometer. Expired gases were measured breath-by-breath with the gas analyser Jaeger Oxycon Pro.

Results: One-way ANCOVA reported a lower peak HR in postmenopausal women (172.4±11.7 bpm) than in eumenorrheic females (180.9±10.6 bpm) (p=0.024). Additionally, postmenopausal women exhibited lower VO_2 (39.1±4.9 ml/kg/min) compared to eumenorrheic females (45.1±4.4 ml/kg/min) in ventilatory threshold 2 (p=0.009). Nonetheless, respiratory variables did not show differences between groups at peak values. Finally, no differences between OC users and eumenorrheic females' cardiorespiratory response were observed in endurance-trained females.

Conclusions: Cardiorespiratory system is impaired in postmenopausal women due to physiological changes caused by age and sex hormones' decrement. Although these alterations appear not to be fully compensated by exercise, endurance training could effectively mitigate them. Additionally, monophasic OC pills appear not to impact cardiorespiratory response to an incremental running test in endurance-trained females.

Keywords: heart rate; blood pressure; oxygen consumption; ventilation; menstrual cycle; oral contraceptive

INTRODUCTION

Females experience cyclical changes in sex hormone levels throughout their menstrual cycle. These fluctuations, especially 17β-estradiol (E2) and progesterone, can affect female athletes in several ways. On the one hand, E2 is known to increase vasodilatation of blood vessels (dos Santos et al., 2014), pulmonary diffusion capacity as well as plasma volume, and this in turn increases blood supply to the heart and muscles (Mattu et al., 2019). Additionally, this sex hormone is associated with increments in growth hormone secretion, epinephrine levels as well as glycogen sparing and fat oxidation (Ashley et al., 2000; Mattu et al., 2019; Packard et al., 2011). Furthermore, E2 regulates mechanical functioning and ventricular myocytes' proteomic profiles (dos Santos et al., 2014) and appears to stimulate parasympathetic tone because of the presence of E2 receptors in the nucleus tractus solitarius of the medulla oblongata (Subhashri, Pal, & Pal, 2019; Weissman et al., 2009). On the other hand, high levels of progesterone have been linked to decrements in respiratory exchange ratio (RER) and lactate values (Burrows & Bird, 2000). Furthermore, this sex hormone enhances water retention, fat utilization, glycogen sparing (Burrows & Bird, 2000; Packard et al., 2011), core temperature and heart rate (HR) (Janse de Jonge, 2003; Lebrun, 1993). Retrospective studies strongly suggested that progesterone increases chemosensitivity of hypothalamus chemoreceptors, lowering the threshold of the medullary respiratory centre, leading to an increase in ventilation (Ve) (Boukari et al., 2017; Constantini et al., 2005; Godbole et al., 2016; Goldsmith & Glaister, 2020; Janse de Jonge, 2003; Samsudeen & Rajagopalan, 2016; Williams & Krahenbuhl, 1997). In addition, these increments in Ve could be accompanied by a rise in oxygen consumption (VO_2) (Goldsmith & Glaister, 2020; Williams & Krahenbuhl, 1997).

Natural endogenous sex hormone secretion is suppressed in women taking oral contraceptive (OC) pills because of the intake of exogenous ethinyl estradiol and progestin (Joyce et al., 2013). In relation to exogenous sex hormones, there is less knowledge about the effects of their administration on females' physiology. Recent studies reported that ethinyl estradiol increases lipid and reduces glucose metabolism (Burrows & Peters, 2007; Mattu et al., 2019; Packard et al., 2011; Rechichi et al., 2009), whereas progestin has been linked with increments in Ve and body temperature (Burrows & Peters, 2007; Rechichi et al., 2009), which may result in an increase in cardiovascular strain (Janse de Jonge, 2003). Similarly, another research study found significantly higher Ve and breath frequency during the exogenous administration phase (Barba-Moreno et al., 2019). In addition, ethinyl estradiol has mineralocorticoid actions, which activates the renin-angiotensin-aldosterone system encouraging Na^+ and fluid retention, and this in turn would increase blood pressure (BP). On the contrary, progestin has anti-mineralocorticoid actions, which antagonizes the effect of Na^+ and fluid retention (Grandi et al., 2014; Meendering et al., 2009; Torgrimson et al., 2007).

Due to the different hormonal environment presented in OC users regarding eumenorrheic females, it is speculated that differences in cardiorespiratory response may exist between both groups. In this sense, some authors found a lower maximal HR (Gordon et al., 2018) with the use of OC pills, whereas some others agree in no effect of monophasic OC pills on cardiovascular system (Giribela et al., 2012; Grandi et al., 2014; Middlekauff et al., 2012; Nisenbaum et al., 2014; Teixeira et al., 2012). Even though a drop in VO_2 max (Casazza et al., 2002; Lebrun, 1993) and glucose metabolism (Burrows & Peters, 2007; Rechichi et al., 2009) has been associated with the use of these pills, there is also some literature concluding that there is no effect on maximal VO_2 and RER with

the use of OC pills (Casazza et al., 2002; Gordon et al., 2018; Mattu et al., 2019; Packard et al., 2011; Vaiksaar et al., 2011). These conflicting findings can largely be explained by methodological shortcomings, such as not considering the OC's formulation and different days of measurements over the menstrual and OC cycle.

Moving on to postmenopausal women, a drastic fall in sex hormone production takes place after menopause due to the loss of the ovarian function (Karsenty, 2012). This in turn elicits some differences in postmenopausal women compared to their premenopausal counterparts, such as a reduction in bone mineral density, muscle mass, strength, aerobic capacity (Bondarev et al., 2018) and HR (Neufeld et al., 2015). Moreover, as E2 enhances vagal activity (Mattu et al., 2019) and vasodilatation of blood vessels (dos Santos et al., 2014; Mattu et al., 2019), its decrease with age is associated with arterial stiffness and vascular resistance, and this in turn increases BP in this population (Farinatti et al., 2018). Exercise is advocated to be one of the best tools to enhance cardiovascular function (Green et al., 2017) and improve respiratory parameters (Moazami & Farahati, 2013). Consequently, sex hormones' influence on the cardiorespiratory system could be covered in trained females due to the positive effect exercise has on these tissues. Furthermore, with regard to cardiorespiratory response to exercise, it has been speculated that sex hormones influence on these physiological variables may be masked when training at high intensities (Barba-Moreno et al., 2019; Janse de Jonge, 2003; Mattu et al., 2019).

As previously mentioned, there are some controversial results when studying the impact of endogenous and exogenous sex hormones on premenopausal females as well as the effect of low concentrations of E2 and progesterone in postmenopausal women

regarding the cardiorespiratory system. Therefore, the aim of this study was to assess the influence of different hormonal environments (eumenorrheic females, low-dose monophasic OC users and postmenopausal women) on endurance-trained females' cardiorespiratory response to exercise.

MATERIAL AND METHODS

Participants

Forty-seven eumenorrheic females (cycles of 24–35 days in length), 38 low-dose monophasic OC users (at least 6 months intaking them) and 13 postmenopausal women (at least one year without menstruation) participated in this study. Brands and formulation of OC pills are presented in Table 10. Exogenous sex hormone concentration mean for the OC group was 0.03±0.01 mg/day of ethinyl estradiol and 1.79±1.28 mg/day of progestin. At the start of the data collection, all participants completed a questionnaire gathering information about training status, health conditions, dietary supplement consumption and type of OC pills when appropriate. All of them were endurance-trained (Table 11). Females with metabolic pathologies, hormonal disorders, smoking habits, intaking supplementation or with injuries/surgeries in the last 6 months were excluded from this study. To be included in the study participants were required to be healthy adult females, without iron deficiency anaemia (serum ferritin <20μg/l, haemoglobin <115 μg/l and transferrin saturation <16%), non-pregnant or oophorectomized, not consuming medication that alters vascular function (e.g., tricyclic antidepressants, α-blockers, β-blockers, etc.) and they had to perform endurance training between 3 and 12 hours per week. An informed consent was obtained from each participant with all the information about the procedures and risks involved. The experimental protocol was approved by the Ethical Committee of the Universidad Politécnica de Madrid and is in accordance

with The Code of Ethics of the World Medical Association (Declaration of Helsinki) (Association, 2002). Lastly, the study was registered on clinicaltrials.gov (ID: NCT04458662).

Table 10: Brand and formulation of OC pills for the OC group.

Number of users	Brand	Ethinyl estradiol (mg)	Progestin (mg)
9	Yasmin	0.03	Drospirenone (3)
4	Diane	0.035	Cyproterone (2)
4	Loette	0.02	Levonorgestrel (0.1)
3	Sibilla	0.03	Dienogest (2)
3	Ceciliana	0.03	Dienogest (2)
2	Linelle	0.02	Levonorgestrel (0.1)
2	Levobel	0.02	Levonorgestrel (0.1)
2	Melodene	0.015	Gestodene (0.06)
1	Edelsin	0.035	Norgestimate (0.25)
1	Drosbelalleflex	0.02	Drospirenone (3)
1	Stada	0.02	Drospirenone (3)
1	Drosure	0.03	Drospirenone (3)

OC: oral contraceptive

Experimental protocol

All measurements were carried out on the same day for each participant. In order to measure all participants under similar low-hormone conditions, eumenorrheic females were evaluated between the 2nd and 5th day of the menstrual cycle, the onset of the cycle being the first day of menstrual bleeding, while OC users were evaluated between the 3rd and the 7th day of the withdrawal phase (Sims & Heather, 2018). Finally, any time was established for postmenopausal women, since their hormonal status does not vary. Volunteers refrain from physical activity and caffeine intake 24 h prior to the test.

Dual-energy X-ray absorptiometry scan

A dual-energy X-ray absorptiometry (DXA) scan (Version 6.10.029GE Encore 2002, GE Lunar Prodigy; GE Healthcare, Madison, WI, USA) was performed between 8 and 10 a.m. in fasting state to obtain body composition variables such as weight, fat mass (FM) and lean mass (LM), considering LM as body weight minus FM and minus bone mineral content. Calibration and evaluation procedures were realized by recommendations of the manufacturer and certified technicians.

Blood samples

To avoid ultradian rhythm variations (Janse de Jonge, 2003), blood sample tests were done at the same time for all volunteers, between 8 and 10 a.m. They were obtained with venipuncture into a vacutainer containing clot activator. Following inversion and clotting, the whole blood was centrifuged (Biosan LMC-3000 version V.5AD) for 10 minutes at 3000 rpm. After that, serum was transferred into Eppendorf tubes and stored frozen at -80 ºC until further analysis. Within 1 to 15 days after testing, the serum samples were delivered to the clinical laboratory of the Spanish National Centre of Sport Medicine (Madrid, Spain) to determine sex hormones in order to verify hormonal profiles. Total E2, progesterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were measured via ADVIA Centaur ® solid-phase competitive chemiluminescent enzymatic immunoassay (Siemens City, Germany). Inter- and intra-assay coefficients of variation (CV) reported by the laboratory for each variable were, respectively: 11.9% and 8.5% at 93.3 pg/ml and 6.8% and 4.7% at 166 pg/ml for E2; 23.1% and 11.8% at 0.7 ng/ml and 5.2% and 2.5% at 9.48 ng/ml for progesterone, 5.3% and 1.8% at 1.2 mIU/ml for FSH and 5.2% and 1.8% at 0.54 mIU/ml for LH.

Maximal aerobic test

At least 2 hours after the last food intake, a maximal aerobic test was performed with a computerized treadmill (H/P/COSMOS 3PW 4.0, H/P/Cosmos Sports & Medical, Nussdorf-Traunstein, Germany) to determine their peak oxygen uptake (VO_2peak). The gradient of the treadmill was set at 1% in order to simulate outdoor running (Goldsmith & Glaister, 2020). Expired gases were measured breath-by-breath with the gas analyser Jaeger Oxycon Pro (Erich Jaeger, Viasys Healthcare, Germany), the validity and reliability of which has been previously demonstrated (James Carter & Asker E Jeukendrup, 2002; Ø. Foss & J. Hallen, 2005). Heart response was continuously monitored with a 12-lead ECG. After a warm-up of 3 minutes at 6 km/h, the test started at 8 km/h. The speed increased 0.2 km/h every 12 seconds up to volunteer's exhaustion. Maximal speed was considered reached at the last completed step of 12 seconds. The highest value from the last 30 seconds of the test was set as peak Ve and RER. Finally, peak HR was the highest value throughout the test. Standing BP was measured both at resting and at volunteers' exhaustion in the maximal aerobic test, always by the same researcher, using the auscultatory method with a calibrated mercury sphygmomanometer. First and second ventilatory thresholds (VT1 and VT2, respectively) were set by the same researcher, following maximum agreement in literature (Rabadan et al., 2011). Finally, 220-age equation was used to analyse the difference between the theoretical maximal HR and the peak HR reached during the maximal aerobic test (theoretical HRmax–HRpeak).

After a recovery phase of 5 minutes (3 minutes walking at 6 km/h and 2 minutes sitting on a chair), a confirmatory test was carried out in order to verify VO_2peak was reached (P. Nolan et al., 2014; David C Poole & Andrew M Jones, 2017). This consisted of 3 minutes' warm-up (2 minutes at 50% followed by 1 minute at 70% of the maximal

speed reached in the maximal aerobic test) (P. Nolan et al., 2014). Then, volunteers ran at the speed of 110% up to exhaustion (Astorino et al., 2018). If volunteers did not run at least 1 minute at this speed, the confirmatory test was not considered for VO_2peak determination and it was obtained only from the maximal aerobic test. Lastly, participants performed a 2 minutes' recovery at 6 km/h.

VO_2peak was determined as the mean of the three highest and continuous 15-second interval VO_2 measurements in the maximal aerobic test (Cortes et al., 2014). This value was considered if its difference with the VO_2peak obtained in the confirmatory test was lower than 3%. If the difference was higher, the value obtained in the confirmatory phase was considered.

Statistical analysis

All data are reported as mean ± standard deviation (SD). Data showed a normal distribution, thus analyses comparing groups (eumenorrheic, monophasic OC users and postmenopausal) were performed by one-way ANCOVA and age was used as a covariable. The Scheffé test was applied to examine the pairwise comparison. Effect size was calculated by partial eta-squared (η_p^2) and small, moderate and large effect corresponded to values equal or greater than 0.001, 0.059, and 0.138, respectively (Cohen, 2013). All tests were conducted with a 5% significance level. Statistical analyses were performed using SPSS software for windows, version 20.1 (SPSS Inc, Chicago, IL, USA).

RESULTS

One-way ANCOVA test showed a mean effect among all groups in age ($F_{2,96}$=138.716) and theoretical maximal HR ($F_{2,96}$=136.211) whereas no differences were reported for height ($F_{2,96}$=1.364), weight ($F_{2,96}$=2.337), body mass index (BMI) ($F_{2,96}$=1.436), FM ($F_{2,96}$=0.179), and LM ($F_{2,96}$=0.420). With regard to training status, no significant differences were found for experience ($F_{2,126}$=0.868), sessions per week ($F_{2,126}$=0.906) or time per session ($F_{2,126}$=0.178) among study groups. FSH ($F_{2,84}$=102.147) and LH ($F_{2,84}$=153.415) were, as expected, different among groups, postmenopausal women presenting higher values than both eumenorrheic females and OC users. Nevertheless, neither E2 ($F_{2,84}$=2.337), progesterone ($F_{2,84}$=2.542) nor E2/progesterone ratio ($F_{2,84}$=0.957) reported differences among study groups (Table 11).

Table 11: Characteristics of the study population

	Eumenorrheic (n=47)	OC users (n=38)	Postmenopausal (n=13)	*p*
	Mean±SD	Mean±SD	Mean±SD	
Age (yrs)	33.1±5.1	26.3±4.9	51.3±3.6	<0.001*
Theorical HR max (bpm)	186.9±5.1	193.7±4.9	168.7±3.6	<0.001*
Height (cm)	163.8±5.7	163.1±6.1	160.8±5.6	0.261
Weight (kg)	59.3±7.1	58.4±5.9	54.1±4.1	0.102
BMI (kg/m^2)	21.4±2.2	21.9±2.1	20.9±1.7	0.243
FM (%)	24.8±7.9	25.4±5.6	24.2±5.2	0.837
LM (%)	71.2±13.9	70.1±5.7	72.9±5.6	0.658
Experience (yrs)	7.7±5.2	6.6±4.5	7.9±3.3	0.422
Sessions per week (days)	3.9±1.1	3.7±1.15	3.9±1.16	0.407
Time per session (hours)	1.3±0.4	1.4±2.1	1.2±0.3	0.837
FSH (mUI/mL)	7.89±3.82	4.98±4.50	81.69±45.69	<0.001¥
LH (mUI/mL)	6.27±2.61	3.11±2.80	44.69±19.06	<0.001¥
E2(pg/mL)	45.15±25.76	26.69±26.64	42.36±78.27	0.103
Progesterone (ng/mL)	0.45±0.56	0.27±0.17	0.20±0.17	0.085
Estradiol/progesterone ratio	0.24±0.34	0.14±0.22	0.18±0.26	0.388

Theorical HR max: theorical maximal HR estimated by 220-age; OC: oral contraceptive; BMI: body mass index; FM: fat mass; LM: lean mass; FSH: follicle-stimulating hormone; LH: luteinizing hormone; E2: Estradiol

* Significant differences between all groups.

¥ Significant differences in postmenopausal women compared to eumenorrheic females and OC users.

Resting values

Cardiovascular resting values (Table 12 and Figure 20) were no different between study groups either for systolic BP (SBP) ($F_{2,71}$=1.110), diastolic BP (DBP) ($F_{2,71}$=0.615) or HR ($F_{2,69}$=0.338; p=0.715; η^2=0.010). Likewise, respiratory resting variables (Table 12 and Figure 21) such as VO_2 ($F_{2,94}$=0.572; p=0.566; η^2=0.012), Ve ($F_{2,94}$=0.473) and RER ($F_{2,94}$=1.145) did not exhibit differences among eumenorrheic females, OC users and postmenopausal women.

Ventilatory threshold 1

Heart response to exercise in VT1 reported differences among the study groups ($F_{2,86}$=3.348; p=0.040; η^2=0.072) (Figure 20). Specifically, postmenopausal women presented lower values than eumenorrheic females (p=0.035; η^2=0.042). The respiratory response to exercise for this threshold (Table 12 and Figure 21) showed no significant differences either for VO_2 ($F_{2,94}$=1.886; p=0.157; η^2=0.039), Ve ($F_{2,94}$=0.804), RER ($F_{2,94}$=2.657) or %VO_2 peak ($F_{2,94}$=0.412). Nonetheless, speed ($F_{2,94}$=6.067) was higher in the postmenopausal compared to the OC (p=0.003; η^2=0.145) and eumenorrheic (p=0.026; η^2=0.077) groups, and eumenorrheic females had higher values than OC users (p=0.016; η^2=0.083).

Ventilatory threshold 2

This threshold did not show differences in HR (Figure 20) among study groups ($F_{2,85}$=2.754; p=0.069; η^2=0.061). Nonetheless, VT2 showed differences in VO_2 ($F_{2,94}$=6.121; p=0.003; η^2=0.115), the postmenopausal group presenting lower values than the eumenorrheic one (p=0.009; η^2=0.111) (Figure 21). Likewise, postmenopausal women reported lower values of %VO_2 peak ($F_{2,94}$=4.680) compared to eumenorrheic

females (p=0.019; η^2=0.102). On the contrary, no differences were reported among study groups either for Ve ($F_{2,94}$=1.229) or RER ($F_{2,94}$=2.250). Finally, although speed reported differences among groups ($F_{2,94}$=3.972), pairwise comparisons were not statistically different.

Peak values

Cardiovascular response at peak values (Table 12) reported no significant differences for SBP ($F_{2,61}$=0.229) and DBP ($F_{2,61}$=0.881), whereas HR (Figure 20) was different among groups ($F_{2,88}$=4.038; p=0.021; η^2=0.084), showing postmenopausal women having lower values than eumenorrheic females (p=0.024; η^2=0.077). In addition, the difference between theoretical maximal HR minus peak HR ($F_{2,88}$=3.968) was lower in postmenopausal women compared to eumenorrheic females (p=0.026; η^2=0.077). Specifically, postmenopausal women reached a peak HR higher than their theoretical values. Finally, neither VO_2 ($F_{2,95}$=1.742; p=0.181; η^2=0.035), Ve ($F_{2,93}$=0.124) RER ($F_{2,93}$=2.917) nor speed ($F_{2,95}$=2.325) showed differences among eumenorrheic females, OC users and postmenopausal women.

Table 12: Cardiorespiratory variables throughout an aerobic maximal test in eumenorrheic females, OC users and postmenopausal women.

		Eumenorrheic (n=47)	OC users (n=38)	Postmenopausal (n=13)	*p*	η^2
		Mean±SD	Mean±SD	Mean±SD		
Rest	SBP (mmHg)	114.7±8.7	114.5±10.4	112.2±12.6	0.335	0.030
	DBP (mmHg)	70.4±8.7	72.2±7.5	71.8±7.5	0.543	0.017
	Ve (L/min)	7.1±2.6	6.8±2.7	6.1±2.4	0.624	0.010
	RER	0.947±1.167	0.771±0.081	0.735±0.096	0.323	0.024
VT1	Ve (L/min)	54.6±12.1	53.4±10.3	50.5±13.1	0.451	0.017
	RER	0.923±0.094	0.924±0.087	0.975±0.067	0.075	0.054
	% VO_2 max (%)	67.9±7.6	66.1±7.9	69.3±12.4	0.664	0.009
	Speed (km/h)	10.7±1.3	10.4±0.9	10.7±1.2	0.003*	0.114
VT2	Ve (L/min)	89.4±12.6	89.4±12.7	75.2±11.2	0.297	0.025
	RER	1.077±0.099	1.077±0.091	1.129±0.050	0.111	0.046
	% VO2 max (%)	90.7±3.6	89.4±5.7	86.1±6.5	0.012£	0.091
	Speed (km/h)	13.9±1.2	13.4±0.9	13.1±1.5	0.022	0.078
Peak	SBP (mmHg)	172.9±16.6	170.2±12.9	171.1±14.7	0.796	0.007
	DBP (mmHg)	76.9±8.5	74.5±7.4	76.8±7.8	0.419	0.028
	Ve (L/min)	109.1±13.8	113.8±15.6	96.4±10.4	0.884	0.003
	RER	1.164±0.108	1.179±0.094	1.232±0.075	0.059	0.059
	Theorical HR_{max} - HR_{peak}(bpm)	5.8±9.1	4.8±6.7	-3.7±10.9	0.022£	0.083
	Speed (km/h)	15.2±1.2	14.8±1.1	14.1±1.6	0.103	0.047

OC: oral contraceptive; VT1: ventilatory threshold 1; VT2: ventilatory threshold 2; SBP: systolic blood pressure; DBP: diastolic blood pressure; Ve: ventilation; RER: respiratory exchange ratio; Theorical HR_{max} - HR_{peak}: difference between theorical maximal heart rate (220-age) and peak heart rate. * Significant differences between all groups. £ Significant differences between postmenopausal females and eumenorrheic women.

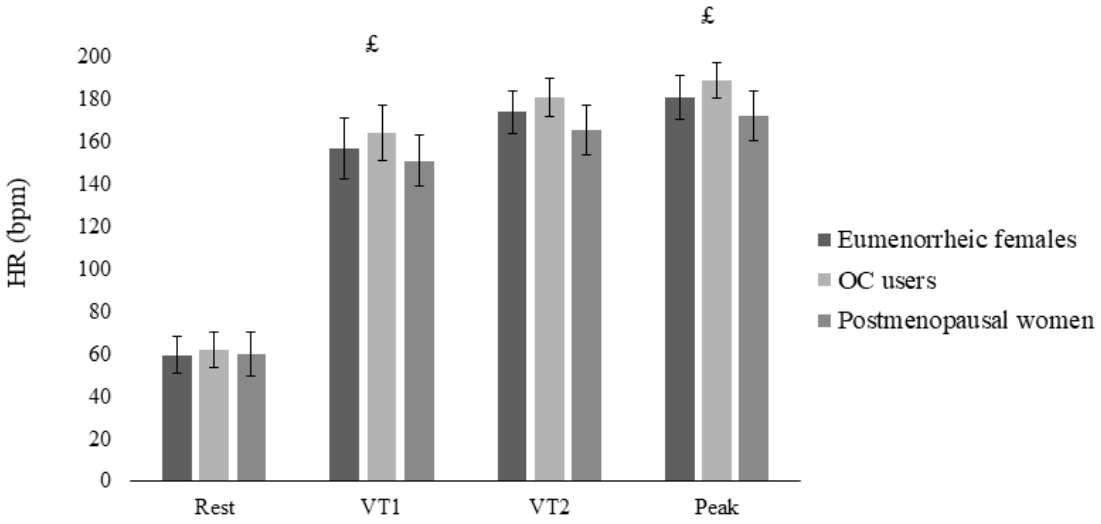

Figure 20: HR response throughout a maximal aerobic test among trained females with different hormonal profile (eumenorrheic females, OC users and postmenopausal women). HR: heart rate; OC: oral contraceptive; VT1: ventilatory threshold 1; VT2: ventilatory threshold 2. £ Significant differences between postmenopausal women and eumenorrheic females.

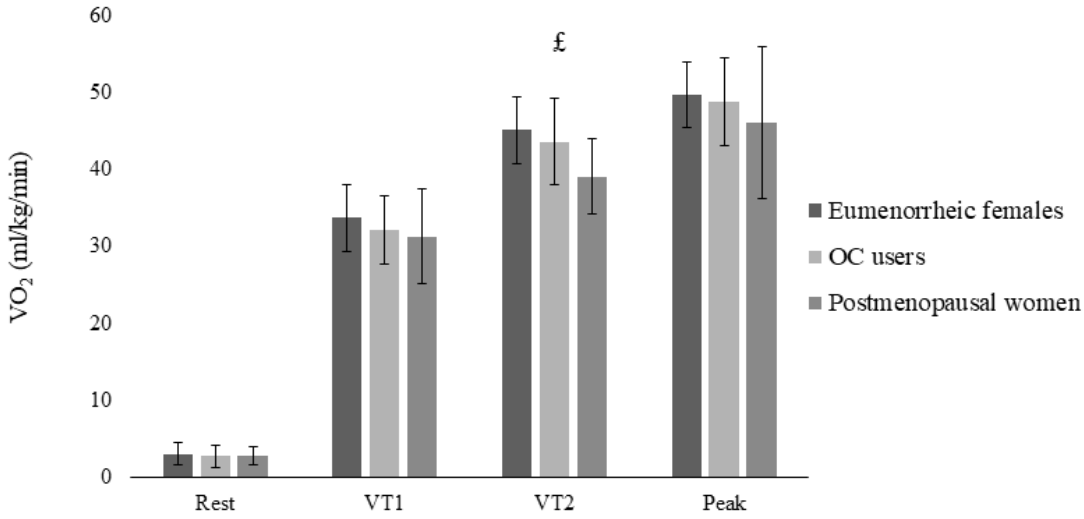

Figure 21: VO_2 response throughout a maximal aerobic test among trained females with different hormonal profile (eumenorrheic females, OC users and postmenopausal women). VO_2: oxygen consumption; OC: oral contraceptive; VT1: ventilatory threshold 1; VT2: ventilatory threshold 2. £ Significant differences between postmenopausal women and eumenorrheic females.

DISCUSSION

The aim of this study was to assess the impact of different hormonal profiles on cardiorespiratory response to exercise in endurance-trained females. The main finding was the similar cardiorespiratory response obtained by postmenopausal and premenopausal endurance-trained females during a maximal aerobic test. In line with this, cardiorespiratory variables were not different in low-dose monophasic OC users compared to eumenorrheic females either at rest or during exercise.

In accordance with previous studies carried out in healthy but sedentary postmenopausal women, this population exhibited increments in resting HR, DBP (Subhashri, Pal, & Pal, 2019; Subhashri, Pal, Papa, et al., 2019) and SBP (Subhashri, Pal, & Pal, 2019; Subhashri, Pal, Papa, et al., 2019; Von Holzen et al., 2016), leading to higher cardiovascular risk and mortality in this population (Subhashri, Pal, & Pal, 2019). The deficiency in ovarian sex hormones after menopause (Karsenty, 2012), along with other hormonal and physiological changes (e.g. bone mineral density, muscle mass, aerobic capacity and HR reduction) caused by age (Bondarev et al., 2018; Neufeld et al., 2015), could explain these increments in resting cardiovascular parameters. The positive and protector effect that ovarian sex hormones, specially E2, have over the cardiovascular system is lost after menopause. Thereby, postmenopausal females appear to suffer HR increments as well as arterial stiffness and vascular resistance, and this in turn increases BP (Farinatti et al., 2018). However, the present study did not show differences in resting cardiovascular parameters when comparing postmenopausal with premenopausal endurance-trained females. As far as we are aware, there is only one study in which active postmenopausal women reported higher HR and SBP than premenopausal women (Tapadar & Tapadar, 2019). In this study, women practised yoga or walking for at least

three months, which may have not been stimulus enough to compensate the cardiovascular system's changes experienced due to the menopause. In fact, a recent review concluded that, in comparison to moderate-intensity exercise, high-intensity interval training elicits superior responses, such as an increase in maximal oxygen uptake and an enhanced capacity for oxidative metabolism owing to an increase in mitochondria (Gibala, 2020).

Discrepancies between literature data and our outcomes could be explained by the key role exercise plays in cardiovascular system protection (Green et al., 2017; Moazami & Farahati, 2013; Roldán et al., 2019), which may compensate physiological changes caused by age and sex hormones' decrement. There is a strong basis for proposing that exercise induces structural changes, such as the growth and stretching of endothelial cells in the walls of the vascular system, leading to a reduction in artery stiffness (Green et al., 2017). Athletes also exhibit a remodelling of conduit arteries, such as an increase in artery diameters to increase blood flow and couple with metabolic demands when exercising (Green et al., 2017). Thus, these physiological effects after menopause appear to be compensated by exercise. Finally, it is worth mentioning the similar low sex hormone concentrations in all groups when testing. Thereby, it could be hypostasized that there may not be a chronic effect of sex hormones on cardiorespiratory response to exercise in endurance-trained females, but there might be an acute effect. Thus, measurements in another phase of the menstrual cycle (e.g., the late follicular phase, when E2 reaches its peak, or the mid-luteal phase, with high levels of E2 and progesterone) or OC cycle (e.g., the active pill phase) could have influenced the results. Retrospective studies strongly suggest that both ovarian sex hormones, specially E2, upregulate very important mediators of vascular relaxation, such as nitric oxide, prostacyclin and endothelium-

derived hyperpolarizing factors (dos Santos et al., 2014). In addition, E2 enhances vagal activity (Subhashri, Pal, & Pal, 2019) and regulates mechanical functioning and ventricular myocytes' proteomic profiles (dos Santos et al., 2014). Consequently, these sex hormones influence on non-reproductive tissues may result in an acute effect on both the cardiovascular and respiratory systems of females.

Cardiovascular response to exercise in postmenopausal endurance-trained women did not report consistent differences compared to premenopausal females, but did so for VT1 and peak HR, where postmenopausal women reported lower values. As previously mentioned, the drastic fall in sex hormones after menopause has been related to increments in myocardial stiffness and drops in myocardial distensibility. With regard to intropic effects, lower β-adrenergic stimulation occurs with aging (Christou & Seals, 2008; Farinatti et al., 2018). Consequently, during vigorous exercise, when great cardiovascular work is required, this could be jeopardised (Christou & Seals, 2008; Farinatti et al., 2018), preventing postmenopausal women from reaching HR values as high as premenopausal females. Nonetheless, a previous study (Farinatti et al., 2018), carried out with light-to-moderate physically active women (65 years), reported maximal HR values of 140 bpm (90.3% of their theoretical maximal HR (220-age)), whereas our endurance-trained females (51 years) achieved 172 bpm (101.8% of their theoretical maximal HR). Furthermore, the present study did not report differences in maximal BP among groups, whereas previous research found higher maximal SBP in healthy sedentary postmenopausal women (Farinatti et al., 2018; Teixeira et al., 2015b). Thereby, some of the changes in maximal cardiovascular parameters after menopause seem to be partially compensated by exercise. Moving on to respiratory response to exercise, the previously lower VO_2 peak reported in postmenopausal women compared to

premenopausal females (Bondarev et al., 2018; Farinatti et al., 2018; Fleg et al., 2005) has not been observed in the present study. Differences in physical activity status should be considered, as most of the previous studies were carried out with sedentary (Fleg et al., 2005) or light-to-moderate physically active women (Farinatti et al., 2018), whereas our postmenopausal participants were endurance-trained women, evidenced by their high oxygen consumption (eumenorrheic females 49.7±4.2; OC users 48.8±5.7; postmenopausal women 46.1±9.9 ml/kg/min) compared to the previously cited studies (Farinatti et al., 2018; Fleg et al., 2005). The cardiovascular adaptations to exercise (e.g., growth and strengthening of endothelial cells in the vessel walls as well as the increase in the arteries' diameter) (Green et al., 2017; Moazami & Farahati, 2013; Roldán et al., 2019) could explain this lack of decay in the respiratory system. It has been suggested that reductions in artery stiffness (Ferreira et al., 2003) and increments in artery diameter (Miyachi et al., 2001) are strongly related to an increase in maximal oxygen consumption. Therefore, the lack of difference in VO_2 peak reported in the present study could be related to the cardiovascular adaptations caused by the regular practice of physical activity. Nonetheless, the lower VO_2 observed in the present study in the postmenopausal group in VT2 should be highlighted. This could be explained by a different buffer system that postmenopausal woman may have, since they also presented at this threshold a higher, but not significant, RER.

Resting cardiovascular response was not different when comparing OC users and eumenorrheic females in the present study. Our results are supported by previous studies, which reported no impact of low-dose OC pills in healthy sedentary females on resting HR (Giribela et al., 2012; Middlekauff et al., 2012; Nisenbaum et al., 2014; Teixeira et al., 2012) and BP (Giribela et al., 2012; Grandi et al., 2014; Nisenbaum et al., 2014). The

same results were obtained when studying physically active OC users (Rebelo et al., 2010). Nonetheless, it is worth highlighting a study carried out with physically active women, where OC pills (no dosages specified) had no impact on HR and DBP, whereas SBP was higher with the use of these pills (Teixeira et al., 2015a). Discrepancies in results regarding SBP could be explained by sex hormone dosages in OC pills and volunteers' training status. Although it is well known that physical activity reduces BP (Green et al., 2017), training status was not analysed in Teixeira's study when comparing OC users and non-OC users. This confounding variable was taken into consideration in the present study, reporting no differences in training status between OC users and eumenorrheic females.

Cardiovascular response to exercise did not report any difference comparing low-dose monophasic OC pill users and eumenorrheic females either. In line with our results, recent studies concluded no impact of these pills on maximal HR and BP (Joyce et al., 2013; Mattu et al., 2019; Rebelo et al., 2010). The lack of OC pills' effect on BP could be explained by the counteraction between ethinyl estradiol (with mineralocorticoid actions) and progestin (with anti-mineralocorticoid actions) contained in these pills (Grandi et al., 2014; Meendering et al., 2009; Torgrimson et al., 2007). Respiratory response to exercise when comparing OC users and eumenorrheic females in the present study is supported by previous studies, which concluded no effect of low-dose monophasic OC pills on maximal VO_2 (Mattu et al., 2019), Ve and RER (Casazza et al., 2002; Gordon et al., 2018; Joyce et al., 2013; Rebelo et al., 2010; Redman et al., 2005; Santos et al., 2008; Vaiksaar et al., 2011) in active females. The absence of influence of OC pills in the female respiratory system could be explained by the current low dosages of exogenous sex hormones in OC pills (White et al., 2011), which might not be enough

to alter all the adjustments that take place in this complex system when exercising (Rebelo et al., 2010). Nonetheless, Lebrun and colleagues observed a decrease in VO_2 max in highly active females with the use of triphasic OC pills (Lebrun et al., 2003). This type of OC pill seems to cause a higher impact on females` physiology than monophasic pills (Burrows & Peters, 2007). Finally, regardless of the OC formulation and training status, it should be pointed out that all groups were measured under the same hormonal environment, low sex hormone levels. Thus, our outcomes suggest that there is no chronic effect with the use of low-dose monophasic OC pills on cardiorespiratory response but there might be an acute effect. In fact, it has been reported that, after oral administration, ethinyl estradiol has an initial peak in plasma after 2 to 4 hours, followed by a secondary peak at about 12 hours and no long detectable in plasma after 24 h (Nilsson & Nygren, 1978; Westhoff et al., 2015). Therefore, if ethinyl estradiol has an acute effect on females' physiology, it was not detectable in the present study, as participants were measured between the 3rd and the 7th day of the withdrawal phase.

The current study attempts to address a gap in the research through the investigation of important cardiorespiratory variables in endurance-trained females. The strengths of our study included different hormonal profiles and the recruitment of a homogeneous group for all of them; eumenorrheic females, OC users and postmenopausal women (active and healthy women with no differences either in training status or in body composition) and the control of OC dosages. Nevertheless, it should be pointed out that there was no difference in sex hormone levels among study groups at the time of testing and, therefore, measuring them in another phase of their OC and menstrual cycle may have reported other results. A limitation of the study might be the use of 220-age to

predict HRmax in an athletic population since it may not be the most accurate method to estimate it. In addition, longitudinal studies with an intra-subject design should be carried out to explore the influence of the hormonal changes throughout the life span. It should be noted that it might also be interesting to analyse different hormonal stages throughout the menstrual cycle and OC cycle in trained females.

CONCLUSION

According to our results, endurance-trained postmenopausal women have a similar cardiorespiratory response to exercise compared to premenopausal females. Due to the age-related physiological changes, along with the sex hormone decrease, postmenopausal maximal HR cannot increase as much as premenopausal values. Therefore, although exercise cannot fully compensate biological changes in postmenopausal women, it could effectively attenuate them. In addition, cardiorespiratory response in low-dose monophasic OC users do not differ from the eumenorrheic response either at rest or during exercise in endurance-trained females. Further research is recommended to provide a better understanding of the potential effects of different hormonal profiles in cardiorespiratory system when studying physically active women, especially with a focus on the different types of OC.

STUDY V

Menstrual cycle phases influence on cardiorespiratory response to exercise in endurance-trained females

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ABSTRACT

Purpose: The aim of this study was to analyse the impact of sex hormone fluctuations throughout the menstrual cycle on cardiorespiratory response to high-intensity interval exercise in athletes.

Methods: Twenty-one eumenorrheic endurance-trained females performed an interval running protocol in three menstrual cycle phases: early-follicular phase (EFP), late-follicular phase (LFP) and mid-luteal phase (MLP). It consisted of 8 × 3-min bouts at 85% of their maximal aerobic speed with 90-s recovery at 30% of their maximal aerobic speed. To verify menstrual cycle phase, we applied a three-step method: calendar-based counting, urinary luteinizing hormone measurement and serum hormone analysis.

Results: Mixed-linear model for repeated measures showed menstrual cycle impact on ventilatory (EFP: 78.61 ± 11.09; LFP: 76.45 ± 11.37; MLP: 78.59 ± 13.43) and heart rate (EFP: 167.29 ± 11.44; LFP: 169.89 ± 10.62; MLP: 169.89 ± 11.35) response to high-intensity interval exercise ($F_{2.59}$ = 4.300; p = 0.018 and $F_{2.61}$ = 4.648; p = 0.013, respectively). Oxygen consumption, carbon dioxide production, respiratory exchange ratio, breathing frequency, energy expenditure, relative perceived exertion and perceived readiness were unaltered by menstrual cycle phase.

Conclusions: Most of the cardiorespiratory variables measured appear to be impassive by menstrual cycle phases throughout a high-intensity interval exercise in endurance-trained athletes. It seems that sex hormone fluctuations throughout the menstrual cycle are not high enough to disrupt tissues' adjustments caused by the high-intensity exercise. Nevertheless, HR based training programs should consider menstrual cycle phase.

Keywords: sex hormones; estradiol; progesterone; eumenorrheic; high intensity interval exercise; athletes

INTRODUCTION

The natural menstrual cycle is perhaps the second most important biological rhythm, next to the circadian one (Constantini et al., 2005), and it is regulated by the hypothalamic-pituitary-ovarian axis and all hormones involved in it (predominantly, follicle-stimulating hormone [FSH], luteinizing hormone [LH], 17β-estradiol [E2] and progesterone). Despite individual variations, female sex hormones fluctuate fairly predictably over 23–38 days (Janse de Jonge et al., 2019), giving rise to the different phases of the menstrual cycle. The first one is the early-follicular phase (EFP), characterised by low concentrations of sex hormones, which starts at the onset of menstruation. Then, E2 starts to rise throughout the mid-follicular phase, reaching its peak in the late-follicular phase (LFP), followed by the peak in LH and FSH, just prior to ovulation. These hormones drastically decrease after ovulation whereas progesterone starts to increase, achieving its peak in the mid-luteal phase (MLP), coinciding with high levels of E2 as well. Finally, during the late luteal phase all sex hormones drop, starting the cycle again (Janse de Jonge, 2003; Janse de Jonge et al., 2019).

Female sex hormones, specially E2 and progesterone, have receptors in several tissues of the body. Thereby, other than reproductive functions, these hormones may influence many other physiological systems such as hypothalamus, cardiovascular system, kidney tubules, liver, skeletal muscle and adipose tissue (Ashley et al., 2000; Constantini et al., 2005; Janse de Jonge, 2003; Oosthuyse & Bosch, 2010), which may have an impact on females' exercise performance. In this sense, an increase in ventilation (Ve) has been reported in sedentary (MacNutt et al., 2012) and active females (Burrows & Bird, 2000; Goldsmith & Glaister, 2020) as well as an increase in heart rate (HR) in

both, sedentary (Brar et al., 2015) and trained females (Barba-Moreno et al., 2019), during the luteal phase. In addition, higher fat utilization in the luteal phase has been observed in active females, resulting in a lower respiratory exchange ratio (RER) during this phase (Ashley et al., 2000). However, other studies concluded no impact of menstrual cycle on maximal oxygen consumption (VO_2max), Ve, RER, lactate and HR in physically active females (Gordon et al., 2018; Packard et al., 2011; Vaiksaar et al., 2011).

These conflicting findings may be explained by methodological shortcomings, mainly the measurements trials carried out in different moments of the menstrual cycle since it has been divided into two (Packard et al., 2011; Vaiksaar et al., 2011), three (Barba-Moreno et al., 2019; Goldsmith & Glaister, 2020) or four (Gordon et al., 2018; MacNutt et al., 2012) phases. An additional limitation is the menstrual cycle verification, as studies often rely on calendar counting (Ashley et al., 2000; Gordon et al., 2018; Packard et al., 2011) or measuring body basal temperature (Ashley et al., 2000), and it is well known than these methods are not accurate enough and should be accompanied by urinary LH tests and serum sex hormone verification, as a recent review concluded (Janse de Jonge, 2003). Therefore, the aim of this investigation was to assess the influence of sex hormone fluctuations throughout the menstrual cycle on cardiorespiratory response to high intensity interval exercise. Based on previous literature, we hypothesis that cardiorespiratory response to exercise is altered by sex hormones fluctuations over the menstrual cycle in endurance-trained females.

MATERIAL AND METHODS

Participants

A total of twenty-one eumenorrheic females (age: 30.5±6.5 years; height: 163.1±6.4 cm; body weight: 58.4±8.7 kg; body fat percentage: 25.2%±6.7%; lean mass, considering it as body weight minus fat mass and minus bone mineral content: 70.38%±6.51%; peak oxygen consumption [VO_2peak]: 48.4±4.4 $mL \cdot min^{-1} \cdot kg^{-1}$) participated in this study. They had regular menstrual cycle, occurring from 23 to 38 days in length during the six months prior the study (Janse de Jonge et al., 2019). Concretely, volunteers´ menstrual cycle ranged from 28 ± 2 to 31 ± 2 days in length. All of them were healthy and well-trained (7.4±5.3 years of endurance training experience with a training volume of 295.9±183.6 min per week during the 6 months prior to recruitment), in endurance activities such as running, obstacle races, triathlon and cycling. Participants were required to meet the following criteria: (a) healthy adult females between 18 and 40 years old; (b) presenting with healthy iron parameters (serum ferritin >20 μg/L, haemoglobin >115 μg/L and transferrin saturation >16%); (c) performing endurance training between 3 and 12 h per week. Exclusion criteria included: (a) irregular menstrual cycles; (b) oral contraceptive use; (c) menopause; (d) smoking; (e) metabolic or hormonal disorder; (f) medication or dietary supplements that alter vascular function (e.g., tricyclic antidepressants, α-blockers, β-blockers, etc.); (g) any surgical interventions (e.g., ovariectomy); (h) pregnancies in the year preceding; (i) any musculoskeletal injury in the last six months. At the start of the data collection, all participants conducted a questionnaire gathering information about training experience, health status, dietary supplements and menstrual cycle aspects. All participants were informed about the procedures and risks involved and informed consent was provided by each participant.

The experimental protocol was approved by the Institutional Ethics Committee and is in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki).

Study Design

The present work is part of the IronFEMME study, an observational cross-sectional study performed by physically active and healthy women. The project consisted of two sections carried out at the same time: iron metabolism (Study I, which exercise protocol was an interval running test) and muscle damage (Study II, which protocol was based on a resistance exercise trial). Concretely, the present work shows data from Study I.

Participants came to our laboratory on four occasions. The initial screening visit was conducted during the EFP (i.e., between 2nd and 5th day of the menstrual cycle with day 1 being onset of menstrual bleeding). Volunteers came to our laboratory between 8 and 10 a.m. in a rested and overnight fasted state. Volunteers did not perform moderate or vigorous physical activity and did not take caffeine, alcohol or any supplementation 24 h prior to the screening day. Firstly, they signed all the informed consents and participant´s weight and height were recorded. Then, baseline blood samples were collected, for a complete blood count, genetic testing, biochemistry and hormonal analyses. Subsequently, an absorptiometry by dual-energy X-ray (DXA) was done. This screening session was completed with a maximal aerobic ramp test on a computerized treadmill (H/P/COSMOS 3PW 4.0, H/P/Cosmos Sports & Medical, Nussdorf-Traunstein, Germany) to determine their VO_2peak. Expired gases were measured breath-by-breath with the gas analyser Jaeger Oxycon Pro (Erich Jaeger, Viasys Healthcare, Friedberg,

Germany) for which validity and reliability have been previously demonstrated (James Carter & Asker E Jeukendrup, 2002; Ø. Foss & J. Hallen, 2005). Heart response was continuously monitored with a 12-lead ECG. Participants began with a warm-up of 3 min at 6 km/h. Once the warm-up finished, the speed was set at 8 km/h and then increased by 0.2 km/h every 12 s until exhaustion. A slope of 1% was set throughout the test to simulate air resistance (Goldsmith & Glaister, 2020). The maximal aerobic speed was considered as the minimum speed required to elicit the VO_2peak (Veronique Billat et al., 1994). To verify that VO_2peak was reached, a confirmatory test was carried out as suggested in previous studies (P. Nolan et al., 2014; David C Poole & Andrew M Jones, 2017) after a 5 min recovery of the maximal aerobic test (David C Poole & Andrew M Jones, 2017). The speed equivalent to 85% of the maximal aerobic speed was calculated to use in the interval running protocol.

After this screening day, participants attended the laboratory to perform the interval running protocol in three different menstrual cycle phases: EFP (day 3.43 ± 0.93), LFP (day 11.95 ± 2.54), and MLP (day 21.86 ± 3.05). In addition, the average day of the positive result in the LH test was 14.02 ± 2.55. In order to avoid learning effects that could influence our results, the order of these running protocols was randomized, and in no case, an order involved evaluating a volunteer in more than two cycles: EFP-LFP-MLP; LFP-MLP-EFP; MLP-EFP-LFP; LFP-EFP-MLP; EFP-MLP-LFP.

Interval Running Protocol

To avoid diurnal variability (Janse de Jonge, 2003), participants came to the laboratory between 8 and 10 a.m., after abstaining from alcohol or caffeine consumption

and any intense physical activity or sport practice the 24 h prior the testing day. Nutritional recommendations were provided to the participants by a nutritionist in order to standardize the diet, and volunteers followed these 24h prior to every test. In addition, participants replicated the same breakfast in each protocol performed in the different menstrual cycle phases. Figure 22 shows the protocol of the testing procedure day. Firstly, a blood sample was collected to analyze sex hormones, followed by a standing blood pressure (BP) measurement, using the auscultatory method with a calibrated sphygmomanometer. Subsequently, participants started the interval running protocol consisting of a 5 min warm-up at 60% of their maximal aerobic speed followed by 8 bouts of 3 min at 85% of their maximal aerobic speed with 90-s recovery at 30% of their maximal aerobic speed between bouts. Finally, 5 min cool down was performed at 30% of their maximal aerobic speed. During this protocol, Ve, breathing frequency (BF), VO_2, carbon dioxide production (VCO_2), RER, HR and energy expenditure (EE) were continuously measured using the same apparatus as mentioned for the maximal aerobic test. Cardiorespiratory values were obtained as the mean of the 5 min warm-up, as well as the mean of the 5 min cool down. Likewise, values over the interval running protocol were elicited as the mean of the 3 min high intensity intervals and the mean of the 90-s recovery intervals.

Additionally, rate of perceived exertion (RPE) and perceived readiness (PR) were respectively measured by RPE Borg 6–20 scale (Gunnar Borg, 1970) and PR Nurmekivi 1–5 scale (Ants Nurmekivi et al., 2001). Participants were asked for RPE in the last 5 s of warm-up and every running bout, and at the end of the cool down. PR scale was applied in the last 5 s of warm-up and active recovery intervals from 1 to 7, and at the end of the cool down.

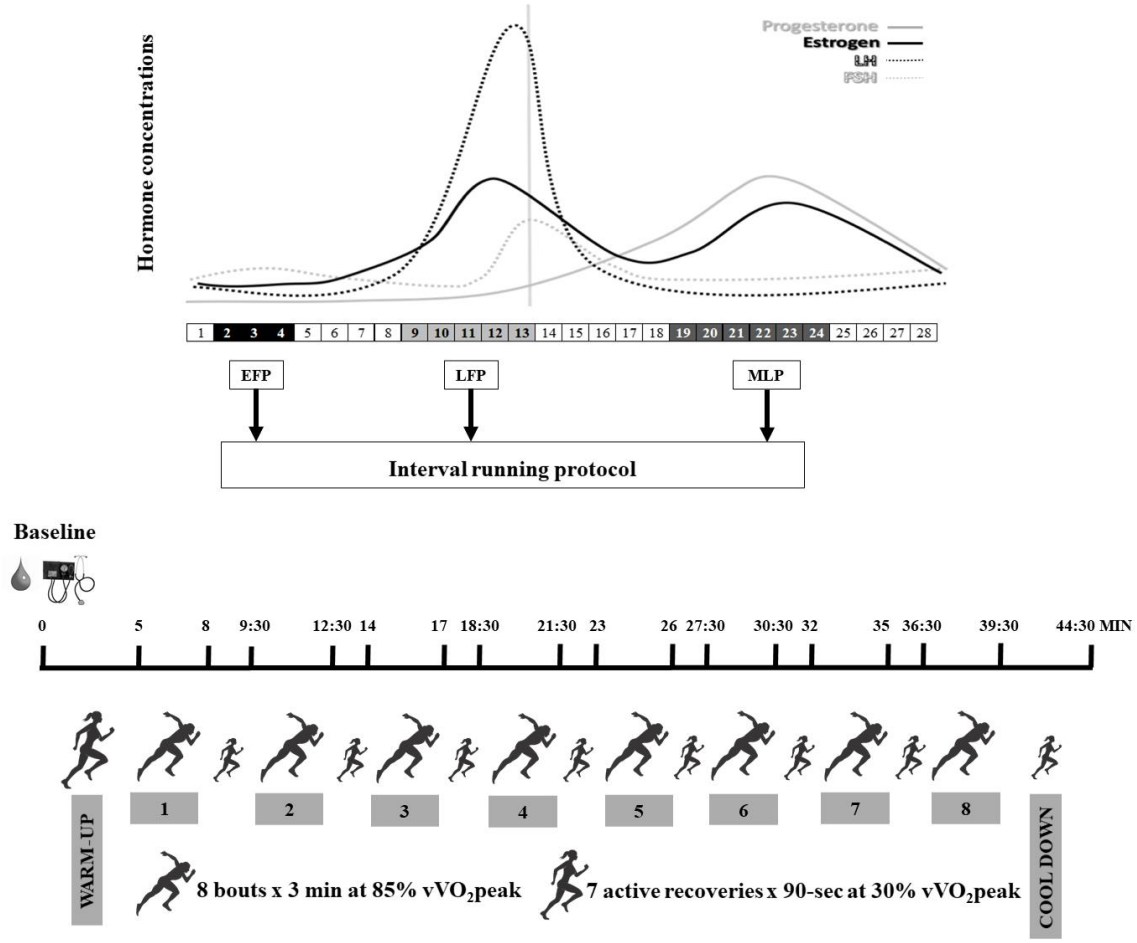

Figure 22: Protocol of the testing procedure day. EFP: early-follicular phase; LFP: late-follicular phase; MLP: mid-luteal phase; v VO_2peak: maximal aerobic speed.

Menstrual Cycle Monitoring and Phase Determination

Considering the first day of the cycle the onset of menstruation, the days of testing were: between the 2nd and the 5th day of the cycle for the EFP, between one and three days before the ovulation day for the LFP and between five and nine days following ovulation for the MLP. These three specific phases were selected in order to analyse different hormonal environments as literature suggests (Janse de Jonge et al., 2019;

Schaumberg et al., 2017): low E2 and progesterone levels in the EFP, low progesterone but high E2 levels in the LFP and elevated levels of both progesterone and E2 in the MLP. In order to meet this and based on the literature (Janse de Jonge, 2003; Janse de Jonge et al., 2019; Schaumberg et al., 2017), we applied a three-step method: calendar-based counting, urinary LH measurement and serum hormone analysis.

Firstly, participants were asked to record information about the length of their last six menstrual cycles. These data were provided to a gynaecologist, who confirmed the menstrual cycles were regular and estimated the ovulation day (the middle day of the menstrual cycle ± 1) as well as the menstrual cycle phases. Then, a hormone ovulation predictor kit (Ellatest, Alicante, Spain) was used to identify the surge of LH in urine. Second morning mid-stream urine sample was collected day to day from three to five days before LFP protocol until LH surge detection, which occurs 14–26 h before ovulation (Janse de Jonge et al., 2019). If LH surge was not detected or was detected more than 3 days after completion of LFP test, this test was discarded and the dates for the LFP test were recalculated to repeat it. Finally, serum sex hormones (LH, FSH, E2 and progesterone) were measured in each of the menstrual cycle phases selected for the study. Minimum progesterone was set at 16 $nmol \cdot L^{-1}$ in the MLP as a reliable indicator of an ovulatory non luteal phase-deficient cycle (Janse de Jonge et al., 2012; Romero-Parra et al., 2020; Vaiksaar et al., 2011).

Blood Samples Analyses

To avoid diurnal variability (Janse de Jonge, 2003), blood samples were taken at the same time for all volunteers, between 8–10 a.m. They were obtained with

venipuncture into a vacutainer containing clot activator. Following inversion and clotting, the whole blood was centrifuged (LMC-3000 version V.5AD, Biosan, Riga, Latvia) for ten minutes at 3000 rpm. After that, serum was transferred into eppendorf tubes and stored frozen at −80 °C until further analysis. Within 1 to 15 days after testing, the serum samples were delivered to the clinical laboratory of the Spanish National Centre of Sport Medicine (Madrid, Spain) to determine sex hormones in order to verify hormonal profiles. Total E2, progesterone, FSH and LH were measured via ADVIA Centaur ® solid-phase competitive chemiluminescent enzymatic immunoassay (IMMULITE 1000 system; Siemens Healthineers AG, Munich, Germany). Inter- and intra-assay coefficients of variation (CV) reported by the laboratory for each variable were, respectively: 11.9% and 8.5% at 93.3 pg/mL and 6.8% and 4.7% at 166 pg/mL for E2; 23.1% and 11.8% at 0.7 ng/mL and 5.2% and 2.5% at 9.48 ng/mL for progesterone, 5.3% and 1.8% at 1.2 mIU/mL for FSH and 5.2% and 1.8% at 0.54 mIU/mL for LH.

Statistical Analysis

Data are presented as mean, standard deviation of the mean (±SD) in tables and standard error of the mean (±SEM) in figures. A Shapiro-Wilk test to assess the normal distribution of the variables was conducted. A linear mixed model for repeated measures was used to analyze menstrual cycle phases (EFP, LFP and MLP), time of measurement (bouts and active recovery intervals) and time* menstrual cycle phase effects on performance variables (HR, VO_2/kg, VCO_2, RER, Ve, BF, EE, RPE, PR). However, the focus of the analysis is on changes over the menstrual cycle phases and the paper will not report changes over time within the protocol. Bonferroni post-hoc tests were conducted where significant differences were found in any of the analyzed factors. Additionally, a

non-parametric Friedman ANOVA for repeated measures was performed to analyze differences in sex hormone concentrations, resting BP, warm-up and cool down variables among the menstrual cycle phases tested. A non-parametric Wilcoxon signed-rank test was performed to obtain post-hoc pairwise comparisons where significant differences were found. Effect sizes were calculated to assess the magnitude of effect in the changes found for non-parametric pairwise comparisons using coefficient r (Rosenthal, 1991), while for Bonferroni post-hoc comparisons, Cohen´s d (Cohen, 2013) were calculated to assess the magnitude of effect in the changes found. In order to unify the effect size under a sole coefficient, r values were converted to d values as proposed by Rosenthal (Rosenthal, 1991). Threshold values were set as small (≥0.2 and <0.5), moderate (≥0.5 and <0.8) and large (≥0.8) (Cohen, 2013). Confidence intervals (95% CI) were also calculated. Statistical significance was set at $p < 0.05$ and all procedures were conducted with SPSS software 21 version (IBM Corp., Armonk, NY, USA).

RESULTS

Firstly, sex hormone concentrations throughout the menstrual cycle phases tested (Table 13) showed significant differences for LH, FSH, E2, progesterone and E2/progesterone ratio.

Baseline

At rest neither SBP (EFP: 106.15 ± 8.44, LFP: 109.00 ± 12.41 and MLP: 111.00 ± 8.97 mmHg) nor DBP (EFP: 65.75 ± 7.66, LFP: 67.75 ± 10.94 and MLP: 65.55 ± 7.56 mmHg) showed differences over the menstrual cycle phases ($c^2 = 5.344$; $p = 0.069$ and $c^2 = 0.781$; $p = 0.677$, respectively). In addition, no difference in initial RPE ($c^2 = 0.269$;

$p = 0.874$) was found among testing days (EFP: 6.95 ± 1.43, LFP: 6.95 ± 1.16 and MLP: 7.05 ± 1.32).

Table 13: Sex hormone concentrations (Mean±SD) on the testing days

	EFP	LFP	MLP	c^2	*p*
LH (mUI/mL)	7.27±3.91	12.56±8.29	5.96±3.26	9.810	0.007[1]
FSH (mUI/mL)	9.14±8.49	6.17±2.95	3.44±1.53	30.095	<0.001[2]
E2 (pg/mL)	38.78±30.39	186.67±154.56	138.11±71.99	25.810	<0.001[3]
Progesterone (ng/mL)	0.33±0.19	0.75±1.79	11.99±5.37	27.494	<0.001[4]
E2/progesterone ratio	0.15±0.17	0.53±0.54	0.03±0.08	26.571	<0.001[5]

EFP: early-follicular phase; LFP: late-follicular phase; MLP: mid-luteal phase; LH: luteinizing hormone; FSH: follicle-stimulating hormone; E2: 17β-estradiol

[1] Significant differences in LFP compared to MLP (p=0.006, d=1.08, CI=0.41 to 1.75).
[2] Significant differences in MLP compared to EFP (p<0.001, d=2.76, CI=1.83 to 3.69) and LFP (p<0.001, d=1.58, CI=0.89 to 2.27).
[3] Significant differences in EFP compared to LFP (p<0.001, d=1.79, CI=0.99 to 2.59) and MLP (p<0.001, d=1.91, CI=1.16 to 2.66).
[4] Significant differences in MLP compared to EFP (p<0.001, d=1.79, CI=0.97 to 2.61) and LFP (p<0.001, d=1.91, CI=1.08 to 2.74).
[5] Significant differences in MLP compared to EFP (p=0.004, d=1.16, CI=0.44 to 1.89) and LFP (p<0.001, d=2.54, CI=1.51 to 3.57).

Warm-up

Most of the measured variables reported to be steady over the different menstrual cycle phases over the warm-up (Table 14), although Ve, VO_2/kg, EE, and PR showed significant difference among menstrual cycle phases. Specifically, lower values of Ve were found in the LFP compared to the EFP (p = 0.034, d = 0.85, CI = 0.56 to 1.14) and MLP (p = 0.001, d = −1.36, CI = −1.84 to −0.88). Moreover, both VO_2/kg and EE exhibited lower values in the LFP than in the EFP (p = 0.013, d = 0.98, CI = 0.58 to 1.39 and p = 0.008, d = 1.05, CI = 0.70 to 1.40, respectively). However, post-hoc pairwise comparisons reported no significant differences among menstrual cycle phases for PR.

Table 14: Performance variables throughout the warm-up across the MC phases.

	EFP	LFP	MLP	c^2	*p*
$\dot{V}e$ (L/min)	48.2±8.7	46.7±8.3	49.0±8.5	13.900	0.001$^{\beta}$
BF (breaths/min)	32.6±6.4	32.7±5.6	33.3±5.9	1.200	0.549
$\dot{V}O_2$/Kg (mL/kg/min)	29.1±2.6	28.0±2.3	28.5±2.3	8.100	0.017$^{\#}$
$\dot{V}CO_2$ (mL/min)	1481.2±215.6	1426.7±202.4	1443.7±189.8	3.600	0.165
RER	0.88±0.05	0.88±0.05	0.88±0.05	0.105	0.949
HR (bpm)	136.0±12.8	136.6±12.2	136.1±16.2	2.923	0.232
EE (Kcal/day)	11834.8±1521.8	11377.7±1598.5	11571.3±1574.3	9.100	0.011$^{\#}$
RPE	9.3±1.8	9.3±2.0	9.5±2.3	0.847	0.655
PR	4.9±0.3	4.6±0.5	4.6±0.5	8.970	0.011

MC: menstrual cycle; EFP: early-follicular phase; LFP: late-follicular phase; MLP: mid-luteal phase; $\dot{V}e$: ventilation; BF: breathing frequency; $\dot{V}O_2$: oxygen consumption; $\dot{V}CO_2$: carbon dioxide production; RER: respiratory exchange ratio; HR: heart rate; EE: energy expenditure; RPE: rate of perceived exertion; PR: perceived readiness.

$^{\beta}$ Significant differences in LFP compared to EFP and MLP.
$^{\#}$ Significant differences in LFP compared to EFP.

Interval Running Protocol

Most variables measured throughout the high intensity exercise, bouts reported to be unchanged when studying menstrual cycle phases and time* menstrual cycle interaction. Even though Ve reported a main effect of menstrual cycle phase, post-hoc pairwise comparisons did not show significant differences among menstrual cycle phases. Additionally, HR exhibited lower values in the EFP compared to the LFP ($p = 0.016$, d = 1.06, CI = 0.41 to 1.71). Menstrual cycle phase and time* menstrual cycle phase effects are shown in Figure 23.

According to the active recoveries throughout the interval running protocol, menstrual cycle phase and time* menstrual cycle interaction showed no effect on cardiorespiratory variables, except for Ve, which reported lower values in the LFP compared to the EFP ($p = 0.019$, d = −0.53, CI = −0.97 to 0.10) and the MLP ($p = 0.019$, d = −0.42, CI = −0.85 to 0.02). Figure 24 shows results regarding menstrual cycle phase and time* menstrual cycle phase effects.

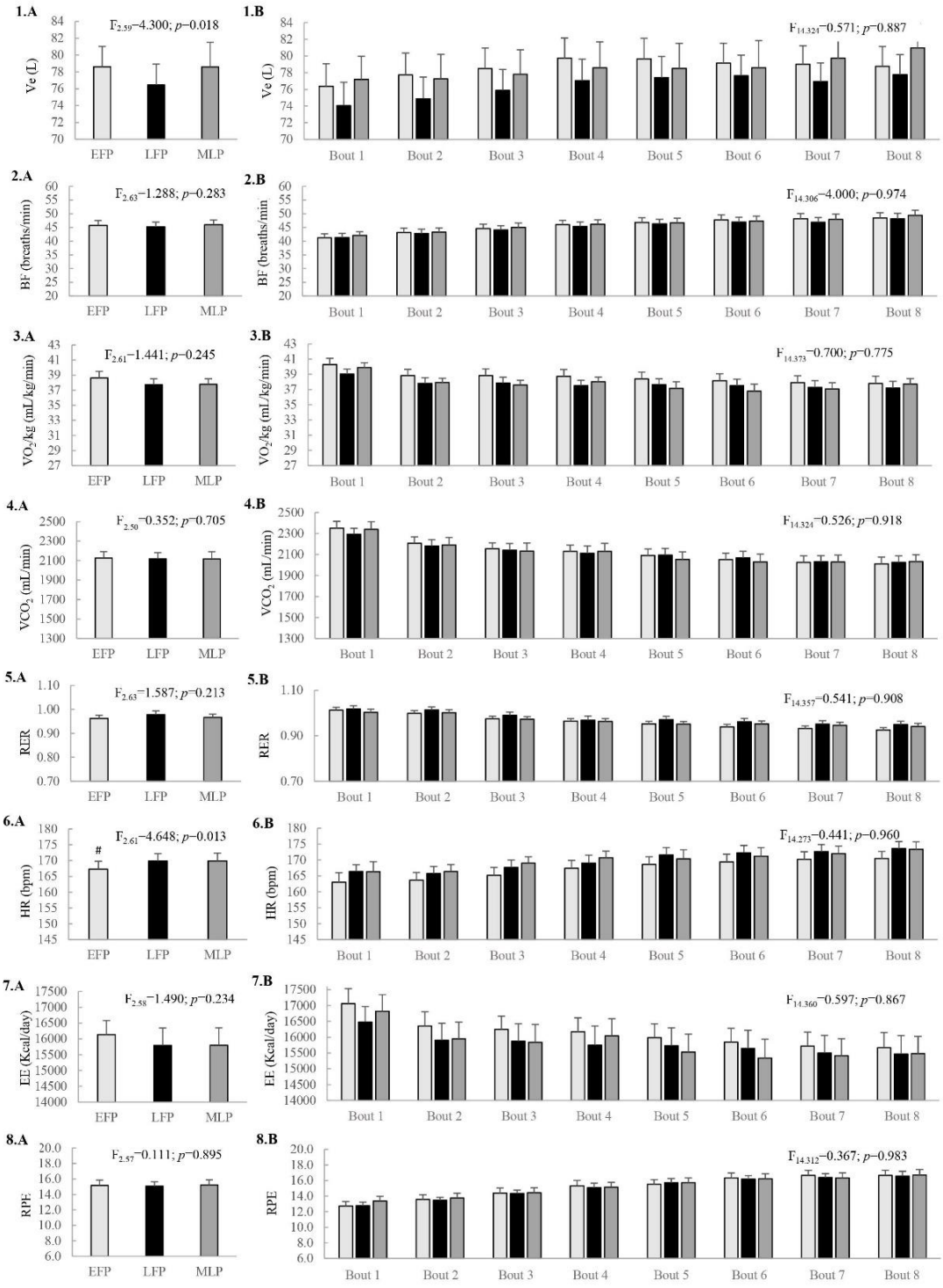

Figure 23: Menstrual cycle phase effect (A) and time* menstrual cycle interaction (B) on performance variables in the bouts throughout the interval running protocol. Ve: ventilation; BF: breathing frequency; VO_2: oxygen consumption; VCO_2: carbon dioxide production; RER: respiratory exchange ratio; HR: heart rate; EE: energy expenditure; RPE: rate of perceived exertion; EFP: early-follicular phase; LFP: late-follicular phase; MLP: mid-luteal phase. # Significant differences in LFP compared to EFP.

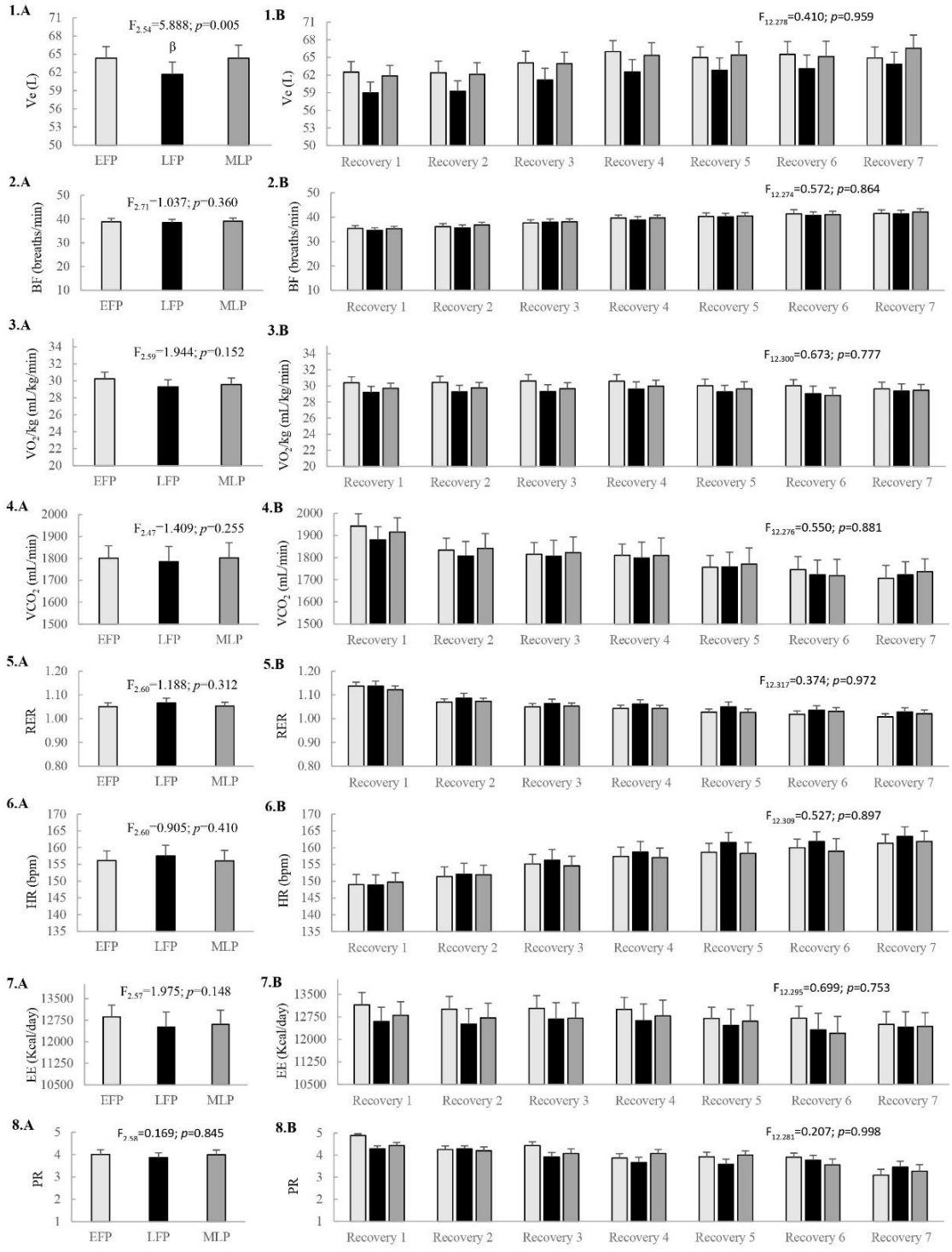

Figure 24: Menstrual cycle phase effect (A) and time* menstrual cycle phase interaction (B) on performance variables in the active recoveries throughout the interval running protocol. Ve: ventilation; BF: breathing frequency; VO_2: oxygen consumption; VCO_2: carbon dioxide production; RER: respiratory exchange ratio; HR: heart rate; EE: energy expenditure; PR: perceived readiness; EFP: early-follicular phase; LFP: late-follicular phase; MLP: mid-luteal phase. $^{\beta}$ Significant differences in LFP compared to EFP and MLP.

Cool Down

Lastly, the response during the cool down (Table 13) were no different among menstrual cycle phases except for the following variables: Ve, VCO_2, BF and EE. Concretely, Ve, VCO_2 and EE showed lower values in the LFP than in the MLP (p = 0.008, d = −1.05, CI = −1.54 to −0.56; p = 0.013, d = −0.98, CI = −1.41 to −0.54; and p = 0.022, d = −0.91, CI = −1.30 to −0.52; respectively). In addition, lower values of BF were found during the EFP compared to the MLP (p = 0.033, d = −0.85, CI = −1.19 to −0.51).

Table 15: Performance variables throughout the cool down across the MC phases.

	EFP	LFP	MLP	c^2	*p*
$\dot{V}e$ (L/min)	43.2±6.4	42.6±6.1	45.9±6.0	10.048	0.007£
BF (breaths/min)	37.2±6.5	37.7±6.4	38.9±6.1	6.723	0.035γ
$\dot{V}O_2$/Kg (mL/kg/min)	19.5±2.7	19.0±2.5	1975±2.0	3.900	0.142
$\dot{V}CO_2$ (mL/min)	1069.4±180.8	1058.4±164.9	1109.6±132.0	9.300	0.010£
RER	0.94±0.06	0.97±0.08	0.97±0.07	2.947	0.229
HR (bpm)	137.9±15.2	138.5±13.6	137.3±13.6	0.824	0.662
EE (Kcal/day)	8046.8±1304.3	7865.7±1390.3	8136.7±1117.6	7.300	0.026£
RPE	9.8±2.9	9.4±2.0	10.0±2.5	2.596	0.273
PR	4.1±1.1	4.3±0.7	4.0±1.0	5.056	0.080

MC: menstrual cycle; EFP: early-follicular phase; LFP: late-follicular phase; MLP: mid-luteal phase; $\dot{V}e$: ventilation; BF: breath frequency; $\dot{V}O_2$: oxygen consumption; $\dot{V}CO_2$: carbon dioxide production; RER: respiratory exchange ratio; HR: heart rate; EE: energy expenditure; RPE: rate of perceived exertion; PR: perceived readiness.

£ Significant differences in LFP compared to MLP.
γ Significant differences in EFP compared to MLP.

DISCUSSION

The hypothesis of the present investigation is that cardiorespiratory response to exercise is altered by sex hormones fluctuations across the menstrual cycle in endurance-trained females. Our hypothesis has been confirmed since the main finding was that menstrual cycle phase effect on Ve and HR when performing a high intensity interval running exercise.

The present study showed a menstrual cycle phase impact on Ve throughout the warm-up, the interval running protocol and the cool down, whereas post-hot comparisons were not statistically different. Outcomes from the present study are supported by previous research, which observed menstrual cycle effect on this variable. Specifically, elevated values of Ve during the MLP compared to the LFP (Goldsmith & Glaister, 2020) and to the EFP (Williams & Krahenbuhl, 1997) were reported. Authors from these studies agree in the fact that increments in cardiorespiratory variables occur during the MLP due to progesterone´s peak in this phase. On the one hand, there is a strong basis in evidence that high levels of progesterone enhance the chemosensitivity of the hypothalamus chemoreceptors, lowering the threshold of the medullary respiratory centre, and this in turn increases Ve (Boukari et al., 2017; Constantini et al., 2005; Godbole et al., 2016; Goldsmith & Glaister, 2020; Janse de Jonge, 2003; Samsudeen & Rajagopalan, 2016; Williams & Krahenbuhl, 1997), which may be accompanied by a rise in VO_2 (Goldsmith & Glaister, 2020; Williams & Krahenbuhl, 1997). In addition, due to the presence of progesterone receptors in the hypoglossal nuclei, this sex hormone relaxes bronchial smooth muscles and reduces respiratory muscles contractions (Boukari et al., 2017), which may account for the increase in flow rate and Ve (Samsudeen & Rajagopalan,

2016). On the other hand, progesterone has been associated with increments in thermoregulatory setpoint, resulting in a rise in body basal temperature 0.3 to 0.5 °C (Janse de Jonge, 2003). In order to dissipate the heat, a redistribution of blood flow occurs, increasing the blood flow to skin whereas decreases the central one. Hence, an increase in HR (Janse de Jonge, 2003; Mattu et al., 2019) and Ve (Janse de Jonge et al., 2012; MacNutt et al., 2012; Williams & Krahenbuhl, 1997) may take place to maintain the cardiac output.

With regard to the cardiovascular system, even though females from the present study did not exhibit menstrual cycle phase impact neither in the warm-up nor in the cool down, HR reported a main effect of menstrual cycle phase throughout the high intensity intervals. Concretely, HR showed lower values in the EFP compared to the LFP. Likewise, a recent research conducted with endurance trained females reported higher values of HR during the MLP compared to the mid-follicular phase (Barba-Moreno et al., 2019). This study also reported increments in body basal temperature during this phase and, as aforementioned, it may be accompanied by an increase in HR (Janse de Jonge, 2003; Lebrun, 1993) and Ve (Janse de Jonge et al., 2012; MacNutt et al., 2012; Williams & Krahenbuhl, 1997). However, some other previous studies reported no effect of menstrual cycle phase on HR response to exercise. They suggested that the increase in cardiorespiratory strain due to high intensity exercise is greater than any possible increase caused by progesterone. Hence, progesterone effect on this physiological variable may be masked by the high intensity exercise (Barba-Moreno et al., 2019; Janse de Jonge, 2003; Mattu et al., 2019). Thus, discrepancies in results could be related with the intensity of the protocols. Studies reporting no menstrual cycle effect on cardiorespiratory response in trained females (Ve, VO_2 and HR) were carried out with protocols such as 30 min

constant load cycling at the MLSS (Mattu et al., 2019), 40 min running at 75% of their maximal aerobic speed (Barba-Moreno et al., 2019), 15 min incremental rowing ergometer test (Vaiksaar et al., 2011), 15 min incremental running protocol utilizing the Bruce Protocol (Packard et al., 2011) and incremental cycling test to exhaustion (Gordon et al., 2018).

Moving on to sex hormones and females' substrate metabolism, females from the present study exhibited different values of EE over the menstrual cycle phases during the warm-up and cool down. Retrospective studies strongly suggest that E2 promotes fat utilization and glycogen sparing. This sex hormone improves epinephrine and growth hormone levels, which has been associated with increments in hormone sensitive lipase secretion and, therefore, fat free acids release (Ashley et al., 2000; Mattu et al., 2019). Moreover, E2 stimulates adenosine monophosphate kinase (Ashley et al., 2000; Burrows & Bird, 2000; Constantini et al., 2005; Janse de Jonge, 2003; Lebrun, 1993; Mattu et al., 2019; Oosthuyse & Bosch, 2010), leading to an increase in lipid oxidation. In addition, progesterone has been also associated with greater fat utilization (Burrows & Bird, 2000; Packard et al., 2011) and glucose sparing (Burrows & Bird, 2000; Constantini et al., 2005; Lebrun, 1993; Packard et al., 2011). However, outcomes from the interval running protocol revealed steady values of RER and EE when analysing sex hormones fluctuations throughout the menstrual cycle, as previous findings pointed out (Gordon et al., 2018; Vaiksaar et al., 2011). Several researchers concluded that substrate availability, training status and diet may have a greater effect on substrate metabolism than sex hormones (Gordon et al., 2018; Janse de Jonge, 2003; Vaiksaar et al., 2011). In fact, there is a study in which no correlation was found between E2 and RER during submaximal runs neither in the MFP nor in the MLP (Ashley et al., 2000). In whole, complex

physiological adjustments are required during high intensity exercise in order to meet physiological demands; so that, it seems that sex hormones fluctuations are not sufficient to disrupt these adjustments. Besides, endurance training cause adaptations which might outweigh any potential differences to sex hormones (Ashley et al., 2000). In this sense, the lack of menstrual cycle impact on substrate metabolism could be related with volunteers training status, since they were physically active females (>30 min per day, 3 days per week) (Gordon et al., 2018), national and international cyclist athletes (Vaiksaar et al., 2011), recreationally trained cyclists (Vaiksaar et al., 2011) and endurance well-trained in the present study. Moreover, the absence of correlation between E2 and RER during submaximal runs was also observed in endurance athletes (40km of running per week or performing equivalent aerobic exercise such as cycling or swimming) (Ashley et al., 2000).

The current study attempts to address a gap in the research through investigation of cardiorespiratory performance in well-trained females. The strengths of our study included its robust methodology, highlighting an accurate menstrual cycle verification, specific hormonal environments selected for the testing days and a homogeneous eumenorrheic group (well-trained and healthy females). Nonetheless, the present study has some limitations such us the uncontrolled of volunteers´ ethnic, daily habits, stress and motivation that may have altered our findings. It should be noted that different hormonal profiles such as menopause, postmenopause and oral contraceptive use might be also interesting to analyse.

CONCLUSIONS

The status of the current research suggests that sex hormone fluctuations throughout the menstrual cycle appear not to be high enough to disrupt physiological adjustments caused by high intensity interval exercise. However, Ve and HR seem to be the most altered variables across the menstrual cycle and, therefore, HR based training programs should consider menstrual cycle phase. Nonetheless, due to high variability in sex hormones concentrations between subjects and from day to day within subjects during any particular phase, individual considerations should be taken into account when training females. Besides, in order to enable a better understanding, further research regarding the effect of the menstrual cycle on cardiorespiratory response and adaptation to exercise is warranted.

STUDY VI

Oral contraceptive phase influence on cardiorespiratory response to exercise in endurance-trained females

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ABSTRACT

Purpose: The aim of this study was to analyse the cardiorespiratory response to exercise throughout an oral contraceptive (OC) cycle in endurance-trained females.

Methods: Sixteen low-dose monophasic-OC users performed an interval running protocol. It consisted of 8x3-min bouts at 85% of their maximal aerobic speed ($v\dot{V}O_2peak$) with 90-seconds recovery at 30% $v\dot{V}O_2peak$ in two OC phases: withdrawal phase (WP) and active pill phase (APP). The non-parametric Wilcoxon test was applied to analyze differences ($p<0.05$) in sex hormones and other performance variables between OC cycle phases.

Results: Throughout the high-intensity intervals, a higher ventilation (WP: 80.90±11.49, APP: 83±13.33 L/min; $p<0.001$) and relative perceive exertion (WP: 14.51±2.58, APP: 15.11±3.11; $p=0.001$) during the APP were found, whereas carbon dioxide production (WP: 2040.92±262.93, APP: 2010.25±305.68 mL/min; $p=0.003$) was higher in the WP. Besides, during the active-recovery intervals, ventilation (WP: 65.78±9.90, APP:67.88±12.66 L/min; $p<0.001$) was higher in the APP, while heart rate (WP: 159.93±10.26, APP:159±12.83 bpm; $p=0.029$) was higher in the WP.

Conclusion: A drive in ventilation occurs during the APP, which is accompanied by a higher perceived exertion. Therefore, coaches and athletes should be aware of these variations, specially perceived exertion, regarding females´ training programs in order to improve their performance, wellness and adherence to physical activity.

Key words: sex hormones, ethinyl estradiol, progestins, exercise, ventilation, athletic performance.

INTRODUCTION

The intake of exogenous sex hormones (ethinyl estradiol and progestin) throughout the oral contraceptive (OC) cycle promotes a negative feedback loop that switches off the hypothalamic-pituitary-gonadal axis, supressing the secretion of follicle-stimulating hormone (FSH) and luteinizing hormone (LH) from the pituitary gland and, therefore, inhibiting the ovulation (Burrows & Peters, 2007; Sims & Heather, 2018). Besides, the inhibition of the hypothalamic-pituitary-gonadal axis downregulates the secretion of endogenous sex hormones (17 β-estradiol [E2] and progesterone) (Burrows & Peters, 2007; Sims & Heather, 2018). Monophasic OC pills, the most common ones, provide the woman seven days of inactive pills or placebo (withdrawal phase [WP]), followed by 21 days of constant dosages of both exogenous sex hormones (active pill phase [APP]) (Burrows & Peters, 2007; Mattu et al., 2019; Rechichi et al., 2009). Notably, ethinyl estradiol remains in plasma for up to two days after ingestion, whilst progestin is detectable for up to five days. Thus, early in the WP both endogenous sex hormones continue supressed, but later in this phase E2 levels increase while progesterone levels remain inhibited (Rechichi et al., 2008; Rechichi et al., 2009).

Exogenous sex hormones may extend beyond the reproductive system and acct over the cardiovascular, respiratory and metabolic systems (Sims & Heather, 2018). On the one hand, there is a strong basis in propose that ethinyl estradiol enhances lipid oxidation and glucose sparing (Burrows & Peters, 2007; Mattu et al., 2019; Packard et al., 2011). On the other hand, progestin has been associated with increments in body basal temperature of approximately 0.3 to 0.5ºC, promoting blood flow redistribution towards the skin in order to dissipate the heat (Burrows & Peters, 2007; Constantini et al., 2005; Packard et al., 2011). Hence, a decrease in central blood flow occurs, leading to a rise in

heart rate (HR) to maintain cardiac output (Constantini et al., 2005). Previous literature reported a higher sensitivity of the hypothalamus chemoreceptors with the administration of progestin, giving rise to an increase in chemosensitivity to hypoxia and hypercapnia. Thus, ventilation ($\dot{V}e$) and respiratory drive is elevated (Cagnacci et al., 2009; Packard et al., 2011; Rechichi et al., 2008; Rechichi et al., 2009; Winkler & Sudik, 2009).

Due to the ethinyl estradiol and progestin influence on female´s physiology, cardiorespiratory response to exercise may be altered throughout the OC cycle. Previous literature reported no differences in maximal oxygen consumption ($\dot{V}O_2max$), $\dot{V}e$ and HR in active females throughout their OC cycle (Burrows & Peters, 2007; Casazza et al., 2002; Gordon et al., 2018; Mattu et al., 2019; Packard et al., 2011; Rechichi et al., 2008; Vaiksaar et al., 2011). Nonetheless, a review that evaluated the physiology and performance within an OC cycle in athletes indicated potential variations in aerobic capacity due to alterations in $\dot{V}e$ response, as well as in anaerobic capacity based on substrate metabolism and buffering mechanisms alterations (Rechichi et al., 2009). This was supported by a recent study conducted in healthy trained females which showed higher values of $\dot{V}e$ and breathing frequency (BF) in the APP compared to the WP (Barba-Moreno et al., 2019).

These conflicting findings can largely be explained by methodological shortcomings. The main problem is the different testing days used since most authors study OC cycles like a natural menstrual cycle (Gordon et al., 2018; Packard et al., 2011; Vaiksaar et al., 2011). Another key factor in these inconsistent conclusions could be the use of different types of OC pills. Previous literature has been carried out with

monophasic OC users (Barba-Moreno et al., 2019; Mattu et al., 2019; Vaiksaar et al., 2011), triphasic OC users (Casazza et al., 2002), mixing both types in the same analyses (Packard et al., 2011) and even without specifying the type of pill used by the volunteers (Gordon et al., 2018). Furthermore, small sample sizes, variation in training status, performance level of the athletes, and different exercise protocols could also explain variations in experimental findings (Rechichi et al., 2009). Therefore, the aim of this investigation was to assess the influence of sex hormones fluctuations throughout the monophasic OC cycle on cardiorespiratory response to exercise using a robust methodology which highlights a specific hormonal environment selected for the testing days and a homogeneous endurance-trained group.

MATERIAL AND METHODS

Participants

Sixteen females taking low-dose monophasic OC pills participated voluntarily. The sample characteristics are shown in Table 16. During the study period, the active hormone pill (APP) was taken at the same time each day over 21 days, followed by seven days of non-active pill (WP). Participants were using OC pills for the last 4.1±4.3 years. OC pills brands and formulations used by the volunteers were previously described (Alfaro-Magallanes et al., 2020). They were healthy and well-trained in endurance activities such as running, obstacle races, triathlon and cycling. All participants were informed about the procedures and risks involved and signed an informed consent. The experimental protocol was approved by the institutional Ethics Committee and was in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) (Association, 2013).

Table 16: Sample characteristics (n=16).

Age (yrs)	25.3±4.7
Height (cm)	162.4±5.7
Weight (kg)	56.0±5.7
Fat Mass (%)	24.8±6.0
Lean Mass (%)	70.9±5.9
$\dot{V}O_2$peak (ml/kg/min)	47.4±5.5
Experience (yrs)	5.8±5.0
Training volume (min/week)	214.9±170.0

Presented as mean±standard deviation. $\dot{V}O_2$peak: peak oxygen consumption

Participants were required to meet the following criteria: (a) healthy adult females between 18 and 40 years old; (b) taking monophasic contraceptive for a minimum of six months prior to commencement of the study; (c) presenting healthy iron parameters (serum ferritin >20μg/L, haemoglobin >115 g/L and transferrin saturation >16%); (d) performing endurance training between five and 12 h per week. Exclusion criteria included: (a) menopause; (b) smoking; (c) metabolic or hormonal disorder; (d) medication or dietary supplements that alter vascular function (e.g., tricyclic antidepressants, α-blockers, β-blockers, etc.); (e) any surgery interventions (e.g., ovariectomy); (f) pregnancies in the year preceding; or (g) any musculoskeletal injury in the last six months.

Study design

The present work is part of the IronFEMME study, an observational cross-sectional study performed by physically active and healthy females. The complete methodology of this project has been well described in a recent publication (Peinado et al., 2021).

Participants came to our laboratory on three occasions. The initial screening visit was conducted during the WP (i.e., between 3rd and 7th day of the cycle). Volunteers did not perform moderate or vigorous physical activity, intake caffeine or any supplementation 24 h previous the screening visit. Volunteers came to the laboratory between 8 and 10 a.m. in a rested and overnight fasted state. Firstly, they signed the informed consent, and the participant´s weight and height were recorded. Then, baseline blood samples were collected, for a complete blood count, genetic testing, biochemistry and hormonal analyses. Subsequently, an absorptiometry by dual-energy X-ray (DXA) was realized. This screening session was completed with a maximal aerobic ramp test on a computerized treadmill (H/P/COSMOS 3PW 4.0, H/P/Cosmos Sports & Medical, Nussdorf-Traunstein, Germany). Expired gases were measured breath-by-breath with the gas analyser Jaeger Oxycon Pro (Erich Jaeger, Viasys Healthcare, Germany) which validity and reliability has been previously demonstrated (James Carter & Asker E Jeukendrup, 2002; Ø. Foss & J. Hallen, 2005). Heart response was continuously monitored with a 12-lead ECG. Participants began with a warm-up of 3 min at 6 km/h. Once the warm-up finished, the speed was set at 8 km/h and then increased 0.2 km/h every 12 s until exhaustion. A slope of 1% was set throughout the test to simulate air resistance (Goldsmith & Glaister, 2020). To verify that $\dot{V}O_2$peak was reached, a confirmatory test was carried out after a 5 min recovery of the maximal aerobic test (David C Poole & Andrew M Jones, 2017), as suggested in previous studies (P. Nolan et al., 2014; David C Poole & Andrew M Jones, 2017). The confirmatory test consisted of a 3-min warm-up (2 min at 50% and 1 min at 70% of the maximal velocity reached in the maximal aerobic test). After the warm-up, velocity was set at 110% of the maximal velocity reached in the maximal aerobic test until participants´ exhaustion. The $\dot{V}O_2$peak was determined as the mean of the three highest $\dot{V}O_2$ measurements in the maximal

aerobic test if it was not less than 3% compared to the one obtained in the confirmatory trial. If the value was less than 3%, $\dot{V}O_2$peak was calculated as the mean of the three highest $\dot{V}O_2$ values recorded during the last 30-s of the confirmatory trial. The maximal aerobic speed (v$\dot{V}O_2$peak) was recorded as the minimum speed required to elicit $\dot{V}O_2$peak (Veronique Billat et al., 1994). The speed equivalent to 85% of the v$\dot{V}O_2$peak was calculated to use in the interval running protocol.

After this screening day, participants attended to the laboratory to perform the interval running protocol in two different OC cycle phases: the WP (day 4.9±1.8) and the APP (day 22.1±5.0). The specific time points for each phase were: (a) "late WP" for highest ovarian activity, because in combined monophasic OC pills, ethinyl estradiol remains in plasma for up to two days after ingestion, whilst progestin is detectable for up to five days. Thus, early in the WP both endogenous sex hormones continue supressed, but later in this phase E2 levels increase while progesterone levels remain inhibited (Rechichi et al., 2008; Rechichi et al., 2009), and (b) "late APP" for downregulated ovarian activity and higher circulating exogenous hormone concentrations because ethinyl estradiol and progestin concentrations continue to increase during OC intake, reaching a steady state concentration after eight or eleven days of active pill consumption (Blode et al., 2012; Endrikat et al., 2002). In order to avoid learning effects that could influence on results, the order of these running protocols was randomized and counterbalanced, and in no case, an order involved evaluating a volunteer in more than two cycles: WP-APP; APP-WP.

Interval running protocol

To avoid diurnal variability (Janse de Jonge, 2003), participants came to the laboratory between 8 and 10 a.m., abstaining from alcohol, caffeine and any intense physical activity or sport practice the 24 hours prior the testing day. Nutritional recommendations were provided to the participants by a nutritionist in order to standardize the diet and volunteers´ followed them 24h before testing days. In addition, participants replicated the same breakfast in each protocol performed during both OC phases. Figure 25 shows the protocol of the testing procedure day. Firstly, a blood sample was collected to analyze sex hormones, followed by a resting blood pressure (BP) measurement using the auscultatory method with a calibrated sphygmomanometer. Subsequently, participants started the interval running protocol consisted of a 5 min warm-up at 60% of the v$\dot{V}O_2$peak followed by 8 bouts of 3 min at 85% of the v$\dot{V}O_2$peak with 90-s recovery at 30% of the v$\dot{V}O_2$peak between bouts. It is worth mentioning that some volunteers were not able to perform all bouts at 85%, hence intensity was downregulated to 80% or 75%. In this scene, 6 volunteers carried out all bouts at 85%, 1 did the last one at 80%, 2 performed bouts 7 and 8 at 80% and other 2 downregulated the intensity to 80% in bouts 6, 7 and 8. Additionally, 2 females ran bout 6 at 80% while bouts 7 and 8 at 75% and 3 volunteers performed bouts 4, 5 and 6 at 80% whereas bouts 7 and 8 at 75%. Finally, five minutes cool down was performed at 30% of the v$\dot{V}$O2peak. During exercise, $\dot{V}$e, BF, $\dot{V}O_2$, carbon dioxide production ($\dot{V}CO_2$), respiratory exchange ratio (RER), energy expenditure (EE) and HR among others ventilatory variables were continuously measured using the same apparatus as mentioned for the maximal aerobic test. Cardiorespiratory variables were obtained as the mean of the five min warm-up, as well as the mean of the five min cool down. Likewise, values over the interval running protocol were elicited as the mean of the three min high intensity intervals and the mean

of the 90-s recovery intervals. Additionally, rate of perceived exertion (RPE) and perceived readiness (PR) were respectively measured by RPE Borg 6-20 scale (Gunnar Borg, 1970) and PR Nurmekivi 1-5 scale (Ants Nurmekivi et al., 2001). Participants were asked for RPE in the last 5-s of warm-up of every running bout, and at the end of the cool down. PR scale was applied in the last 5-s of warm-up of every active recovery interval, and at the end of the cool down.

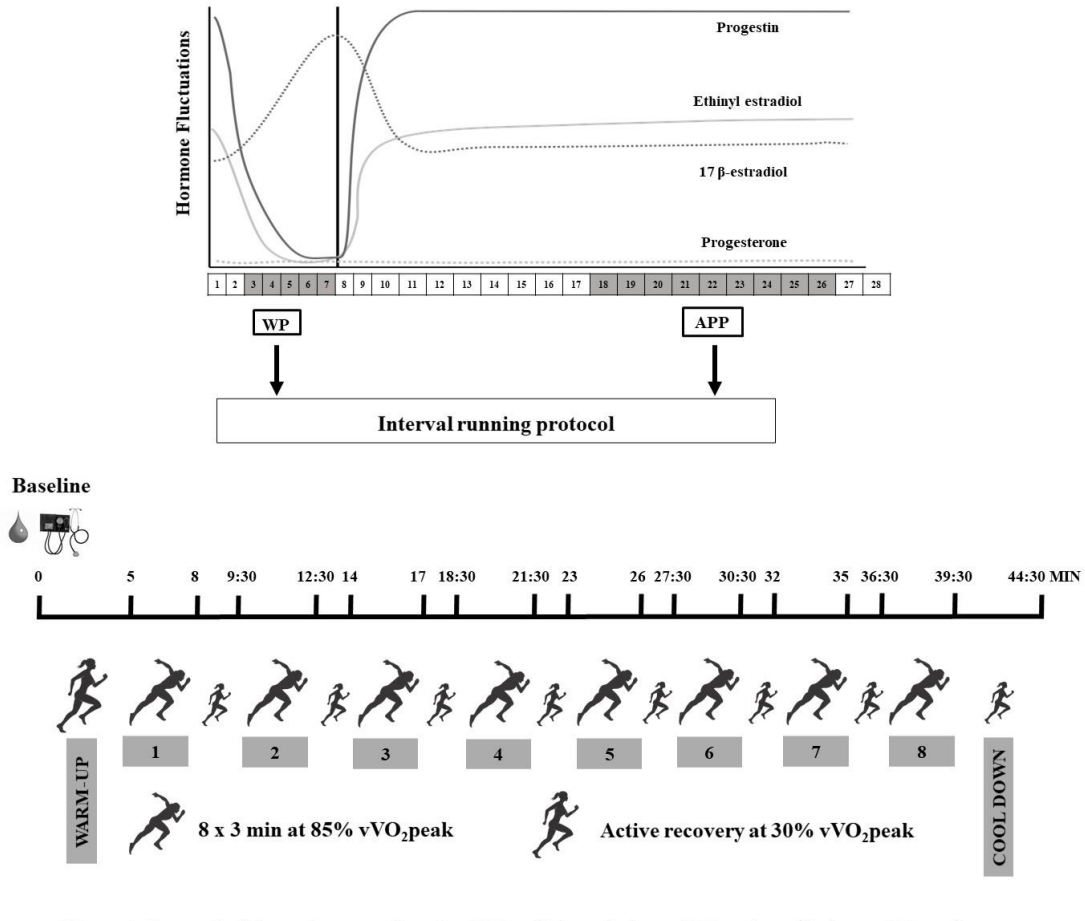

Figure 25: Protocol of the testing procedure day. WP: withdrawal phase; APP: active pill phase; $v\dot{V}O_2peak$: maximal aerobic speed.

Blood samples analyses

As previously mentioned, to avoid diurnal variability (Janse de Jonge, 2003), blood samples were collected at the same time for all volunteers, between 8-10 a.m. They

were obtained by venipuncture into a vacutainer containing clot activator. Following inversion and clotting, the whole blood was centrifuged (Biosan LMC-3000 version V.5AD) for ten minutes at 1610 g. After that, serum was transferred into eppendorf tubes and stored at -80ºC until further analysis. Within one to 15 days after testing, the serum samples were delivered to the clinical laboratory of the Spanish National Centre of Sport Medicine (Madrid, Spain) to determine sex hormones in order to verify hormonal profiles. Total E2, progesterone, FSH and LH were measured via ADVIA Centaur ® solid-phase competitive chemiluminescent enzymatic immunoassay (Siemens city, Germany). Inter- and intra-assay coefficients of variation (CV) reported by the laboratory for each variable were, respectively: 11.9% and 8.5% at 93.3 pg/mL and 6.8% and 4.7% at 166 pg/mL for E2; 23.1% and 11.8% at 0.7 ng/mL and 5.2% and 2.5% at 9.48 ng/mL for progesterone, 5.3% and 1.8% at 1.2 mIU/mL for FSH and 5.2% and 1.8% at 0.54 mIU/mL for LH.

Statistical analysis

Data are presented as mean and standard deviation (±SD) in tables and mean and standard error of the mean (±SEM) in figures. A Shapiro-Wilk test was conducted to assess the normality of the variables. The non-parametric Wilcoxon test was applied to analyze differences between OC cycle phases tested (WP and APP) in terms of sex hormones (FSH, LH, E2 and progesterone**),** resting BP as well as warm-up, high intensity intervals, active recovery intervals and cool down variables (HR, $\dot{V}O_2$/kg, $\dot{V}CO_2$, RER, $\dot{V}e$, BF, EE, RPE and PR. Effect sizes for non-parametric pairwise comparisons were calculated using coefficient r (Rosenthal, 1991). Then, as Rosenthal proposed (Rosenthal, 1991) r values were converted to Cohen´s d values. Threshold values were set as small ($\geq$0.2 and <0.5), moderate ($\geq$0.5 and <0.8) and large ($\geq$0.8) (Cohen, 2013). In addition, 95% confidence intervals (CI) were calculated. Statistical significance was set at $p<0.05$

and all procedures were conducted with SPSS software 21 version (IBM Corp., Armonk, NY, USA).

RESULTS

Firstly, sex hormone concentrations throughout the OC phases tested (Table 17) showed significant differences for LH, FSH and E2, which values were lower during the APP than in the WP. Nonetheless, progesterone levels were unaffected by OC phases.

Table 17: Sex hormone concentrations (mean±SD) on the testing days.

	WP	APP	Z	p	d	CI
LH (mIU/mL)	4.17±4.57	1.79±2.82 *	-2.981	0.03	0.63	-0.08 to 1.34
FSH (mLU/mL)	5.22±4.22	1.51±1.66 *	-3.233	<0.001	1.16	0.41 to 1.91
E2 (pg/mL)	29.36±25.94	7.91±7.24 *	-3.180	0.001	1.22	0.38 to 1.87
Progesterone (ng/mL)	0.33±0.20	0.32±0.12	-0.465	0.642	0.06	-0.63 to 0.75

APP: active pill phase; WP: withdrawal phase; CI: confidence interval; FSH: follicle-stimulating hormone; LH: luteinizing hormone; E2: 17 β-estradiol.

* Significantly different from WP.

Secondly, neither resting systolic BP (WP: 112.81±11.82 and APP: 111.56±6.51 mmHg) nor diastolic BP (WP: 71.56±11.65 and APP: 69.69±8.85 mmHg) showed differences between both OC phases tested (Z=-0.514; *p*=0.607; d=0.13; CI=-0.56 to 0.83 and Z=-0.494; *p*=0.621; d=0.18; CI=-0.51 to 0.88, respectively). Regarding the warm-up (Table 18), cardiorespiratory variables showed no differences between OC phases except for $\dot{V}e$, exhibiting higher values in the APP than in the WP. Lastly, cool down variables (Table 18) reported to be unaffected by OC phase.

Table 18: Performance variables throughout the warm-up and cool down between the OC phases tested.

		WP	APP	Z	p	d	CI
Warm-up	$\dot{V}e$ (L/min)	46.36±6.41	48.69±8.37*	-2.120	0.034	0.31	-1.01 to 0.39
	BF (breaths/min)	33.66±5.33	34.54±4.70	-1.241	0.215	0.18	-0.87 to 0.52
	$\dot{V}O_2$/kg (mL/kg/min)	27.58±2.97	27.41±2.88	-0.827	0.408	0.06	-0.64 to 0.75
	$\dot{V}CO_2$ (mL/min)	1387.74±184.42	1391.20±233.83	-0.310	0.756	0.02	-0.71 to 0.68
	RER	0.884±0.069	0.884±0.070	-0.155	0.877	0.00	-0.69 to 0.69
	HR (bpm)	141.26±20.18	145.30±9.49	-0.874	0.382	0.26	-0.95 to 0.44
	EE (Kcal/day)	11093.53±1431.07	11071.25±1660.58	-0.621	0.535	0.01	-0.68 to 0.71
	RPE	8.44±1.67	8.81±1.97	-0.960	0.337	0.20	-0.90 to 0.49
	PR	4.81±0.51	4.78±0.36	-0.378	0.705	0.07	-0.63 to 0.76
Cool down	$\dot{V}e$ (L/min)	46.02±7.48	47.87±9.63	-1.396	0.163	0.22	-0.91 to 0.48
	BF (breaths/min)	40.52±5.38	41.29±6.24	-1.293	0.196	0.13	-0.83 to 0.56
	$\dot{V}O_2$/kg (mL/kg/min)	18.86±2.34	18.98±2.60	0.000	1.000	0.05	-0.74 to 0.65
	$\dot{V}CO_2$ (mL/min)	1062.47±137.29	1053.89±172.77	-0.414	0.679	0.06	-0.64 to 0.75
	RER	0.992±0.023	0.972±0.091	-0.207	0.836	0.30	-0.40 to 1.00
	HR (bpm)	139.79±10.77	143.72±13.08	-1.789	0.074	0.33	-1.03 to 0.37
	EE (Kcal/day)	7746.24±1038.53	7791.84±1249.21	0.000	1.000	0.04	-0.73 to 0.65
	RPE	8.88±1.96	8.40±1.92	-0.834	0.404	0.25	-0.45 to 0.94
	PR	4.38±0.74	4.33±0.79	-0.365	0.715	0.07	-0.63 to 0.76

OC: oral contraceptive; WP: withdrawal phase; APP: active pill phase; CI: confidence interval; $\dot{V}e$: ventilation; BF: breathing frequency; $\dot{V}O_2$: oxygen consumption; $\dot{V}CO_2$: carbon dioxide production; RER: respiratory exchange ratio; HR: heart rate; EE: energy expenditure; RPE: rate of perceived exertion; PR: perceived readiness.

* Significantly different from WP.

According to the bouts during the interval running protocol (Figure 26), APP reported higher values for $\dot{V}e$ (Z=-4.546; p<0.001; d=0.18; CI=-0.87 to 0.52) and RPE (Z=-3.438; p=0.001; d=0.21; CI=-0.90 to 0.49) and lower values for $\dot{V}CO_2$ (Z=-2.932; p=0.003; d=0.11; CI=-0.59 to 0.80) in comparison with WP. The rest of variables measured during the bouts reported to be unaffected throughout the OC cycle. Comparing each bout in both hormonal conditions, only $\dot{V}e$, $\dot{V}CO_2$ and RPE reported differences. Concretely, $\dot{V}e$ showed higher values in the APP in the 1st and 3rd bout (Z=-2.146; p=0.032; d=0.27; CI=-0.97 to 0.43 and Z=-2.069; p=0.039; d=0.23; CI=-0.92 to 0.47, respectively), $\dot{V}CO_2$ exhibited higher values in the WP in the 6th and 8th bout (Z=-2.068; p=0.039; d=0.27; CI=-0.42 to 0.97 and Z=-2.068; p=0.039; d=0.21; CI=-0.49 to 0.90, respectively) and RPE reported to be higher in the APP in the 5th bout (Z=-2.112; p=0.035; d=0.45; CI=-0.26 to 1.15) (Figure 26).

Considering the active recovery throughout the interval running protocol, Figure 27 shows the comparison of cardiorespiratory variables and perceived readiness on each OC phase. The APP registered higher values for $\dot{V}e$ (Z=-4.145; p<0.001; d=0.19; CI=-0.88 to 0.51) and lower for HR (Z=-2.185; p=0.029; d=0.02; CI=-0.68 to 0.71) than WP. The rest of the parameters measured during the recovery periods showed no variation between OC cycle phases. Regarding OC phase influence in each active recovery interval, $\dot{V}e$ exhibited higher values in the APP in the 1st (Z=-2.120; p=0.034; d=0.30; CI=1.00-0.40), 2nd (Z=-2.172; p=0.030; d=0.28; CI=-0.97-0.42) and 3rd (Z=-2.331; p=0.020; d=0.24; CI=-0.94-0.45) active recovery interval.

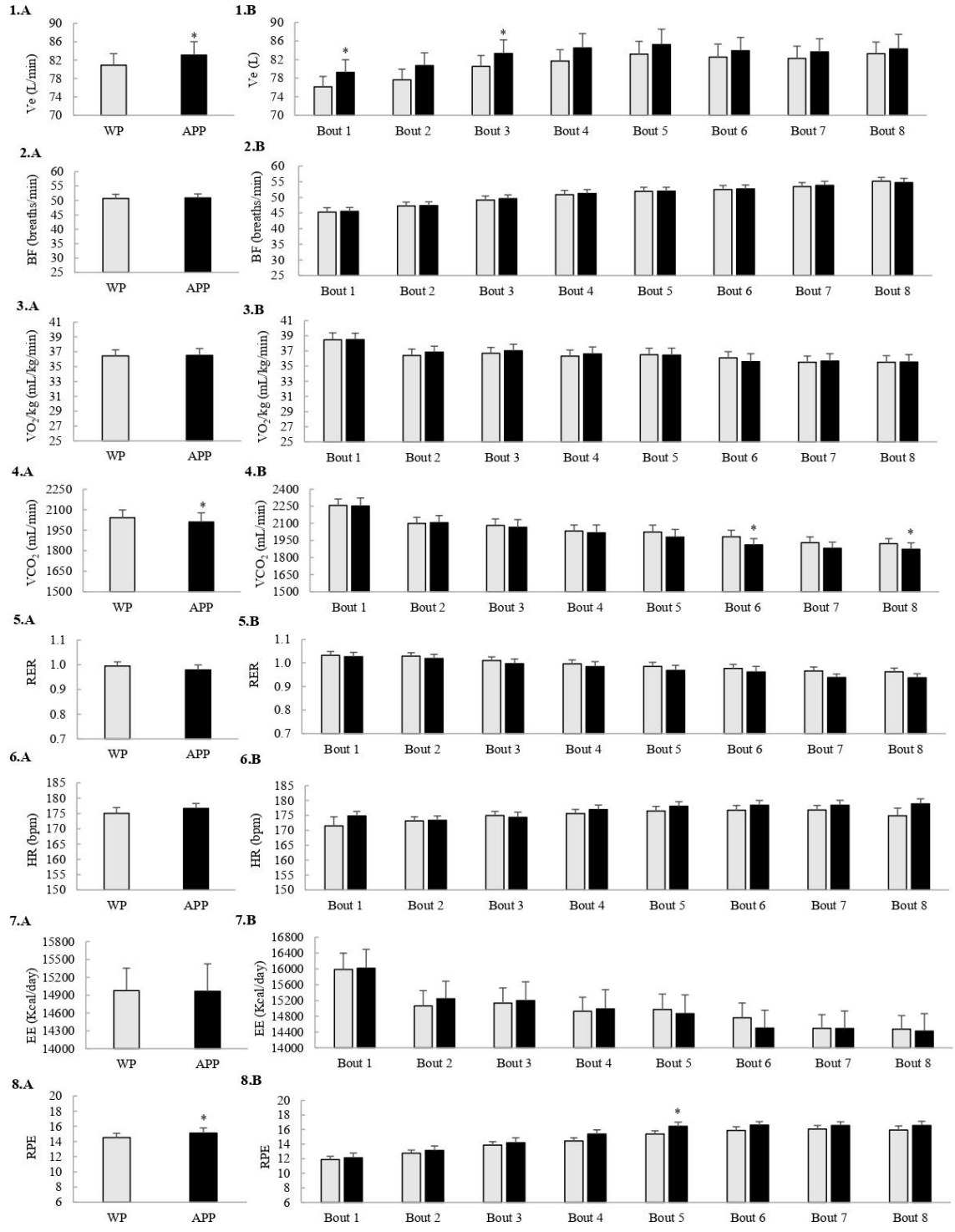

Figure 26: Mean OC phase (A) and OC phase in each bout (B) effect on performance variables throughout the high intensity intervals. $\dot{V}e$: ventilation; BF: breath frequency; $\dot{V}O_2$: oxygen consumption; $\dot{V}CO_2$: carbon dioxide production; RER: respiratory exchange ratio; HR: heart rate; EE: energy expenditure; RPE: relative perceived exertion; OC: oral contraceptive; WP: withdrawal phase; APP: active pill phase. *Significantly different from WP.

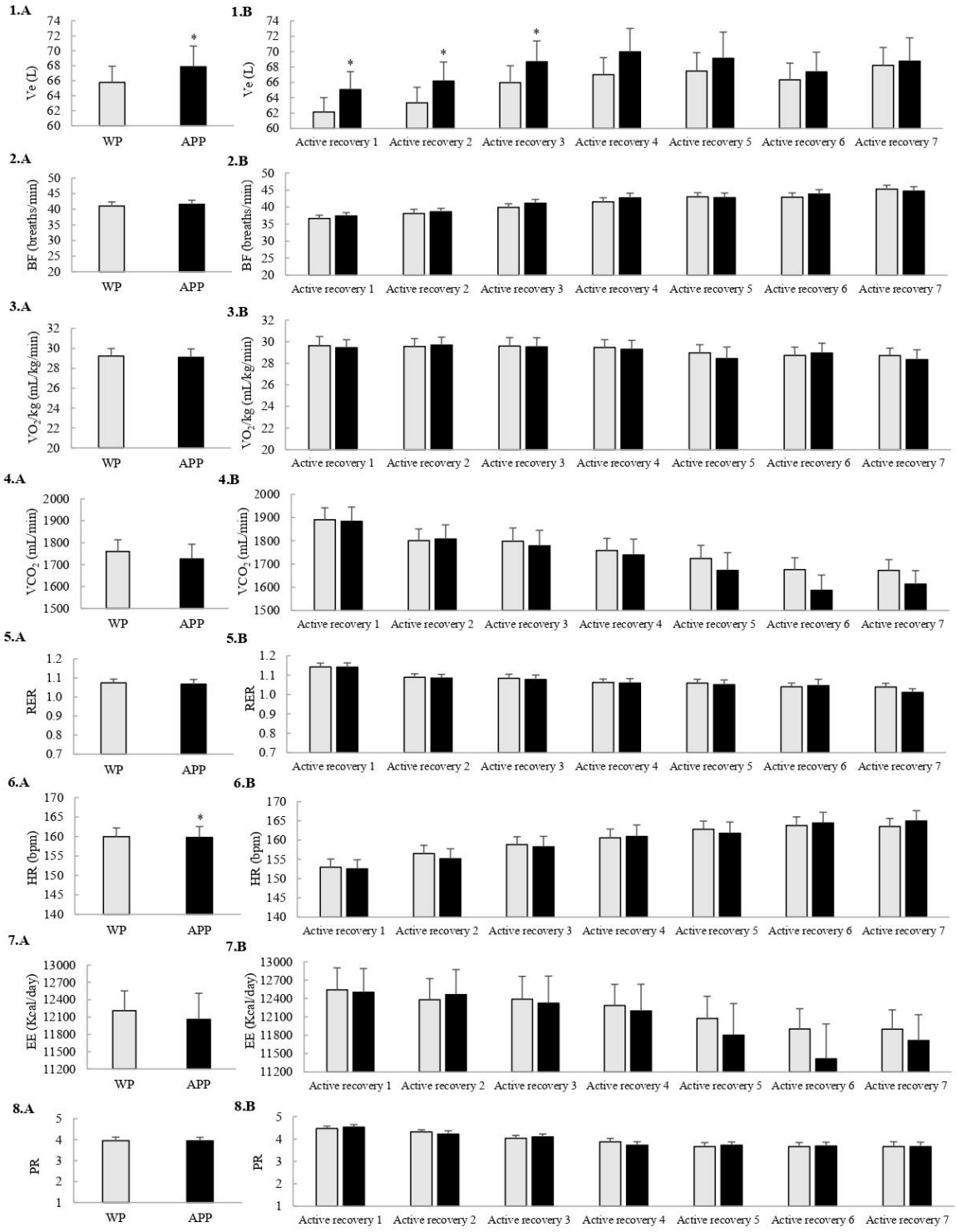

Figure 27: Mean OC phase (A) and OC phase in each interval (B) effect on performance variables throughout the active recovery intervals. $\dot{V}e$: ventilation; BF: breath frequency; $\dot{V}O_2$: oxygen consumption; $\dot{V}CO_2$: carbon dioxide production; RER: respiratory exchange ratio; HR: heart rate; EE: energy expenditure; PR: perceived readiness; OC: oral contraceptive; WP: withdrawal phase; APP: active pill phase. *Significantly different from WP.

DISCUSSION

This study investigated the possible influence of sex hormones fluctuations throughout the low-dose monophasic OC cycle on cardiorespiratory response to exercise, perceive exertion and readiness in endurance trained females. The main findings are the increase in $\dot{V}e$ and RPE during the bouts in the APP as well as higher values of $\dot{V}e$ during the active recovery intervals in this phase, which may suggest a different physiological response between OC phases.

Females from the present study exhibited higher $\dot{V}e$ during the warm-up and the interval running protocol (in both, bouts and active recovery) in the APP, when continuous dosages of progestins were administered. These results are backed up by previous studies carried out with cyclists (Rechichi et al., 2008), runners (Reilly & Whitley, 1994) and endurance-trained (Barba-Moreno et al., 2019) females, which concluded an increase in $\dot{V}e$ during the OC consumption. There is a strong basis in evidence that progestin enhances the sensitivity of chemoreceptors of the hypothalamus, lowering the threshold of the medullary respiratory centre and, therefore, increasing the chemosensitivity to hypoxia and hypercapnia. Consequently, a rise in respiratory drive occurs with the administration of progestin (Cagnacci et al., 2009; Packard et al., 2011; Rechichi et al., 2008; Rechichi et al., 2009; Winkler & Sudik, 2009). Besides, outcomes from the present study showed higher RPE and lower $\dot{V}CO_2$ during the APP, coinciding with previous research (Barba-Moreno et al., 2019). These findings could be linked with the higher values of $\dot{V}e$ also observed in the APP. On the one hand, a strong association between $\dot{V}e$ and RPE have been observed during maximal effort exercises, regardless the nature of the protocol (Nicolò et al., 2016). It is well known that the premotor and motor areas of the brain regulate $\dot{V}e$ and RPE during exercise. Along with the discharge of these

areas to the skeletal muscle, a projection of it occurs to the medullary respiratory centres and to the sensory areas of the brain, where perceive exertion is generated. Thus, a drive in $\dot{V}e$ and a higher RPE, respectively, takes place almost simultaneously during exercise (Nicolò et al., 2014; Nicolò et al., 2016). On the other hand, the physiological explanation for the lower $\dot{V}CO_2$ coinciding with higher $\dot{V}e$ has not been previously described. It is hypothesized that higher $\dot{V}e$ could be linked with an alkalosis breathing because of the decrease in body CO_2, since more $\dot{V}CO_2$ is expired (Abbiss et al., 2007; Kowalchuk et al., 1984). Nonetheless, the present findings are not in line with this hypothesis. Thus, further research is needed in order to elucidate the better association between $\dot{V}e$ and $\dot{V}CO_2$ during the APP.

In contrast, some previous OC research concluded no differences in $\dot{V}e$ over the OC cycle in active females (Casazza et al., 2002; Gordon et al., 2018; Packard et al., 2011; Vaiksaar et al., 2011). The findings could be limited because testing days were conducted at two times when the synthetic hormone intake was similar (during OC consumption) (Packard et al., 2011; Vaiksaar et al., 2011) as opposed to measure during the WP and APP, where there is a greater contrast in hormone levels. Furthermore, the only study analysing the WP (Gordon et al., 2018), tested during the early stage of this phase (day 1-3), when exogenous sex hormones are still detectable in plasma (Rechichi et al., 2008; Rechichi et al., 2009). Finally, the other study concluding no differences in $\dot{V}e$ over the OC cycle in active females (Casazza et al., 2002) was conducted with females taking triphasic OC pills; hence, differences in OC type could explain discrepancies in results.

With regard to the other cardiorespiratory variables measured in the current study, they seemed to be unaffected by OC phases tested, except for HR. Nonetheless, the tiny increase found in HR in the WP during the active recovery intervals (WP: 159.93±10.26 and APP: 159.74±12.83 bpm) lacks of clinical relevance on females´ response. This is also manifested by the small effect size reported. Likewise, previous literature concluded no differences in cardiorespiratory response to exercise over a monophasic (Barba-Moreno et al., 2019; Gordon et al., 2018; Mattu et al., 2019; Packard et al., 2011; Vaiksaar et al., 2011) and triphasic (Casazza et al., 2002) OC cycle in active females. Besides, a recent review with meta-analysis concluded that OC phases do not affect females´ response to exercise (Elliott-Sale et al., 2020). The lack of influence might be explained by the dosages of sex hormones concentrations in OC formulations (White et al., 2011). Currently, ethinyl estradiol and progestin levels in OC pills are lower than those used to be in the past (e.g., ethinyl estradiol concentration was 150 mg/day but today is 15 mg/day; progestin concentration was 9.85 mg/day but today is 0.35 mg/day) (White et al., 2011). Therefore, they appear not to affect female´s physiology response to exercise (Mattu et al., 2019; Rechichi et al., 2008).

The current study attempts to address a gap in the research through investigation of cardiorespiratory performance in well-trained females. The strengths of this study include its robust methodology, highlighting the specific hormonal environments selected for the testing days and a homogeneous low dose monophasic OC group (endurance well-trained and healthy females). However, longitudinal studies with an intra-subject design should be carried out to explore the influence of the hormonal changes throughout the season and the life span. Moreover, further research is recommended to provide a better understanding of the potential effects of exogenous sex hormones on cardiorespiratory

response to high intensity exercise in physically active women, with a particular focus on the different types and formulations of OC pills.

Based on the present study, OC phase appears to have a small effect on cardiorespiratory response to exercise. A drive in $\dot{V}e$ occurs during the APP, which is accompanied by a higher perceived exertion during this phase. Therefore, coaches and athletes should be aware of these variations, specially perceived exertion, regarding females´ training programs in order to improve not only their performance but also their wellness and adherence to physical activity.

STUDY VII

Cardiorespiratory response to high intensity interval exercise in endurance-trained postmenopausal women

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ABSTRACT

Objective: To evaluate the cardiorespiratory response to high-intensity interval exercise in endurance-trained postmenopausal women and compare it with their counterparts eumenorrheic females.

Methods: Twenty-one eumenorrheic (30.5±6.5 years, 58.4±8.7 kg, 25.2±6.7% fat mass, 48.4±4.4 ml/kg/min $\dot{V}O_2$peak) and thirteen postmenopausal (51.3±3.6 years, 54.1±4.1 kg, 24.2±5.2% fat mass, 46.01±9.8 ml/kg/min $\dot{V}O_2$peak) endurance-trained women performed a high-intensity interval running protocol consisted of 3-min at 85% with 90-s recovery at 30% of their maximal aerobic speed. It was carried out in the early-follicular phase for the eumenorrheic group and at any time for the postmenopausal group. Cardiorespiratory variables were continuously monitored throughout the protocol.

Results: The Mann–Whitney U test reported lower values ($p<0.05$ for all variables) in postmenopausal women compared to eumenorrheic females for ventilation (66.9±10.1 vs 78.6±11.1 l/min), oxygen consumption (33.7±3.9 vs 38.6±4.1 ml/kg/min), % maximal oxygen consumption (79.6±5.3 vs 76.0±10.6 %), heart rate (154.6±9.5 vs 167.3±11.4 bpm) and carbon dioxide production (1914.8±248.9 vs 2127.5±296.8 ml/min). On the contrary, % maximal carbon dioxide production (60.6±15.0 vs 65.3±8.9 %), respiratory exchange ratio (1.03±0.08 vs 0.96±0.06) and % maximal respiratory exchange ratio (75.4±19.0 vs 83.3±8.2 %) were higher in the postmenopausal group. Finally, % maximal heart rate (91.9±1.7 vs 91.1±2.4 %, $p=0.443$) and % maximal ventilation (71.9±6.7 vs 71.1±8.4 %, $p=0.138$) lacked of difference between study groups.

Conclusions: Postmenopausal women appear to have a lower cardiorespiratory response to high-intensity interval exercise than eumenorrheic females, because of the age-related physiological changes, along with the chronic sex hormone decrease. Nonetheless, trained postmenopausal women present a similar cardiac strain when comparing to

eumenorrheic females in relative values, which could be associated to the regular practice of physical activity.

Key words: eumenorrheic, exercise, heart rate, menopause, oxygen consumption, sex hormones.

INTRODUCTION

Physical fitness performance is reduced with aging. However, its pattern differs by sex showing women a more rapid decline than men during middle age (Bondarev et al., 2018). This sex difference might be related to the hormonal changes that women experience during their menopausal years. Menopause is characterised by the loss of the ovarian function along with dramatic changes in endogenous sex hormones secretion (Karsenty, 2012). Based on previous research, follicle-stimulating hormone (FSH) concentrations rises approximately 68% the following 7 to 10 months after the last menstruation, with a concomitant drop of 60% in 17β-estradiol (E2) level (Rannevik et al., 2008).

Endogenous sex hormones change after menopause, specially E2 decrease, may have an impact on women´s physiology. Previous research reported body composition adaptations such as an increase in fat mass (Santosa & Jensen, 2013) as well as a decrease in muscle mass (Abildgaard et al., 2013; Bondarev et al., 2018) and bone mineral density (Clarke & Khosla, 2010) in postmenopausal women. Besides, some cardiorespiratory shifts have also been observed in this population such as a rise in arterial stiffness and blood pressure as well as a drop in heart rate (HR) and oxygen consumption ($\dot{V}O_2$) (Bondarev et al., 2018; Farinatti et al., 2018; Neufeld et al., 2015). Indeed, literature showed a HRmax reduction of 6 beats/min per decade (Loe et al., 2013) and a maximal oxygen consumption ($\dot{V}O_2max$) decrease of 1% per year after the third decade of life in women (Posner et al., 1995). Apart from sex hormones influence, it has been suggested that postmenopausal women have a more sedentary lifestyle than premenopausal females (Karine et al., 2013). Hence, the reduction in cardiorespiratory fitness observed in this population may partially occur because of the decrease in E2 (Lynch et al., 2002; Mercuro

et al., 2006; Rannevik et al., 2008) as well as the decline un physical activity level (Karine et al., 2013). All this factors that postmenopausal women experience at this stage enhance the risk of suffering cardiovascular diseases and several types of cancer (Kokkinos & Myers, 2010; Ross et al., 2016). However, impairments on cardiorespiratory system caused by both, age and sex hormones change after menopause, may be partially offset in trained females because of the positive effect that physical activity has on these systems, especially high intensity exercise (Batacan et al., 2017; Green et al., 2017; Kessler et al., 2012; Moazami & Farahati, 2013; Ramos et al., 2015; Weston et al., 2014).

In this regard, few authors have analysed the cardiorespiratory response to high intensity interval exercise in postmenopausal sedentary women (Coswig et al., 2020; Farinatti et al., 2018) and, as far as we are concerned, none has evaluated it in endurance-trained postmenopausal women. Therefore, the aim of the present study was to assess the cardiorespiratory response to high intensity interval exercise in endurance-trained postmenopausal women and compare it with their counterparts premenopausal females.

MATERIAL AND METHODS

Participants

A total of twenty-one eumenorrheic females and thirteen postmenopausal women (at least one year without menstruation (Lara Delamater & Nanette Santoro, 2018)) participated in this study. All of them were healthy and well-trained in endurance activities such as running, obstacle races, triathlon, and cycling. Eumenorrheic females had regular menstrual cycles (MC), occurring from 23 to 38 days in length, during the six months prior the study (Janse de Jonge et al., 2019). Characteristics of the study population are described in Table 19.

Participants were required to meet the following criteria: (a) healthy adult females between 18 and 40 years old for the eumenorrheic group and under 60 years old for the postmenopausal group; (b) presenting healthy iron parameters (serum ferritin >20μg/l, haemoglobin >115 μg/l and transferrin saturation >16%); (c) performing endurance training at least 3 h per week. Exclusion criteria included (a) oral contraceptive users; (b) smoking; (c) metabolic or hormonal disorder; (d) medication or dietary supplements that alter vascular function (e.g., tricyclic antidepressants, α-blockers, β-blockers, etc.); (e) any surgery interventions (e.g. ovariectomy); (f) pregnancies in the year preceding; (g) any musculoskeletal injury in the last six months. At the start of the data collection, all participants conducted a questionnaire gathering information about training experience, health status and dietary supplements. All participants were informed about the procedures and risks involved and informed consent was provided by each participant. The experimental protocol was approved by the institutional Ethics Committee board of the Universidad Politécnica de Madrid and is in accordance with The Code of Ethics of the World Medical Association (Declaration of Helsinki) (Association, 2001).

Study design

The present work is part of the IronFEMME study, an observational cross-sectional study performed by physically active and healthy women which methodology has been recently published (Contract DEP2016-75387-P) (Peinado et al., 2021).

Participants came to our laboratory on two occasions. To avoid diurnal variability (Janse de Jonge, 2003), participants came to the laboratory between 8 and 10 a.m., abstaining from alcohol, caffeine and any intense physical activity or exercise practice the 24 hours prior the testing day. Nutritional recommendations were provided to the

participants by a nutritionist to standardize the diet, and volunteers followed them 24h prior every protocol. Volunteers underwent a screening (first visit) and interval running protocol (second visit), which were conducted any time for postmenopausal women and during the early follicular phase for the eumenorrheic group (i.e., between 2^{nd} and 5^{th} day of the menstrual cycle with day 1 being the onset of menstrual bleeding), to measure them under similar hormonal environments (low estrogen and progesterone levels). Regarding postmenopausal women, at least one rest day was between the first and the second visit.

On the first visit, volunteers came to our laboratory between 8 and 10 a.m. in a rested and overnight fasted state. Volunteers did not perform moderate or vigorous physical activity, intake caffeine or any supplementation 24 h prior to the test. Firstly, they signed all the informed consents and participant´s weight and height were recorded. Then, baseline blood samples were collected, for a complete blood count, genetic testing, biochemistry, and hormonal analyses. Subsequently, a dual-energy X-ray absorptiometry was done with a GE Lunar Prodigy apparatus using GE Encore 2002 software (version 6.10.029; GE Healthcare, Madison, WI). Finally, after eating and resting for a minimum of 2 hours, participants completed a maximal aerobic ramp test on a computerized treadmill (H/P/COSMOS 3PW 4.0, H/P/Cosmos Sports & Medical, Nussdorf-Traunstein, Germany) to determine their $\dot{V}O_2$peak. Expired gases were measured breath-by-breath with the gas analyser Jaeger Oxycon Pro (Erich Jaeger, Viasys Healthcare, Germany), which validity and reliability has been previously demonstrated (J. Carter & A. E. Jeukendrup, 2002; O. Foss & J. Hallen, 2005), whilst heart response was continuously monitored with a 12-lead ECG. Participants began with a warm-up of 3 min at 6 km/h. Once the warm-up finished, the speed was set at 8 km/h and then increased by 0.2 km/h every 12 s until exhaustion. A slope of 1% was set throughout the test to simulate air

resistance (Goldsmith & Glaister, 2020). To verify that $\dot{V}O_2$peak was reached, a confirmatory test was carried out as suggested in previous studies (P. B. Nolan et al., 2014; D. C. Poole & A. M. Jones, 2017) after a 5 min recovery of the maximal aerobic test (D. C. Poole & A. M. Jones, 2017). The confirmatory test consisted of a 3 min warm-up (2 min at 50% and 1 min at 70% of the maximal speed reached in the maximal aerobic test). Then, speed was set at 110% of the maximal speed reached in the maximal aerobic test until volunteers´ exhaustion. The $\dot{V}O_2$peak was determined as the mean of the three highest $\dot{V}O_2$ measurements in the maximal aerobic test if it was not less than 3% compared to the one obtained in the confirmatory trial. If the value was less than 3%, $\dot{V}O_2$peak was calculated as the mean of the three highest $\dot{V}O_2$ values recorded during the last 30-s of the confirmatory trial. The maximal aerobic speed (v$\dot{V}O_2$peak) was recorded as the minimum speed required to elicit $\dot{V}O_2$peak (V. Billat et al., 1994). Then, the speed equivalent to 85% of the v$\dot{V}O_2$peak was calculated to use in the interval running protocol.

Interval running protocol

After this screening day, eumenorrheic participants attended to the laboratory to perform the interval running protocol. The protocol of the testing procedure day has been previously described (Peinado et al., 2021; Rael, Alfaro-Magallanes, et al., 2021). Firstly, a blood sample was collected to analyze sex hormones, followed by a resting blood pressure (BP) measurement, using the auscultatory method with a calibrated sphygmomanometer. Subsequently, participants started the interval running protocol, which consisted of a 5 min warm-up at 60% of the v$\dot{V}O_2$peak followed by 8 bouts of 3 min at 85% of the v$\dot{V}O_2$peak, with 90-second recovery at 30% of the v$\dot{V}O_2$peak between bouts. Finally, 5 min cool down was performed at 30% of the v$\dot{V}O_2$peak. During exercise, ventilation ($\dot{V}e$), $\dot{V}O_2$, carbon dioxide production ($\dot{V}CO_2$), respiratory exchange ratio

(RER), and HR among others ventilatory variables were continuously measured using the same apparatus as mentioned for the maximal aerobic test. Besides, maximal cardiorespiratory values percentage (%$\dot{V}e$ max, %$\dot{V}O_2$/Kg max, %$\dot{V}CO_2$ max, %RER max and %HR max) throughout the interval running protocol was calculated considering maximal values, previously obtained in the maximal aerobic test, as 100%. Cardiorespiratory values were obtained as the mean of the 5 min warm-up, as well as the mean of the 5 min cool down. Likewise, values over the interval running protocol were elicited as the mean of the 3 min high intensity bouts and the mean of the 90-second recovery intervals.

Additionally, Rate of Perceived Exertion (RPE) and Perceived Readiness (PR) were measured by RPE Borg 6-20 scale (G. Borg, 1970) and PR Nurmekivi 1-5 scale. (A. Nurmekivi et al., 2001) Participants were asked for RPE in the last 5 s of warm-up, of every running bout, and at the end of the cool-down. PR scale was applied in the last 5 s of warm-up, of every active recovery interval, and at the end of the cool down.

Blood samples analyses

Blood samples were obtained with venepuncture into a vacutainer containing clot activator. Following inversion and clotting, the whole blood was centrifuged (Biosan LMC-3000 version V.5AD) for ten minutes at 1610 g to obtain the serum (supernatant). After that, serum was transferred into eppendorf tubes and stored at -80ºC until further analysis. Within 1 to 15 days after testing, the serum samples were delivered to the clinical laboratory of the Spanish National Centre of Sport Medicine (Madrid, Spain) to determine sex hormones and verify hormonal profiles. Total E2, progesterone, FSH and luteinizing hormone (LH) were measured via ADVIA Centaur® solid-phase competitive

chemiluminescent enzymatic immunoassay (Siemens city, Germany). Inter- and intra-assay coefficients of variation (CV) reported by the laboratory for each variable were previously described (Peinado et al., 2021).

Statistical analysis

Data are presented as mean and standard deviation (±SD). A Saphiro-Wilk test to assess the normality of the variables was conducted. The Mann–Whitney U test was applied to analyze differences in sex hormones (FSH, LH, E2 and progesterone) and cardiorespiratory variables (BP, $\dot{V}e$, $\dot{V}O_2$/kg, $\dot{V}CO_2$, RER, HR, RPE, PR) throughout the interval running protocol between both groups tested. Then, Cohen´s d (Cohen, 2013) and their 95% confidence intervals (CI) were calculated to assess the magnitude of effect on the changes found. Threshold values were set as small (≥0.2 and <0.5), moderate (≥0.5 and <0.8) and large (≥0.8). (Cohen, 2013) Statistical significance was set at $p<0.05$ and all procedures were conducted with SPSS software 21 version (IBM Corp., Armonk, NY, USA).

RESULTS

Firstly, it is worth mentioning that a homogeneous group of females have been studied since no differences in descriptive variables (height, weight, FM percentage, LM percentage, training status and $\dot{V}O_2$peak), other than age, were observed between eumenorrheic and postmenopausal women (Table 19). Regarding sex hormone concentrations in the testing day, significant differences between groups were observed, presenting postmenopausal women higher values of LH and FSH, whereas eumenorrheic females reported higher E2 and progesterone levels (Table 19). Eumenorrheic volunteers´

menstrual cycles ranged from 28±2 to 31±2 days in length, and their early follicular phase was at day 3.43±0.93.

Table 19: Characteristics of the study population (mean±SD).

	Eumenorrheic in the EFP	Postmenopausal	Z	*p*	d	CI
Age (years)	30.5±6.5	51.3±3.6	-5.059	<0.001	3.37	2.31 to 4.43
Height (cm)	163.1±6.4	160.8±5.6	-0.755	0.450	-0.49	-1.19 to 0.21
Weight (kg)	58.4±8.7	54.1±4.1	-0.562	0.574	-0.55	-1.25 to 0.16
Fat mass (%)	25.2±6.7	24.2±5.2	-0.471	0.637	-0.16	-0.86 to 0.53
Lean mass (%)	70.4±6.5	72.9±5.6	-1.145	0.252	0.41	-0.29 to 1.10
Experience (years)	7.4±5.3	7.9±3.3	-1.297	0.195	0.11	-0.59 to 0.80
Training volume (mins/week)	295.9±183.6	258.5±90.45	-0.273	0.785	-0.24	-0.94 to 0.45
$\dot{V}O_2$peak (ml/kg/min)	48.4±4.4	46.01±9.8	-1.577	0.115	-0.35	-1.04 to 0.35
LH (mIU/ml)	6.70±2.71	41.22±12.26	-4.790	<0.001	4.42	3.16 to 5.68
FSH (mIU/ml)	7.15±2.36	81.99±38.20	-4.739	<0.001	3.19	2.16 to 4.22
E2 (pg/ml)	48.60 ±32.23	33.03±57.34	-2.433	0.015	-0.36	-1.06 to 0.34
Progesterone (ng/ml)	0.32±0.19	0.17±0.13	-2.250	0.024	-0.88	-1.61 to -0.16

EFP: early-follicular phase; $\dot{V}O_2$peak: peak oxygen consumption; FSH: follicle-stimulating hormone; LH: luteinizing hormone; E2: 17 β-estradiol.

Speed throughout the interval running protocol was lower for the postmenopausal group compared to eumenorrheic females in the warm-up (8.2±0.7 and 9.0±0.7 km/h, respectively; Z=-2.463; *p*=0.014; d=1.14; CI=0.40 to 1.89), high intensity intervals (11.7±1.1 and 12.8±0.9 km/h, respectively; Z=-2.428; *p*=0.015; d=1.12; CI=0.38 to 1.86), active recovery intervals (4.2±0.5 and 4.6±0.5 km/h, respectively; Z=-2.218; *p*=0.027; d=0.80; CI=0.08 to 1.52) and cool down (4.2±0.4 and 4.5±0.4 km/h, respectively; Z=-2.304; *p*=0.021; d=0.75; CI=0.04 to 1.46).

Secondly, neither resting systolic blood pressure (eumenorrheic group: 106.15±8.44 and postmenopausal group: 107.85±8.09 mmHg; Z=-1.004; *p*=0.316; d=0.21; CI=-0.49 to 0.90) nor diastolic blood pressure (eumenorrheic group: 65.75±7.66 and postmenopausal group: 68.57±7.48 mmHg; Z=-1.815; *p*=0.070; d=0.37; CI=-0.33 to 1.07) showed differences between both groups tested.

Regarding the warm-up, $\dot{V}e$, $\dot{V}O_2$/Kg, $\dot{V}CO_2$ and HR exhibited lower values in postmenopausal women than in eumenorrheic females; while RER, RPE and PR did not show differences between study groups. However, when comparing relative values, only %HR max was lower in postmenopausal women throughout the warm-up, since %$\dot{V}e$ max, %$\dot{V}O_2$/Kg max, %$\dot{V}CO_2$ max and %RER max reported no differences between study groups (Table 20). Lastly, cool down outcomes showed lower values for $\dot{V}e$ and $\dot{V}O_2$/Kg in the postmenopausal group compared to the eumenorrheic one, whereas RER was higher. Besides, no differences between study groups were observed for $\dot{V}CO_2$, HR, RPE, PR, %$\dot{V}e$ max, %$\dot{V}O_2$/Kg max, %$\dot{V}CO_2$ max and %RER max and %HRmax (Table 20).

Table 20. Performance variables (Mean±SD) throughout the warm-up and cool down between eumenorrheic females and postmenopausal women.

		Eumenorrheic in the EFP	Postmenopausal	Z	*p*	d	CI
Warm-up	$\dot{V}e$ (l/min)	48.24±8.65	39.38±6.58	-2.746	0.006	-1.12	-1.86 to -0.38
	%$\dot{V}e$ max (%)	44.71±5.49	42.65±5.63	-1.085	0.291	-0.37	-1.09 to 0.34
	$\dot{V}O_2$/Kg (ml/kg/min)	29.07±2.56	23.41±2.19	-3.739	<0.001	-2.33	-3.22 to -1.45
	%$\dot{V}O_2$/Kg max (%)	60.18±3.87	55.86±7.20	-1.683	0.096	-0.82	-1.55 to -0.08
	$\dot{V}CO_2$ (ml/min)	1481.21±215.57	1235.88±131.10	-2.395	0.017	-1.30	-2.06 to -0.54
	%$\dot{V}CO_2$ max (%)	42.23±10.86	44.65±6.85	-0.112	0.927	0.25	-0.46 to 0.96
	RER	0.88±0.05	0.94±0.11	-1.953	0.051	0.65	-0.06 to 1.36
	%RER max (%)	69.20±18.26	76.69±9.75	-1.235	0.228	0.48	-0.24 to 1.19
	HR (bpm)	135.95±12.83	117.50±16.03	-2.208	0.027	-1.31	-2.07 to -0.55
	%HR max (%)	75.62±8.57	70.10±7.77	-2.533	0.011	-0.73	-1.44 to -0.02
	RPE	9.33±1.77	10.86±1.68	-1.822	0.068	0.85	0.13 to 1.57
	PR	4.86±0.28	4.70±0.49	-0.573	0.566	-0.43	-1.13 to 0.27
Cool down	$\dot{V}e$ (l/min)	43.19±6.35	42.58±6.06	-2.463	0.014	-0.10	-0.79 to 0.59
	%$\dot{V}e$ max (%)	39.61±5.11	39.76±6.66	-0.299	0.782	0.03	-0.68 to 0.74
	$\dot{V}O_2$/Kg (ml/kg/min)	19.49±2.67	19.01±2.47	-2.948	0.003	-0.19	-0.88 to 0.51
	%$\dot{V}O_2$/Kg max (%)	38.44±9.91	37.66±6.13	-1.045	0.309	-0.09	-0.80 to 0.62
	$\dot{V}CO_2$ (ml/min)	1069.41±180.80	1058.41±164.94	-1.658	0.097	-0.06	-0.76 to 0.63
	%$\dot{V}CO_2$ max (%)	30.44±8.05	31.94±5.24	0.000	1.000	0.21	-0.50 to 0.92
	RER	0.94±0.06	0.97±0.08	-2.505	0.012	0.33	-0.37 to 1.02
	%RER max (%)	73.64±18.75	81.01±7.49	-1.385	0.175	0.47	-0.25 to 1.19
	HR (bpm)	137.91±15.16	138.54±13.55	-1.239	0.215	0.04	-0.65 to 0.74
	%HR max (%)	75.99±7.51	74.65±6.91	-0.713	0.476	-0.28	-0.97 to 0.42
	RPE	9.81±2.91	9.44±1.97	-0.921	0.357	-0.14	-0.84 to 0.55
	PR	4.09±1.13	4.28±0.71	-0.343	0.732	0.19	-0.50 to 0.88

$\dot{V}e$: ventilation; %$\dot{V}e$ max: maximal ventilation percentage; $\dot{V}O_2$: oxygen consumption; %$\dot{V}O_2$ max: maximal oxygen consumption percentage; $\dot{V}CO_2$: carbon dioxide production; %$\dot{V}CO_2$ max: maximal carbon dioxide production percentage; RER: respiratory exchange ratio; %RER max: maximal respiratory exchange ratio percentage; HR: heart rate; %HR max: maximal heart rate percentage; RPE: rate of perceived exertion; PR: perceived readiness.

According to the interval running protocol (Table 21) postmenopausal women exhibited lower values of $\dot{V}e$, $\dot{V}O_2$/Kg, %$\dot{V}O_2$/Kg max, $\dot{V}CO_2$, HR and RPE, whereas %$\dot{V}CO_2$ max, RER and %RER max were higher for this group throughout the high intensity bouts compared to the premenopausal one. Nonetheless, no differences in %$\dot{V}e$ max and %HRmax were reported between study groups across the high intensity bouts. Moreover, postmenopausal women reported lower values of $\dot{V}e$, $\dot{V}O_2$/Kg, %$\dot{V}O_2$/Kg max, $\dot{V}CO_2$, and HR while %$\dot{V}CO_2$ max, RER, %RER max and PR were higher for this group during the active recovery. Finally, no differences in %$\dot{V}e$ max, %$\dot{V}CO_2$ max and %HRmax were observed between study groups during the active recovery intervals.

Table 21. Performance variables (Mean±SD) throughout the interval running protocol.

		Eumenorrheic in the EFP	Postmenopausal	Z	*p*	d	CI
Bouts	$\dot{V}e$ (l/min)	78.61±11.09	66.95±10.08	-7.906	<0.001	-1.09	-1.83 to -0.35
	%$\dot{V}e$ max (%)	71.91±6.65	71.11±8.36	-1.485	0.138	-0.11	-0.82 to 0.60
	$\dot{V}O_2$/Kg (ml/kg/min)	38.62±4.04	33.74±3.95	-8.270	<0.001	-1.22	-1.97 to -0.47
	%$\dot{V}O_2$/Kg max (%)	79.64±5.26	75.98±10.64	-2.980	0.003	-0.48	-1.20 to 0.24
	$\dot{V}CO_2$ (ml/min)	2127.48±296.78	1914.77±248.91	-5.634	<0.001	-0.76	-1.48 to -0.05
	%$\dot{V}CO_2$ max (%)	60.62±15.04	65.33±8.91	-2.564	0.010	0.36	-0.36 to 1.07
	RER	0.962±0.060	1.031±0.083	-6.623	<0.001	1.03	0.29 to 1.76
	%RER max (%)	75.35±19.02	83.33±8.21	-4.499	<0.001	0.50	-0.22 to 1.22
	HR (bpm)	167.29±11.44	154.59±9.48	-7.578	<0.001	-1.18	-1.93 to -0.44
	%HR max (%)	91.86±1.73	91.07±2.44	-0.767	0.443	-0.39	-1.09 to 0.31
	RPE	15.15±3.18	13.97±1.81	-4.753	<0.001	-0.43	-1.13 to 0.27
Active recovery intervals	$\dot{V}e$ (l/min)	64.34±8.77	55.06±9.47	-6.669	<0.001	-1.03	-1.76 to -0.29
	%$\dot{V}e$ max (%)	58.96±6.33	58.54±8.53	-0.033	0.974	-0.06	-0.77 to 0.65
	$\dot{V}O_2$/Kg (ml/kg/min)	30.23±3.60	26.05±3.12	-8.395	<0.001	-1.22	-1.97 to -0.47
	%$\dot{V}O_2$/Kg max (%)	62.36±5.59	58.39±9.36	-3.296	0.001	-0.56	-1.28 to 0.17
	$\dot{V}CO_2$ (ml/min)	1801.27±257.15	1615.70±220.42	-5.878	<0.001	-0.76	-1.48 to -0.05
	%$\dot{V}CO_2$ max (%)	51.30±12.72	54.20±8.22	-0.555	0.579	0.26	-0.46 to 0.97
	RER	1.050±0.075	1.121±0.104	-5.579	<0.001	0.80	0.08 to 1.51
	%RER max (%)	82.11±20.36	90.51±9.83	-3.680	<0.001	0.48	-0.23 to 1.20
	HR (bpm)	156.17±13.12	142.96±13.93	-6.607	<0.001	-0.98	-1.71 to -0.25
	%HR max (%)	84.86±2.24	82.63±5.17	-0.889	0.374	-0.62	-1.32 to 0.09
	PR	4.00±0.99	4.15±0.82	-2.979	0.003	0.16	-0.53 to 0.85

$\dot{V}e$: ventilation; %$\dot{V}e$ max: maximal ventilation percentage; $\dot{V}O_2$: oxygen consumption; %$\dot{V}O_2$ max: maximal oxygen consumption percentage; $\dot{V}CO_2$: carbon dioxide production; %$\dot{V}CO_2$ max: maximal carbon dioxide production percentage; RER: respiratory exchange ratio; %RER max: maximal respiratory exchange ratio percentage; HR: heart rate; %HR max: maximal heart rate percentage; RPE: rate of perceived exertion.

DISCUSSION

The purpose of this study was to examine cardiorespiratory response to high intensity interval exercise in postmenopausal endurance-trained women and compare it with their counterparts eumenorrheic females. The findings of the present work suggest a lower aerobic fitness in postmenopausal women than in premenopausal females. However, it is wort mentioning the similar cardiorespiratory response between groups when comparing relative values.

The lack of difference in %HRmax means a similar cardiac strain between eumenorrheic and postmenopausal endurance-trained women. This finding might be explained by the positive effect exercise has on cardiac function. It is well known that cardiac myocytes are increased and strengthened due to the regular practice of exercise, leading to a better cardiac function and lower myocardial stiffness in this population (Green et al., 2017). Although very few studies have evaluated this variable, a recent study carried out with active (3 times per week during the last 3 months) postmenopausal (62 years old) women reported a 65% HRmax throughout a cycle ergometer test at 75% of their $\dot{V}O_2max$, while postmenopausal volunteers from the present study reported a 91% HRmax throughout a high intensity interval running protocol (Stathokostas et al., 2008). Discrepancies in %HRmax between studies could be explained by differences in exercise protocols as well as volunteers´ training status and age.

The lower cardiovascular response with aging previously documented (Farinatti et al., 2018; Kaminsky et al., 2015; Loe et al., 2013; Rossi Neto et al., 2019), is in line with the findings from the present study since postmenopausal women reported lower HR response throughout the interval running protocol compared to premenopausal females.

An age-related decline in heart function has been long time accepted (Christou & Seals, 2008; Vigorito & Giallauria, 2014). On the one hand, a pivotal aspect of the aging heart is the increase in myocardial stiffness, leading to a drop in myocardial distensibility and, thereby, cardiac filling is impaired (Milia et al., 2015). Meanwhile, the decrease in cardiac compliance limits the recruitment of the Franck-Starling mechanism and reduces the possibility of increasing the systolic volume and, therefore, cardiac output (Milia et al., 2015). On the other hand, E2 enhances cardiac contractility (dos Santos et al., 2014) as well as vasodilation of the coronary and peripheral arteries (Mendelsohn, 2002); thereby, its drastic decrease after menopause may compromise cardiac function.

Turning on to the respiratory system in elderly women, the lower values observed in relative and absolute values observed in the postmenopausal group from the present study agrees with previous research (Farinatti et al., 2018; Kaminsky et al., 2015; Loe et al., 2013; Rossi Neto et al., 2019). This system also undergoes a measurable decline in the physiological function. With advancing age, the thoracic cage stiff and airways resistance increase, and this in turn elevates the work of breathing (Robergs & Roberts, 2000). In addition, sex hormones shifts occurring in women at this stage have also been linked to impairment of respiratory function (Hayatbakhsh et al., 2011). For instance, a cross-sectional study found a significantly lower spirometric measures and more respiratory symptoms in postmenopausal women compared to women of the same age but with regular menstruations (Real et al., 2008). Besides, it appears that E2 concentrations can increase pulmonary blood volume and pulmonary diffusion capacity (Mattu et al., 2019; Smith et al., 2015); thus, its fall after menopause could compromise pulmonary function.

Nonetheless, it should be pointed out that, in the present study the interval running protocol speed was lower for the postmenopausal group. Consequently, the lower respiratory response observed in this group might be related to this factor. Besides, a recent publication carried out with these eumenorrheic and postmenopausal endurance-trained women reported no differences in most cardiorespiratory values either at resting or at peak values (Rael, Barba-Moreno, et al., 2021). Thus, outcomes from the present study should be taken with cautious since resting and peak values lack of differences between eumenorrheic and postmenopausal women (Rael, Barba-Moreno, et al., 2021) and the cardiorespiratory response to a high intensity interval exercise might be altered by differences in speed.

Finally, according to RER and %RER max, the present study showed higher values in the postmenopausal group throughout the high intensity interval protocol, indicating a higher glycogen consumption and a lower fat oxidation in this population. Women´s metabolism could also be affected by the fall in E2 levels after menopause, since this sex hormone enhances glycogen sparing and fat oxidation by promoting lipolysis in the muscles (Ashley et al., 2000; Constantini et al., 2005; D'Eon & Braun, 2002; Mattu et al., 2019).

The current study attempts to address a gap in the research through the investigation of important cardiorespiratory variables in endurance-trained postmenopausal women. The strengths of our study included the recruitment of a homogeneous group, regardless the age, of premenopausal and postmenopausal endurance-trained women, since most of previous research have evaluated healthy

sedentary women. Thus, longitudinal studies with an intra-subject design should be carried out to explore the influence of the hormonal changes over the life span.

CONCLUSIONS

This investigation suggests that postmenopausal cardiorespiratory response to exercise cannot be as high as premenopausal one when performing a high intensity interval training. This fact appears to be associated with age-related physiological changes, along with the chronic sex hormone decrease after menopause. Nonetheless, postmenopausal women present a similar cardiac strain when comparing to eumenorrheic females in relative values, which could be associated to the regular practice of physical activity. Further research is recommended to provide a better understanding of the potential effects of different hormonal profiles in cardiorespiratory system when studying physically active women.

5. CONCLUSIONS

CONCLUSIONS

The overall aim of this thesis was to evaluate whether MC phases, low-dose monophasic OC cycle phases and postmenopause affect BC and cardiorespiratory response to exercise. Therefore, the general conclusions of this thesis are the following:

(1) Sex hormone fluctuations throughout the MC phases appear not to influence BC in well-trained females. Nonetheless, Ve and HR might be slightly altered across the MC during high intensity exercise, reporting the LFP and the EFP lower values of Ve and HR, respectively.

(2) Sex hormone fluctuations throughout the low-dose monophasic OC cycle do not alter BC in well-trained females. Nonetheless, when performing high intensity exercise, a drive in $\dot{V}e$ accompanied by a higher perceived exertion occurs during the APP.

(3) Sex hormones decline after menopause do not alter BC in well-trained postmenopausal women. Nevertheless, endurance-trained postmenopausal women present a lower cardiorespiratory response to exercise than premenopausal females.

These conclusions are fully developed below alluding to the seven contributions of this thesis represented by each of the studies of this work.

Conclusions of Study I:

Postmenopausal women showed lower values of BMD compared to eumenorrheic females in spite of the regular practice of exercise. Interestingly, maximal oxygen consumption did not correlate to BMD in this population; while1RM back squat reported

a slight association to BMD. Hence, strength training may be the best choice to prevent BMD loss.

Conclusion of Study II:

Sex hormones from different hormonal profiles (eumenorrheic, low-dose monophasic OC users and postmenopausal females) do not influence body composition in physically active women. Interestingly, android FM do not vary in active postmenopausal female, which could be explain by the positive effect exercise has on body composition, and this in turn on female´s health.

Conclusion of Study III:

Different female sex hormone environments throughout the MC and OC cycle do not influence BC variables measured through bioimpedance analysis in well-trained females.

Conclusion of Study IV:

Endurance-trained postmenopausal women have a similar cardiorespiratory response to exercise compared to premenopausal females. In addition, cardiorespiratory response in low-dose monophasic OC users do not differ from the eumenorrheic response either at rest or during exercise in endurance-trained females.

Conclusion of Study V:

Sex hormone fluctuations throughout the MC appear not to be high enough to disrupt physiological adjustments caused by high intensity interval exercise. However, Ve

and HR seem to be the most altered variables across the MC and, therefore, HR based training programs should consider menstrual cycle phase.

Conclusion of Study VI:

OC phase appears to have a small effect on cardiorespiratory response to exercise. A drive in $\dot{V}e$ occurs during the APP, which is accompanied by a higher perceived exertion during this phase.

Conclusion of Study VII:

Postmenopausal cardiorespiratory response to exercise cannot be as high as premenopausal one when performing a high intensity interval training. Nonetheless, postmenopausal women present a similar cardiac strain when comparing to eumenorrheic females in relative values, which could be associated to the regular practice of physical activity.

6.STRENGTHS AND LIMITATIONS

STRENGTHS

The IronFEMME Study was aimed to examine the influence of sex hormonal environment on BC and cardiorespiratory response to exercise by considering the different reproductive status present throughout well-trained females' life span. The strengths from the present thesis should be highlighted:

1. This is the first study analyzing BC and cardiorespiratory response to exercise in premenopausal females with a robust methodology based on:
    - Using the three-step method to verify menstrual cycle phases consisting of retrospective calendar counting, blood analysis of sex hormones and urine-based tests to predict ovulation.
    - Measuring volunteers on three menstrual cycle phases coinciding with the most pronounced changes in sex hormones over the menstrual cycle or on the two phases of a low-dose monophasic oral contraceptive cycle.
    - Following an intra-subject design.
2. Participants from the OC group used low-dose monophasic OC pills in order to homogenize the exogenous hormonal concentrations.
3. This is the first study comparing BC and cardiorespiratory response to exercise in postmenopausal women and premenopausal females under similar hormone environments, low sex hormones.
4. Phases were randomized and counter-balanced to avoid learning effect and repeated bout effect.
5. The adequate design of exercise protocol using the same durations and intensity during different phases of the MC and OC cycle ensured an accurate comparison among phases for both study groups.

6. Volunteers from the present thesis were a homogeneous group of healthy and well-trained females.

LIMITATIONS

After carrying out the study, data analysis, discussing and comparing our results with previous studies, we present a critical view of our work, listing the main limitations below:

1. Despite participants were carefully selected and methodology was robust, the variability between subjects was high. This could have influenced the lack of differences between phases.
2. In the study with oral contraceptive users, all the preparations did not provide the same dosage of exogenous sex hormones.
3. Hormonal variability could be high for the same woman between different menstrual cycles and even within one menstrual cycle. Sex hormone concentrations could also vary between days within the same oral contraceptive cycle especially during the withdrawal phase. Hence, it would be interesting to perform daily measurements of sex hormones in both groups.

7. FUTURE RESEARCH LINES

FUTURE RESEARCH LINES

This project and thesis have given the possibility to study the influence sex hormones on BC and cardiorespiratory response to exercise in well-trained females. Learning from it and having a wider view of similar projects, future studies should take into consideration the following points:

1. To perform a long distance protocol in order to evaluate sex hormone influence on cardiorespiratory response to other type of exercise.
2. To include the study of qualitative measurements related to the psychological aspects, symptoms and moods derived from the hormonal changes throughout the menstrual cycle in order to show a more comprehensive perspective.
3. To organize multi-center collaborations in order to obtain a large sample size to ensure power and reduce intra-subject variability.
4. To consider the analysis of different forms and types of hormonal contraception and their influence on BC and cardiorespiratory exercise response.
5. To include in the postmenopausal study, sedentary females as well as hormone replacement therapies users.

8. PRACTICAL APPLICATIONS

PRACTICAL APPLICATIONS

Nowadays, experts in sports science and coaches still apply the same methodology to train both women and men. To date, hormonal variations are not taken into account when doing a training program or session. The best way to develop a well-designed, controlled and supervised exercise is to deeply know how women physiology and their hormone fluctuations can influence their performance.

Despite more literature is necessary to deeply understand female physiology and its responses and adaptations to exercise over female´s life span, in light of our findings some practical applications from this work are worth mentioning which could be helpful for coaches:

1. Sex hormone fluctuations throughout the MC and OC cycle appear not to influence BC variables measured by bioelectrical impedance in well-trained females. Therefore, it seems that bioimpedance analysis can be performed at any moment of the cycle, both for eumenorrheic females and for OC users. Although no differences have been found throughout the MC and the OC cycle, it is worth mentioning that BC individual variations may take place in trained females due to their hormonal fluctuations. Thus, coaches and athletes should be aware of these individual differences, especially regarding FM in eumenorrheic females.

2. Sex hormone fluctuations throughout the MC appear not to be high enough to disrupt physiological adjustments caused by high intensity interval exercise. However, HR based training programs should consider MC phase since it seems that this variable is the most affected one.

3. Although OC phase appears to have a small effect on cardiorespiratory response to exercise, an increase in V̇e and perceived exertion occurs during the APP. Therefore, coaches and athletes should be aware of these variations, specially perceived exertion, regarding females´ training programs in order to improve not only their performance but also their wellness and adherence to physical activity.

4. Exercise plays a key role in the maintenance of healthy bones, BC variables and cardiorespiratory system after menopause. Hence, coaches and females should be aware of the importance of the regular practice of resistance and endurance training in order to achieve a healthy postmenopausal stage.

5. On the basis of our findings, despite being all participants healthy and well-trained, a high variability in BC and cardiorespiratory response between individuals was observed. This means that coaches should have the patience to monitor every athlete over some MC to properly understand how much hormone fluctuations affect her performance. Decisions on training adaptations should be made according to this registry. Additionally, getting familiarized with calendar counting and urine-based ovulation tests would be of use, as these are easy and not expensive methods not only to adequately verify MC phases but also to detect possible menstrual dysfunctions, which are very common in female athletes.

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10. APPENDIX I:

CURRICULUM VITAE

BEATRIZ RAEL DELGADO

PhD Candidate in Sports Sciences

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EDUCATION

2017 - present PhD Sport Sciences. Universidad Politécnica de Madrid (UPM), Madrid (Spain).

2020 - present Master of Education. Universidad Complutense de Madrid (UCM), Madrid (Spain).

2016 - 2017 Master of Research in Sport Sciences. Universidad Politécnica de Madrid (UPM), Madrid (Spain).

2012 - 2016 Sport Sciences Degree. Universidad Politécnica de Madrid (UPM), Madrid (Spain).

2010 - 2014 Official School of Languages.

WORK EXPERIENCE

2016 – present Self-employed as a teacher of Sports Science Degree:

- Statistics (1st course of Sports Science Degree)
- Human Physiology (2nd course of Sports Science Degree)
- Biomechanics (2nd course of Sports Science Degree)

- Exercise Physiology (3rd course of Sports Science Degree)

2016 – present Laboratory assistant in ergoespirometry testing at Laboratorio de Fisiología del Esfuerzo (LFE) - INEF, at Universidad Politécnica de Madrid (UPM, Madrid (Spain).

2017 – present Member of LFE Research Group at Universidad Politécnica de Madrid (UPM, Madrid (Spain).

2019 Teacher at **"**Exercise is medicine: from theory to practice", at Universidad Politécnica de Madrid (UPM), Madrid (Spain).

2019 Research Stay under supervision of Dr. Xanne Janse de Jonge. February- June 2019. The University of Newcastle (UON). NSW, Australia.

RESEARCH PROJECTS

2017 – present IronFEMME Project. Influence of menstrual cycle on iron metabolism and muscle damage in female athletes. Funded by the Spanish Ministry of Economy, Industry and Competitiveness.

2019 Talent development in NSW Sports High School, Australia.

2015 - 2016 UPCYCLING. Physiological profile of elite cyclists during a time-trial. Funded by University Camilo José Cela, Madrid (Spain).

2015 - 2016 IronFEMME Pilot Study: Influence of menstrual cycle on iron metabolism in female athletes.

2014 - 2015 PREFIT Project. FITness assessment inf PREschoolers. Funded by the Spanish MInistry of Science and Innovation.

PUBLICATIONS

Rael, B., Alfaro-Magallanes, V. M., Romero-Parra, N., Barba-Moreno, L., Butragueño, J., Cupeiro, R., & Peinado, A. B. (2021). Body composition in premenopausal and postmenopausal well-trained females. Rev Int Med Cienc Act Fís Deporte, Online ahead of print.

Rael, B., Alfaro-Magallanes, V. M., Romero-Parra, N., Castro, E. A., Cupeiro, R., Janse de Jonge, X., Wehrwein, E. A., & Peinado, A. B. (2021). Menstrual Cycle phases influence on cardiorespiratory response to exercise in endurance-trained females. Int J Environ Res Res Public Health, 18(3), E860. https://doi.org/10.3390/ijerph18030860.

Rael, B., Barba-Moreno, L., Romero-Parra, N., Alfaro-Magallanes, V. M., Castro, E. A., Cupeiro, R., & Peinado, A. B. (2021). Cardiorespisratory response to exercise in endurance-trained premenopausal and postmenopausal females. Eur J Appl Physiol, 121(3), 903-913. https://doi.org/10.1007/s00421-020-04574-4.

Rael, B., Cupeiro, R., Alfaro-Magallanes, V. M., Romero-Parra, N., Barba-Moreno, L., Castro, E. A., & Peinado, A. B. (2021). Bone mineral density in well-trained females with different homonal profiles. Arch Med Deporte, 38(2). https://doi.org/10.18176/archmeddeporte.00029.

Rael, B., Romero-Parra, N., Alfaro-Magallanes, V. M., Barba-Moreno, L., Cupeiro, R., Janse de Jonge, X., & Peinado, A. B. (2021). Body composition over the menstrual and oral contraceptive cycle in trained females. Int J Sports Physiol Perform, 16, 375-381. https://doi.org/10.1123/ijspp.2020-0038.

Alfaro-Magallanes, V. M., Benito, P. J., Rael, B., Barba-Moreno, L., Romero-Parra, N., Cupeiro, R., Swinkels, D. W., Laarakkers, C. M., & Peinado, A. B. (2020). Menopause delays the typical recovery of pre-exercise hepcidin levels after high-intensity interval running exercise in endurance-trained women. Nutrients, 12, 1-13. https://doi.org/10.3390/nu12123866.

Alfaro-Magallanes, V. M., Barba-Moreno, L., Rael, B., Romero-Parra, N., Rojo-Tirado, M. A., Benito, P. J., Swinkels, D. W., Laarakkers, C. M., Díaz, Á. E., & Peinado, A. B. (2021). Hepcidin response to interval running exercise is not affected by oral contraceptive phase in endurance-trained women. Scand J Med Sci Sports, 31(3), 643-652. https://doi.org/10.1111/sms.13894.

Peinado, A. B., Alfaro-Magallanes, V. M., Romero-Parra, N., Barba-Moreno, L., Rael, B., Maestre-Cascales, C., Rojo-Tirado, M. A., Castro, E. A., Benito, P. J., Ortega-Santos, C. P., Santiado, E., Bustragueño, J., García-de-Alcaraz, A., Rojo, J. J., Calderón, F. J., García-Bataller, A., & Cupeiro, R. (2021). Methodological Approach of the Iron and Muscular Damage: Female Metabolism and Menstrual Cycle during Exercise Project (IronFEMME Study). Int J Environ Res Public Health, 18(2), 735. https://doi.org/10.3390/ijerph18020735.

Romero-Parra, N., Alfaro-Magallanes, V. M., Rael, B., Cupeiro, R., Rojo-Tirado, M. A., Benito, P. J., & Peinado, A. B. (2020). Indirect markers of muscle damage throughout the menstrual cycle. Int J Sports Physiol Perform, Online ahead of print. https://doi.org/10.1123/ijspp.2019-0727.

Romero-Parra, N., Barba-Moreno, L., Rael, B., Alfaro-Magallanes, V. M., Cupeiro, R., Díaz, Á. E., Calderón, F. J., & Peinado, A. B. (2020). Influence of the menstrual cycle on blood markers of muscle damage and inflammation following eccentric exercise. Int J Environ Res, 17(5), 1618. https://doi.org/10.3390/ijerph17051618.

Romero-Parra, N., Cupeiro, R., Alfaro-Magallanes, V. M., Rael, B., Rubio-Arias, J. Á., Peinado, A. B., & Benito, P. J. (2021). Exercise-induced mucle damage

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Romero-Parra, N., Rael, B., Alfaro-Magallanes, V. M., Janse de Jonge, X., Cupeiro, R., & Peinado, A. B. (2021). The effect of the oral contraceptive cycle phase on exercise-induced muscle damage after eccentric exercise in resistance-trained women. J Strenght Con Res, 35(2), 353-359. https://doi.org/10.1519/JSC.0000000000003897.

CONFERENCE PRESENTATIONS

Rael, B., Alfaro-Magallanes, V. M., Barba-Moreno, L., Romero-Parra, N., De Castro, E. A., Rojo Tirado, M. A., Cupeiro, R., & Peinado, A. B. (2020). Cardiorespiratory variables during a maximal running test in well-trained females with different hormonal profiles. Medicine & Science in Sports & Exercise, 52 (7S) (5), 189-189. https://doi.org/10.1249/01.mss.0000675552.43474.28.

Rael, B., Alfaro-Magallanes, V. M., Romero-Parra, N., Cupeiro, R., Castro, E. A., & Peinado, A. B. (2018, September). Differences in physiological variables between eumenorrheic females and oral contraceptive users: IronFEMME project VI NSCA International Conference, Madrid, Spain.

Rael, B., Alfaro-Magallanes, V. M., Romero-Parra, N., Peinado, A. B., & Cupeiro, R. (2018, 19th and 20th October). Composición corporal y ciclo menstrual en mujeres deportistas. IronFEMME project. VI Symposium Exernet. Investigación en Ejercicio, Salud y Bienestar. Exercise is Medicine, Pamplona, Spain.

Rael, B., Castro, E. A., Cupeiro, R., Barba-Moreno, L., Barrionuevo, E., & Peinado, A. B. (2016, 06-07-2018). Body composition and menstrual cycle in endurance-trained females. A pilot study XXI Annual Congress of the European College of Sport Science, Vienna.

Rael, B., Romero-Parra, N., Alfaro-Magallanes, V. M., Barba-Moreno, L., García-de-Alcaraz, A., Cupeiro, R., & Peinado, A. B. (2019). Bone mineral density in well-trained females XII International Symposium in Strength Training & IronFEMME Study, Madrid, Spain.

Rael, B., Romero-Parra, N., Alfaro-Magallanes, V. M., Cupeiro, R., & Peinado, A. B. (2018, 14th-15th December). Impacto de la toma de anticonceptivos orales sobre variables respiratorias durante el ejercicio. XI Internacional Symposium in Strenght Training, Madrid.

Rael, B., Romero-Parra, N., Cupeiro, R., Alfaro-Magallanes, V. M., Couceiro, J., Butragueño, J., & Peinado, A. B. (2018, 4th-7th July). Sex hormones and bone mineral density in females athletes with different hormonal profiles. XXIII Annual Congress of the European College of Sport Science, Dublín, Ireland.

CONTRIBUTIONS IN CONFERENCE PRESENTATIONS

Alfaro-Magallanes, V. M., Barba-Moreno, L., Benito, P. J., Cupeiro, R., Romero-Parra, N., Rael, B., Benítez, J. A., & Peinado, A. B. (2019, 3rd-6th July). Low ferritin status and hepcidin response to exercise in female athletes. XXIV Annual Congress of the European College of Sport Science, Prague, Czech Republic.

Alfaro-Magallanes, V. M., Barba-Moreno, L., Cupeiro, R., Romero-Parra, N., Rael, B., Maestre-Cascales, C., Peinado, A. B., Sánchez, C., Bodoque, M., & Salmerón, C. (2018, 4th-7th July). Iron metabolism regulation in women after an endurance protocol depending on ferritin status. XXIII Annual Congress of the European College of Sport Science, Dublín.

Alfaro-Magallanes, V. M., Barba-Moreno, L., Romero-Parra, N., Rael, B., Cupeiro, R., & Peinado, A. B. (2018, 14th-15th December). Iron metabolism regulation in monophasic oral contraceptive users after an endurance protocol depending on ferritin status. XI Simposio internacional de actualizaciones en el entrenamiento de la fuerza, Madrid.

Alfaro-Magallanes, V. M., Cupeiro, R., Barba-Moreno, L., Romero-Parra, N., Rael, B., Maestre-Cascales, C., & Peinado, A. B. (2018). Respiratory and perceived exertion variables during an intervallic endurance protocol through menstrual cycle phases VI NSCA International Conference, Madrid, Spain.

Barba-Moreno, L., Alfaro-Magallanes, V. M., Cupeiro, R., Romero-Parra, N., Rael, B., Benítez, J. A., Maestre-Cascales, C., & Peinado, A. B. (2019, 3rd-6th July). Influence of menstrual cycle and oral contraceptive rating of percieve exertion throughout an endurance exercise in female athletes. XXIV Annual Congress of the European College of Sport Science, Prague, Czech Republic.

Barba-Moreno, L., Alfaro-Magallanes, V. M., Romero-Parra, N., Rael, B., Benítez, J. A., Cupeiro, R., Castro, E. A., & Peinado, A. B. (2019). Menstrual cycle influence on oxygen consumption and ventilation in physically active women during an intervallic running protocol. XII International Symposium in Strength Training & IronFEMME Study, Madrid, Spain.

Barba-Moreno, L., Cupeiro, R., Díaz, Á. E., Alfaro-Magallanes, V. M., Santiago, E., Rael, B., & Peinado, A. B. (2018, 30th May-5th June). Menstrual cycle influence on hepcidin secretion and inflammatory reponses in female athletes. IronFEMME Pilot Study. ACSM Annual Meeting, Minneapolis, USA.

Benítez, J. A., Alfaro-Magallanes, V. M., Barba-Moreno, L., Benito, P. J., Cupeiro, R., Romero-Parra, N., Rael, B., & Peinado, A. B. (2019, 3rd-6th July). Effects of oestrogens and progesterone on substrate oxidation and respiratory variables. XXIV Annual Congress of the European College of Sport Science, Prague-Czech Republic.

Peinado, A. B., Rael, B., Romero-Parra, N., Alfaro-Magallanes, V. M., Barba-Moreno, L., Bodoque, M., & Cupeiro, R. (2019, 3rd-6th July). Sex hormones influence in respiratory variables in female athletes with different hormonal profiles. XXIV Annual Congress of the European College of Sport Science, Prague, Czech Republic.

Romero-Parra, N., Cupeiro, R., Alfaro-Magallanes, V. M., Rael, B., Barba-Moreno, L., Maestre-Cascales, C., Castro, E. A., & Peinado, A. B. (2020). Menstrual cycle and menopause influence on creatine kinase response after exercise- induced muscle damage. Medicine & Science in Sports & Exercise, 52 (7S) (5). https://doi.org/10.1249/01.mss.0000675552.43474.28.

Romero-Parra, N., Cupeiro, R., Alfaro-Magallanes, V. M., Rael, B., Maestre-Cascales, C., & Peinado, A. B. (2018, 14th-15th December). Muscle soreness and range of movement after exercise-induced muscle damage in eumenorrheic women. XI International Symposium in Strength Training, Madrid.

Romero-Parra, N., Peinado, A. B., Alfaro-Magallanes, V. M., Rael, B., Barba-Moreno, L., Rojo-Tirado, M. A., Maestre-Cascales, C., & Cupeiro, R. (2019, 3rd-6th July). Exercise induced muscle damage throughout the menstrual cycle. XXIV Annual Congress of the European College of Sport Science, Prague, Czech Republic.

Romero-Parra, N., Peinado, A. B., Alfaro-Magallanes, V. M., Rael, B., Benito, P. J., & Cupeiro, R. (2018, 4th-7th July). Influence of menstrual cycle on muscle function and jump performance: IronFEMME project. XXIII Annual Congress of the European College of Sport Science.

Romero-Parra, N., Peinado, A. B., Alfaro-Magallanes, V. M., Rael, B., Karabas, S., Navarro-García, J. M., & Cupeiro, R. (2018, September). Influence of sex hormones on muscle function and jump performance in oral contraceptive users: IronFEMME Project VI NSCA International Conference, Madrid, Spain.

AWARDS

2020 National Research Award. SEMEDE-FEMEDE research award 2020.

COMPLEMENTARY BACKGROUND

2020 Radiodiagnostic Operator Certificate. STERICYCLE (Company approved by the Nuclear Safety Council).