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ABSTRACT

Purpose: The aim of this study was to investigate within-cycle differences in nocturnal heart rate (HR) and heart rate variability (HRV) in naturally menstruating women (NM) and women using combined hormonal contraceptives (CU) or progestin-only hormonal contraceptives (PU). **Methods:** Physically active participants were recruited into three groups: NM (n=19), CU (n=11), and PU (n=12). Participants' HR and HRV (with Bodyguard 2 HRV monitor), and blood hormones were monitored during one menstrual cycle (MC) (NM-group) or for 4 weeks (CU and PU-groups). Estradiol, progesterone, and luteinizing hormone were analyzed from fasting blood samples collected four times in the NM (M1=bleeding, M2=follicular phase, M3=ovulation, and M4=luteal phase) and PU groups (M1 = lowest E_2; M2 = M1+7 days; M3 = M1+14 days; M4 = M1+21 days) and twice in the CU group (active and inactive pill phases). After every blood sample, nightly HR and HRV were recorded and examined as an average from two nights. **Results:** Hormonal concentrations differed ($p<0.05$) between MC phases in the NM- and PU-groups, but not ($p\geq0.116$) between the active and inactive phases in the CU-group. In the NM- and PU-groups, some of the HRV values were higher, while in the NM-group, HR was lower during M2 compared to M3 ($p<0.049$) and M4 ($p<0.035$). In the CU-group, HRV values ($p=0.014$-0.038) were higher, and HR was lower ($p=0.038$) in the inactive phase compared to the first week of the active phase. **Conclusions:** The MC and hormonal cycle phases influence autonomic nervous system balance, which is reflected in measurements of nocturnal HR and HRV. This should be considered when monitoring recovery in physically active individuals.

Key Words: MENSTRUAL CYCLE, SEX HORMONES, RECOVERY, AUTONOMIC NERVOUS SYSTEM

INTRODUCTION

The effects of the menstrual cycle (MC) and the hormonal contraceptive cycle (HC) on physical performance and recovery (e.g. the autonomic nervous system, ANS) are unclear. Meta-analytical data shows trivial effects of MC and HC on performance (1, 2), and slightly inferior performance in women who use HC compared with women who menstruate naturally (2). In addition, it has been suggested that MC phase might affect recovery after intermittent activity (3) and consistent moderate intensity running (4).

Briefly, the MC is characterized by fluctuations in female sex steroid hormones. In a normal MC, estradiol (E2) levels are lowest during bleeding, in the early follicular phase, and rise thereafter until ovulation, while a surge of both luteinizing hormone (LH) and follicle-stimulating hormone (FSH) occurs just before ovulation (5). A second E2 peak and progesterone (P4) peak occurs during the mid-luteal phase (5) for comprehensive information on the MC and perspective on E2 and P4 reference ranges see Elliott-Sale et al. (5).

Approximately half of female athletes report HC use, with 70-75 % using oral contraceptive pills (6–8). Hormonal contraceptives include combined and progestin-only formulations (6) that influence endogenous hormonal milieus. Combined hormonal contraceptives (CHC), including oral contraceptive pills (COC-pills) and contraceptive rings, are typically administered for a 21 to 24 day active phase followed by a 4 to 7 day inactive phase (7–9). The use of CHC reduces endogenous hormone production, leading to substantially or completely attenuated peaking of all of the female sex hormones (E2, P4, LH, and FSH) (9). Progestin-only contraceptives, including hormonal IUDs and minipills, may also affect

endogenous hormone production and inhibit ovulation although hormonal profiles are variable (10–12). Fluctuation of endogenous sex hormones are suggested to mediate variation in recovery and performance during MC.

Heart rate variability (HRV) has become a widely used non-invasive tool for monitoring ANS activity, which appears to be effective for determining the recovery and training status of an athlete (13) because HRV works as a marker of both physical and mental stress (14). For example, sympathetic drive increases, and parasympathetic drive decreases, after an exercise session, which can be noticed as decreased variation in variability. This decreased variation in variability has been found to be greater after higher intensity exercise sessions (15). Nevertheless, high individual variability in HRV measures were found in female soccer players during a competitive season (16, 17). Meta-analytical data has revealed negative changes in HRV from the follicular to the luteal phase of the MC and from bleeding to the late luteal phase in naturally menstruating women (14). The literature also includes contradictory results, which may be explained by different methods used to determine MC phases as well as different HRV monitors and monitoring durations (14). At present, most published studies include only daytime monitoring of HRV (14). Thus, quality studies assessing HRV that include hormonal and nocturnal HRV analyses are needed to help inform guidelines for monitoring female athletes' training and recovery.

The aim of this study was to investigate within-cycle differences in nocturnal heart rate (HR) and HRV as well as perceived recovery, in three groups: naturally menstruating women (NM), women using combined hormonal contraceptives (CU), and women using

progestin-only hormonal contraceptives (PU). We hypothesized that HRV would decrease from the follicular phase to the luteal phase in NM. Furthermore, we hypothesized that the same pattern in hormonal fluctuations and HRV would be observed in women using PU. In addition, it was hypothesized that there would be no differences in HRV during the HC in CU.

METHODS

Participants

Participants were recruited through social media as well as official university information channels. Forty-two women met the inclusion criteria: healthy and normal weight (BMI 18-30 $kg \cdot m^{-2}$) and trained (Tier 2 in the classification on a scale of 0–5, according to McKay et al. 2022) (18). Furthermore, women reported that they were neither pregnant nor breastfeeding and they did not report any known polycystic ovary syndrome, endocrine disorders, or other diseases or medications that may affect ovarian or ANS function. Participants were allowed to continue physical activity as they normally would during the research period, and no marked changes in daily physical activity and exercise were observed. Competitions during the research period were prohibited to avoid the possible influence of physical and mental stress on hormonal and ANS function. The participants were informed about the possible risks of all study procedures before providing written informed consent. The Ethical Committee of the University of Jyväskylä granted ethical approval (7/2019).

The participants were recruited into three groups: NM, CU, and PU (Table 1). Participants in the NM-group reported a regular menstrual cycle based on bleeding for the previous 6 months. The participants in the PU-group used hormonal IUDs and the participants in

the CU-group used either third or fourth generation CHC-pills, or contraceptive rings (Table 2). Active pills used by participants in the CU-group contained similar amounts of estrogenic (0.02–0.03 mg ethinyl estradiol or 1.5 mg bioidentical estradiol hemihydrate) and progestogenic (0.075–3.0 mg) compounds. Taken together, participants' prescriptions in the CU group provided a relatively stable hormonal condition for 21–24 active phase days followed by 4–7 hormone-free (inactive phase) days.

Study design

Participants' nocturnal HR and HRV, menstrual symptoms and pain, subjective recovery, and hormonal changes were monitored during the research period i.e. one menstrual cycle in the NM-group and for 4 weeks in the CU-group and PU-groups.

During the study period, participants used an HRV-monitor (see methods) every night. In addition, they filled-in sleep and MC/HC diaries, which included information about subjective perception of recovery as well as bleeding days. The participants in the NM-group assessed the occurrence of ovulation using an ovulation kit (Sofi, Finland), which is described later. This study uses HRV and subjective recovery data as an average from two nights and days after the blood sample. Furthermore, in the CU-group, data were analyzed from two nights of the active hormone weeks, where blood samples were not collected because endogenous sex hormone profile tends to remain stable during exogenous CU use (19, 20).

The participants in the NM-group and PU-group gave a fasted blood sample four times during the research period. In the NM-group, blood samples were collected during bleeding (measurement 1 = M1; 2 to 3 days from the beginning of the MC), the follicular phase (M2; 7 to 10 days from the beginning of the MC), the ovulation phase (M3; the day after the positive ovulation test), and the luteal phase (M4; 5 to 7 days after ovulation). The participants in the PU-group gave a fasting blood sample four times separated by approximately 7 days (range: 5-9 days). In the PU-group, MC phases (M1 = lowest E2; M2 = M1+7 days; M3 = M1+14 days; M4 = M1+21 days) were arranged based on hormonal values and bleeding according to the methods described by Elliott-Sale et al. (3). In the CU-group, the MC phases were divided into inactive phase (M1) and active phases (M2; the first week of the active phase, M3; the second week of the active phase, and M4; the third week of the active phase). In the CU-group, one blood sample was collected during the active phase (during M2 n=1, M3 n=9, and M4 n=1) and the other during the inactive phase. Measurement time points in the CU- and PU-groups are hereafter referred to collectively as HC phases.

Sleep and menstrual diary

Every morning, participants filled in a sleep diary, which included bedtime, perception of recovery, alcohol use, and open comments about other observations that affected sleep (21). Participants also filled in a MC/HC diary. Items in the diary included morning body temperature, question about possible bleeding, and ordinal variables on MC/HC associated symptoms (e.g. mood, behavior, pain, and physical symptoms), scored on 5-point scales (modified from Freeman et al. 1996 (22)).

Heart rate and heart rate variability monitoring

An electrocardiogram-based HRV monitor (Bodyguard 2, BG2; Firstbeat Technologies Ltd., Jyväskylä, Finland) was used to record HR and HRV. The BG2 monitor used two spot electrodes, which were placed under the right collarbone and on the left rib cage. Participants attached the monitor before going to bed and removed the device after waking up. HRV data were analyzed for each night over a four-hour period beginning 30 minutes after reported bedtime. Alcohol and melatonin use was discouraged, but if their use was recorded during the day before sleep, this sleep data was excluded from the analysis. Participants were instructed to maintain their habitual levels of training during measurement days. The BG2 monitor records electrocardiogram and converts the signal into R-R intervals using an algorithm with an accuracy of 1 ms. Based on previous studies, the BG2 device is accurate for HRV analysis (23). The gathered data was transferred to Kubios software (Kubios Oy, Kuopio, Finland), which was used to remove artifacts and obtain the HRV variables presented in Table 3. Kubios is a validated software that is widely used for the analysis of biological signals in scientific research (24).

Ovulation test

The duration of previous menstrual cycles was used in combination with a urinary ovulation kit (Sofi, Finland) to determine the timing of M3 and M4 in the NM-group. The urinary ovulation test identifies the LH-peak before ovulation. Participants began performing ovulation tests 4-6 days before the mid-point determined from previous menstrual cycles. Participants were asked not to perform the test using the first urine of the day, to avoid drinking

excessive amounts of fluids for 2 hours before the test, and to repeat the test at the same time every day. The blood sample for M3 was collected 1-2 days after the positive ovulation test.

Blood sampling and biochemical analysis

Blood samples were collected to analyze serum hormone concentrations between 7-10 a.m., after 10 hours of fasting. Blood samples were taken from the antecubital vein into serum tubes (Vacuette, Greiner Bio One International GmbH, Kremsmünster, Austria) using standard laboratory procedures. Whole blood was centrifuged at 2245 G (Megafuge 1.0 R, Heraeus, Hanau, Germany) for 10 min. After separation of serum, samples were frozen at -20 °C until the final analysis. Serum estradiol (E2), progesterone (P4), and luteinizing hormone (LH) levels were measured with chemiluminescence immunoassays (IMMULITE 2000 XPi, Siemens Healthcare Diagnostics, UK) and hormone specific immunoassay kits (Siemens, New York City, USA). The sensitivity of the E2, P4, and LH assays were 5.5 $nmol \cdot l^{-1}$, 0.3 $nmol \cdot l^{-1}$, and 0.05 $IU \cdot l^{-1}$, respectively. The intra-assay coefficients of variation (CV%) for E2, P4, and LH were 9.9 %, 7.4 % and 1.6 %, respectively.

Body composition

Body composition was measured by Dual-energy X-ray absorptiometry (DXA; Lunar Prodigy Advance, GE Medical Systems -Lunar, Madison WI USA) at the beginning of the study period. A whole-body scan was used with automatically allocated regions of interest.

Incremental Treadmill Running Testing

Maximal oxygen uptake (VO_{2max}) was used to assess and describe the physical performance level of the participants. A treadmill test was performed using a standard incremental protocol 2-4 weeks before the beginning of the study period similar to Taipale-Mikkonen et al. (19). Briefly, for the first 3-min stage of the test, treadmill speed was 6-7 $km \cdot h^{-1}$ after which it was increased by 1 $km \cdot h^{-1}$ every third minute until volitional exhaustion. Treadmill incline remained constant at 0.5° for the entire test. Oxygen consumption (VO_2) was measured breath-by-breath using a gas analyzer (Vyntus CPX, Vyaire Medical GmbH, Hoechberg, Germany) and VO_{2max} was defined as the highest average 60-s VO_2 value.

Statistical Analyses

Data were analyzed using IBM SPSS 28.0 (SPSS Inc., Chicago, IL). The effects of the MC or HC phase on HR, HRV variables, and measured hormones were analyzed separately for each of the three groups using generalized estimating equations (GEEs). GEE were introduced by Liang and Zeger in 1986 as an extension of generalized linear models that facilitates the analysis of data collected in repeated measures designs (25). GEE is capable of handling missing data that was not collected due the Covid19-pandemic (7.5% of blood samples and 2.4% of HRV data were missing) (26). In the univariate models, the main factor was the MC or HC phase.

In the multivariate models, HR and HRV variables were adjusted with the changes in E2 and LH concentrations (ΔE2, ΔLH) from M1 to M3 and the change in P4 concentration (ΔP4) from M1 to M4. Furthermore, the interactions between MC or HC phase and the changes

in hormonal concentrations were analyzed. To further assess significant interactions between hormonal changes and MC or HC, ΔE2 was divided into thirds: low <230 pmol·l^{-1}, moderate = 295-605 pmol·l^{-1}, and high >680 pmol·l^{-1} changes; and ΔP4 was divided into thirds: low <7.0 nmol·l^{-1}, moderate = 16-24 nmol·l^{-1}, and high >25 nmol·l^{-1} changes. In the CU-group, the associations between the measured hormones and HR and HRV variables were not analyzed, because there were no significant differences in the hormonal concentrations between the HC phases. Regression coefficients (B), standard errors (SE), p-values and 95% CI are reported for each model. The models were adjusted for age, $\dot{V}O_{2max}$, body fat percentage, and body mass index, but these covariates did not change the results. Thus, non-adjusted models are reported. When significant differences between the MC or HC phases were found, post hoc LSD-adjusted comparisons of time differences were performed. Estimated mean and 95% confidence intervals (95% CI) are presented. The perception of recovery between the MC or HC phases were analyzed separately for each of the three groups using Friedman's test. From those variables, mean and 95% CI are presented.

RESULTS

Hormonal concentrations

Hormonal concentrations differed ($p<0.05$) between the MC and HC phases in the NM- and PU-groups (Figure 1; for individual data, see Supplemental Figure 1, Supplemental Digital Content, http://links.lww.com/MSS/C823) in post hoc analyses. The lowest E2 concentrations were found during M1 and the highest E2 concentrations at M3 in both groups. The lowest P4 concentrations were found during M1 and M2 and the highest P4 concentrations in M4 (CV% of P4 during M4 in NM=61% and PU=85%). The highest LH concentrations were

found at M3 in the NM- and PU groups. There were no differences ($p \geq 0.116$) in hormonal concentrations between M1 and M3 in the CU-group based on post-hoc analyses (Figure 1) or in the univariate GEE models.

In the univariate GEE models, significant B-values for E2 concentrations were found between M2 (follicular phase as reference) and other MC phases in the NM-group (M2-M1: B=-147, p=0.001; M2-M3: B= 311, p<0.001; M2-M4: B=193, p<0.001). In the PU-group, there were significant B-values for E2 concentrations between M2 (reference) and M3 (B=374, p=0.009), and M2 and M3 (B=-132, p=0.049). Significant B-values for P4 concentrations were found between M2 and M3 (B=3.4, p=0.006), and M2 and M4 (B=18.1, p<0.001) in the NM-group. In the PU-group, there was a significant B-value for P4 concentrations between M2 and M4 (B=14.5, p<0.001). Significant B-values for LH concentrations were found between M2 and M3 (B=8.2, p<0.001) in the NM-group, and between M2 and M1 (B=-5.0, p=0.025) in the PU-group.

Heart rate and heart rate variability

The HR and HRV-variables differed between MC and HC phases in the NM- and CU-groups, respectively (Figure 2; for individual data, see Supplemental Figure 2, Supplemental Digital Content, http://links.lww.com/MSS/C823). In the NM-group, RMSSD values were higher during M2 compared to M3 (p=0.013) and M4 (p=0.009), while SDNN values were higher in M2 compared to M4 (p=0.035). HR was lowest in M2 and highest in M4. In the NM-group, no differences (p=0.060-0.992) were observed in the frequency domain analysis.

However, there was a trend towards lower HF-power from M1 to M3 (p=0.060) and from M2 to M3 (p=0.067).

In the CU-group, RMSSD (p=0.014), SDNN (p=0.038), and HF-power (p=0.025) were higher in M1 compared to M2. Furthermore, HR was lower during M1 compared to M2 (p=0.038) and M4 (p=0.020). In the CU-group, no differences were found in LF-power (p=0.149-0.848) and LF/HF-ratio (p=0.070-0.584). However, there was a trend (p=0.070) towards lower LF/HF-ratio from M1 to M3.

There were no differences ($p \geq 0.107$) in HR and LF/HF-ratio between cycle phases in the PU-group, but RMSSD (p=0.042) and HF-power (p=0.049) values were higher in M2 compared to M3 (Figure 2). Furthermore, SDNN (p=0.021-0.030) and LF-power (p=0.004-0.021) values were higher in M2 compared to M3 and M4, and LF-power values were higher ($p<0.001$) during M1 compared to M4 in the PU-group.

The univariate GEE models revealed significant B-values between M2 in the NM and PU-groups or M1 in the CU-group and other MC and HC phases that reflected results found in the post hoc analysis (see Supplemental Tables 1-3, Supplemental Digital Content, Association of menstrual cycle stages and hormones with HRV and HR in the NM-group, CU-group, and PU-group, http://links.lww.com/MSS/C823).

Association of menstrual cycle stages and hormones with HRV and HR

The associations between the measured hormones with HRV and HR were investigated using GEE-models presented in Supplemental Tables 1 (NM-group) and 3 (PU-group) (see Supplemental Digital Content, http://links.lww.com/MSS/C823). In the NM-group, ΔP4 was positively associated only with HR (B=0.35, p<0.001; Supplemental Figure 3A, Supplemental Digital Content, http://links.lww.com/MSS/C823) in multivariate model 2. In addition, the interaction of MC phase and ΔP4 was significantly associated with HR (B=0.37-0.58, p<0.001) in all the phases, and with RMSSD during M3 (B=-0.95, p=0.043). ΔP4 was not significantly associated with HC phases in the PU group.

In the NM-group, ΔE2 from M1 to M3 was negatively associated with RMSSD (B=-0.05, p<0.001) and SDNN (B=-0.03, p=0.007), and positively associated with HR (B=0.01, p<0.001) in multivariate model 1. Furthermore, the interaction between MC phase and ΔE2 was significantly associated with RMSSD (B=-0.05 - -0.06, p≤0.001; Supplemental Figure 3B, Supplemental Digital Content, http://links.lww.com/MSS/C823), HR (B=0.01-0.02, p<0.001), and SDNN (B=-0.04, p≤0.002) in all the phases. ΔE2 was not significantly associated with HC phases in the PU group.

ΔLH was not associated with HR or any of the HRV variables. The interaction between MC phase and ΔLH was associated with HR during M4 (B=0.67, p=0.010) in the NM-group. In the PU-group, there were no associations between ΔLH and HC phases.

Perception of recovery

Sleep duration on average was 8.5 ± 0.6 h, 8.4 ± 0.9 h, and 8.1 ± 1.0 h in NM, CU, and PU respectively. There was no difference in sleep duration between the MC or HC phases in any of the groups. Perceived feeling of recovery was reported to be 3.7 ± 0.7, 3.4 ± 0.6, and 3.3 ± 0.8 in NM, CU, and PU respectively. There was no difference in perception of recovery ($p>0.635$) between the MC or HC phases in any of the groups.

DISCUSSION

Our aim was to investigate nocturnal HR and HRV during different phases of the MC in naturally menstruating women, and in different phases of the HC in women using combined hormonal contraceptives or progestin-only hormonal contraceptives. The main finding was that MC and HC phases influenced HR or HRV in all three groups. In addition, the change in E2 concentrations were associated with HR and HRV during the MC in NM. Despite these observations, there were no changes in subjective perception of recovery between the MC or HC phases in any of the groups.

As expected, measured hormones fluctuated during the MC in the NM-group and during the HC in the PU-group, although there was substantial heterogeneity in individual hormone concentrations in both groups (5). Despite positive ovulation tests, some participants in the NM group had lower levels of E2 and P4 at the luteal phase measurements than might be expected in a eumenorrheic cycle (5, 27). In the PU-group sex steroid response was variable with only some participants apparently experiencing consistently lower concentrations of E2 and P4 (27). No differences in E2, P4, or LH concentrations were observed between the inactive and

active phase in the CU-group, consistent with a reduction in endogenous female hormone production while using CHCs (27). This nuance in hormonal variation may be important to consider when analyzing the present results particularly in NM and PU groups.

In NM, higher RMSSD and SDNN values were observed during the follicular phase (M2) compared to the luteal phase (M4). Furthermore, higher HR was observed at ovulation (M3) and during the luteal phase compared to the bleeding (M1) and follicular phases. Similar results have been reported in naturally menstruating female athletes and physically active participants (28-30). Regrettably, these previous studies did not confirm MC phases with hormonal analyses. Furthermore, where most previous studies have used day-time measurements (14) nocturnal measurements seem to have greater sensitivity for detecting disturbances in cardiovascular homeostasis after exercise sessions (31). Our results, based on nocturnal HRV monitoring and confirmation of MC phase with hormonal analyses, support previous observations that HRV is higher during the follicular phase compared to the luteal phase, which may indicate that parasympathetic modulation decreases from the follicular to the luteal phase (28). This pattern may be important to take into consideration when monitoring training and recovery in athletes.

It has been suggested that female sex hormones influence sympathetic and parasympathetic neural activity (32, 33). In the present study, the change in E2 concentrations were associated with variations in HR and HRV during the MC in the NM-group. Thus, when HR and HRV results were adjusted for the change in E2, the RMSSD and SDNN differences between MC phases disappeared in multivariate model 2. In HR, the E2 adjustment had no effect

on the difference between the follicular phase (M2) and ovulation (M3) or luteal phases (M4). The significant interaction between the changes in E2 and MC phase in RMSSD and SDNN was due to heterogeneity in E2 concentrations within the NM-group from ovulation to the luteal phase. The RMSSD and SDNN values increased from ovulation to the luteal phase for participants who had small changes in E2 concentration during the MC. In contrast, the RMSSD and SDNN values decreased from ovulation to the luteal phase for participants who had moderate or high changes in E2. Furthermore, the change in P4 was positive associated with HR during the MC in NM. Importantly, the significant interaction between changes in P4 and MC phase was due to individual changes. In previous studies Leicht et al. (34) found a correlation between E2 and HRV during ovulation, but Schmalenberger et al. (35) did not. Schmalenberger et al. (35) found a significant correlation between P4 and HRV, but in other studies P4 and LH did not correlate with HRV (33, 34). It should be noted that unlike the present study, most of these studies did not report differences in HRV between MC phases (34, 36). Taken together, endogenous E2 appeared to affect HR and HRV during the MC in NM.

It has been speculated that endogenous and exogenous hormones may exert different effects on the autonomic nervous system (ANS) (30, 33, 37). In women using CHC, RMSSD, SDNN, and HF-power were higher, and HR was lower in the inactive phase compared to the first week of the active phase. Sims et al. (30) reported a similar decline in HRV in CHC users at the beginning of the active phase. They also reported an increase in HRV values in the second and third weeks of the active phase (M2 and M3) (30). In other studies, HRV did not significantly differ between the active and inactive phases although, HRV monitoring was completed at the end of the second or third week of the active phase, in which a possible decline

in HRV during the first half of active phase was not measured (38, 39). In addition, RMSSD, SDNN, LF-power, and HF-power were higher in M2, representing the follicular phase, compared to M3, representing ovulation, in PU. Furthermore, SDNN and LF-power values were higher in M2, representing the follicular phase compared to M4 representing the luteal phase. The effects of progestin-only contraceptive use on HRV has received only limited attention. Nevertheless, Sims et al. (30) reported that a progestin-only group showed similar changes in HRV as naturally menstruating women except that the late-luteal phase did not include as large a decline in HRV as observed in the naturally menstruating group. Similarly, we did not find as large a decline in RMSSD and HF-power from the M2 to the M4 as during M3. Overall, exogenous hormones (via endogenous hormonal changes) may have small influence on HR and HRV in HC users.

Although we did not observe any significant hormonal changes in CU, it is important to consider that exogenous hormones are not consumed during the inactive period. Lack of exogenous hormones influences endogenous hormone secretion (9) where e.g. FSH and LH secretion rapidly resumes during the inactive phase (27), indicating that CHC dosage patterns may influence the effects of endogenous hormones on the ANS. During the active phase, a stable hormone profile emerges and the inhibitory effects of both the E2 and the P4 components on endogenous hormones are established (30). Thus, it seems that the inhibitory effects of exogenous hormones could influence HRV during the first week of the active phase, which decreases vagal activity.

In the PU-group, we observed the greatest decline in HRV at M3, even though P4 concentrations were highest at M4. The progestin-only contraceptives in this study used levonorgestrel, which differs from endogenous progesterone and may attenuate the magnitude of the phase-based increase in sympathetic activity caused by endogenous progesterone during the late-luteal phase noticed with naturally menstruating women (30). Stadler et al. (40) reviewed the effects of endogenous and exogenous progesterone on the ANS and hypothalamic–pituitary–adrenal (HPA) axis, which are both activated by stressors. They noticed that HPA-axis activity was not affected by endogenous progesterone exposure across the MC but might be reduced by exogenous progestin. In contrast, the ANS has a sympathetic predominance in the progesterone-dominated luteal phase, but has not yet been evaluated under exogenous progestin exposure (40).

To our knowledge, this is the first study that reports data from three groups with concurrent measurements of female sex hormones and HRV. It should be noted as that all women in the NM group had a regular menstrual cycle (no amenorrhea) and returned a positive ovulation test during the measurement period. Regrettably, an ovulation test was not used in the PU group to identify ovulating progestin-only HC users. A strength of this study is that hormonal analysis was used to assess the cycle phase in NM and PU. In addition, nocturnal analysis of HRV was completed using reliable monitoring and analysis methods. Unfortunately, because of the relatively long research period (one MC or four weeks) all variables that might possibly affect HR and HRV, such as psychological stress factors and dietary patterns, could not be controlled. Furthermore, the time-intensive nature of well-controlled studies with hormonal analyses across the MC commonly result in a low sample size (14), and this study is no exception. Further studies with larger samples and hormonal analyses are needed to confirm our

findings while the present results should be generalized to e.g. elite athletic populations with caution as the participants were not all highly trained but classified as trained (Tier 2 in the classification on a scale of 0–5) (18).

CONCLUSIONS

In physically active women, the hormonal fluctuation associated with MC and HC phases appear to affect autonomic nervous system balance. Our study indicates that between-phase differences in autonomic activity can be explained by within-individual differences in the studied hormonal concentrations, it is important to account for within-individual differences in hormone production when assessing phase differences on a group level. Therefore, MC and HC status should be considered when assessing nocturnal HR and HRV. Considering hormonal status, including MC and HC phases, may help to promote quality training and recovery in physically active individuals.

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Conflict of Interest

No conflicts of interest, financial or otherwise, are declared by the authors. The results of this study are presented clearly, honestly and without fabrication, falsification, or inappropriate data manipulation. The results of the present study do not constitute endorsement by the American College of Sports Medicine.

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FIGURE LEGENDS

Figure 1. Estimated mean and 95 % confidence intervals of female hormone concentrations. A) estradiol; B) progesterone; C) luteinizing hormone. NM = naturally menstruating group (M1=bleeding, M2=follicular phase, M3=ovulation, M4=luteal phase), CU = combined hormonal contraceptives group (M1=inactive, M3=active); PU = progestin-only contraceptives group; (M1 = lowest E2; M2 = M1+7 days; M3 = M1+14 days; M4 = M1+21 days).

Significant difference compared to M2, *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$.

Significant difference compared to M1, $^{\#}$, $p<0.05$, $^{\#\#\#}$, $p<0.001$.

Significant difference compared to M3, §, $p<0.05$, §§§, $p<0.001$.

Figure 2. Estimated mean and 95 % confidence intervals of heart rate and heart rate variability variables. A) RMSSD; B) HR; C) SDNN; D) HF-power; E) LF-power; F) LF/HF-ratio. NM = naturally menstruating group (M1=bleeding, M2=follicular phase, M3=ovulation, M4=luteal phase), CU = combined hormonal contraceptives group (M1=inactive phase, M2= active phase week 1, M3=active phase week 2, M4= active phase week 3); PU = progestin-only contraceptives group; (M1 = lowest E2; M2 = M1+7 days; M3 = M1+14 days; M4 = M1+21 days).

Significant difference compared to M2, *, $p<0.05$, **, $p<0.01$, ***, $p<0.001$.

Significant difference compared to M1, $^{\#}$, $p<0.05$, $^{\#\#}$, $p<0.01$, $^{\#\#\#}$, $p<0.001$.

Significant difference compared to M3, §§, $p<0.01$.

SUPPLEMENTAL DIGITAL CONTENT

SDC 1: Supplemental Digital Content.docx

Table S1 - Association of menstrual cycle stages and hormones with HRV and HR in the NM-group

Table S2 - Association of menstrual cycle stages with HRV and HR in the CU-group

Table S3 - Association of menstrual cycle stages and hormones with HRV and HR in the PU-group

Figure S1. Individual profiles of estradiol (E2) and progesterone (P4) for normally menstruating (NM; M1=bleeding, M2=follicular phase, M3=ovulation, M4=luteal phase), combined hormonal contraceptive using (CU; M1=inactive, M3=active), and progestin-only contraceptive using (PU; M1 = lowest E2; M2 = M1+7 days; M3 = M1+14 days; M4 = M1+21 days) participants.

Figure S2. Individual profiles of heart rate (HR) and heart rate variability (rMSSD) for normally menstruating (NM; M1=bleeding, M2=follicular phase, M3=ovulation, M4=luteal phase), combined hormonal contraceptive using (CU; M1=inactive, M3=active), and progestin-only contraceptive using (PU; M1 = lowest E2; M2 = M1+7 days; M3 = M1+14 days; M4 = M1+21 days) participants.

Figure S3. A) Mean and SD of heart rate in different progesterone (P4) concentrations in the naturally menstruating group. Low = change in P4 from bleeding to luteal phase -0.5-7.0 $nmol \cdot L^{-1}$; Medium = change in P4 from bleeding to luteal phase 16-24 $nmol \cdot L^{-1}$; High = change in P4 from bleeding to luteal phase >25 $nmol \cdot L^{-1}$ B) Mean and SD of RMSSD in different estradiol (E2) concentrations in the naturally menstruating group.

Figure 1

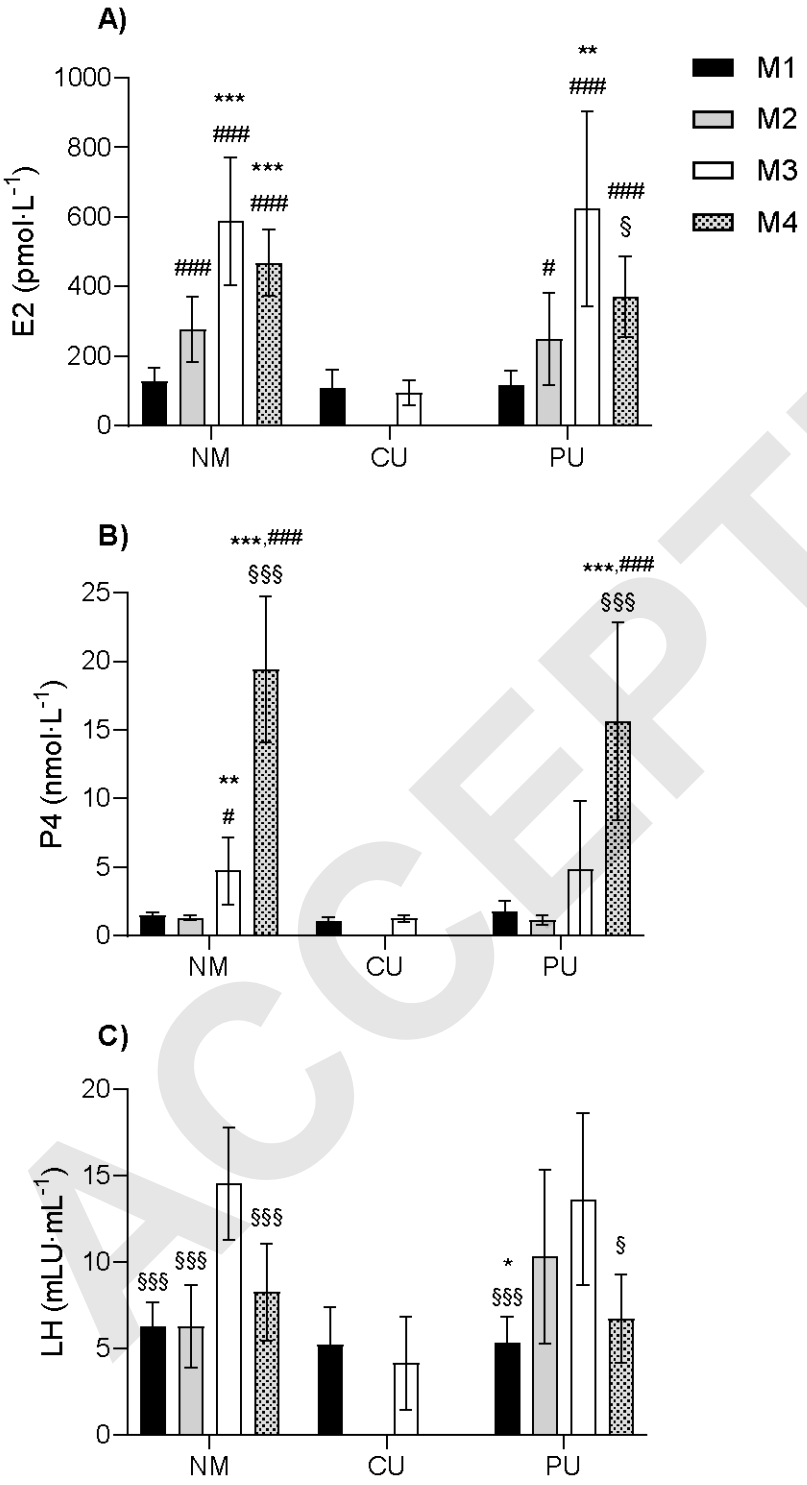


Figure 2

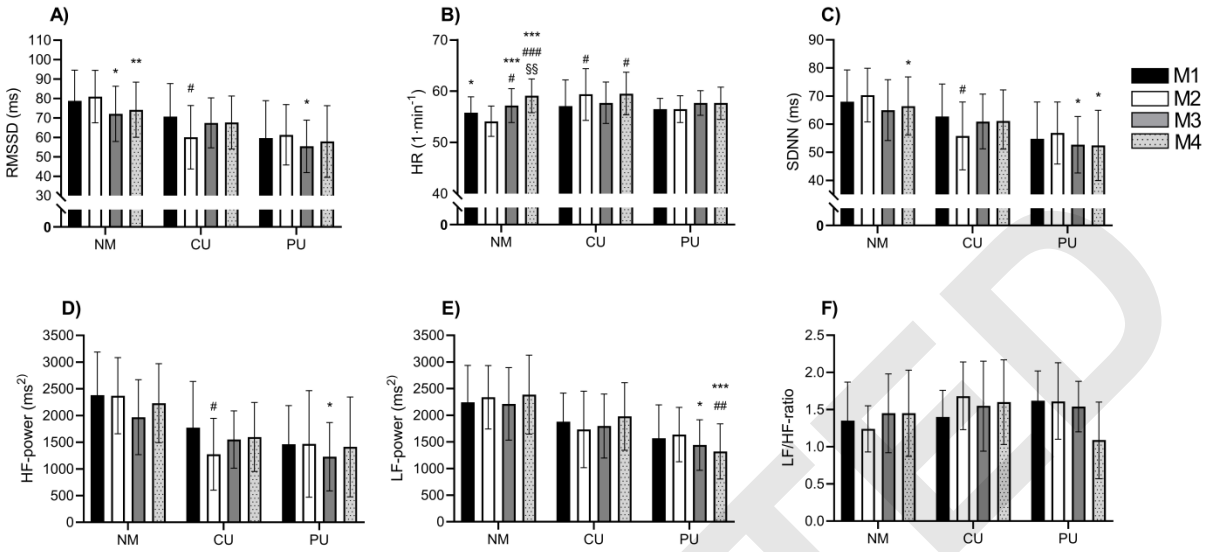


Table 1. Participant characteristics in all three groups. NM = naturally menstruating group, CU = combined hormonal contraceptives group; PU = progestin-only contraceptives group; BMI = body mass index; VO_{2max} = maximal oxygen uptake.

Group	n	Age (year)	Height (m)	Body mass (kg)	BMI ($kg \cdot m^{-2}$)	Body fat percentage (%)	VO_{2max} ($m \cdot kg^{-1} \cdot min^{-1}$)
NM	19	25.5 ± 4.4	1.66 ± 0.07	63.2 ± 6.7	22.8 ± 2.4	25.2 ± 6.1	45.5 ± 5.2
CU	11	25.4 ± 2.5	1.68 ± 0.05	66.1 ± 5.0	23.5 ± 1.7	27.7 ± 8.0	43.0 ± 4.8
PU	12	28.5 ± 6.4	1.69 ± 0.05	62.6 ± 8.8	21.9 ± 3.0	23.7 ± 6.8	45.8 ± 5.8

Table 2. Active hormone components and the brand names of combined oral contraceptive pills, contraceptive rings and in progestin-only hormonal contraceptives group.

Pills/rings/IUD included	Brand names
CU: Third generation pills	
Ethinyl estradiol coupled with gestodene (n=1)	Meliane
Ethinyl estradiol coupled with desogestrel (n=2)	Mercilon and Lumivela
CU: Fourth generation pills	
Ethinyl estradiol coupled with drospirenone (n=3)	Yasminelle, Stefaminelle, and Tasminetta
Ethinyl estradiol coupled with dienogest (n=1)	Dienorette
Estradiol hemihydrate coupled with nomegestrol acetate (n=1)	Zoely
CU: Contraceptive ring	
Ethinyl estradiol coupled with etonogestrel (n=3)	Ornibel and Vagiprev
PU: Hormonal IUD	
Levonorgestrel 13.5 mg (n=2)	Jaydess
Levonorgestrel 19.5 mg (n=4)	Kyleena
Levonorgestrel 52 mg (n=6)	Mirena

CU = combined hormonal contraceptive users, PU = progestin only hormonal contraceptive users, IUD = intrauterine device.

Table 3. The variables that were analyzed from the BG2-monitor.

Variable	**Explanation**
Heart rate (BPM)	Average heart rate during measurement
RMSSD (ms)	The root mean square of successive differences between normal heartbeats
SDNN (ms)	Standard deviation of normal-to-normal R-R intervals
HF-power (ms^2)	Average power of high frequency HRV during measurement (0.15 – 0.40 Hz)
LF-power (ms^2)	Average power of low frequency HRV during measurement (0.04 – 0.15 Hz)
LF/HF-ratio	Ratio of low and high frequency HRV (LFm^2/HFm^2)

Supplemental Tables and Figures

Supplemental Table 1. Association of menstrual cycle stages and hormones with HRV and HR in the NM-group.

	RMSSD models		**HR models**		**SDNN models**		**HF-power models**		**LF/HF-ratio models**	
NM-group	**B (SE)**	**95 % CI**	**B (SE)**	**95 % CI**	**B (SE)**	**95 % CI**	**B (SE)**	**95 % CI**	**B (SE)**	**95 % CI**
Univariate model										
M2 (ref)										
M3	**-8.96 (3.59)***	-15.99, -1.92	**3.08 (0.62)*****	1.87, 4.29	-5.30 (2.80)	-10.79, 0.20	-402 (220)	-833, 28	0.21 (0.14)	-0.07, 0.49
M4	**-6.78 (2.60)****	-11.87, -1.69	**4.94 (0.62)*****	3.72, 6.16	**-3.87 (1.83)***	-7.46, 0.28	-140 (135)	-404, 125	0.21 (0.18)	-0.14, 0.56
M4	-2.21 (6.88)	-8.46, 4.05	**1.66 (0.72)***	3.06, 5.34	-2.32 (2.19)	-6.1, 1.98	11 (191)	-363, 385	0.11 (0.14)	-0.17, 0.39
Multivariate model 1										
M2 (ref)										
M3	-4.94 (3.65)	-12.10, 2.23	**2.49 (0.62)*****	1.28, 3.70	-2.38 (2.95)	-8.16, 3.41	-137 (197)	-524, 249	0.06 (0.06)	-0.06, 0.17
M4	-5.17 (2.93)	-10.92, 0.58	**4.76 (0.73)*****	3.32, 6.19	-2.18 (1.84)	-5.79, 1.43	-89 (155)	-392, 214	0.04 (0.06)	-0.08, 0.17
M4	-0.60 (3.71)	-7.88, 6.68	1.00 (0.72)	-0.41, 2.41	-1.34 (2.54)	-6.32, 3.64	78 (229)	-370, 526	-0.04 (0.07)	-0.18, 0.10
Change of E2 (M3 vs. M1)	**-0.05 (0.01)*****	-0.08, -0.02	**0.01 (0.00)*****	0.01, 0.02	**-0.04 (0.01)*****	-0.06, -0.02	1.48 (1.10)	-3.65, 0.68	0.00 (0.0004)	-0.001, 0.000
*Time * Change of E2*										
M2	**-0.05 (0.08)****	-0.08, -0.02	**0.01 (0.00)*****	0.00, 0.01	**-0.04 (0.01)****	-0.06, -0.01	-1.02 (1.55)	-4.06, 2.02	0.00	-0.001, 0.000

									(0.0004)	
M3	**-0.05 (0.01)*****	-0.08, -0.03	**0.01 (0.00)*****	0.01, 0.02	**-0.04 (0.01)****	-0.06, -0.02	-1.16 (1.57)	-4.23, 1.92	0.00 (0.0004)	-0.001, 0.001
M4	**-0.06 (0.01)*****	-0.08, -0.03	**0.02 (0.00)*****	0.01, 0.02	**-0.04 (0.01)*****	-0.06, -0.02	-1.21 (1.58)	-4.31, 1.90	0.00 (0.0004)	-0.001, 0.000
M1	**-0.05 (0.01)*****	-0.07, -0.02	**0.01 (0.00)*****	0.01, 0.02	**-0.04 (0.01)*****	-0.06, -0.02	-0.93 (1.66)	-4.18, 2.32	0.00 (0.0004)	-0.001, 0.000
Multivariate model 2										
M2 (ref)										
M3	**-7.83 (3.61)***	-14.90, -0.75	**3.07 (0.63)*****	1.84, 4.29	-4.66 (2.84)	-10.23, 0.90	-312 (210)	-722, 99	0.21 (0.15)	-0.09, 0.51
M4	**-5.92 (2.60)***	-11.01, -0.83	**4.91 (0.66)*****	3.62, 6.20	-3.34 (1.86)	-6.98, 0.30	-91 (133)	-352, 170	0.21 (0.19)	-0.16, 0.58
M4	-1.79 (3.21)	-8.08, 4.49	**1.64 (0.72)***	0.22, 3.05	-2.08 (2.20)	-6.40, 2.25	35 (192)	-341, 411	0.11 (0.15)	-0.18, 0.41
Change of P4 (M4 vs. M1)	-0.78 (0.47)	-1.70, 0.14	**0.35 (0.08)*****	0.20, 0.51	-0,52 (0.40)	-1.30, 0.26	-43 (31)	-104, 18	0.01 (0.01)	-0.01, 0.04
*Time * Change of P4*										
M2	-0.64 (0.48)	-1.85, 0.31	**0.37 (0.08)*****	0.21, 0.54	-0.43 (0.41)	-1.23, 0.37	-16.8 (38.1)	-91.4, 57.8	0.02 (0.01)	-0.01, 0.04
M3	**-0.95 (0.47)***	-1.87, -0.03	**0.51 (0.08)*****	0.36, 0.66	-0.63 (0.41)	-1.43, 0.18	-28.3 (39.7)	-106.0, 49.4	0.02 (0.01)	0.00, 0.05
M4	-0.89 (0.48)	-1.82, 0.04	**0.58 (0.08)*****	0.42, 0.73	-0.58 (0.40)	-1.37, 0.20	-21.2 (39.7)	-99.0, 56.7	0.02 (0.01)	-0.01, 0.05

M1	-0.68 (0.50)	-1.66, 0.31	**0.44 (0.07)*****	0.30, 0.59	-0.49 (0.42)	-1.31, 0.34	-13.2 (42.0)	-95.6, 69.1	0.02 (0.01)	-0.01, 0.05

Significant B-value, *, p<0.05, **, p<0.01, ***, p<0.001. M1=bleeding; M2=follicular phase; M3=ovulation; M4=luteal phase.

Supplemental Table 2. Association of menstrual cycle stages with HRV and HR in the CU-group.

	RMSSD models		**HR models**		**SDNN models**		**HF-power models**		**LF/HF-ratio models**	
CU-group	**B (SE)**	**95 % CI**	**B (SE)**	**95 % CI**	**B (SE)**	**95 % CI**	**B (SE)**	**95 % CI**	**B (SE)**	**95 % CI**
Univariate model										
M1 (ref)										
M2	**-10.59 (4.29)***	-19.00, -2.18	**2.25 (1.09)***	0.12, 4.38	**-6.86 (3.31)***	-13.35, -0.38	**-499 (223)***	-936, -62	0.28 (0.15)	-0.02, 0.58
M3	-3.24 (5.50)	-10.16, 4.23	0.62 (0.82)	-0.99, 2.22	-1.76 (3.29)	-8.20, 4.69	-223 (322)	-854, 407	0.14 (0.23)	-0.30, 0.59
M4	-2.96 (3.67)	-9.51, 0.37	**2.41 (1.04)***	0.38, 4.44	-1.07 (2.19)	-5.37, 3.23	-175 (193)	-555, 204	0.19 (0.20)	-0.20, 0.59

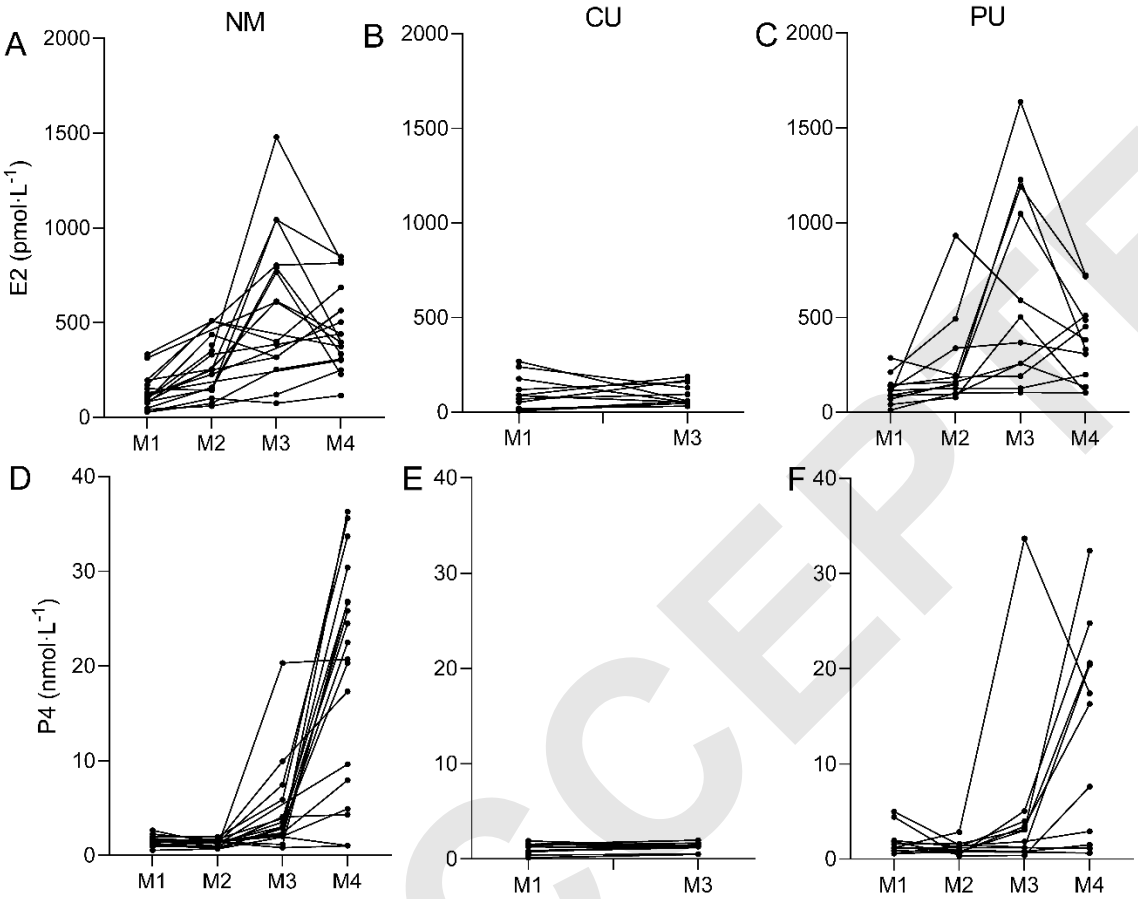
Significant B-value, *, $p<0.05$. M1=inactive phase; M2= active phase week 1; M3=active phase week 2; M4= active phase week 3.

Supplemental Table 3. Association of menstrual cycle stages and hormones with HRV and HR in the PU-group.

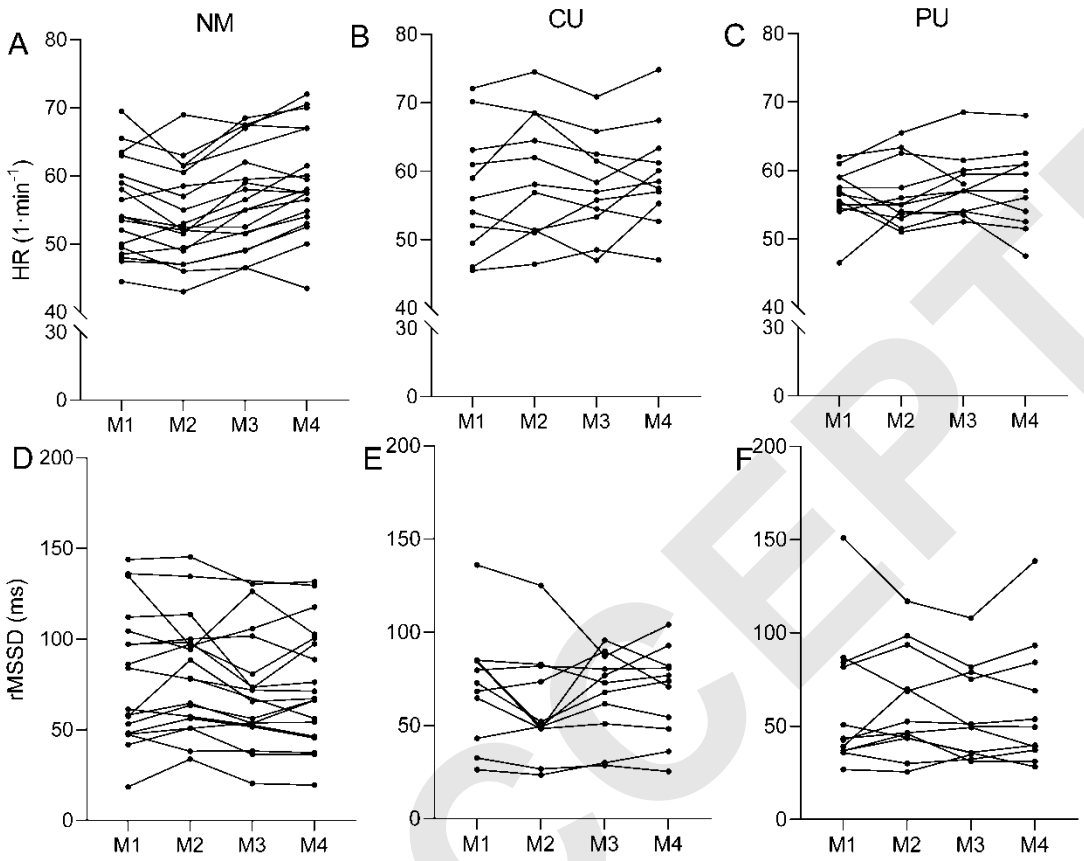
	RMSSD models		**HR models**		**SDNN models**		**HF-power models**		**LF/HF-ratio models**	
PU-group	**B (SE)**	**95 % CI**	**B (SE)**	**95 % CI**	**B (SE)**	**95 % CI**	**B (SE)**	**95 % CI**	**B (SE)**	**95 % CI**
Univariate model										
M2 (ref)										
M3	**-5.93 (2.91)***	-11.63, -0.22	1.22 (0.73)	-0.22, 2.65	**-4.22 (1.83)***	-7.81, -0.62	**-234 (119)***	-467, -1	-0.07 (0.18)	-0.43, 0.29
M4	-3.45 (3.46)	-10.23, 3.33	1.17 (0.97)	-0.77, 3.10	**-4.44 (2.05)***	-8.46, -0.42	-50 (166)	-376, 276	-0.52 (0.44)	-1.39, 0.35
M4	-1.61 (4.60)	-10.63, 7.40	-0.04 (1.11)	-2.22, 2.14	-2.05 (2.97)	-7.87, 3.76	7 (221)	-426, 440	0.01 (0.14)	-0.26, 0.28
Multivariate model 1										
M2 (ref)										
M3	**-5.93 (2.91)***	-11.63, -0.22	1.22 (0.73)	-0.22, 2.65	**-4.22 (1.83)***	-7.81, -0.62	**-234 (119)***	-467, -1	-0.07 (0.18)	-0.43, 0.29
M4	-3.67 (3.26)	-10.06, 2.73	1.12 (0.99)	-0.84, 3.08	**-4.44 (2.05)***	-8.46, -0.42	-63 (151)	-358, 233	-0.48 (0.41)	-1.28, 0.31
M4	-1.61 (4.60)	-10.63, 7.40	-0.04 (1.11)	-2.22, 2.14	-2.05 (2.97)	-7.87, 3.76	7 (221)	-426, 440	0.01 (0.14)	-0.26, 0.28
Change of E2 (M3 vs. M1)	0.02 (0.02)	-0.02, 0.06	0.002 (0.004)	-0.01, 0.01	0.01 (0.01)	-0.01, 0.04	1.20 (1.11)	-0.96, 3.37	0.00 (0.0004)	-0.001, 0.000
*Time * Change of E2*										
M2	0.02 (0.02)	-0.02, 0.06	0.01 (0.00)	-0.002, 0.01	0.02 (0.01)	-0.01, 0.04	1.07 (0.96)	-0.83, 2.96	0.00 (0.0006)	-0.001, 0.001

M3	0.02 (0.02)	-0.02, 0.05	0.01 (0.00)	-0.001, 0.01	0.01 (0.01)	-0.01, 0.04	0.85 (0.86)	-0.84, 2.54	0.00 (0.0006)	-0.001, 0.001
M4	0.02 (0.02)	-0.02, 0.07	0.01 (0.00)	-0.003, 0.01	0.01 (0.02)	-0.02, 0.04	1.22 (1.21)	1.15, 3.59	0.00 (0.0006)	-0.001, 0.001
M1	0.03 (0.02)	-0.02, 0.07	0.004 (0.00)	-0.003, 0.01	0.02 (0.02)	-0.01, 0.05	1.43 (1.28)	-1.08, 3.93	0.00 (0.0006)	-0.001, 0.001
Multivariate model 2										
M2 (ref)										
M3	**-5.93 (2.91)***	-11.63, -0.22	1.22 (0.73)	-0.22, 2.65	**-4.22 (1.83)***	-7.81, -0.62	**-234 (119)***	-467, -1	-0.07 (0.18)	-0.43, 0.29
M4	-3.67 (3.26)	-10.06, 2.73	1.19 (0.98)	-0.72, 3.11	**-4.44 (2.05)***	-8.46, -0.42	-55 (158)	-365, 255	-0.52 (0.44)	-1.39, 0.35
M4	-1.61 (4.60)	-10.63, 7.40	-0.04 (1.11)	-2.22, 2.14	-2.05 (2.97)	-7.87, 3.76	7 (221)	-426, 440	0.01 (0.14)	-0.26, 0.28
Change of P4 (M4 vs. M1)	-0.03 (0.62)	-1.25, 1.19	-0.15 (0.09)	-0.32, 0.03	-0.05 (0.46)	-0.94, 0.85	-6 (25)	-54, 43	0.01 (0.01)	-0.01, 0.04
*Time * Change of P4*										
M2	0.14 (0.65)	-1.14, 1.18	0.03 (0.11)	-0.19, 0.25	0.08 (0.48)	-0.86, 1.02	1.5 (24.6)	-46.6, 49.6	-0.01 (0.02)	-0.04, 0.02
M3	-0.18 (0.62)	-1.40, 1.03	0.13 (0.11)	-0.08, 0.34	-0.14 (0.46)	-1.03, 0.76	-10.0 (22.4)	-53.9, 33.8	-0.01 (0.02)	-0.04, 0.03
M4	-0.13 (0.64)	-1.39, 1.13	0.13 (0.11)	-0.09, 0.35	-0.15 (0.47)	-1.07, 0.76	-5.8 (23.6)	-52.2, 40.5	-0.01 (0.02)	-0.04, 0.02
M1	0.06 (0.58)	-1.07, 0.07	0.03 (0.08)	-0.14, 0.19	0.00 (0.43)	-0.84, 0.84	-0.9 (21.6)	-43.1, 41.4	-0.01 (0.02)	-0.04, 0.02

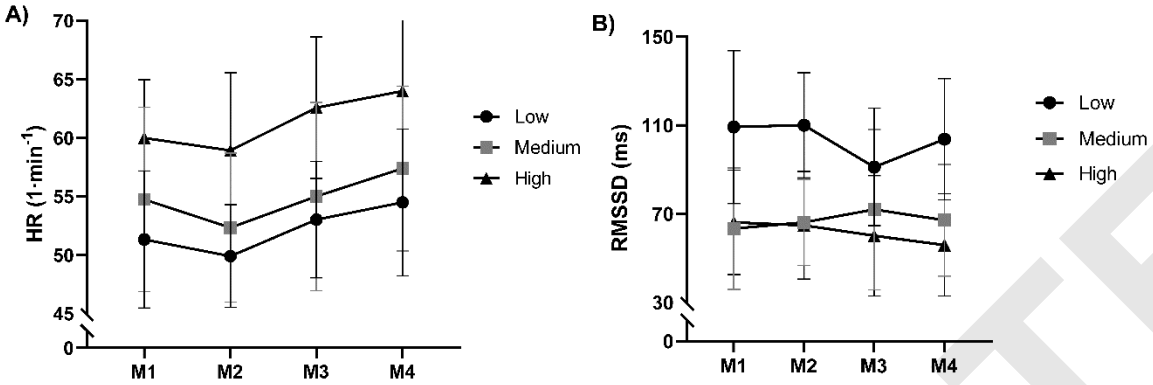
Significant B-value, *, $p<0.05$. M1 = lowest E2; M2 = M1+7 days; M3 = M1+14 days; M4 = M1+21 days.



Supplemental Figure 1. Individual profiles of estradiol (E2) and progesterone (P4) for normally menstruating (NM; M1=bleeding, M2=follicular phase, M3=ovulation, M4=luteal phase), combined hormonal contraceptive using (CU; M1=inactive, M3=active), and progestin-only contraceptive using (PU; M1 = lowest E2; M2 = M1+7 days; M3 = M1+14 days; M4 = M1+21 days) participants.



Supplemental Figure 2. Individual profiles of heart rate (HR) and heart rate variability (rMSSD) for normally menstruating (NM; M1=bleeding, M2=follicular phase, M3=ovulation, M4=luteal phase), combined hormonal contraceptive using (CU; M1=inactive, M3=active), and progestin-only contraceptive using (PU; M1 = lowest E2; M2 = M1+7 days; M3 = M1+14 days; M4 = M1+21 days) participants.



Supplemental Figure 3. A) Mean and SD of heart rate in different progesterone (P4) concentrations in the naturally menstruating group. Low = change in P4 from bleeding to luteal phase -0.5-7.0 nmol·L^{-1}; Medium = change in P4 from bleeding to luteal phase 16-24 nmol·L^{-1}; High = change in P4 from bleeding to luteal phase >25 nmol·L^{-1} B) Mean and SD of RMSSD in different estradiol (E2) concentrations in the naturally menstruating group. Low = change in E2 from bleeding to ovulation <230 pmol·L^{-1}; Medium = change in estradiol from bleeding to ovulation 295-605 pmol·L^{-1}; High = change in E2 from bleeding to ovulation >680 pmol·L^{-1}; M1=bleeding; M2=follicular phase; M3=ovulation; M4=luteal phase.