HEART RATE VARIABILITY-BASED RECOVERY IN HORMONAL CONTRACEPTIVE USERS AND NATURALLY MENSTRUATING FEMALES DURING AN ENDURANCE TRAINING INTERVENTION

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Master's Thesis
Science of Sport Coaching and Fitness testing
Faculty of Sport and Health sciences
University of Jyväskylä
Spring 2024
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TIIVISTELMÄ

Kurtén V. 2024. Kestävyysharjoitteluintervention aikainen sykevälivaihteluun perustuva palautuminen hormonaalista ehkäisyä käyttävillä ja luonnollisen kuukautiskierron omaavilla naisilla. Liikuntatieteellinen tiedekunta, Jyväskylän Yliopisto. Valmennus- ja testausopin pro gradu -tutkielma, 72 s.

On tärkeää ottaa huomioon naisten erilaiset hormooniprofiilit ja niiden ominaisuudet, kun tavoitteena on saavuttaa paras mahdollinen fyysinen suorituskyky ja palautuminen. Kuukautiskierron ja hormonaalisen ehkäisyn vaiheiden välinen sukupuolihormonitasojen vaihtelu on suurta. Hormonaalisen ehkäisyn käytöstä on tullut suosittua sekä yleisessä naisväestössä, että urheilijoiden keskuudessa. Tutkimustuloksissa on kuitenkin havaittu epäjohdonmukaisuutta koskien hormonaalista ehkäisyä ja sen mahdollisia vaikutuksia palautumiseen ja harjoitteluun. Joissakin tutkimuksissa on havaittu, että endogeenisten ja eksogeenisten hormonien vaihtelut vaikuttavat aerobiseen kapasiteettiin ja autonomisen hermoston modulaatioon. Sykevälivaihtelun mittaaminen on loistava tapa autonomisen hermoston toiminnan mittaamiseksi, kuvaamalla yksilön palautumistilaa. Tämän opinnäytetyön tavoitteena oli verrata yön sykettä ja sykevälivaihtelua hormonaalista ehkäisyä käyttävien ja luonnollisen kuukautiskierron omaavien naisten välillä. Lisäksi tutkittiin kohtalaisen intensiteetin kestävyysharjoittelun (MIET) mahdollista vaikutusta sykevälivaihteluun perustuvaan palautumiseen.

Tämän tutkimuksen osallistujat olivat nuoria terveitä naisia (n=16, ikä 27,9 ± 4,2 vuotta). Osallistujat jaettiin kahteen ryhmään, joista toiseen kuului yksivaiheisen hormonaalisen ehkäisyn käyttäjät (n=8) ja toiseen luonnollisen kuukautiskierron omaavat naiset (n=8). Osallistujat tekivät 8 viikon MIET-jakson, josta noin 4 ensimmäistä viikkoa (yksi kierto) analysoitiin tätä opinnäytetyötä varten. Syke ja sykevälivaihtelu tallennettiin Garmin Venu 2S kelloilla, joita osallistujia pyydettiin pitämään kädessä 24/7. Kierron ajalta valittiin kahdeksan yötä yöllisiä sykeanalyysejä varten, neljä yötä olivat ehkäisyn aktiivisessa vaiheessa tai luonnollisen kierron follikulaarisessa vaiheessa, sekä neljä inaktiivisessa tai luteaalivaiheessa. Sykeanalyysit tehtiin Kubios ohjelmiston (Kubios Oy, Kuopio, Suomi) avulla. Yöt valittiin mahdollisimman korkean intensiteetin ja keston omaavien harjoitusten ympärille. Neljän tunnin jakso valittiin harjoituksia edeltävästä ja sen jälkeisestä yöstä, jota käytettiin analysointiin. Syketietojen tilastollinen analyysi tehtiin käyttämällä riippumattomia t-testejä. Mahdolliset erot yön sykkeissä, sykevälivaihtelun (RMSSD ja HF teho) ryhmien välillä ja harjoitusten ympäröivien öiden välillä, tehtiin käyttämällä kaksisuuntaista toistettujen mittausten varianssianalyysiä (ANOVA).

Tilastollisia eroja yön HR-, RMSSD- tai HF-tehossa hormonaalisen ehkäisyvälineiden tai kuukautiskierron vaiheiden tai ryhmien välillä ei havaittu. MIET:llä ei ollut tilastollisesti merkitsevää vaikutusta yön syke-, RMSSD- tai HF-tehoon hormonaalista ehkäisyä käyttävillä tai luonnollisen kuukautiskierron omaavilla naisilla. Pieni otoskoko, lyhyt tiedonkeruujakso ja aerobisen harjoittelun intensiteetti, voivat myös olla syitä sille, ettei muutoksia autonomisen hermoston käytöksessä havaittu. Tutkimuksen havainnot indikoivat, että hormonaalisella vaihtelulla ei ole tilastollisesti merkitsevää vaikutusta autonomiseen modulaatioon, mutta aihetta käsitteleviä tutkimuksia tarvitaan lisää toteamuksen vahvistamiseksi.

Asiasanat: kuukautiskierto, hormonaalinen ehkäisy, sykevälivaihtelu, palautuminen

ABSTRACT

Kurtén. V. 2024. Heart rate variability-based recovery in hormonal contraceptive users and naturally menstruating females during an endurance training intervention. Faculty of Sport and Health Sciences, University of Jyväskylä. Science of Sport Coaching and Fitness Testing, Master's Thesis, 72 p.

Considering different hormonal profiles and their effects on physiology may be important when females want to achieve the best possible training adaptations and recovery outcomes. There are notable differences between fluctuating sex hormones across the menstrual cycle (MC) and hormonal contraceptive (HC) cycle. HC has become popular in both the general female population and athletes. However, there has been inconsistency in research findings regarding HC and the possible effects on recovery and training adaptations. Some studies have reported that the fluctuations of endogenous and exogenous hormones impact aerobic capacity and the modulation of the autonomic nervous system (ANS). Heart rate variability (HRV) is a great method to assess the ANS's function, thereby describing an individual's recovery state. The objective of this Master's Thesis was to compare nocturnal HR and HRV between HC users and naturally menstruating females. Furthermore, to investigate the possible influence of moderate-intensity endurance training (MIET) on HRV-based recovery.

Healthy young females (n=16*,* age 27.9 ± 4.2 years) were recruited for this study. The participants were divided into two groups, one for monophasic HC users (n=8) and another for naturally menstruating females (n=8*)*. The participants did an 8-week MIET period, of which approximately the first 4 weeks ((one HC or menstrual cycle (MC)) were analyzed for this thesis. HR and HRV were recorded with Garmin Venu 2S watches, which participants were asked to wear 24/7. Eight nights were chosen from the cycle for further analysis of nocturnal HR and HRV (RMSSD and HF). Four of the nights were in the active phase (AP) of HC or follicular phase (FP) of MC, and four were in the inactive phase (IP) or luteal phase (LP). The nights were chosen around training sessions of the highest intensity and duration possible, the night before and after the sessions. Kubios software (Kubios Oy, Kuopio, Finland) was used for the analysis of the 4-hour interval of HR data. Statistical analysis for the descriptive data was done using independent t-tests. Possible differences in nocturnal HR, RMSSD, and HF power between groups and between pre-post workout nights were done using two-way repeated measures analysis of variance (ANOVA).

Statistical differences in nocturnal HR, RMSSD, or HF power between HC or MC phases or between the groups were not observed. MIET did not have a statistically significant influence on nocturnal HR, RMSSD, or HF power in either HC users or normally menstruating females. The small sample size, short data collection period, and the intensity of the aerobic training may also be reasons why no changes in the behavior of the autonomic nervous system were observed. The findings of the study indicate that hormonal variation does not have a statistically significant effect on autonomic modulation, but more studies on the subject are needed to be able to confirm the statement.

Keywords: menstrual cycle, hormonal contraceptives, heart rate variability, recovery

ABBREVIATIONS

ANOVA	analysis of variance
ANS	autonomic nervous system
BP	blood pressure
BRS	baroreflex sensitivity
CK	creatine kinase
COC	combined oral contraceptive
EE	ethinyl estradiol
E2	estradiol
FP	follicular phase
FSH	follicle-stimulating hormone
HC	hormonal contraceptive
HF	high frequency
HH	high hormone
HPA-axis	hypothalamic-pituitary-adrenal axis
HPO-axis	hypothalamic-pituitary-ovarian- axis
HR	heart rate
HRV	heart rate variability
IL-6	interleukin-6
LF	low frequency
LH	low hormone/luteinizing hormone
LP	luteal phase
MC	menstrual cycle
MIET	moderate-intensity endurance training
OC	oral contraceptive
P4	progesterone
RR interval	time interval between successive heartbeats
RMSSD	root mean square of RR differences
RCT	randomized controlled trial
SDNN	standard deviation of NN intervals
VO_2max	maximal oxygen uptake
VO_2peak	peak oxygen uptake

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1 INTRODUCTION

In the past, sports and exercise science research has primarily focused on males due to the more complex physiology of females (Sims and Heather, 2018). Recommendations for females have been generalized based on these male studies, which leaves a huge gap in the field that needs to be filled with high-quality research including females (Elliott-Sale et al. 2013). The dynamic hormonal profiles of females should not be a reason to exclude them from exercise science research. On the contrary, by investigating the specific effects of sex hormones and their effect on physiological function, performance, and recovery, we may be able to help female athletes reach their true potential in sports (Elliott-Sale et al. 2021).

Menstrual cycle (MC) can be considered a health parameter and it is an essential part of female reproductive physiology. During one cycle (approximately 28 days), different endogenous sex hormones fluctuate in the body (Davis & Hackney, 2017). The use of hormonal contraceptives (HC) has become popular worldwide with more than 150 million females using oral contraceptives (OC) around the world (United Nations, Department of Economic and Social Affairs, Population Division 2019). The HC cycle for OC users is prescribed usually 24+4 or 21+7 days, where the inactive phase can be modified if necessary. The differences between hormonal profiles need to be considered when researching females. Endogenous and exogenous hormones act differently in the body, even though exogenous hormones try to mimic the endogenous ones (Sims and Heather 2018). Exogenous hormones enter the body's circulations differently and the concentration of the circulating hormones fluctuates at different timeframes. (Sims and Heather 2018). Additionally, the exogenous hormone levels lead to suppressed endogenous hormone levels in HC users (Romero-Parra et al., 2021; Gborienemi et al. 2022).

The activity of the autonomic nervous system (ANS) can be measured quantitatively with HRV, reflecting the heart-brain interaction and dynamics of the ANS (Shaffer et al. 2014). The autonomic control of the cardiovascular system is influenced by external and internal factors (Dong et al. 2016). Parasympathetic activity and high HRV levels can be reached when maintaining an active lifestyle and keeping a high level of physical fitness (Sammito & Böckelmann, 2016). Also, an association between aerobic fitness and increased autonomic control of HR has been found (Hautala et al. 2009). Female sex hormones influence the sympathetic (activating) and parasympathetic (calming) activity of the ANS (von Holzen et al. 2016; Ahokas et al. 2023). Some evidence indicates that the active phase of HC would affect

recovery negatively (Sims et al. 2021; Ahokas et al. 2023). However, there are also studies indicating no significant differences between active or inactive pill phases (Teixeira et al. 2015). In naturally menstruating females the evidence suggests that the parasympathetic activity is dominant in the follicular phase (FP) when compared to the luteal phase (LP) (Tenan et al. 2014; Ahokas et al. 2023; Sims et al, 202), hence, other studies did not find any significant differences in HRV metrics between the MC phases (Leicht et al. 2003; Ylidirir et al. 2002).

Exercise is good for our overall mental and physical health (Ruegsbegger and Booth, 2018). Endurance exercise influences the cardiovascular system on many levels, most importantly by increasing oxygen delivery around the body (Hackney, 2019). Endurance capacity is often determined by maximal oxygen uptake (VO2max). Moderate-intensity endurance training (MIET) is enough to get positive physiological training adaptations (McArdle et al. 2014). HRV can be used to evaluate stress and recovery, as well as recovery from training (Zecchin, 2021). When the parasympathetic nervous system is dominating as much as possible, the individual can get the most out of the recovery.

Hormonal profiles and endurance training both possibly influence the ANS, combining these into a study seemed intriguing. Therefore, this master's thesis aimed to investigate the possible differences in HR and parameters of HRV between HC users and naturally menstruating females, and whether these differences could be observed across the cycles between HC/MC phases. Additionally, the possible influence of hormones during MIET on HRV-based recovery was examined. The findings of this thesis may increase the knowledge about possible alterations the different hormonal profiles may have on the ANS modulation and recovery from MIET and may help with the choice of contraceptive method.

2 FEMALE MENSTRUAL PHYSIOLOGY

Female physiology is complex due to its many components and cyclical hormonal fluctuations. A functioning reproductive system is important not only for reproduction, but for overall health in females (Davis & Hackney, 2017). This chapter will include an introduction to female menstrual physiology starting from the neuroendocrine basics and then describing the menstrual cycle and female sex hormones. Then we will dig into what hormonal contraceptives are and how they affect the female endocrine system. Finally, we will investigate how the hormonal profiles affect exercise and recovery.

2.1 Female reproductive system

The female reproductive system consists of the hypothalamus, the pituitary, the ovary, and the uterus (Davis & Hackney, 2017). This axis is called the hypothalamic-pituitary-ovarian- axis (HPO-axis) (Davis & Hackney, 2017). Complex signaling between neuroendocrine glands regulates the reproductive functions and the release of various hormones, which all have their function in the body. This complex process starts when gonadotropin-releasing hormone is released from the hypothalamus to the pituitary gland through the bloodstream. The pituitary gland reacts by releasing several gonadotropin hormones (especially luteinizing hormone (LH) and follicle-stimulating hormone (FSH)). FSH and LH activate the ovarian receptors for FSH and LH, to release the two major sex hormones, estrogen, and progesterone. Both estrogen and progesterone are primarily produced in the ovaries of females (Davis & Hackney, 2017).

2.2 Female sex hormones

The menstrual cycle is characterized by fluctuation of these sex hormones; estrogen, progesterone (P4), FSH and LH. All hormones have their role in the female reproductive system and the imbalance of these hormones can lead to health concerns and increase the risk for various diseases.

Estrogen and progesterone. For females of reproductive age, most endogenous estrogen is produced in the ovaries and corpus luteum, while during pregnancy the placenta also secretes estrogen. Additionally, some smaller amounts of estrogen are produced in other organs such as the liver, heart, and brain. (Cui et al. 2013). Increased E2 levels during the menstrual cycle cause maturation and release of the egg as well as the thickening of the endometrial wall for a possible fertilized egg to implant. (Davidge-Pitts and Burt Solorzano, 2022) Estrogens can be divided into three different types which all play an important role across the lifespan. Estradiol is the most common type of estrogen during the reproductive age, estriol is the type produced during pregnancy, and estrone is the only type that the body produces after menopause. (Davidge-Piits and Burt Solorzano, 2022) Estradiol (E2) is the hormone that drives puberty, the change from a girl to a woman. The main physical changes of puberty are the growth of breasts and pubic hair, and the start of the menstrual cycle. E2 also plays an important role in bone health, skin health, and sexual desire. (Davidge-Piits and Burt Solorzano, 2022) When females experience menopause, E2 levels decrease because the ovaries stop producing it. Postmenopausal females have lower E2 levels than premenopausal females, which affects bone health and increases the risk of getting osteoporosis. The side effects of low E2 levels can be treated with exogenous estradiol in the form of hormone replacement therapy. (Davidge-Piits and Burt Solorzano, 2022)

Progesterone (P4) is produced by the adrenal cortex and the ovaries (Cable & Grider, 2023) and is secreted by the corpus luteum, the endocrine gland (collection of cells) formed on an ovary after ovulation (Cable & Grider, 2023; Davidge-Pitis and Burt Solorzano, 2022). P4 affects endometrial changes in the uterus (proliferating the endothelial lining and thickening the endometrial wall), making it possible for implantation of a fertilized egg to occur. If no implantation occurs, the endometrium is removed causing bleeding, and a new menstrual cycle begins. (Cable & Grider, 2023) However, if implantation does occur, P4 continues to have an important role during pregnancy.

FSH and LH. FSH and LH are both released from the pituitary (Davis & Hackney, 2017). Both hormones peak mid-cycle before ovulation occurs. A rise in LH from the urine has been considered highly accurate to mark the timing of ovulation. When E2 and P4 concentrations decrease towards the end of the menstrual cycle, there is a smaller inter-cycle rise in FSH. (Baker et al. 2020).

FSH has an important role in female reproduction because it stimulates the production of ovarian follicles and sex hormones. These affect fertilization, implantation, and pregnancy. (Marshall, 2004). LH is essential for ovulation to occur and important for the late stages of follicle development (Bulun, 2016).

2.3 Menstrual Cycle

A normal menstrual cycle lasts approximately 28 days but ranges from 21 to 35 days in healthy females. The first day of the cycle is the onset of menstruation and the start of the FP. At this point, FSH is elevated, and estradiol (E2) is low. (Davis & Hackney, 2017) Many females experience menstrual symptoms, which means symptoms associated with menstruation (Schoep et al. 2019). In the middle of the cycle (around day 14) E2 levels reach their peal whereafter LH reaches its peak; FSH has a smaller peak as well, and ovulation should occur. (Davis & Hackney, 2017) Ovulation can be described as when an oocyte is released from the follicle, corpus luteum is developed from the ruptured follicle, where progesterone (P4) and E2 are secreted (Baker et al. 2020). Ovulation and the couple of days leading to it is called the fertile window (Fehring et al. 2006). Knowledge about the length of the fertile window is often of particular interest to individuals who want to avoid or achieve pregnancy. The fertile window can be estimated in various ways, but Fehring et al. (2006) determined it from the day of ovulation and the 5 days before. However, an individualized approach is recommended to determine the fertile window (Bull et al. 2019). After that, the LP begins. At the beginning of this phase, E2 levels can be high (Ahokas et al. 2023) but drop drastically and then slowly increase once more before decreasing towards the end of the cycle. Post ovulation, P4 increases and reaches its peak around the mid-LP before decreasing towards the end of the cycle as well (Chidi-Ogbolu and Baar, 2019). An adequate LP phase is determined by P4 concentrations being >16 nmol/l (Elliott-Sale et al. 2021). Figure 1 illustrates a typical natural MC including the changes in the ovaries, fluctuations in sex hormones, MC cycles, and the thickening of the endometrial wall.

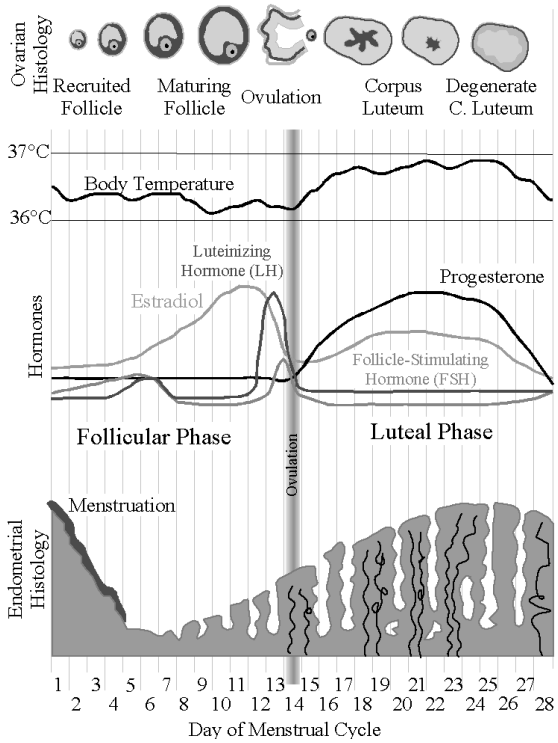


FIGURE 1. A typical menstrual cycle is divided into follicular and luteal phases including the ovarian cycle, body temperature, fluctuations of the reproductive hormones, and the endometrial wall and its thickness. Modified from physiopedia.com.

2.3.1 Menstrual symptoms and menstrual cycle characteristics

An adequate hormonal function (concentrations of sex hormones) is important for bone health and several other health outcomes. Hormonal fluctuations of the menstrual cycle may affect physical and emotional well-being. Schoep et al. (2019) studied menstrual symptoms and their impact on everyday life. They concluded that dysmenorrhea (painful cramps of the uterus during menses) was the most common symptom of menstruation with a prevalence of 85%. Psychological complaints with a 77% prevalence and tiredness with 71%, were also common among the more than 42 000 females who participated in the study. In addition to these symptoms, headache, back pain, and heavy menstrual bleeding were reported menstrual symptoms. During the menstrual bleeding, 38% of the participants reported not being able to do their daily activities. (Schoep et al. 2019) Menstrual symptoms and disorders need to be considered since they have an impact on the quality of life of the female population.

Hormonal fluctuations of the menstrual cycle may affect physical and mental well-being, in both the general population and athletes (Martin et al. 2018). The most frequent physical symptoms for athletes were abdominal pain, cramps, back pain, bloating, and headache. The emotional symptoms were not as frequent as the physical ones, but 4% of the participants reported mood swings as an emotional symptom of the menstrual cycle. (Martin et al. 2018)

Premenstrual syndrome (PMS) is the behavioral, emotional, and physical symptoms that some females experience during the luteal phase, especially one week before menstruation (de Zimbotti et al. 2013). De Zimbotti (2013) did a study examining how HR and HRV changed during the menstrual cycle in females with and without PMS symptoms. No differences were found between the groups in HRV indices. However, for both groups (PMS and control) they found a reduction in HF power in the mid-luteal phase during REM sleep. Progesterone levels are highest at this point of the MC phase. Progesterone has a thermogenic effect which increases HR and might impact the HF power. The core body temperature has been studied to generally be a bit higher (0,3-0,7°C) during the luteal phase of the menstrual cycle (Baker et al. 2020) (see figure 1). This increase in temperature is caused by the thermogenic effect of progesterone, which increases during this phase of the menstrual cycle. The increase in body temperature correlates with an increase in HR (Baker et al. 2020). The P4 levels in LP have shown a correlation with HF power, indicating a decrease autonomic control in LP compared to FP (de Zambotti et al. 2013). Autonomic control will be discussed more precisely in the next chapter.

It is important to highlight that the phase lengths vary a lot among the general population. Bull et al. (2019) did a study where they investigated 600,000 MCs and got important information about the characteristics of MCs. This study demonstrated that shorter cycles had a shorter LP when compared to normal length cycles. On the contrary, the very long cycles had longer FP, but the luteal phase length was not affected much in relation to the normal length of the LP. Another interesting finding from this study was the strong linear correlation between MCcycle length and FP length with an increase with age. This study used data from a mobile app, which works as a platform for educational purposes and MC tracking. Measuring the basal body temperature and doing ovulation tests helps to track the menstrual cycle and possibly determine a fertile window. (Bull et al. 2019)

2.3.2 Concentrations of estrogen and progesterone in females of reproductive age

Female sex hormones fluctuate, and concentrations of these hormones vary a lot across the lifespan. Fluctuations during adulthood may appear due to the menstrual cycle, clinical conditions, or external factors such as hormonal contraceptive use or exercise as well as pregnancy (Elliott-Sale et al. 2021). The menstrual cycle is mainly controlled by estrogen and if the estrogen levels are too low it may affect the menstrual periods making them less frequent or even stopping. Other symptoms of low estrogen levels can be sleeping troubles, hot flashes, and vaginal dryness. Moreover, too high concentrations of estrogen in females can cause symptoms such as weight gain, mood swings, low sexual desire, and breast swelling or tenderness. (Davidge-Piits and Burt Solorzano, 2022).

Low progesterone levels decrease fertility and result in impaired ability for implantation of a fertilized egg (Cable & Grider, 2023). If progesterone levels are too low during pregnancy, it increases the risk of miscarriage and early delivery of a fetus. During the menstrual cycle, low progesterone levels can lead to unopposed estrogen in the uterus which may lead to endometrial hyperplasia (irregular thickening of the endometrial lining in the uterus) which is a risk factor for endometrial cancer. In contrast, excessive concentrations of progesterone increase the risk of granulosa cell tumors (in the ovaries) and breast cancer. (Cable & Grider, 2023).

2.4 Hormonal contraceptives

Hormonal contraceptives (HC) are a reliable and safe option to prevent unwanted pregnancy and mitigate symptoms associated with PMS and menstruation (Speroff & Darney, 2011). HCs include a combination of the two hormones: estrogen and progestin, or only progestin. HC types may differ by the concentration of the hormones or by the method, meaning if it's taken daily (e.g. oral contraceptives) or if it is more permanent (e.g. intrauterine devices (IUDs) and implants). An individual should discuss the different contraceptive options with a health professional before making decisions about them. Even if contraception would be the main reason to use HC, there might be several other reasons to start with HC. These will be discussed later in this chapter.

Prevalence of hormonal contraceptive use. Contraceptives have developed a lot during the last decades and are commonly used across the whole world. When looking at all possible contraceptive methods, the prevalence of contraceptive use in reproductive females (18-49 years old) was 48,5% in 2019 (United Nations, Department of Economic and Social Affairs, Population Division 2019). The most common HCs can be divided into two groups; combined and progestin-only. All the combined HCs contain exogenous estrogen, and exogenous progesterone (progestin). Common combined HCs include the combined pill (oral contraceptive), the patch, and the vaginal ring. (Lewis et al. 2019). The prevalence of oral contraceptives (OC) was 8% in the world (United Nations, Department of Economic and Social Affairs, Population Division 2019). These numbers in Finland were even higher, 78% for any method of contraceptive and 32% for OC. This shows that a significant number of reproductive females are familiar with contraceptives. According to a United Nations report (2019), OC use increased between years 1994 and 2019 from 97 million users to 151 million females in the world. Martin et al. (2018) reported the prevalence in elite female athletes, was relatively even between HC use and non-use (49,5% of the athletes were currently using HCs and 50,5% were not). Combined contraceptives, including both estrogen and progestin, were the most common type of HC (68,5 %) in elite female athletes.

2.4.1 Combined oral contraceptives

Generally, monophasic combined oral contraceptives (COCs) include a dose of exogenous versions of estradiol and progestin and are most often taken for 21 days (active phase) followed

by 7 days of placebo pill (inactive phase) (Sims & Heather, 2018). The daily proportion of hormones in the monophasic COCs remains the same throughout the active pill phase and the elevated exogenous hormone levels lead to suppressed endogenous hormone levels in the body (Romero-Parra et al., 2021; Gborienemi et al. 2022). COCs control the reproductive cycle by manipulating the hypothalamic-pituitary-ovarian axis (HPO-axis) such that E2 and P4 are not released from the ovaries (Sims & Heather 2018). Additionally, when there is no pre-ovulatory surge of LH, COCs stall ovarian follicular maturation and prevent ovulation and pregnancy (Gborienemi et al. 2022). Also, an increase in cervical mucus occurs, which hinders sperm transportation (Casado-Espada et al. 2019).

Even though the hormones in HC are supposed to mimic endogenous E2 and P4, exogenous hormones work differently in the body (Sims & Heather 2018). For example, endogenous and exogenous hormones enter circulation via differing pathways. When the exogenous hormones are taken orally, they enter hepatic circulation before they enter systemic circulation, while endogenous hormones enter directly into systemic circulation when they are secreted (Sims and Heather, 2018). Consequently, OCs and exogenous estrogens have been linked to several liver-related diseases and complications. (Bethesda, 2012). The concentration of circulating hormones is also affected by daily variation in fluctuation. OC is taken as a dose once a day, while endogenous hormones are secreted by the ovaries and fluctuate during the entire cycle (Sims and Heather, 2018). The differences between the daily fluctuations of hormones between HC and MC can eb seen in figure 2. However, there is still fluctuation in ormones during an HC phase, but it looks different than the fluctuations during an MC phase. The molecular structure of exogenous hormones is made to be bioactive for a longer period (Sims and Heather, 2018). During the inactive phase of the COC cycle, withdrawal bleeding occurs due to the drop in exogenous hormone levels in the body (Fleishman et al. 2010). This scheduled bleeding should not be confused with menstrual bleeding, because the HC cycle is manipulated by exogenous hormones and does not function naturally.

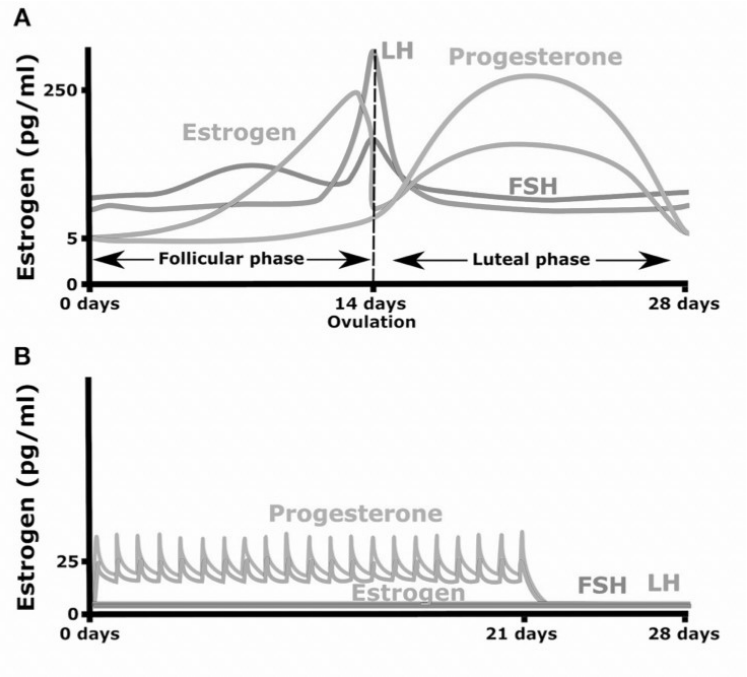


FIGURE 2. An illustration comparing the hormonal fluctuations during a typical natural menstrual cycle (A) and when using combined hormonal contraceptives (B). Modified from Chidi-Ogbolu & Baar 2019.

In a combined oral contraceptive (COC), there can be three types of exogenous estrogens used; ethinyl estradiol (EE), estradiol valerate, and 17 beta-estradiol (Casado-Espada et al. 2019). Several different progestin types are also used in COCs, and they are classified based on their chemical structure. The concentrations of hormones in COCs may differ between brands, and additionally, they can differ in how the concentrations change during the active pill phase, for instance, monophasic meaning one-phase of the same amount of exogenous hormones (van Vliet et al. 2011). There are also biphasic and triphasic with two- and three different phases with differing concentrations of hormones (Vliet et al. 2011). In Ahokas et al. (2023) they found no significant differences in E2 concentrations between the inactive and active pill phase. Also, Teixeira et al. (2015) found no significant differences between the HC phases (inactive and active pill phases) in E2 concentrations. Yet, Romero-Parra et al. (2021) found that E2 was significantly higher in the inactive pill phase than in the active phase. Notably, the statistical significance does not tell the whole story of the physiological phenomenon.

2.4.2 Reasons for hormonal contraceptive use

The main reasons for hormonal contraceptive use in addition to pregnancy prevention are regulation of menstrual cycle, treatment of acne, and management of hormonal imbalance (De Leo et al. 2016). It is possible to manipulate your menstrual cycle with hormonal contraceptives to get it scheduled or to skip the menstrual bleeding altogether (Buck et al. 2023). exogenous estrogen in COCs helps with irregular menses (Gborienemi et al. 2022)., Irregular menses can for instance be the result of stress, low energy availability, or gynecological illnesses (Ravi et al. 2021). If menstrual suppression is the goal, a gynecologic care provider should be contacted to ensure that menstrual suppression is safe. Some special populations such as military service members, athletes, and transgender individuals might often consider this option, but any female could benefit from menstrual suppression. (Buck et al. 2023).

Martin et al. (2018) reported that for elite female athletes (n=430) the main non-contraceptive reason for HC was the "ease of use" (18.8%), referring exactly to the aspect of being able to predict, schedule or manipulate the menses. Martin et al. (2018) stated that regular, less frequent, and lighter periods were on top of the list of positive effects of HC use. This study was based on elite athletes and therefore the ability to predict and change your cycle can be handy for example for an upcoming competition. Other reasons were the attempt to reduce fluctuations in performance and fatigue (Martin et al. 2018).

2.4.3 Side effects of combined oral contraceptives

Several side effects have been recorded by users of combined oral contraceptives (COCs). Some of the most common side effects are vaginal spotting and abnormal bleeding, breast tenderness, nausea, and bloating (Speroff and Darney, 2011). In addition to these, headaches, weight gain, and irritability are also common side effects (De Leo et al. 2016). However, the side effects of COCs are difficult to recognize due to subjective feelings and aches. The symptoms may vary between individuals and products, and they can challenge the individual in finding the dose/product that suits them.

The most frequent medical conditions linked with combined HC use are increased risk for venous thromboembolism (blood clots), arterial thrombosis (for example myocardial infarction

and stroke), and breast and cervical cancer (Brynhildsen, 2014). The risk of venous thromboembolism may be enhanced for an individual if there is a hereditary factor for the condition. A higher age and obesity will also increase the risk (Brynhildsen, 2014). These aspects make it even more important for the individual to get proper information and go through screening before starting to take oral contraceptive pills.

3 THE AUTONOMIC NERVOUS SYSTEM AND HEART RATE VARIABILITY

Heart rate variability (HRV) is a quantitative measure that reflects the heart-brain interactions and the dynamics of the ANS (Shaffer et al. 2014). HRV describes both the variation in time between successive heartbeats (RR intervals) and instantaneous heart rate (Task Force 1996). In the following chapter, first, the ANS, cardiac cycle and the heart's electrical activity will be introduced. Hereafter, the methods to measure and factors affecting HRV will be presented. Lastly, HRV as a recovery metric and scientific evidence of how exercise and the female hormonal profiles might influence it will be investigated.

3.1 The autonomic nervous system

The nervous system is a system of conducting electrochemical stimuli that come from cells around the body (mostly neurons) reaching the brain and spinal cord. These neural impulses are then channeled back where the response occurs (Rudge et al. 2024). The nervous system can be divided into two main parts, the central nervous system (brain and spinal cord) and the peripheral nervous system (system around the body that carries the impulses to and from the central nervous system. (Rudge et al. 2024)

The heart and the circulatory system are controlled by the higher brain center (central command) and the cardiovascular control center in the brain stem (Dong, 2016). This happens through the ANS, which is more exactly mediated in the hypothalamus and medulla oblongata (lowest part of the brainstem). The ANS regulates nearly every tissue in the human body, e.g. smooth muscle, cardiac muscle, endocrine and exocrine glands, and adipose tissue (Wehrwein et al. 2016). The ANS controls important physiological processes such as blood pressure, blood flow, digestion, energy balance, sexual function, and inflammatory processes. These processes happen without conscious effort, which makes ANS the involuntary nervous system. However, the ANS's main responsibility is to ascertain that homeostasis (meaning a stable equilibrium) is maintained (including cells, tissues, and organs) regardless of disturbances from internal and external environments (Wehrwein et al. 2016).

The ANS is anatomically and functionally divided into the activating sympathetic nervous system, also called the fight or flight system, and the calming parasympathetic nervous system, also called the rest and digest system (Mulroney & Myers 2015; Johnson 2013). The sympathetic nervous system emerges from the spinal cord and acts predominantly by the neurotransmitter norepinephrine. This activates tissues around the body and increases blood flow in organs such as the heart, brain, and muscles (Johnson 2013). The parasympathetic nervous system emerges from specific cranial and sacral nerves, acting predominantly by the neurotransmitter acetylcholine. This regulates the resting activities in the body such as salivation, urination, and digestion. (Johnson 2013) In figure 3 you can see the autonomic nervous system and how the parasympathetic and sympathetic nervous systems control important physiological processes.

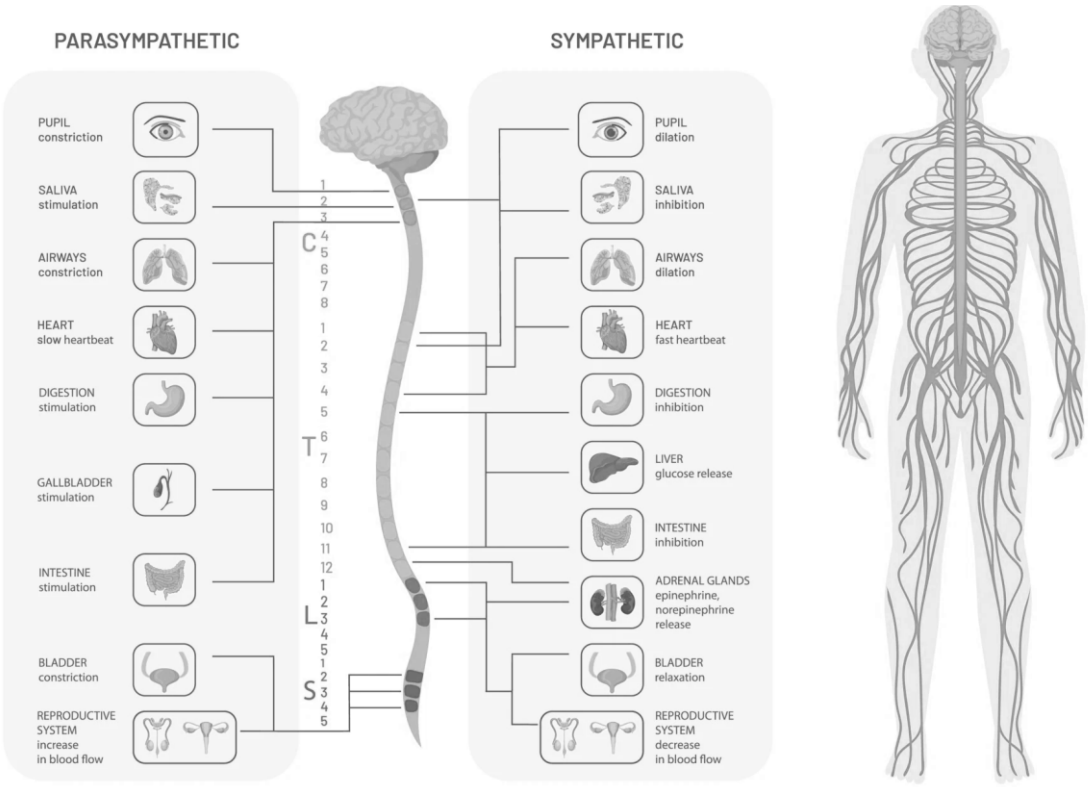


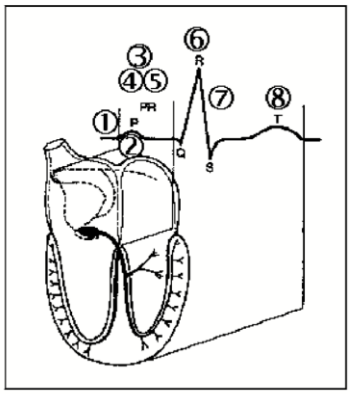
FIGURE 3. Illustration of the ANS divided into parasympathetic and sympathetic nervous systems and how they are responsible for certain bodily functions. In the middle, the brain and the vertebrae of the spine show their territory of bodily functions (Picture from simplyspychology.org, modified).

3.1.1 Cardiac cycle and electrical activity of the heart

The heart is predominantly controlled by the ANS. The sinoatrial node (SA node) in the heart works as a pacemaker that has its own rate of neural firing (McArdle et al. 2014). From the SA node, an electrical impulse goes throughout the atria to the atrioventricular node (AV node). Thereafter, the atria contract forcing blood to the ventricles, and the AV bundle (also called the bundle of His) transports the impulse further through the ventricles by the Purkinje system. The contraction of both ventricles will follow, and the cardiac cycle begins again from the SA node. (McArdle et al. 2014). The SA node is influenced by the parasympathetic and sympathetic nervous systems. The nucleus tractus solitarius (sensory nuclei i.e., cluster of nerve cell bodies) placed in the brainstem, receives sensory inputs, and provokes cardiovascular feedback for physical stress and emotion (Dong, 2016).

When the parasympathetic nervous system is influencing the heart rate, acetylcholine is released by the vagus nerve (tenth cranial nerve). This hinders the potential of the SA node and decelerates the HR (Task Force, 1996; Dong, 2016). Cardiac vagal outflow (from the heart) has been increasing the prevalence of increase in HF power (Kiviniemi et al. 2006). When the sympathetic nervous system dominates, epinephrine and norepinephrine are rereleased and the heart rate increases (Task Force, 1996; MacCraty & Shaffer, 2015).

The electrical activity of the heart can be monitored with an electrocardiogram (ECG). The most important parts of one cycle of ECG are the P wave, the QRS complex, and the T wave (see figure 4) (Reed et al. 2005). The P wave symbolizes depolarization of the atria followed by atrial contraction. The QRS complex represents depolarization of the ventricles, followed by contraction of the ventricles. The QRS complex usually masks atrial repolarization. (McArdle et al., 2014) Finally, the interval from the end of the QRS-complex to the T wave indicates ventricular repolarization (Ozimek et al. 2021).



	Physiologic Event	ECG Evidence
1.	SA node initiates impulse	Not visible
2.	Depolarization of atrial muscle	P wave
3.	Atrial contraction	Not visible
4.	Depolarization of AV node & Common Bundle	Not visible
5.	Repolarization of atrial muscle	Not visible
6.	Depolarization of ventricular muscle	QRS complex
7.	Contraction of ventricular muscle	Not visible
8.	Repolarization of ventricular muscle	T wave

FIGURE 4. Illustration and table of the physiological events of the cardiac cycle and which of these events can be observed on an ECG. (From Becker 2006).

The QRS complex includes a Q-wave, an R-wave (peak), and an S-wave. Typically, the R-wave is used to determine heart rate (HR) in beats per minute (bpm). The time between two of these R waves is called the R-R interval. Heart rate variability (HRV) can be calculated using the variation between R-R intervals. (Reed et al., 2005) An example of a typical ECG can be seen in figure 5.

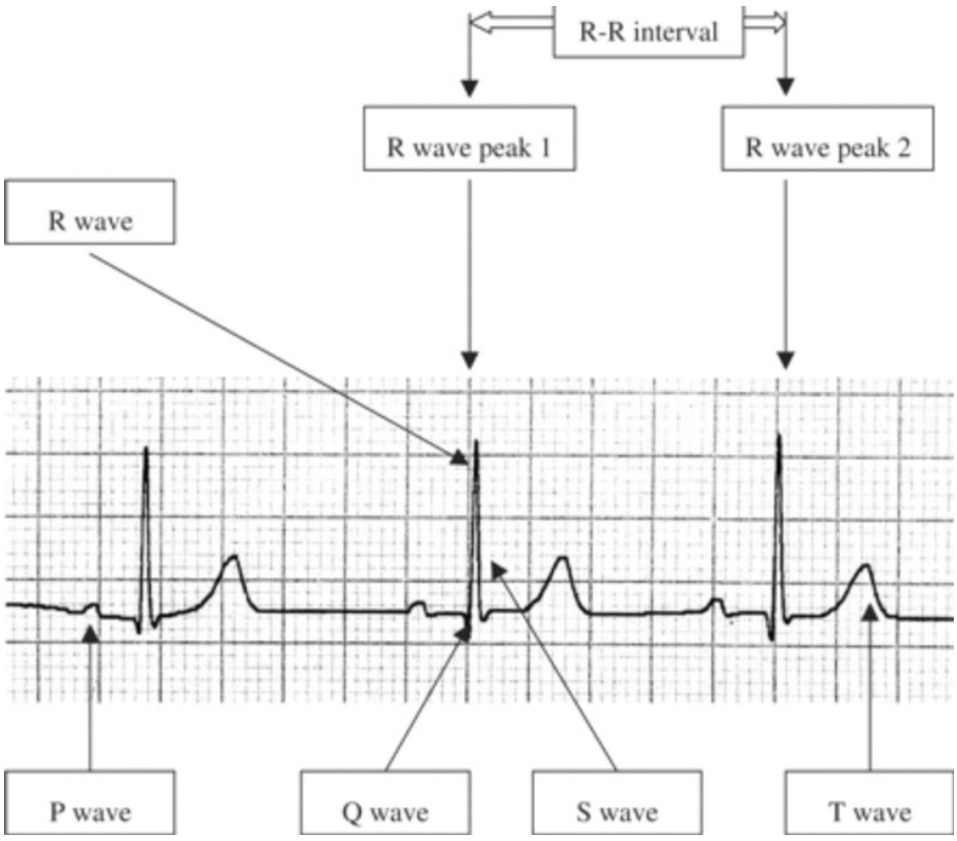


FIGURE 5. A typical ECG tracing of a healthy individual identifying the P-wave (atrial depolarization), the QRS complex (ventricular depolarization), and T-wave (ventricular repolarization). Note the peak R-wave identifying the heart rate, and the R-R interval used to determine HRV. Image borrowed from Reed et al. 2005.

Resting heart rate. The cardiovascular system is responsible for uninterrupted blood flow to meet the requirements of bodily demands and heart rate acts as a critical determinant of cardiac output (Olshansky et al. 2023). Resting heart rate (RHR) is mainly determined by the vagus nerve activity affecting pacemaker cells in the sinus node and is to some extent also determined by genetics. RHR is dynamic and is regulating due to factors such as diet, caffeinated drinks, circadian rhythm, and smoking. (Olshansky et al. 2023) RHR has also been reported to decrease with regular physical activity (Reimer et al. 2018).

RHR has been positively related to mortality (Reimers et al. 2018) and can predict various health outcomes, e.g. cardiac sudden death (Olshansky et al. 2023). An ideal resting heart rate differs between individuals but if RHR exceeds 70 bpm, one should be concerned especially if some sort of disease is present (Olshansky et al. 2023). In research, RHR is often used as an additional parameter together with HRV metrics.

3.2 Heart rate variability

The heart does not work as a metronome and in healthy individuals the autonomic tone, describing the balance or dominance between either parasympathetic or sympathetic tone, is the primary reason for a change in heart rate (Kleiger et al. 2005; Sigurdsson et al. 2018). Previous studies have shown that a decrease in HRV has been correlated with an increase in mortality (Sammito & Böckelmann, 2016). The autonomic control of the cardiovascular system is affected by external factors from the surrounding environment, and internal factors such as circulating hormones, muscles, and tissue metabolism (Dong, 2016). There are different methods to measure the autonomic tone and it is important to consider them when choosing a method for HRV research. RR intervals are the intervals between R-wave peaks (see figure 5) and can also be described as intervals between normal heartbeats (Kleiger et al. 2005).

3.2.1 Methods to measure heart rate variability

One possible way to measure HRV is to use time-domain measures. Of these measures, the root mean square of successive differences (RMSSD) between RR intervals is often used to calculate HRV. This measure shows the parasympathetic tone of normal RR intervals and is determined by ventilation. (Kleiger et al., 2005). In Myllymäki et al. (2012) they decided to use RMSSD as the only time-domain measure for HRV. They investigated how exercise intensity and duration affect the nocturnal cardiac autonomic activity and sleep quality.

Another way to measure HRV is to use frequency domain measures. These give us information about the power, meaning the variance, in spectral analysis of RR intervals (Task Force, 1996). The heart's rhythm can be divided into four different frequency bands: high-frequency (HF), low-frequency (LF), very-low-frequency (VLF), and ultra-low-frequency (ULF) (McCraty & Shaffer, 2015). HF bands range between 0.15-0.4 Hz and LF in 0.04-0.15 Hz. High frequency represents vagal, parasympathetic dominance, whereas LF symbolizes some vagal activity with sympathetic dominance. (Task Force, 1996)

Lower levels of HF power have been associated with anxiety, stress, and worry (McCraty & Shaffer, 2015). A decrease in HF demonstrates a decrease in parasympathetic activity. In Castaldo et al. (2019) study they expressed that LF could not be seen during an ultra-short-term HRV measurement of fewer than 2 minutes. However, HF was noticed already during the 1-minute measurement.

Lengths of HRV measurements. HRV measurements can be done during a variety of timeframes from only a few minutes to continuous 24-hour measurements, which do not replace one another but are used depending on the aim of the measurement (Shaffer and Ginsberg, 2017). While ECG is the "golden standard" for detecting the heart's electrical activity, other equipment can be used for this. Many companies have produced health monitors to promote awareness of physical activity and health (Spierer et al. 2015). The scientific communities are doing their best to examine their validity and reliability to improve the technology to give as high-quality data as possible Spierer et al. 2015).

Nocturnal HRV measurements might be a more time-efficient method compared to measurements taken when woken up, specifically when measures are taken during deep sleep

phases (Herzig et al., 2017). Nocturnal measurements of HR and RMSSD have been shown to give reliable values of the average HRV on a weekly basis (Mishica, 2022). During night-time, the body goes into a rested state where it can recover from strain. HRV can be used as an indicator of objective recovery (Föhr et al. 2017). However, to be able to see a pattern or a change in a pattern of HRV, it is important to consider an individualized approach and develop a long-term baseline of HRV (Föhr et al. 2017).

3.3 Factors affecting heart rate variability

HRV has great potential in the role of examining autonomic nervous activity in healthy individuals and patients with various disorders (Task Force, 1996). A variety of physiological factors affect HRV levels such as hormonal reactions, metabolic processes, stress, cognitive processes, and respiratory arrhythmia (Task Force, 1996).

Lifestyle factors and physical activity. Various lifestyle factors may influence autonomic balance and HRV. Chronic alcohol abuse and elevated body weight, especially free fat mass, decrease HRV (Sammito & Böckelmann, 2016). An increase in parasympathetic activity and HRV can be reached when maintaining an active lifestyle and keeping a high level of physical fitness (Sammito & Böckelmann, 2016). Also, Hautala et al. (2009) study showed an association between aerobic fitness and increased vagal modulation of HR, which in this case means higher HF. Additionally, HRV reacts to intensive sports activities such as competitions and overtraining. In these situations, HRV decreases, and sympathetic activity predominates (Sammito & Böckelmann, 2016).

Emotionally or physiologically challenging or overwhelming situations can often be described as stressful (McEwen, 2007). The spectrum from bad to good stress is subjective and refers often to the amount of control an individual has over the task or the situation. A physiological indicator of stress is the activation of the ANS and HPA-axis. (McEwen, 2007) As humans, we are constantly exposed to different environments, noise, situations, and conflicts, that affect our mental and physical state. We need to learn and find our patterns to calm down every now and then, to reduce chronic stress. Parasympathetic activation of the cardiac vagal nerve occurs when the body and mind relax. In a meta-analysis by Kim et al. (2018), it was reported that HRV is sensitive to changes in the ANS related to stress. A stressful state is characterized by

low parasympathetic activity, with increased LF and decreased HF. Schmalenberger et al. (2019) found that decreased cardiac vagal activation was related to inferior cognitive control, emotional regulation, and social engagement. Additionally, the reduction of vagal activity has been linked to depression and anxiety.

Myllymäki et al. (2012) did a study where subjects did 5 days of running sessions with 48 hours in between. The cardiac autonomic activity was analysed during the night for 4 hours, starting 30 minutes after falling asleep. To measure their activity rates, the subjects wore a heart monitor (Alive Technologies, Pty Ltd., Australia) to find their ECG RR intervals and an Actiwatch on their wrist. The main outcome of this study is that an increase in exercise intensity elevated nocturnal HR and decreased relaxation percentage but had no effect on HRV during sleep. The longest exercise duration (90 minutes) had the greatest effect on HR, HRV, and relaxation, compared to the shorter durations with the same intensity (30 and 60 minutes). (Myllymäki et al., 2012)

In a study by Costa et al. (2018), they examined the sensitivity of two different methods to measure nocturnal cardiac autonomic activity in female soccer players. The methods they used were slow-wave sleep episodes (SWSE) and hour-by-hour methods. The SWSE method 10-min records of normal RR intervals are analyzed. Whereas, in the hour-by-hour method the entire sleep time is divided into 1-hour periods and then analyzed. No significant changes were found in the participants' HRV measures between training and non-training days. However, the hour-by-hour method showed some interesting comparisons regarding increased HR during the first hours of sleep, on the nights when the participants had late-night training.

3.3.1 HRV-guided training

HRV-guided training has shown greater improvements in the 5 km running test when compared to standardized fixed training (da Silva et al. (2019) Thus study was done on untrained females, and the training plan was based on an HRV measurement of 3 min in a standing position. RMSSD was used as the HRV measure in this study due to its reliability and because it is a great predictor of changes in endurance performance (Buchheit et al., 2004). The HRV value was compared with the mean (SD) of the previous 10 values. The training was guided through analysis of HRV values and if the HRV value was < mean -1 SD of the previous measures it

implied weakened parasympathetic activity and moderate-intensity continuous training (MICT) was recommended. High-intensity interval training was recommended if the value was the same or > of the mean. The training sessions were controlled, which made adherence to the program possible. (da Silva et al. 2019)

3.3.2 HRV across the lifespan and chronic diseases

HRV increases at night and decreases significantly in the morning (Sammito & Böckelmann, 2016). After the age of 15, HRV starts to decrease over the years, and function of the the ANS differs between genders. The results regarding genetics and their effect on HRV have been inconsistent (Sammito & Böckelmann, 2016). People with various chronic diseases (e.g. heart disease and cardiac insufficiency, lung diseases, and diabetes) have been shown to have lower HRV than healthy individuals (Sammito & Böckelmann, 2016).

When studying and diagnosing psychological disorders (psychopathology), HRV can be a great asset for the process (Beauchaine & Thayer, 2015). Higher HRV has been shown to correlate positively with cheerfulness, calmness, and life satisfaction (Geisler et al. 2010). High-frequency HRV has been shown to affect positively on psychological adjustments, such as empathic responding, behavioural regulation, and attachment security (Beauchaine & Thayer, 2015). Low resting HF as well as decreased HF as a reaction to emotional challenges, has been associated with an extensive range of psychopathological syndromes including autism, anxiety, depression, and attention problems. Beauchaine & Thayer (2015) also describe a model for how high-frequency HRV could be used as a transdiagnostic biomarker of self-regulation and how the individual differences in motivational approaches evolve through the subcortical neural circuits to the cortical neural circuits affecting our behavioral patterns.

3.3.3 Female sex hormones and heart rate variability

Generally, female sex hormones affect the sympathetic and parasympathetic activity of the ANS (von Holzen et al. 2016). Ahokas et al. (2023) support this with their main finding that both MC and HC phases affected HR and HRV. Estrogen (E2) has been shown to regulate the ANS by increasing parasympathetic (vagal) activity, and progesterone the opposite, elevating

norepinephrine release and activating the sympathetic nervous system (Sims et al. 2021). Cardiac vagal activity has been shown to dominate in the early FP (Leicht et al. 2003) and decrease from FP to LP (Schmalenberger et al. 2019). Leicht et al. (2003) propose that vagal dominance in early FP and a decrease in LP might be due to increasing levels of FSH, LH, and P4 in LP). A positive relationship was also seen between E2 levels and vagal activity at ovulation. In other menstrual cycle phases, the decreased vagal activity might be a result of FSH, LH, and P4 inhibiting E2 of cardiac autonomic control, which means that E2 might have a cardioprotective effect by reflecting vagal activity. Despite these results, Leicht et al (2003) did not find any significant differences in HRV metrics between MC phases.

Wilczak et al. (2013) investigated autonomic balance and baroreflex sensitivity (BRS) between OC users and eumenorrheic females. The main finding was that BRS was lower during the FP compared to LP in eumenorrheic females and during the active pill phase compared to the inactive phase in the OC group. The authors discussed that OCs might be unfavorable for health when they hinder the baroreflex regulation of the cardiovascular system. Additionally, Sims et al. (2021) demonstrated that females who used COCs had reduced recovery rates during the active pill phase. Recovery rate was considering HRV, resting heart rate, respiratory rate, and sleep duration. They had a sample size of 3870 naturally cycling and 455 OC users. Minson et al. (2000) examined how BRS changes in females using monophasic OC. BRS explains the autonomic control of the cardiovascular system, more specifically neural regulation of the sinus atrial (SA) node. The baroreflex can control heart rate on a beat-to-beat basis when utilized through parasympathetic, but not sympathetic activity, since the time delay of reaction to cardiac action by parasympathetic activation is much faster than the sympathetic ones. (La Rovere et al. 2008). Minson et al. (2000) found that sympathetic and cardiovagal BRS were both greater in the low hormone phase (inactive) than in the high hormone phase (> 2 weeks of taking OC pills). Yet, in normally menstruating females the only sympathetic BRS is greater in the mid-luteal phase than in the early FP. In this phase, both E2 and P4 are more elevated compared to the early FP.

3.3.4 Menstrual cycle and heart rate variability

As mentioned in the previous section, female sex hormones have been shown to affect vagal control (von Holzen et al. 2016; Ahokas et al. 2023). The endogenous hormones (especially E2) could have a cardioprotective effect (Leicht et al. 2003). Brar et al. (2015) also support this by suggesting that sympathetic activity dominates during the LP of the menstrual cycle. This suggestion was made based on their results showing that mean RR, VLF, and LF power, mean HR and low-frequency normal units (LFnu) were higher in the luteal phase compared to the other two phases.

However, Yildirir et al. (2002) did not report any significant differences between HR, LF, or HF between menstrual cycle phases. Thus, LFnu was increased in the luteal phase and HFnu in the FP. These indices are normalized spectral HRV measures, which are often used to remove large within-and across-subject variation and to get more exchangeable values (Burr, 2007). On the contrary, Blake et al. (2023) study showed higher HF power in early LP versus early FP, indicating parasympathetic modulation post ovulation early in the LP.

In contradiction, Ahokas et al. (2023) reported that the RMSSD values were higher in the FP than in the luteal phase for eumenorrheic females. However, they reported heterogeneity in the E2 concentrations in the eumenorrheic females from ovulation to the luteal phase. This change in E2 likely affected the HRV metrics (RMSSD). The subjects who had smaller changes in their E2 concentrations had increased HRV metrics from ovulation to the luteal phase. Hence, subjects who had moderate to high E2, had decreased HRV values from ovulation to the luteal phase.

Tenan et al. (2014) did a study where they examined 13 eumenorrheic females and their HRV across the menstrual cycle. They did a resting ECG five times during their menstrual cycle. They did a piecewise function for breathing rate and spectral HRV measures, and these demonstrated a decrease in standard deviation of NN intervals (SDNN), which is a time-domain HRV metric, and an increase in HR. These led to a decrease in parasympathetic control (HF) of the ANS. The HRV was higher prior to ovulation and decreased during the cycle until the onset of menstruation. Simultaneously, the heart rate increases over the cycle, indicating parasympathetic withdrawal during the luteal phase. The reason for parasympathetic withdrawal and the physiological responses to it might originate in the simple mechanism of

increased P4 in the luteal phase causing an increase in body temperature, metabolic rate (Zhang et al. 2020), and ventilation (Tenan et al. 2014). The authors discussed if the main neurotransmitter, acetylcholine, could stimulate E2 and P4, but this is contradictory to the previous statement about P4 being a reason for parasympathetic withdrawal. Tenan et al. (2014) point out that it is an intertwined ecosystem between ANS and the endocrine system, and that further study is needed in this area.

In Sims et al. (2021) HRV and recovery metrics were decreased in early and mid-FP in naturally menstruating females. A possible explanation for this could be that estrogens modulate the ANS by increasing vagal activity. However, in this study they included participants with a regular natural MC lasting 25-35 days, but no other measurements to support the phases were done. Identification of the generation of progestin used in the COC users was not completed either. Yazar et al. (2016) did not find any significant differences between HRV (RMSSD) in different menstrual cycles. They did however conclude that SDNN decreased in LP, possibly indicating dominance of the sympathetic nervous system during this phase. Therefore, when planning and monitoring training loads in females, understanding the possible impact of endogenous and exogenous hormones on the ANS and recovery, is important but a personalized approach is recommended (Sims et al. 2021). A summary of the most important evidence about the effect of the MC and HRV seen in table 1.

TABLE 1. Summary of the results from studies that have investigated the effect of the menstrual cycle and heart rate variability metrics.

Study	Change in HRV and HR	Variables used	Phase of MC where change in HR or HRV was seen
Leicht et al. (2003)	No significant differences	LF, HF, TP, LF/HF,	Early FP and ovulation
Sims et al. (2021)	Lower HRV higher HR and RR	HRV, HR, RR and sleep duration	Early and mid FP

Brar et al. (2015)	Sympathetic predomination		LP
Ylidirir et al. (2002)	No significant differences	HR, LF, HF	
Ahokas et al. (2023)	Higher HRV and lower HR	RMSSD, SDNN, HR	FP
Tenan et al. (2014)	Higher HRV and lower HR	SDNN, HF, HR	FP
Yazar et al. (2016)	Lower HRV (some metrics)	SDNN, SDANN	LP
Blake et al. (2023)	Higher HF	HF power	Early LP

HR = heart rate, HRV = heart rate variability, LP = luteal phase, FP = follicular phase, SDNN = standard deviation of NN intervals, RMSSD = root men square of successive differences, LF = low-frequcney, HF = high frequency, LF/HF = LF/HF ratio, TP = total power, RR = respiratory rate.

3.3.5 Hormonal contraceptives and heart rate variability

Sims et al. (2021) did a study where they investigated the patterns of endogenous and exogenous ovarian hormone modulation on recovery metrics. They included both normally menstruating females and females using hormonal contraceptives (n=4594). One of the main findings in this study was that the exogenous hormones reduce adaptation to stress at all pill stages, which might impact day-to-day recovery. Females who used combined HC had elevated HRV and improved recovery during the inactive phase.

Ahokas et al. (2023) findings support the suggestion by Sims et al. (2021) that endogenous and exogenous hormones have different effects on the ANS. Females who used OCs had higher RMSSD, SDNN, and HF power and lower HR in the inactive pill phase compared to the first week of the active phase. During the inactive pill week, exogenous hormones are not consumed, so OCs may influence ANS.

Teixeira et al. (2015) investigated if OC-use influences the autonomic control of the heart. Seventeen young females using HCs did a resting ECG in both low hormone (LH) and high hormone (HH) phases of OC. They did not discover any differences between HR, BP, or HRV values. Pereira et al. (2023) did a systematic review where they found inconsistencies in the literature regarding OC use and its effect on autonomic indices. They mention that the OC formulations and concentrations of sex hormones, as well as the stressors and intensity of the stressor, might be the reasons behind the inconsistencies.

These studies included participants using different COC brands. In Ahokas et al. (2023), 5 different brands of COCs were used, but in Teixeira et al. (2015) the hormonal concentration in the COC was standardized, however, the individual responses to the dose needed to be considered and noted. Romero-Parra et al. (2020) included COCs with 4 different combinations of exogenous hormones. A summary of the three main articles regarding the possible effect of OC on HRV can be seen in table 2.

TABLE 2. Summary of the results from studies that have investigated hormonal contraceptive use and heart rate variability metrics.

Study	Change in HRV and HR	Variables used	Phase of HC	Considerations
Sims et al. (2021)	Higher HRV and recovery	HRV, RHR, RR and sleep duration	Inactive	Nocturnal Different formulas of HC used
Teixeira et al. (2015)	No significant differences	HR, BP and HRV	High hormone vs. low hormone	Supine position, metronome-controlled breathing rate Different formulas of HC used
Ahokas et al. (2023)	Higher HRV and lower HR	RMSSD; SDNN, HF, HR	Inactive	Nocturnal Different formulas of HC used

HR = heart rate, HRV = heart rate variability, SDNN = standard deviation of NN intervals, RMSSD = root men square fo successive differences, LF = low-frequency, HF = high frequency, LF/HF = LF/HF ratio, TP = total power.

4 ENDURANCE TRAINING AND RECOVERY

Exercise is good for our overall health and is associated with a reduced risk or delay of the onset of several chronic diseases. Exercise improves not only cardiorespiratory fitness but many other parts of health (e.g. mental health), which increase the quality of life. (Ruegsegger & Booth, 2018). Endurance training can be defined as activity at 60-80% of maximal heart rate (HRmax) lasting for at least 20 minutes (Carter et al. 2003). Long-term endurance training leads to several physiological adaptations, of which the most important for our study is the positive effects on the cardiovascular system and autonomic nervous activity.

4.1 Physiology of endurance training

Endurance exercise influences the cardiovascular system, most importantly by having a positive effect on oxygen delivery (Hackney, 2019). The three main factors affecting endurance exercise performance are maximal oxygen uptake (VO_2max), exercise economy (relationship between oxygen consumption and speed/power), and exercise efficiency (meaning the ratio between energy produced during and energy cost of exercise. (Hackney, 2018)

Endurance capacity is often determined by measuring VO_2max. VO_2max is generally achieved during maximal performance where large muscle mass is in use, for example, while running (Joyner and Coyle, 2008). High-level endurance athletes have higher values of VO_2max than the general population. In high-level endurance athletes, the most remarkable physiological adaptation affecting the VO_2max values is an increased cardiac stroke volume (Joyner and Coyle, 2008). The definition of stroke volume is the volume of blood pumped out from the heart's left ventricle during each systolic contraction (beat) (Bruss and Raja, 2022). Cardiac output (the product of HR and stroke volume) has a key role in VO_2max improvement and the increased capacity of ventricular filling. (Hackney, 2018) Additional adaptations include increased blood volume (at rest) and capillary and mitochondrial density (size) in trained skeletal muscles (Joyner and Coyle, 2008; Hackney, 2018).

HR increases linearly with exercise intensity and VO_2max. The maximal heart rate (HRmax) is often defined as the highest heart rate reached during maximal physical exertion (Lach et al.

2022). until exhaustion and it is usually achieved and measured at the same time as VO_2max (for example at a graded exercise test) (McArdle et al. 2014). A relatively easy way to determine exercise intensities is to define them with % of HRmax. In Meyer et al. (1999) they concluded that exercise intensity recommendations should not be based only on VO_2max or HRmax, but also take into consideration other aspects such as ventilatory and/or lactate levels. The levels of 60 and 70% HRmax showed to be aerobic intensities in this study. (Meyer et al, 1999). Notably, the participant in this study did exhaustive incremental tests on a bike and the HR behaviour might differ between activities.

Training at different training intensities is essential for specific sports, influencing specific physiological adaptations in the body (MacInnis and Gibala, 2017). Not only the intensity but also the duration and frequency of the training influence the physiological adaptations to endurance training. Aerobic capacity has shown to be improved already when exercising at a heart rate of 55-70% of HRmax. (McArdle et al. 2014) Moderate-intensity endurance exercise is enough to induce positive physiological training adaptations and can be continued for a longer period because the intensity is kept below LT (McArdle et al. 2014). In Tabata et al. (1996) participants exercised 5 days a week for 6 weeks at moderate intensity (70% of VO_2max) for 60 min. The results showed a significant improvement in VO_2max of 5 ml/kg/min and no change in anaerobic capacity (determined from the maximal accumulated oxygen deficit).

Energy systems of endurance training. During physical activity, different energy-transfer systems are activated depending on the length of the effort. First, adenosine triphosphate (ATP) and phosphocreatine (PCr) deliver immediate energy to get the working muscles going. Then, the glycolytic pathways are activated to continue the ATP resynthesis. If the activity continues for a longer time, the aerobic system takes more and more responsibility for the energy transfer. (McArdle et al. 2014). Short-term maximal efforts require more energy than the hydrogen oxidation of the respiratory chain can produce, which leads to glycolytic ATP production and lactate appears as a side product in active muscles and eventually in the blood (McArdle et al. 2014). This type of activity is called anaerobic activity using non-oxidative systems.

The long-term energy system is called the aerobic energy system, and it utilizes oxygen as an energy source. This type of energy production is slower than the glycolytic one, but it is not as fatiguing, because the body can remove the lactate, so the concentration does not increase much

from the baseline (McArdle et al. 2014). However, when the exercise level increases and hypoxia occurs (not enough oxygen in the tissues), it leads to increased blood lactate concentrations. (McArdle et al. 2014) When the level of exercise reaches this point, it is called the lactate threshold (LT) and determines the oxidative capacity of the skeletal muscle (Joyner and Coyle, 2008). The rise in blood lactate levels does not usually occur before 60% of maximal oxygen uptake (VO_2max) is reached, or 75-90% in trained individuals (Joyner and Coyle, 2008).

With endurance training, the main metabolic adaptation is the increased capacity of the working skeletal muscles to oxidize fuel for energy (Hackney, 2018). Additionally, during exercise, the body utilizes fats as fuel for longer, which spares the glycogen stores and potentially prolongs fatigue (Hackney, 2018). To clarify, these different energy systems work simultaneously, but some are just dominating in specific types of exercise more than others.

4.2 Recovery from endurance training

Even though exercise is good for us, it also strains our bodies, which requires recovery. Recovery is often used as an umbrella term; however, it can be defined as a time-relative restorative process (Kellmann et al. 2018). Recovery can be regeneration from daily strain, stress, training, or other activities. Recovery plays a key role in sports performance and the prevention of injuries and overtraining, and if an individual's recovery is disturbed it can lead to fatigue (Kellmann et al. 2018; Hackney et al. 2018).

Recovery and training state can be assessed by looking at several different variables. Nuuttila et al. (2021) looked at recovery from endurance training by examining performance, physiological markers, and subjective feedback finding that increased training intensity and volume both improved endurance performance. Regarding recovery, nocturnal HR and perceived (subjective) recovery were affected by training, but HRV was not.

4.2.1 Exercise and recovery with different hormonal profiles

There is a lack of studies investigating cardiovascular adaptations to endurance training in females (Carter et al. 2003). Females have different metabolism and hormone levels than men, as well as smaller lean body mass, heart size, and blood volume. (Carter et al. 2003). These

need to be taken into consideration and results from studies done on males should not always be not generalized for the female population.

Menstrual cycle, exercise performance and recovery. In normally menstruating females, Hackney (1999) found that E2 and P4 levels were significantly greater before and after exercise in the LP compared to the FP. The exercise was seen to result in a decrease in glycogen, however, the muscle glycogen storages were lower in FP post-exercise, compared to LP post-exercise. These results indicate that elevated E2 and P4 levels in LP may affect exercise metabolism by sparing glycogen and using lipids as the primary energy source for a longer period. McNulty et al. (2020) examined the possible effects of the MC phase on exercise performance in eumenorrheic females. The result of the study showed that exercise performance might be trivially reduced during the early FP compared to other phases. However, this meta-analysis included studies with quite large variance, and the quality in many of the studies was classified as low for instance due to variability in methods to identify MC phase.

Hackney et al. (2019) investigated inflammation and muscle damage during recovery after aerobic exercise between different phases of the menstrual cycle (mid-FP and mid-LP). The results showed that at 24- and 72-hours post-exercise creatine kinase (CK) levels were significantly higher in the mid-FP than in the mid-LP. The Interleukin-6 (IL-6) levels were significantly higher immediately, 24- and 74 hours after exercise in the mid-FP than in the mid-LP. IL-6 is a useful marker for the immune system and increased CK levels can explain muscle damage. These findings indicate that recovery from aerobic exercise when looking at inflammation and muscle damage variables, might be slower when sex hormones are reduced during the FP of MC.

Hormonal contraceptives, exercise performance, and recovery. There are contradictory results regarding the impact of hormonal contraceptive phases (inactive vs. active phase) on athletic performance. Taipale-Mikkonen et al. (2021) observed no systematic differences between the HC cycle phases in body mass, maximal performance, or other physiological responses when investigating COC users (n=12) doing maximal running tests until exhaustion in four different parts of the HC phase. Barba-Moreno et al. (2019) investigated OC users (n=8) who performed running at 75% of their maximal aerobic speed during different HC phases. Ventilation and breathing frequency were significantly higher during the active pill phase compared to the

inactive pill phase. However, no significant differences were seen in VO2max. These studies were quite different from each other, but still looked at endurance capacity and performance, hence included in this section.

Romero-Parra et al. (2021) found that creatine kinase (CK) concentrations were higher in the inactive phase than in the active pill phase of OC in resistance-trained females. Something to add regarding exercise while using HC is that Minahan et al. (2017) observed that thermoregulation is altered during exercise in females who use HC. The participants were divided into OC and non-OC-users and performed three-stage cycling trials in two different conditions: temperate (22°C) and heat (35°C). In the OC group, core temperature was already elevated at baseline and during the first stages of exercise, when compared to the normally menstruating group. Furthermore, the OC group had an increased skin blood flux (measured from the forearm) caused by cutaneous vasodilation in the heat, and they reported higher RPE values at all stages. The higher RPE values indicate that the OC users perceived exercising in the heat condition as more demanding, and the altered thermoregulation presumably led to delayed sweating. However, they did not have any measures for the actual performance in the heat, so how and if HC influences that, still needs further investigation.

Lebrun et al. (2003) completed a double-blinded randomized controlled trial (RCT) investigating the effects of OC use on athletic performance in highly trained females (N = 14). Those who took triphasic OC showed a decrease (-2,7 ml/kg/min) in maximal oxygen uptake (VO_2max compared) with the placebo group (+0,8 ml/kg/min) and the difference between the groups was significant. However, all participants were first without contraceptives to get baseline measurements, subsequently one of the groups got OC and the control group got a placebo pill. This means that the participants were in the early phase of the start of the OC pill, which may influence the individual differences and adaptations to aerobic capacity (Schumpf et al. 2023). To reduce the risk of including participants with hormonal profiles not fitting into the characteristics of HC users, a minimum of 3 months of HC use before recruitment is recommended (Elliott-Sale et al. 2021). In Ahokas et al. (2023) There was no mention of how long their participants had been on HC before recruitment and Sims et al. (2021) included only participants who had used HC within the last 9 months without stopping.

OC users have been found to have lower improvements in peak maximal oxygen uptake (VO2peak) after a sprint interval training period of 4 weeks when compared to naturally

menstruating females (Schaumberg et al. (2017). The participants in this study were recreationally active OC users (n = 25) and naturally menstruating females (n = 22). Dalgaard et al. (2019) did a study on 28 females, 14 OC users, and 14 non-users who performed a 10-week resistance training program. They found that OC use had a potent anabolic effect as they had greater muscle mass and showed a significant increase in their type 1 fibers. However, no differences were observed in muscle strength between the groups. These results explain that OC use might negatively affect endurance capacity (VO_2max) compared to naturally menstruating females.

Regarding recovery from exercise, HC users have been shown to have a slower recovery from exercise-induced muscle damage (Mackay et al. 2019) which is illustrated by higher CRP concentrations (Larsen et al. 2020) compared to naturally menstruating, eumenorrheic females.

4.3 Autonomic adaptations to endurance exercise

Good aerobic fitness has a positive effect on ANS regulation (Hautala et al. 2009), which may include decreasing HR during rest and submaximal exercise. These adaptations are the product of intrinsic changes in different parts of the heart. (Hautala et al. 2009; Carter et al. 2003) Acute intense aerobic exercise might decrease cardiac vagal outflow at first, but after rest or a lighter training period, it might rebound to better cardiac vagal outflow than before. Reimers et al. (2018) did a meta-analysis and found that aging is associated with a reduction in the parasympathetic control of the heart. However, with regular long-term endurance training, this reduction can be slowed down (Carter et al. 2003.)

Heart rate variability and recovery. HRV is seen as a valid method to evaluate stress and recovery in athletes. Training loads and responses can be monitored with HRV responses, which can help both the athlete and the coach (Zecchin, 2021). When the parasympathetic nervous system is dominating as much as possible, especially during the night, an individual can get the most out of the resting state and recover both mentally and physically.

Recovery from endurance training. Cardiovascular autonomic regulation measured by HRV, is an important tool to monitor training adaptations. A high HRV at baseline has been associated with increased training adaptations after high-intensity training, whereas a low HRV baseline demonstrates a poor adaptation or possibly fatigue or unreasonable training load (Vesterinen et

al. 2013). A functioning ANS has been shown to determine the response to aerobic training among healthy subjects (Hautala et al. 2009). HRV should increase or remain stable as a product of positive adaptations to training (Sims et al. 2021).

Vesterinen et al. (2013) indicated that high-intensity training is more effective for positive ANS adaptations and endurance performance than constant load training at low-intensity (below the aerobic threshold). Higher training intensity and frequency improved HRV more than training done at lower intensities. In Nummela et al. (2016) HR decreased and nocturnal HRV showed an increase with a high-intensity training program, compared to a constant load program. The constant load program included moderate-intensity training including 3x 40 min sessions/week at an intensity between aerobic and anaerobic thresholds. However, the HR and HRV differences were not observed until the second 4-week period of the training. The HRV measurements were done during the night-time after to training. Additionally, a greater increase in maximal oxygen uptake (VO2 max) was observed in the high-intensity group than in the control group. This study shows that high-intensity training is more effective on the ANS modulation and endurance performance than constant load training.

During exercise, a fast withdrawal of parasympathetic activity occurs, and sympathetic activity dominates, resulting in greater catecholamine circulating in the blood. In response, HR increases and HRV decreases (Breuer et al. 1993). Barrero et al. (2019) analyzed daily HRV in well-trained female cyclists during the Tour de France circuit and compared these results with training load and perceived exertion. Significant increases were seen in low-frequency normal units (LFnu) and decreases in (high-frequency normal units (HFnu), meaning an increase in sympathetic input and a decrease in parasympathetic input during the event. This HRV imbalance was associated with RPE and training impulse and the kilometers the participants biked. However, it took only one week for the HRV values to return to baseline values.

Kaikkonen et al. (2008) investigated immediate (5 min) and slow (30 min) HRV recovery after high-intensity exercise interventions in endurance athletes. The participants performed two different interval interventions, one with 3 min intervals at 93% of maximal velocity at maximal oxygen uptake (vVO2max) and the other with 85% vVO2max. At the end of each exercise, almost no HRV was seen. However, during immediate 5 min recovery, LF power and total power (TP) recovered significantly, whereas recovery of HF power was not seen at all, indicating almost no vagal reactivation. When the exercise intensity increased, lower TP and

LF power were seen during the 5 min recovery. In the slow HRV recovery (30 min), even small changes in the exercise intensity made a difference in HF power metrics. Nuuttila et al. (2022) examined changes in HRV metrics after maximal exercise. Results showed a significant increase in HR and a decrease in RMSSD and HF power. They concluded that recordings of a 4 h period (starting 30 min after going to bed) and a full night are highly reliable methods to detect disruptions in cardiovascular homeostasis, meaning disruption in the balance between different parts of the cardiovascular system, and the recovery process. Nuuttila et al. (2022) concluded in their study that there were large interindividual variations in the HRV recovery although all participants did the same training plan in this study. This highlights the importance of an individualized approach and the usefulness of HRV monitoring.

In conclusion of this chapter, endurance exercise has a positive effect on the ANS regulation (Hautala et al. 2009) and HRV is an effective tool to monitor the recovery of the ANS. Even though the female sex hormones have been shown to affect the ANS (von Holzen et al. 2016), the effect of these hormones on recovery from endurance exercise is not entirely clear and requires further investigation.

5 RESEARCH QUESTIONS AND HYPOTHESES

According to the literature, it is important to consider hormonal concentrations when performing research, especially in the female population (Ahokas et al. 2023). When we want to plan and monitor training loads for females, the understanding of endogenous and exogenous hormones and their possible impact on the ANS is essential (Sims et al. 2021). Further research is required to improve and clarify the contradictory findings regarding how sex hormones affect HRV and recovery after training in females. Therefore, the main objective of this thesis is to evaluate how nocturnal HRV and HR change between cycles in HC users and naturally menstruating females during a moderate intensity endurance training intervention.

RQ1: Is there a difference in nocturnal HRV and HR between phases of one HC/MC cycle?

H1: Yes. HRV measures (RMSSD and HF power) will be higher and HR lower during the inactive phase (IP) in HC users (Ahokas et al. 2023). HRV will be higher in FP (Tenan et al. 2014; Brar et al. 2015; Ahokas et al. 2023; Leicht et al. 2003) and HR lower (Tenan et al. 2014; Ahokas et al. 2023) in naturally menstruating females.

RQ2: Do nocturnal HR and HRV metrics (RMSSD, HF power) differ between HC users and naturally menstruating females?

H2: Yes. The HC group will have reduced HRV measures and a higher HR when compared to naturally menstruating females due to exogenous hormones in the HC (Ahokas et al. 2023; Sims et al. 2021).

RQ3: Can moderate-intensity endurance training (MIET) influence recovery as reflected in nocturnal HRV metrics (RMSSD, HF power) and HR?

H3: No. Moderate-intensity continuous exercise has not been shown to influence nocturnal HR or HRV (Nummela et al. 2016) regardless of the evidence that endurance exercise decreases HR at rest (Reimers et al. 2018; Hautala et al. 2009).

6 METHODS

This study was an observational study. The study was part of a larger Women's menstrual cycle and endurance training study (NaisQs) funded by the Finnish Ministry of Education and Culture (OKM/21/626/2021, OKM/101/626/2021, OKM/82/626/2022) and Firstbeat Analytics Oy where Garmin Venu 2S watches and Garmin HRM-dual HR belt and HR monitors were also provided by Firstbeat Analytics Oy. The JYU Ethical Committee approved the study. Participants provided written informed consent before the study, and they could withdraw from the study at any time. The informed consent was signed at the university prior to the familiarization session.

6.1 Participants

Healthy young females ($N = 16$) between the ages of 18 – 35 years were recruited for this study. Participants (mean age 27.9 ± 4.2 years; mean height 167.4 ± 6.4 cm; mean weight 66.7 ± 9.1kg; BMI 23.8 ± 2.6 kg/m^2, table 3) were either naturally menstruating with a 28-35-day cycle who had been without HC for at least 12 months before the study or had been taking monophasic HC for at least 12 months. The contraceptives used were monophasic hormonal contraceptive pills including different amounts of Ethinyl Estradiol (EE) and progestin. In this study, we included six different brands of COC (see table 4). HC users took a pill including these exogenous hormones every day for 21 days (active phase = AP), followed by a 7-day break or placebo pills (inactive phase = IP).

Other inclusion criteria for participants in this study were that they were not allowed to be smokers or competitive athletes, pregnant, or breastfeeding. They should not have any chronic diseases or medications that could influence the outcome of the study or have any injuries or disabilities that would prevent running. The participants were recruited into two groups according to their use of hormonal contraceptives or self-report of having a normal cycle. One group used monophasic HC (HC; $n = 8$) while the other reported a natural menstrual cycle (NM; $n = 8$).

TABLE 3. Descriptive data of the participants divided by group.

	HC group	NM group	Total
n	8	8	16
Age (years)	26.8 ± 4.0	29.1 ± 4.5	27.9 ± 4.2
Height (cm)	168.9 ± 6.5	165.9 ± 6.3	167.4 ± 6.4
Weight (kg)	68.0 ± 4.7	65.9 ± 12.2	66.7 ± 9.1
BMI (kg/m^2)	23.9 ± 1.7	23.7 ± 3.4	23.8 ± 2.6
Body fat (%)	28.8 ± 5.3	25.5 ± 6.6	27.2 ± 6.1

HC = hormonal contraceptive group, NM group = normally menstruating group. All results are presented as means ± standard deviation. BMI = body mass index

TABLE 4. Combined hormonal contraceptives (including both estradiol and progestin) that were used in the HC group. Number of users (n) for each product, name, and hormone content for each product.

n	Product	Hormone Content
2	Gestinyl	EE 20 µg / gestoden 75 µg
1	Daisynelle	EE 20 µg / desogestrel 150 µg
1	Tasminetta	EE 0.03 mg / drospirenone 3 mg
2	Dienorette	EE 0.03 mg / dienogest 2 mg
1	Yasmin	EE 0.03 mg / drospirenone 3 mg
1	Yasminelle	EE 0.02 mg / drospirenone 3 mg

EE = ethinylestradiol, µg =microgram, mg =milligram

6.2 Study design

Groups were recruited to be of similar size. The HC group was the experimental group and the NM group was the control group. The entire study took approximately 6 months and during this period the subjects visited the laboratory 7 times (see figure 6). First, they had a 4–8-week control period (1 menstrual cycle/HC cycle), followed by an 8-week (2 cycles) moderate-intensity endurance training (MIET) period, and an 8-week (2 cycles) high-intensity interval (HIIT) period. Figure 6 illustrates the study design with group 1 being the HC group, and group

2 the NM group. Before the control period started, the participants had their first visit to the laboratory where they were familiarized with the study protocol, signed an informed consent, and gathered all the equipment and materials needed to start the study (including ovulatory kits, the watch and the HR strap, and the menstruation- and training diary templates). During the control period, the participants were asked to live their lives as normal as possible and monitor their menstrual- or hormonal contraceptive cycles. The possibility of an LH surge was assessed with a urinary ovulation test (Clearblue® Advanced Digital Ovulation Test, described later).

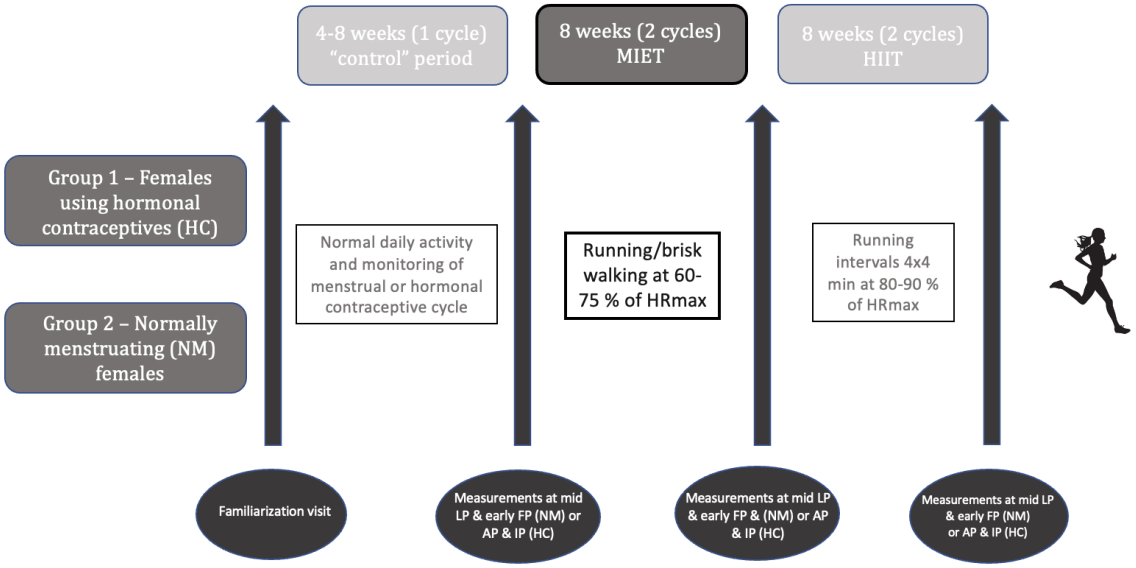


FIGURE 6. The study design for the groups included in this study focused only on the MIET period (middle, darker color).

There were 6 measurement points during the entire study period: after the control period, after the MIET period, and after the HIIT period. The time points for these measurements were planned according to the participant's menstrual/HC cycles, with one measurement time point at the mid-luteal phase (LP) or active phase (AP) and the other one at the early follicular phase (FP) or inactive phase (IP). In more detail, in the NM group, the mid-LP measurements were done 4-9 days after ovulation (OV 4-9) while early FP measurements were done 1-5 days after the start of menstruation. In the HC group, the inactive phase (IP) measurements were completed on days 2-4 of the placebo pill day. Whereas the active phase (AP) measurements were completed on pill days 2-5. There were some minor variations in the scheduling of the measurement days due to various reasons (illness, personal reasons, etc.). In this thesis, the hormonal profiles between groups are compared so that in the HC group AP is compared to the

NM group FP and the HC group IP to the NM group LP. The reason behind this was that these hormonal environments were meant to be quite similar between the two groups. Elliott-Sale et al. (2021) suggest that when the HC group is the experimental group in the study, which part of the HC cycle is compared can be chosen according to how high/low the hormonal profiles regarding the exogenous and endogenous hormones desired for the study. Nevertheless, in this thesis it was not possible to choose the specific part of HC or MC for the HRV analyses, so we stuck to AP and IP, and FP and LP, as the different phases within the HC and MC cycle. In this thesis, we focused entirely on the first phase (HC/MC) of the MIET period and did not consider any other phases of the NaisQs project.

6.3 Monitoring of ovulation and serum hormones

The time points of measurements were booked by monitoring menstruation and ovulation. The monitoring of the menstrual cycle phases was done in line with Janse de Jonge et al (2019) and Elliott-Sale et al. (2021) recommendations, including urinary LH surge testing, measurement of serum estrogen and progesterone concentrations, and a calendar-based counting on top of these to keep track of each cycle. The subjects in the NM group were given ovulation kits and monitored their ovulation with urinary ovulation tests (Clearblue® Advanced Digital) according to manufacturer instructions every menstrual cycle. This ovulation test accurately measures both LH and estrogen and therefore identifies a wider fertility window than other tests on the market (clearblue.com). The test is done by putting the reusable part under the urine stream or dipping it in a urine sample. The urine needs to be the first urine after sleep. The test shows a 48-hour peak of fertility when LH and estrogen are peaking, and ovulation occurs. (Clearblue.com)

Blood samples were collected to analyze serum hormone concentrations. The samples were taken on day one of the measurements. Samples were taken between 6.30 and 8.30 in the morning, after 12 hours of fasting and 24 hours without strenuous activity. Blood samples were taken from the antecubital vein using sterile needles and put into serum tubes (Venosafe, Terumo Medical Co., Leuven, Belgium). The whole blood was centrifuged at 2500g for 10 min after the serum was separated and stored at -80°C until final analysis. Serum estradiol (E2) and Progesterone (P4), levels were measured and analyzed with chemical luminescence techniques, and hormone-specific immunoassay kits were used. These analyses were done at four time

points: two at the beginning of the MIET period and two at the end of the MIET period, to assess differences in the hormonal concentrations between MC and HC phases in the two groups. To verify the luteal phase in NM, a limit of >16 nmol was set (Janse de Jonge, 2019; Elliott-Sale et al. 2021). More detailed information about the hormonal concentrations can be found in table 5.

TABLE 5. Endogenous hormone concentrations for all participants.

Participant	Group	E2 AP or FP pmol/L	P4 AP or FP nmol/L	E2 IP or LP pmol/L	PRG IP or LP nmol/L
1	HC	59,5	3,47	119	3,09
2	HC	197	1,13	24,9	1,35
3	HC	13,3	1,31		
4	HC	16,8	1,61	134	0,85
5	HC	48,8	0,67	65,3	0,43
6	HC	60,2	0,96	85,9	0,57
7	HC	67,2	1,02	126	0,60
8	HC	20,9	0,992	125	1,75
9	NM	174	1,26	218	19,7
10	NM	573	23,00	712	29,2
11	NM	712	27,70	866	69,00
12	NM	459	8,20	1109	29,5
13	NM	87,4	0,79	536	28,60
14	NM	114	1,70	430	23,60
15	NM	114	0,77	290	1,35
16	NM	185	0,78		

HC = hormonal contraceptive group, NM = naturally menstruating group, E2 = estradiol, P4 = progesterone, AP = active phase of the hormonal contraceptive cycle, FP = follicular phase of the menstrual cycle, IP = inactive phase of the hormonal contraceptive cycle, LP = luteal phase of the menstrual cycle.

6.4 Training plan

The participants started a moderate-intensity endurance training (MIET) program after the control period. At the end of the control period, the participants had their first performance tests with a maximal test to determine maximal oxygen consumption (VO_2max test), an isometric leg press, and a countermovement jump. The heart rate max (HRmax) was determined after the first VO2 max test, and then percentages and HR zones that were used during the intervention were calculated. During the MIET period, the participants did 2-3 running sessions per week (see Table 5) with a HR between 60 and 75% of HRmax. For this thesis, HC/MC one cycle of MIET was included for analysis. The participants were advised to wear a Garmin® Venu 2 Series, (Garmin Ltd., Taiwan) wrist-worn sports watch 24 hours a day during the entire study period and asked to record training and testing with the additional chest strap HR sensor Garmin® HRM-dual (Garmin Ltd., Taiwan). All the training sessions were saved in the smartwatch and the data from the watch was uploaded every 2 weeks into an open cloud space where researchers were able to collect the data. In addition, participants kept training and menstrual diaries during the entire training period. In these diaries, participants recorded their physical activity, symptoms, and subjective feelings on scales from 1 to 10 of the rate of perceived exertion (RPE), recovery, and stress. They also described their workouts including duration and distance. The participants were instructed to follow the training plan, but due to different personal reasons, the dates did not always match with the planned ones.

TABLE 6. Training plan for the MIET period with total time spent for the sessions per week described in minutes (min). This thesis focuses on approximately the first 4 weeks (one cycle), bolded.

Week	HC & NM
Week 1	**Testing**
	2 MIET sessions = 90 min
Week 2	3 MIET sessions = 150 min
Week 3	3 MIET sessions = 180 min
Week 4	3 MIET sessions = 195 min
Week 5	3 MIET sessions = 165 min
Week 6	3 MIET sessions = 195 min
Week 7	3 MIET sessions = 210 min
Week 8	**Testing**
	1 MIET session = 60 min

HC = hormonal contraceptive group, NM = normally menstruating group.

6.5 Monitoring of heart rate

HR and HRV were recorded with Garmin Venu 2S and Garmin HRM-dual HR belts. These watches have sensors that work with the help of LED light that detect changes in blood flow. The sensors work at two different sensing frequencies, depending on the activity level. At rest, the HR sensor is functioning at lower frequencies. When an individual is physically active, the watch changes to higher-frequency sensing. Continuously, the sensor reflects green, red, or infrared light to the wrist's skin through photoplethysmography (PPG). PPG changes light to electrical signals and measures blood oxygen density. HR data is then filtered, and external noise is excluded with the help of G-sensors. (Garmin.com). Based on previous studies, Garmin optical smartwatches are more reliable at rest than at activity, and data can be inaccurate depending on tracker type, -fit, or user characteristics (Evenson et al. 2020). HR measurements form the skin might be more inaccurate during activities where intensity and speed increase. Additionally, skin pigment might impact the sensitivity of the PPG measurements. (Spierer et al. 2015)

6.6 Data analysis

When the participants were done with their MIET phase of the project, analysis of 24/7 HR data commenced. Each participant had separate folders for recordings of training and general HR data. In the present study, from the HR data, we were only interested in the nocturnal RR intervals, and from the training diaries, we were able to inspect the correct dates for the nights we wanted to include in the analysis. These nights were chosen around four MIET sessions during one cycle, two in the active phase (HC) or follicular phase (NM) and two in the inactive phase (HC) or luteal phase (NM).

The criteria for choosing training sessions for analysis were the following: rest day/lighter day the day before the session (if possible), preferably a session with higher intensity % (70-75% instead of 60-65% of HRmax), and longer instead of shorter sessions (e.g. 60 or 75 min instead of 45 min). According to Myllymäki et al. (2012), higher exercise intensity elevated nocturnal HR compared to lower, and longer exercise duration induced nocturnal HRV and shorter did not. These criteria were not always met due to diverse reasons, but at least the aim was to meet them as well as possible. One night before and one night after the previously chosen training session were analyzed. To minimize possible measurement errors, two workouts in each HC- and MC phase were chosen for the analysis. In total, eight nights per participant were analyzed. Microsoft Excel (Microsoft Corporation, US) software was used to exclude all erroneous RR intervals. A four-hour sleep interval was chosen for analysis (Nuuttila et al. 2022) and because the bedtime was not recorded, we decided to analyze each night from 0.00-04.00. A file with four hours of RR intervals was exported as a CSV file into Kubios software (Kubios Oy, Kuopio, Finland) for further analysis. In Kubios software, the file was corrected from noise with the artifact correction using the medium threshold correction of beats, and the percentage (%) of corrections made on each file was reported (Alcantara et al. 2020). The percentage was mostly around 0,1- 0,5%, with a maximum of 1,28% of corrections made on a specific file of nocturnal RR intervals.

6.7 Statistical Analysis

Statistical analyses were done with IBM SPSS 29.0 (SPSS Inc, Chicago, IL.). To check the normality of the data, the Shapiro-Wilk test was used. An independent t-test was done to see if the descriptive data was homogenous or not. Possible differences in nocturnal HR, RMSSD,

and HF power between groups were tested with two-way repeated measures analysis of variance (ANOVA) with a level of significance set at $p<0.05$.

When looking at the differences between pre-and post-workout nights for the dependent variables, we calculated separately the differences for the nights pre- and post-workouts and then took the mean of the two values for each phase. To see if there were any significant differences in the change in nocturnal HR, RMSSD, and HF power values in different HC or MC phases between groups, another two-way repeated measures ANOVA was done. To identify possible differences in the analyses, post hoc tests were done with Bonferroni corrections and a level of significance was chosen as $p<0.05$. All results are presented as means ± standard deviations.

7 RESULTS

No statistically significant differences were observed in descriptive statistics (age, height, weight, body mass, fat %, or BMI) between HC and NM (table 3 presented in the methods section). According to an independent t-test, the groups are homogenous ($p>0.05$). The descriptive hormonal data for each participant can be found in table 5 (in the methods). These act as descriptive data and are not further analyzed in this thesis. The differences in HR and HRV between pre and post workout nights in the different MC/HC phases were not statistically significant ($p>0.05$).

7.1 Descriptive statistics for dependent variables

Descriptive statistics for dependent variables (HR, RMSSD, and HF power) are seen in figure 6 and 7, and table 7. Eight nights of HR values are presented in figure 6. The multivariate test results can be quite robust when we have equal sample size in out groups, so we looked at the tests of within-subjects effects in our analyses. No statistically significant differences were found in HR between nights or groups. The main effect of night on HR is not statistically significant ($p=0.457$) and when looking at the effect of night X group interaction, no significant findings were found ($p=0.395$).

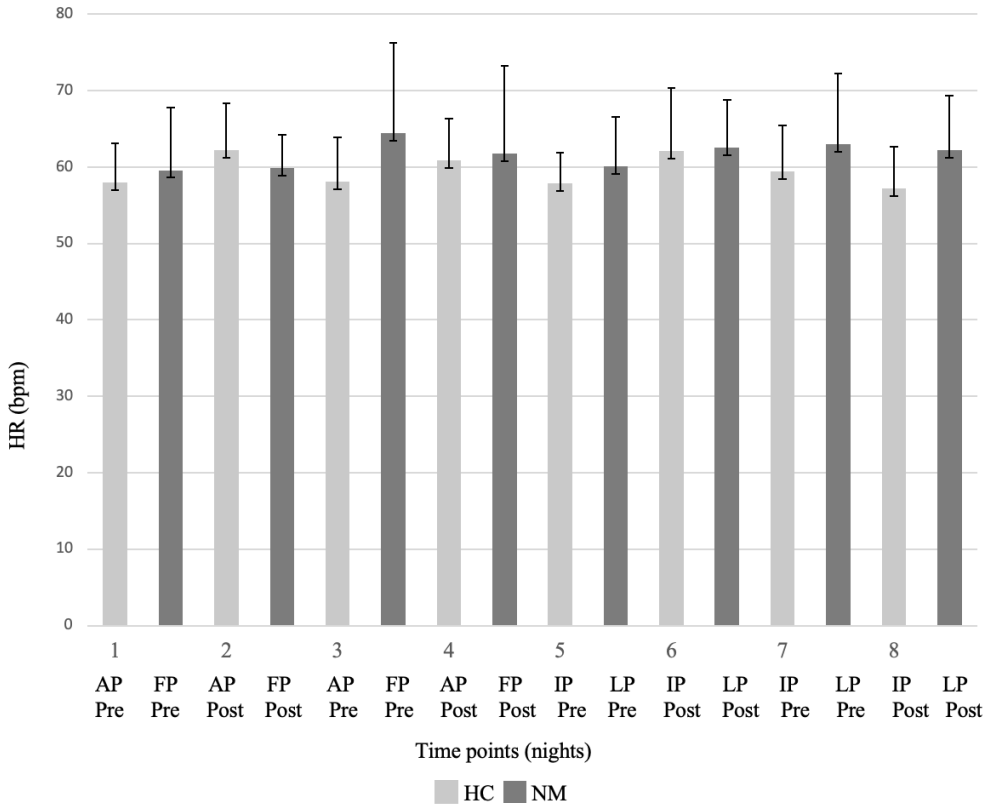


FIGURE 6. Nocturnal heart rate (HR) in bpm for the hormonal contraceptive (HC) group and normally menstruating (NM) group for eight nights during one HC/MC cycle. Results are presented as means and standard deviations of 16 participants, n=8 (HC) and n=8 (NM). AP=active phase (HC) or FP=follicular phase (NM), IP = inactive phase (HC) or LP=luteal phase (NM). All results are presented as means ± standard deviation. Pre = pre workout night, post = post workout night.

The means of RMSSD values are presented in figure 7. The test of within-subject effects showed that the main effect of night on RMSSD was not significant (p=0.223) and the effect of the night X group interaction was not significant either (p=0.380).

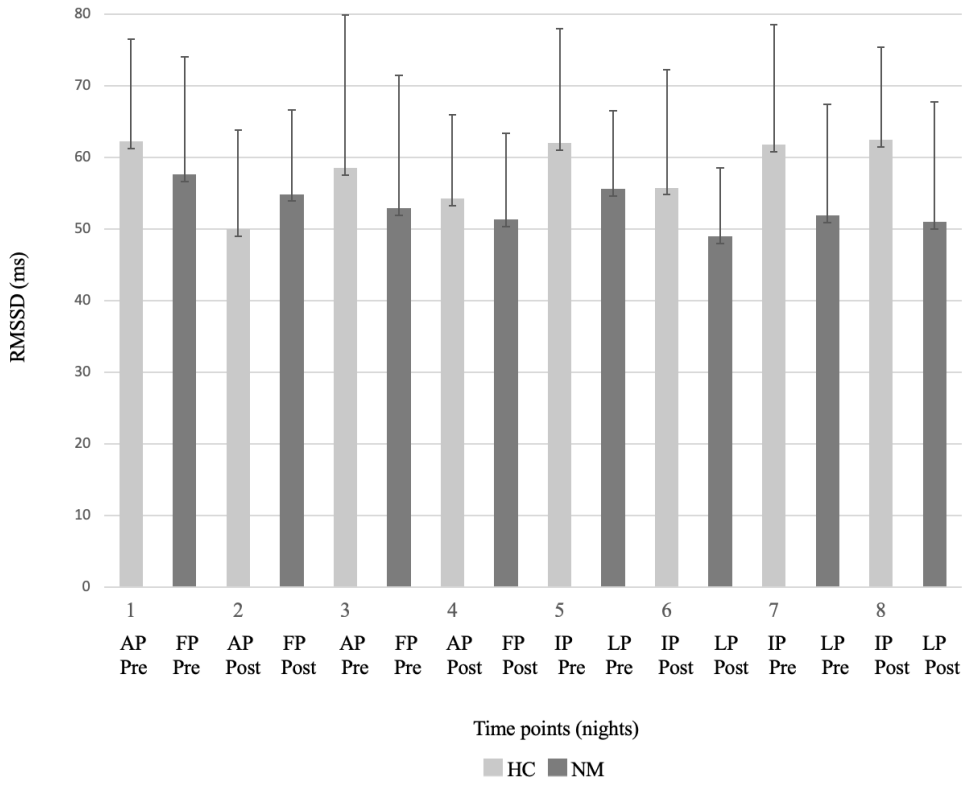


FIGURE 7. Nocturnal RMSSD in ms for the hormonal contraceptive (HC) group and normally menstruating (NM) group for eight nights during one HC/MC cycle. Results are presented as means and standard deviations of 16 participants, n=8 (HC) and n=8 (NM). AP=active phase (HC) or FP=follicular phase (NM), IP = inactive phase (HC) or LP=luteal phase (NM). All results are presented as means ± standard deviation. Pre = pre workout night, post = post workout night.

The descriptives of high-frequency (HF) power for eight nights are shown in table 6. In table 7 the effect of night on HF power was not statistically significant (p=0.239) and the effect of night X group was not significant either (p=0.301). Pairwise comparisons of groups and for all nights separately with Bonferroni corrections did not show any significance for any of the dependent variables.

TABLE 7. Nocturnal high-frequency power (HF power) for eight nights divided by group.

Night	Group	Mean
Night 1: HF pre workout 1 in AP or FP	HC	1217.4 ± 593.0
	NM	1063.3 ± 556.8
Night 2: HF post workout 1 in AP or FP	HC	813.7 ± 420.0
	NM	949.8 ± 436.5
Night 3: HF pre workout 2 in AP or FP	HC	1106.0 ± 797.4
	NM	913.0 ± 575.9
Night 4: HF post workout 2 in AP or FP	HC	944.5 ± 405.4
	NM	825.9 ± 385.0
Night 5: HF pre workout 1in IP or LP	HC	1268.5 ± 690.0
	NM	955.6 ± 270.8
Night 6: HF post workout 1 in IP or LP	HC	1102.4 ± 746.5
	NM	749.1 ± 284.9
Night 7: HF pre workout 2 in IP or LP	HC	1281.5 ± 680.7
	NM	853.3 ± 473.5
Night 8: HF post workout 2 in IP or LP	HC	1273.5 ± 567.1
	NM	847.6 ± 523.6

HC group=hormonal contraceptive group, NM group=normally menstruating group. AP=active phase (HC) or FP= follicular phase (NM), IP=inactive phase (HC) or LP=luteal phase (NM). All results are presented as means ± standard deviation.

TABLE 8. Results from tests of within-subjects effects from Two-Way ANOVAs of night and night X group for HR, RMSSD, and HF power.

Effect	df	F	Significance (p-value) Greenhouse-Geisser
HR night	3.672	0.913	0.457
HR night X group	3.672	1.034	0.395
RMSSD night	3.869	1.479	0.223
RMSSD night X group	3.869	1.069	0.380
HF power night	3.716	1.432	0.239
HF power night X group	3.716	1.253	0.301

HR=heart rate, RMSSD=Root mean square of successive differences between normal heartbeats, HF=high-frequency power, df=degree of freedom. All results are presented as means ± standard deviation.

7.2 Pre-post workout night differences in HR, RMSSD, and HF power between phases

The differences between pre and post workout nights in HR, RMSSD, and HF power in the two phases (HC/M) are presented in table 9. There were no statistically significant differences in HR, RMSSD, or HF power between pre- and post-nights in either of the phases within or between groups. The within-subject tests of ANOVA showed no significant effects of phase on pre-post workout nights HR (p=0.879) or phase X group (p=0.159). The phase did not affect the RMSSD difference between pre-post workout nights (p=0.542) or the phase X group between pre-post workout nights (p=0.267). Similar results were seen for HF values. No statistical significance for the effect of phase on the difference in HF power between pre-post workout nights (p=0.387) or the phase X group (p=0.359). The within-subject effects results from the ANOVA can be seen in table 10.

TABLE 9. Differences between pre and post-training night values between MC/HC phases for HR, RMSSD, and HF power, divided by group.

Variable (unit)	Group	Phase	Mean ± SD
HR difference (bpm)	HC	AP	3.5 ± 4.0
HR difference (bpm)	NM	FP	-1.2 ± 9.5
HR difference (bpm)	HC	IP	1.1 ± 5.9
HR difference (bpm)	NM	LP	0.8 ± 5.0
RMSSD difference (ms)	HC	AP	-8.3 ± 10.8
RMSSD difference (ms)	NM	FP	-2.1 ± 11.4
RMSSD difference (ms)	HC	IP	-2.8 ± 11.0
RMSSD difference (ms)	NM	LP	-3.8 ± 9.2
HF power difference (ms2)	HC	AP	-282.6 ± 414.8
HF power difference (ms2)	NM	FP	-100.3 ± 373.8
HF power difference (ms2)	HC	IP	-87.0 ± 390.1
HF power difference (ms2)	NM	LP	-106.1 ± 299.1

HC group = hormonal contraceptive group, NM group = naturally menstruating group, AP = active phase of HC cycle, FP=follicular phase of menstrual cycle, IP=inactive phase of HC

cycle, LP=luteal phase of menstrual cycle, HR=heart rate, RMSSD=Root mean square of successive differences between normal heartbeats, HF power=high-frequency power.

TABLE 10. Results from tests of Within-subjects effects from Two-Way ANOVAs of night and night X group for HR, RMSSD, and HF power.

Effect	df	F	Significance Greenhouse-Geisser
HR night	1.000	0.024	0.879
HR night X group	1.000	2.215	0.159
RMSSD night	1.000	0.390	0.542
RMSSD night X group	1.000	1.337	0.267
HF power night	1.000	0.798	0.387
HF power night X group	1.000	0.900	0.359

HR=heart rate, RMSSD=Root mean square of successive differences between normal heartbeats, HF power =high-frequency power.

8 DISCUSSION

The main object of this master's thesis was to examine how nocturnal HR and HRV change between cycles in HC users and naturally menstruating females during an endurance training intervention. An additional aim was to investigate the changes in nocturnal HR and HRV between pre and post MIET nights and the possible influence of MIET on these variables. According to hypothesis 1, HRV measures would've been higher and HR lower during the inactive phase in HC users and FP in naturally menstruating females. Against hypothesis 1, no significant differences in HR or HRV (RMSSD or HF power) were observed between HC/MC phases ($p>0.05$). The multivariate test results revealed, rejected hypothesis 2, that the HC group would have reduced HRV measures and higher HR compared to naturally menstruating females. No between-group differences in HR or HRV (RMSSD or HF power) were detected. In line with hypothesis 3, MIET did not influence recovery enough to cause significant differences in HR or HRV (RMSSD or HF power) between pre and post workout nights. Neither phase (AP vs. IP in HC or FP vs LP in NM) or group showed significant differences in these variables. Despite no significant differences being found in this study, the results give valuable information and might provoke discussion about how hormonal profiles, autonomic tone, and recovery from endurance exercise affect one another.

8.1 Discussion on female physiology, heart rate variability, and endurance training

In the present study, we did not focus on the sex hormone concentrations but did present them as descriptive data in table 5. Female sex hormones have been shown to affect the ANS, which can be observed in nocturnal HRV metrics (von Holzen et al. 2016; Ahokas et al. 2023). Previous literature indicate that E2 might have a cardioprotective effect on vagal activity (Leicht et al. 2003) and many studies suggest that the sympathetic nervous system is dominating during LP (Brar et al. 2015; Ahokas et al. 2023; Tenan et al. 2014; Sims et al. 2021). Some previous studies have compared specific parts of MC cycles in their study, for instance, Leicht et al. (2003) compared early FP and ovulation and found no differences. Blake et al. (2023) reported higher HF power specifically in early FP. The addition of the early-, mid-, or late-time of the phase adds specificity to a study. It also makes it easier to match possible occasions of blood serum samples measuring hormonal concentrations. This kind of matching was unfortunately not possible in the present study due to the training sessions and the nocturnal HRV not being at a particular part of the phases. The nights were chosen by being in either the

FP or LP phase but could be quite close to each other or far away from each other. This analysis was done one by one, which took a lot of time, so compromises had to be made. The fluctuations of hormones during an MC vary a lot from one individual to another (Fehring et al. 2006), which makes it even more difficult to make general conclusions about hormonal concentrations and autonomic modulation.

In the present study, we only included COCs as the possible HC. This makes it easier to generalize the result to the entire group of COC users. However, it is important to ask which brand of COCs are used, so that the hormonal concentrations of the pills can be reported. Pereira et al. (2023) found inconsistencies in the literature regarding OC use and its effect on autonomic indices and mentioned OC formulations, hormone concentrations, as well as the stressors and intensity of the stressors as possible reasons behind the inconsistencies. Both Sims et al. (2021) and Ahokas et al. (2023) included many different types of hormonal contraceptives, not only COCs. This potentially leads to an even bigger variety in the hormonal profiles between participants and gives a bigger picture of how they affect the outcomes of the study.

The results of this study indicate that the hormonal profile or phase of HC/MC does not affect nocturnal HR or HRV. One positive outcome of this finding is that an individual might be more open to finding the contraceptive that best meets their needs, without being too stressed about how and if it impacts their recovery (referring to HR and HRV). Endurance training is shown to have an impact on nocturnal autonomic modulation (Myllymäki et al. 2012; Vetserinen et al. 2011). However, these studies are done on male participants and should not automatically be applied to females, because it increases the risk of never finding the true potential of females in sport and how the sex hormones influence the performance and recovery from it (Elliott-Sale et al. 2021). Hence, in some cases, the male studies can be applied to females as well. Hormonal contraceptive use is popular in both the general population and athletes (United Nations, Department of Economic and Social Affairs, Population Division 2019; Martin et al. 2018). Personalization of HC choice is important (De Leo 2022), and the more research is done in the field, the better recommendations the specialists can give to individuals. For naturally menstruating females the results of this study might help with listening to their subjective feelings regarding recovery, by understanding the individual differences in hormonal concentrations and how they might impact HR and HRV metrics.

The variety of the level of activity of the female participants in previous studies has been ranging from sedentary (Nummela et al. 2016) to recreationally active (Schaumberg et al. 2017) and elite athletes or highly trained females (Lebrun et al. 2003; Vesterinen et al. 2013). The participants in the present study were healthy females and were not allowed to be competitive athletes. This makes it difficult to compare the studies because the endurance adaptations to a training program might be quite different if you are an athlete or a novice trainer. The autonomic adaptations of aerobic exercise (e.g. decreased HR at rest and submaximal training) are a product of intrinsic changes in the heart (Hautala et al. 2009; Carter et al. 2003). These are long-term adaptations, which might lead to the lack of significant changes during the present study of one MC or HC cycle taking approximately one month.

There are many modern devices out there that measure HRV. There is limitations to the PPG If an individual wants to do this, it is important to remember to let the device have enough time to get a baseline metric of HRV (Föhr et al. 2017; Nummela et al. 2016). This helps the device to detect possible changes from the personalized baseline of HRV.

8.2 Discussion on recovery from endurance training

The main finding in this study regarding MIET influencing nocturnal HR or HRV differences was that MIET did not significantly impact HR or HRV. Some possible reasons for this finding could be that the intensity was not high enough to see differences in HR or HRV. Previous literature shows that high-intensity training has increased nocturnal HRV and decreased nocturnal HR (Vesterinen et al. 2013; Nummela et al. 2016). Both studies compared high-intensity training to constant lower-intensity training. Nummela et al. (2016) compared one group that did high-intensity training with a group that did moderate-intensity training, which was described as 3x 40 min sessions/week at the intensity between aerobic and anaerobic thresholds. Vesterinen et al. (2013) compared high-intensity training with low-intensity (below aerobic threshold) training. Low-intensity training showed no effect on the homeostasis of the cardiovascular system. They did suggest that moderate or high-intensity training is needed to see significant changes in autonomic function, but the present study did not support this.

The length of this study could also be one reason for the lack of differences in the autonomic function. Vesterinen et al. (2013) and Nummela et al. (2016) were both longitudinal studies and looked at the overall changes in HR and HRV. It is a limitation that this study was only

approximately one month long (one cycle). In addition to looking at the HR and HRV of 8 nights of data during the entire cycle, we looked at the possible differences between pre-post workout nights, to see differences in the HR or HRV between them. Myllymäki et al. (2012) study compared nocturnal HR and HRV of nights after moderate-intensity exercise with nights after control day. Their results indicate that higher intensity of exercise had an impact on HR and a longer duration of exercise (90 min compared to 30 or 60 min) had an impact on HRV. Therefore, in this study when choosing nights to analyze, was based on the highest intensities and durations. Nevertheless, there appeared to be no statistically significant changes between pre-post workout nights HR or HRV in this study.

In this thesis, we used PPG as a method to measure HRV. This decision was based on user friendliness with the risk of getting data that is not as accurate as measurements done with ECG based equipment (Evenson et al. 2020). For instance, skin pigmentation (Spierer et al. 2015) and the fit of the device might reasons for possible inaccuracy in data. Using some additional ECG based equipment during the nights could possibly make our data even more reliable. HRV is sensitive to changes in the ANS related to stress (Kim et al. 2018). During intensive sports activities, sympathetic activity predominates which can also decrease HRV (Sammito & Böckelmann, 2016). Hence, HRV can be a useful tool to monitor or even make training load focusing on the recovery of the ANS.

8.3 Methodological considerations

Current evidence regarding the female sex hormones and their impact on HRV and recovery from exercise are contradictory. Some evidence shows that the different hormonal phases HC/MC) have an impact on autonomic modulation (Sims et al.; 2021; Ahokas et al. 2023; Tenan et al. 2014; Yazar et al. 2016). Other studies showed no significant differences in autonomic modulation between phases (Teixeira et al. 2015; Leicht et al. 2003; Ylidirir et al. 2002). The reasons for these inconsistencies are the different hormonal profiles (HC users and NM females), differences in sample sizes, and most importantly differences in methodological considerations between studies.

In the present study, however, the determination of the menstrual cycle was supported with ovulation tests (urinary ovulation test by Clearblue®) and blood serum samples, which increases the validity of the study. Menstrual cycle determination is an important

methodological consideration to discuss. To get high-quality research in exercise science including female participants, the method used for menstrual cycle determination is important to be presented (Elliott-Sale et al. 2021). There is substantial variability between normal MC lengths and hormone fluctuations, and it is common to have menstrual disturbances during the reproductive years (Fehring et al. 2006). Hence, important to measure serum hormone concentrations in menstrual cycle studies, as disturbances and anovulation are more likely to be detected (Janse de Jonge 2019; Elliott-Sale et al. 2021). For instance, Sims et al. (2021) did not meet this standard, they included participants with a regular natural MC lasting 25-35 days but did no other measurements to support the phases. Identification of the generation of progestin used in the COC users was not feasible. However, they had a large sample size of 455 individuals which is a strength of their study.

Another aspect that requires discussion is the possibility of having temperature recorded during the menstrual cycle, supporting the determination of menstrual cycle phases (Baker et al. 2020). An increase in temperature has been studied to generally be higher in the luteal phase. According to Baker et al. (2020), this is caused by the thermogenic effect of progesterone, which rises during this phase of the menstrual cycle. The rise in body temperature has been shown to correlate with an increase in HR during the luteal phase (Baker et al. 2020), which implies a possible influence on autonomic control (de Zambotti et al. 2013). The possibility to record temperature would've been a great addition to this study, also due to altered thermoregulation in females who use HC (Minahan et al. 2017). However, in Minahan et al. (2017) study thermoregulatory differences shown in OC users were detected during exercise in heat. Adding the body temperature could increase the validity of a study and increase discussion about the thermoregulatory differences between different hormonal profiles and the possible influence on autonomic control.

Regarding hormonal contraceptives, there are several aspects to take into consideration in research. In this study, we included six different brands of COC (reported in table 4). This study was quite demanding with the length of the entire study (NaisQs) being 5-6 months with several visits and a training program that needed to be followed carefully. With the sample size already being quite small, the requirement of having a specific type of OC might have decreased this amount even more. Hormonal contraceptives of different brands have different hormonal concentrations (van Vliet et al. 2011). The hormonal contraceptives also affect the endogenous hormone concentrations in HC users (Sims and Heather, 2018). Previous studies have shown

contradictory results regarding the differences in E2 levels between inactive and active pill phases (Ahokas et al. 2023; Teixeira et al. 2015; Romero-Parra et al. 2021). In the studies that did not find any significant differences in E2 levels between HC phases, Ahokas et al. (2023), 5 different brands of COCs were used, but in Teixeira et al. (2015) the hormonal concentration in the COC was standardized, and Romero-Parra et al. (2021) included 4 different combinations of the exogenous hormones. The criteria for HC were for it to be monophasic, but we were not able to meet the Elliott-Sale et al. (2013) suggestion about avoiding the usage of many brands of OC within one sample. We know that the hormone concentrations may vary a lot between brands and might cause errors in the results. However, it is important to take into consideration the individual differences in endogenous hormones and the variation in the effect HC has on hormone concentrations depending on the product, regardless of being considered to have the same mechanisms of action (Elliott-Sale et al. 2013).

In this thesis, the HC group was the experimental group since we wanted to see if the exogenous hormones would impact the HRV and recovery from endurance training. Elliott-Sale et al. (2013) suggest that when the HC group is the experimental group, the HC phases should be put into four different ones instead of only looking at the differences between AP and IP. This was unfortunately not possible due to us having only one cycle to analyze and a limited number of nights to analyze around MIET workouts of high enough intensity and duration. Previous studies (Sims et al. 2021; Ahokas et al. 2023) also only used AP and IP phase, which makes the present study still valuable in the field next to these studies, despite its limitations.

The nocturnal HRV was compared between nights after MIET training and nights after a day of rest or light activity, in a similar way as done by Myllymäki et al. (2012). However, the criteria of having a rest day before the pre-night was not always met due to individual differences in following the training plan. The menstrual cycle phases did not always include many pieces of training to choose from, so we just had to decide and stick to the same pattern for the entire analysis process. Thus, we chose the training sessions of the highest intensity and duration to possibly get an impact on HRV (Myllymäki et al. 2012; Vesterinen et al. 2013). Additionally, when late-night training affects HR during the first hours of sleep (Costa et al. 2018) the possibility to standardize the timing of the workouts before the nights analyzed would have given more reliability to the results. Having controlled training sessions makes it possible for the research group to get adherence and be in control of what the participant does (da Silva et al. 2019). In the present study, the training session control was not possible, which gives the

participant the responsibility to do the training sessions and report them back to the research group.

In the present study, we compared groups with different hormonal profiles (HC vs. NM) to each other and also the possible differences within the groups. We ensured a steady hormonal profile in the HC group by including participants who had used monophasic HC for a minimum of 12 months before recruitment. This is in line with Elliott-Sale et al. (2021) recommendation of a minimum of 3 months of HC use before recruitment to ensure their characteristics match the HC user profile. However, Lebrun et al. (2003) compared participants who first did a baseline measurement period without any HC and then randomized them into two groups, one HC group and one placebo group. This means that the HC users were at the start of their HC, not at a steady hormonal state yet, which may impact the results for aerobic capacity (Schumpf et al. 2023). Regarding two of our main articles in the field of female hormonal profiles and HRV, Ahokas et al. (2023) did not mention how long their participants had been on HC before recruitment and Sims et al. (2021) included only participants who had used HC within the last 9 months without stopping. As the present study ensures a steady HC profile, increases the reliability of the hormonal profiles in the HC group in this thesis study. On the other hand, it can make it difficult to compare results with previous literature.

8.4 Strengths and limitations

One strength of this study was that the participants were homogenous in both groups and groups were the same size. This increases the reliability of the study results. Another strength of the study was that the endurance training sessions were constant during the entire study period. This helps when analyzing the results and sharing recommendations for future research based on the study. Finally, one more strength to mention is that the nocturnal HRV analysis was based on a 4-hour period, which has been a highly reliable method to detect disturbances in homeostasis (Nuuttila et al. 2022).

When it comes to limitations, the sample size in this study was relatively small (n=16) and highly likely affected the possibility of finding significant differences within or between groups. Despite having reliable methods to determine menstrual cycle phases (ovulation tests and blood samples), when checking the endogenous hormone concentrations, the criteria limit of >16 mmol of progesterone in LP was not always met (one participant did not reach this criteria). Also, the progesterone levels were very high for a few participants (10 and 11 in table 5), which might be due to the wrong timing of measurement or that the values were put into the database in the wrong order. At this point, these participants were included in the study and most of the data analysis was done. Therefore, to clarify terminology to describe the hormonal profile of the control group, it is called the naturally menstruating group instead of the eumenorrheic group. A limitation that is important to mention here once more, is the fact that we were not able to choose the nights at specific parts of the phases but had to choose the best possible ones. There are both strengths and weaknesses with the PPG method for HRV analysis. PPG is not as accurate as other ECG-based devices (Evenson et al. 2020), however, PPG in a smartwatch is a user-friendly way to measure HRV.

8.5 Future research

Based on the present study and previous literature, some additional variables could have made this study more valuable. Some ideas for future research would be to plan training with higher intensity, collect data for a longer period (e.g. 2-3 cycles instead of one) from a larger sample, add aerobic capacity (VO2max) as descriptive data, and measure temperature during the entire study period.

8.6 Conclusions

Based on this study, there are no significant differences between nocturnal HR, RMSSD, or HF power between HC users and naturally menstruating females during an endurance training intervention. There are no significant differences in nocturnal HR, RMSSD, or HF power between HC or MC phases either. Additionally, no significant differences are detected in nocturnal HR or HRV between pre and post MIET training session nights. However, these findings indicate that the hormonal profiles between HC users and normally menstruating females may not influence autonomic modulation. Measuring nocturnal HRV is a useful tool to monitor the training load and recovery of the ANS. However, the possible effects of female sex hormones or HC on recovery still need to be approached on an individual level before more research is done in the field.

8.7 Practical applications

The results from this thesis indicate that the hormonal profile does not affect HRV-based recovery from endurance training during an individual MC/HC. This might give individuals the possibility to be a bit more open towards finding the right contraceptive for them. Personalization of HC choice is very important and the growing information and research about them and their effect on physiology and performance is valuable for finding the true potential of females in sport.

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