Changes in resting heart rate variability across the menstrual cycle

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Abstract

Heart rate variability (HRV) is a noninvasive indicator of autonomic control. This study examines HRV changes across a normal menstrual cycle and proposes a novel piecewise function controlling for the effects of breathing on HRV spectral parameters. A resting ECG was collected from 13 women at five points in their menstrual cycle. Both heart rate and breathing rate increased across the cycle (p < .01) while time-domain variability decreased (p = .04). Use of the piecewise function for breathing rate in HRV spectral analysis was confirmed by a substantial increase in model goodness-of-fit. HRV spectral parameters, controlled for breathing with the piecewise function, confirm that the decrease in variability is likely due to a parasympathetic withdrawal, since high frequency HRV decreases (p = .02).

Descriptors: Heart rate variability, Autonomic nervous system, Menstrual cycle, Estrogen, Progesterone

The analysis of heart rate variability (HRV) is a commonly used noninvasive measure of autonomic cardiovascular control. Since the early work by Akselrod et al. (1981), the oscillatory (frequency-domain) components of HRV have been described in the high frequency (HF) band (0.15–0.40 Hz) and the low frequency (LF) band (0.04–0.15). At rest, time-domain HRV (SDNN) and HF power are metrics of parasympathetic tone (Hayano et al., 1991). Early studies suggested that LF, particularly when normalized or in ratio with HF, was an indicator of sympathetic tone (Malliani, Pagani, Lombardi, & Cerutti, 1991; Pagani et al., 1997). Current literature presents a compelling argument that LF is primarily the product of phasic baroreceptor activity (Goldstein, Bentho, Park, & Sharabi, 2011). Multiple techniques, including the gold standard Oxford technique, have indicated that LF is predominately modulated by the baroreceptor-cardiovagal gain (Rahman, Pechnik, Gross, Sewell, & Goldstein, 2011), though this has also been disputed (Reyes del Paso, Langewitz, Mulder, Roon, & Duschek, 2013). Although the exact physiologic underpinnings of the LF band are still being delineated, the use of HRV as a measure has increased as a non-invasive way to gain insight into various physical and mental perturbations in both men and women.

Investigations using HRV in women may have unrecognized inconsistency if HRV cannot be considered stable across the menstrual cycle. An inconsistency in HRV readings across the menstrual cycle may lead to inappropriate conclusions based upon unstable baseline readings or a narrowed difference between baseline and physiologic ceiling. An increase in sympathetic drive or decrease in parasympathetic activity after ovulation is reasonable because studies have consistently shown an increase in metabolic rate in this period (Bisdee, James, & Shaw, 1989; Solomon, Kurzer, & Calloway, 1982; Webb, 1986), implicating luteal phase progesterone. Recently, several studies have investigated the effect of the menstrual cycle on HRV (Bai, Li, Zhou, & Li, 2009; Leicht, Hirning, & Allen, 2003; McKinley et al., 2009; Sato, Miyake, Akatsu, & Kumashiro, 1995; Yildirim, Kabakci, Akgul, Tokgozoglu, & Oto, 2002). Previous research has reported a decrease in time-domain HRV in the luteal phase compared to the follicular phase (McKinley et al., 2009) and frequency-domain changes, indicating decreases in parasympathetic and/or increases in sympathetic activity from the follicular to luteal phases (Bai et al., 2009; Sato et al., 1995; Yildirim et al., 2002). However, there has also been reported to be no change in frequency-domain HRV across the menstrual cycle (Leicht et al., 2003). All previous studies examining HRV across the menstrual cycle have utilized only two or three testing sessions. Two or three testing points may be an oversimplification of the menstrual cycle, which exhibits a number of hormonal periodicities. Furthermore, the known increase in ventilation from the follicular to luteal phases (Girija & Veeraiah, 2011; Slatkovska, Jensen, Davies, & Wolfe, 2006) may account for the frequency-domain changes across the menstrual cycle.
cycle, possibly indicating that the observed HRV changes across the menstrual cycle are artifactual in nature.

Breathing frequency substantially impacts HRV spectral parameters, whereas the time-domain parameters are not affected by breathing frequency or tidal volume (Brown, Beightol, Koh, & Eckberg, 1993). Total spectral power decreases when breathing rate increases; and when people breathe at rates between 7–10 breaths per minute, the respiratory sinus arrhythmia obscures the HF and LF divide, making interpretation of the autonomic properties difficult (Beda, Jandre, Phillips, Giannella-Neto, & Simpson, 2007; Brown et al., 1993; Cammann & Michel, 2002). The respiratory sinus arrhythmia peak in the power spectrum is less prominent in spontaneous breathing than in metronome-controlled breathing at the same rate (Bloomfield et al., 2001). However, controlled metronome breathing can be a stressor unto itself (Patwardhan, Vallurupalli, Evans, Bruce, & Knapp, 1995), potentially confounding the assessment of autonomic balance.

Therefore, the goal of this study is two-fold: (1) to record HRV at five time points across the menstrual cycle to more effectively track the changes throughout the cycle, and (2) to use a novel piecewise analysis of covariance (ANCOVA) function analysis to control for changes in breathing rate that may obscure actual changes in frequency-domain HRV. The piecewise function for breathing rates higher and lower than 10 breaths per minute will allow for the unbiased examination of frequency-domain HRV by accounting for the effects of breathing rate without the addition of methodological stressors (i.e., controlled metronome breathing). We hypothesize that HRV variables will indicate less parasympathetic control after ovulation and that the use of the piecewise function will contribute explanatory information when examining HRV across the menstrual cycle.

**Method**

**Participants and Ethical Approval**

Thirteen young eumenorrheic women (ages 20–31 years) participated in this study. All participants were free from cardiovascular, neurologic, endocrine, or metabolic disorders, had a self-reported history of normal menstrual cycles, no previous history of pregnancy, and were contraception naïve for at least 6 months prior to testing. Additional participant demographic information is provided in Table 1. All participants gave their informed consent in accordance with the Helsinki Declaration, and all experimental procedures were approved by the University of Texas at Austin Institutional Review Board.

**Determination of Study Visit Days and Ovulation**

Data were collected from each participant at five points in the menstrual cycle, corresponding to the early follicular, late follicular, ovulatory, midluteal, and late luteal menstrual phases.

<table>
<thead>
<tr>
<th>Table 1. Subject Demographics</th>
<th>Average</th>
<th>Minimum</th>
<th>Maximum</th>
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</thead>
<tbody>
<tr>
<td>Height (cm)</td>
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<td>152.4</td>
<td>177.8</td>
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<td>BMI</td>
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<tr>
<td>Cycle length (days)</td>
<td>29.5</td>
<td>25</td>
<td>32</td>
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Note. BMI = body mass index.

The first point of data collection for each subject was randomized and resulted in a pseudocounterbalanced design. Three participants started collection in early follicular, three in late follicular, two in ovulation, two in midluteal, and three in late luteal phase. The average cycle length for participants during data collection is shown in Table 1. All data were collected in the morning, and time was standardized within each participant. Our use of the basal body temperature map (BBT) to determine menstrual phase has been previously reported (Tenan, Peng, Hackney, & Griffin, 2013). The BBT has been used extensively for the determination of normal menstrual cycles and menstrual phases (Barrett & Marshall, 1969; Bisdee et al., 1989; Broverman et al., 1981; Cassidy, Bingham, & Setchell, 1994; Dunson, Colombo, & Baird, 2002; Lebrun, McKenzie, Prior, & Tauntion, 1995; Prior, Cameron, Yuen, & Thomas, 1982; Viergiver & Pomerenke, 2013).

Procedurally, participants obtained their body temperature via oral thermometer for one month prior to data collection. Participants were instructed to take their oral temperature (BD Basal, Franklin Lakes, NJ) every morning before arising. Ovulation was operationally defined as the BBT nadir before the temperature rise in the luteal phase (de Mouzon, Testart, Lefevre, Pouly, & Frydman, 1984). Previous research has shown the BBT to be 90% effective in determining ovulation when compared against luteinizing-hormone determinations (Martinez et al., 1992). This biphasic response in the BBT is characteristic of a normal menstrual cycle in which ovulation occurs. If the temperature map from the first month was not clearly defined, the participant performed a second cycle map before admission to the data collection portion of the study. If the second temperature map was not well defined, the participant did not enter data collection. The early follicular and late follicular phases are equally spaced before the 3-day ovulatory phase; the midluteal and late luteal phases are equally spaced after the ovulatory phase. The data collection point was equally spaced within the middle of each determined menstrual phase. The BBT was first assessed and then subsequently confirmed independently by two trained investigators. Two participants exhibited short luteal defects during the data collection portion of the study; another participant was anovulatory (no biphasic BBT) upon entry for their last data collection (ovulatory phase). Therefore, these three participants only completed four study visits. The data from subjects with a short luteal defect were included in the analysis as it has been shown that cycle length has little association with luteal progesterone or estradiol levels (Barrett, Thune, Lipson, Furbeg, & Ellison, 2013). The ovulation date for the anovulatory participant was interpolated from the previous 3 months of normal BBT maps since menstrual cycle length was consistent. The follicular phase data for the anovulatory participant was also entered into analysis as it has been shown that women occasionally presenting as anovulatory (oligomenorrhea) have estradiol concentrations within expected normal limits (Laven & Fauser, 2006). Finally, consistent overall cycle length (±2 days) and BBT were confirmed upon completion of data collection; mean intraindividual variation in menstrual cycle length of ±2.5 days is considered normal (Treloar, Boynton, Behn, & Brown, 1967). All participants collected at least three BBT maps over the course of the study.

**Experimental Protocol**

All data collection was performed in the Neuromuscular Physiology Laboratory at the University of Texas at Austin. Participants were instructed to not perform strenuous physical activity or ingest food containing large amounts of phytoestrogens 48 h prior to
testing. Additionally, the participants were instructed to avoid alcohol and caffeine for 8 h prior to the visit and any food or beverage, except water, 2 h prior to their study visit. No further dietary restrictions or controls were applied to the participants.

The participants were seated in a padded adjustable chair with their relative hip and knee angles at 90 degrees. A seated position was used because this may more effectively represent daily human activity than the supine position. A standard three-lead electrocardiogram (ECG) (Coulbourn Instruments, Allentown, PA), sampled at 1000 Hz (Micro 1401, Cambridge Electronics Design, Cambridge, UK), was used to obtain QRS wave timing. A piezoelectric chest harness (ADI Instruments, Sydney, Australia) was affixed inferior to the sternum to record the expansion and contraction of the chest cavity for determination of breathing rate.

After appropriate placement of the instruments was verified, the participant performed 20 min of seated rest. During the quiet rest period, the participant remained seated with limited movement and was instructed not to speak. After completion of the rest period, 5 min of ECG and spontaneous breathing data were collected. The participants were instructed to face forward and keep their hands in their lap for the duration of the collection. No ectopic heart beats were observed.

**Electrocardiogram and Breathing Data Reduction**

The expansion–contraction of the chest cavity data obtained from the piezoelectric transducer was visually assessed and counted by one investigator. The breathing rate (breaths per minute) was determined by dividing the number of breathing events by the total length of the observation (in minutes). This method was used to determine the breathing rate.

The primary data reduction of the ECG signal was performed in ECGlab (de Carvalho, da Rocha, de Oliveira Nascimento, Neto, & Junqueira, Jr., 2002). The ECG waveforms were manually assessed by one investigator to ensure correct identification of the QRS wave. The R-R interval tachogram was also visually inspected for stationarity and aberrant classification. From R-R interval tachogram, the heart rate (HR) and SDNN were determined. The following frequency-domain metrics were obtained using the fast Fourier transform: total spectral power (TP, \( \leq 0.4 \) Hz), LF (0.04–0.15 Hz), HF (0.15–0.4 Hz), and the ratio of LF to HF (LF/HF). The LF/HF ratio was assessed because it is commonly reported in the literature; however, the ratio is unlikely to represent sympathetic–parasympathetic balance since the LF band does not effectively convey information on sympathetic activity (Goldstein et al., 2011; Reyes del Paso et al., 2013).

**Assessment of Normality**

All data were first assessed to determine normality. Using a visual assessment of a bell-curve distribution, a Q-Q plot, and the Shapiro-Wilk test for normality, only HR and breathing rate were determined to be normally distributed. All other variables were determined to be normally distributed after performing a natural logarithmic transform (ln); therefore, all further statistical analysis and modeling was performed on the transformed data. A skewed HRV distribution has been reported previously in a large-scale study, and the natural log transform has been applied to normalize the distribution (Tsiu et al., 1996). To aid in the interpretation of our findings and comparison with previous studies, we have exponentiated the data in the presented figures back to their original units; however, all analysis and regression were performed on the transformed data.

**Statistical Analysis**

The primary interest in this study was the effect of menstrual cycle on HRV. To assess the changes in HRV across menstrual cycles of different lengths, the collection points were analyzed as the number of days from ovulation (BBT nadir), and total cycle length for each individual was included in the models as a covariate. All statistical analyses were performed in SAS 9.2 (Cary, NC).

**Statistical Analysis: Time Domain**

To assess HR, breathing rate, and SDNN, a multilevel regression model was constructed using the maximum-likelihood estimation technique. This model used a first-order autoregressive covariance structure to account for the repeated measures nature of the study design. Both the intercept and the slope of the variable of interest were allowed to vary (unstructured covariance structure) at the subject level. This modeling approach allows each participant to have their own regression line, which is then compiled to a model-wide regression (see Figures 1–3). The fit of this mixed effects model can be assessed via the concordance correlation coefficient (\( r_c \)) and interpreted similarly to the \( R^2 \) criterion (Vonesh, Chinchilli, & Pu, 1996). All models were fit with the linear time variable as well as a quadratic time variable; however, the addition of a quadratic term did not increase model fit nor were the terms statistically significant. Therefore, the quadratic time variable was not included in the final analyses.

**Statistical Analysis: Frequency Domain**

To assess TP, HF, LF, and LF/HF, a multilevel regression model was constructed using the maximum-likelihood estimation technique. Because breathing rates higher and lower than 10 breaths per minute are known to impact spectral parameters of HRV (Brown et al., 1993), an a priori piecewise function was used as a covariate within the model. This piecewise function statistically controls for any variability introduced by changes in breathing rate by assigning two different covariate slopes, one when the breathing rate is less than or equal to 10 breaths per minute and one when breathing rate is greater than 10 breaths per minute. This model used a first-order autoregressive covariance structure to account for the repeated measures nature of the study design. Because model convergence could not be attained when both the slope and intercept varied at the subject level, only the intercept was allowed to vary from the overall model. The goodness-of-fit for each model was assessed via \( r_c \). Similar to the time-domain analysis, all models were originally fit with the linear time variable and a quadratic time variable, but the quadratic term did not increase model fit nor were the terms statistically significant. Therefore, the quadratic time variable was not included in further analyses. A reduced model, without the piecewise function, was fit as well as the full model for all variables to examine the utility of the function when analyzing spectral variables of HRV.

**Results**

**Time Domain**

HR and breaths per minute increased (\( p = .01 \) and \( p < .01 \), respectively) as a function of days from ovulation. Ten days after ovulation, HR increased 2.9 beats per minute (Figure 1), and breaths per minute increased by 0.8 breaths per minute (Figure 2). Variability (lnSDNN) decreased (\( p = .02 \)) across the menstrual cycle. Ten days
after ovulation, lnSDNN decreased by 0.069 (exponentiated SDNN in Figure 3). The fit of all time-domain models as days from ovulation was good (see $r_c$ in Figures 1–3).

**Frequency Domain**

In the reduced model, without accounting for breathing rate, both lnTP ($p < .01$) and lnLF ($p = .01$) decreased across the menstrual cycle. No significant changes in lnHF ($p = .14$) and lnLF/HF ($p = .31$) were observed across the menstrual cycle. The fit of all reduced models was moderate to good (see $r_c$ in Figure 4).

In the full model, accounting for breathing rate with a piecewise function, lnTP ($p = .02$) and lnHF ($p = .03$) decreased across the menstrual cycle; however, no significant change was observed in lnLF or lnLF/HF ($p = .12$ and $p = .40$, respectively). The fit of all full models was good (see $r_c$ in Figure 5).

**Discussion**

The results of this study demonstrate that HRV is higher prior to ovulation and that this variability decreases until the onset of new menses. HRV spectral parameters indicate that this effect is likely due to withdrawal of parasympathetic control of the autonomic nervous system. The changes are independent of changes in breathing rates, which were observed to increase across the menstrual cycle (i.e., follicular $\rightarrow$ luteal). HR also increased across the menstrual cycle, a likely result of the decreased parasympathetic control.

**Evaluating the Piecewise Function for Spectral HRV Analysis**

This study introduced a novel method of accounting for the effect of breathing rate on HRV spectral assessment. The piecewise function allowed different slopes of change when breathing rates were
above and below 10 breaths per minute. Though HF is commonly termed the “respiratory frequency,” spontaneous breathing below a 10 breaths-per-minute average result in increased HRV signal power in the LF band (Beda et al., 2007; Cammann & Michel, 2002). Spontaneous breathing rates below 10 breaths per minute are not uncommon (Beda et al., 2007; Hoit & Lohmeier, 2000), and the rise in LF signal power appears to become exponential below 9 breaths per minute (Beda et al., 2007). The piecewise function introduced a negligible change in the fit and assessment of TP. However, in HF, LF, and LF/HF, the fit of the model was discernibly increased, and the resulting changes alter the interpretation of the data. Without use of the piecewise function, the spectral data results in the finding that no significant change in parasympathetic control (HF) is observed while the mixed parasympathetic/sympathetic/baroreflex frequency band (LF) decreases. An obviously erroneous interpretation of this result is that sympathetic activity decreases in later phases of the menstrual cycle without changes in parasympathetic activity. This interpretation is counterintuitive given the observed decrease in SDNN and increase in HR across the cycle.

The piecewise function for breathing rate maintains internal validity within the data. The results of the full ANCOVA model clearly indicate that the decrease in SDNN and increase in HR are largely mediated by decreases in parasympathetic control (HF) of the autonomic nervous system. The observed trend towards decreases in LF may be due to the nature of the frequency band encompassing parasympathetic, sympathetic, and/or baroreflex activity since preliminary evidence suggest that baroreflex sensitivity is modified across the menstrual cycle (Tanaka, Sato, Umehara, & Nishikawa, 2003), though this has been disputed (Cooke, Ludwig, Hogg, Eckberg, & Convertino, 2002). As with all new analytic techniques, further validation of the piecewise func-

Figure 2. Breathing rate changes across the menstrual cycle. Time point zero is BBT nadir (ovulation). A, B: Data for two individuals with their subject-specific regression lines. C: Individual lines for each subject (thin lines) and the model-wide regression line (thick line).
tion is needed to confirm that the HF and LF bands still represent their proposed autonomic analogues.

Decreases in Heart Rate Variability Across the Menstrual Cycle

Our study is supported by previous reports indicating that HR increases (Girija & Veeraiah, 2011; McKinley et al., 2009) and SDNN decreases when examining two data points in the follicular and luteal menstrual phases (McKinley et al., 2009). However, other studies have failed to find changes in HR (Sato et al., 1995; Tousignant-Laflamme & Marchand, 2009; Yildirir et al., 2002) across the menstrual cycle, and some show SDNN increases in later menstrual phases (Vallejo, Márquez, Borja-Aburto, Cárdenas, & Hermosillo, 2005). The discrepancy with the previous literature may arise, in part, from previous studies not maintaining consistent data collection times (Vallejo et al., 2005; Yildirir et al., 2002), utilizing controlled metronome breathing (Patwardhan et al., 1995; Sato et al., 1995), and having a less stringent criteria for study participation (Tousignant-Laflamme & Marchand, 2009). The present study, examining five time points in the menstrual cycle, offers a more comprehensive view of HR and SDNN changes.

The results of this study indicate that the increase in HR and decrease in SDNN are mediated by a withdrawal of parasympathetic control of the autonomic nervous system, evidenced by a decrease in HF. Interestingly, previous research has been unable to consistently correlate sex hormone levels with changes in LF or HF (Bai et al., 2009; Leicht et al., 2003). Controlling for breathing rate with a metronome or failing to control for breathing rate at all may contribute to the failure to find changes in HRV spectral parameters across the menstrual cycle, an issue we have successfully resolved with our piecewise function. Nevertheless, the ability to find HRV changes across the menstrual cycle (Bai et al., 2009; McKinley

Figure 3. SDNN changes across the menstrual cycle. Time point zero is BBT nadir (ovulation). A, B: Data for two individuals with their subject-specific regression lines. C: Individual lines for each subject (thin lines) and the model-wide regression line (thick line).
et al., 2009; Yildirir et al., 2002) but not in correlation with the sex hormones oscillating across the menstrual cycle is surprising. Two phenomena may contribute to this discrepancy: (1) the HRV changes observed across the menstrual cycle result from interactive effects of multiple sex hormones, and (2) the HRV effects observed may not occur in temporal continuity with observed sex hormone levels in the serum. The results of the present study should not be affected by either of these phenomena because the goal of this study was to characterize the changes across the cycle without regard for contribution of specific hormones.

Implications of Decreased Parasympathetic Activity Across the Menstrual Cycle

The parasympathetic withdrawal observed later in the menstrual cycle corroborates previous research demonstrating increases in

Figure 4. The reduced model for power spectrum changes occurring across the menstrual cycle. Time point zero is BBT nadir (ovulation). The left y axis is the frequency content for total power, high frequency, and low frequency spectrums. The right y axis is the ratio of low frequency-to-high frequency spectrums.

Figure 5. The full model for power spectrum changes occurring across the menstrual cycle, which controls for breathing rate with the piecewise function. Time point zero is BBT nadir (ovulation). The left y axis is the frequency content for total power, high frequency, and low frequency spectrums. The right y axis is the ratio of low frequency-to-high frequency spectrums.
basal body temperature (Buxton & Atkinson, 1948; Lundy et al., 1974; Zuspan & Rao, 1974), increases in basal metabolic rate (Solomon et al., 1982; Webb, 1986), and increases in ventilation during luteal phases of the menstrual cycle (Girija & Veeraiah, 2011; Slatkowska et al., 2006). Progesterone appears to be the main driver for increases in ventilation in the luteal phases, although there is a notable secondary effect of estrogen as well (Regensteiner et al., 1989). Indeed, progesterone has a higher uptake in the hypothalamus than in other portions of the brain in rats (Seiki et al., 1968). A simple mechanism could be proposed whereby increased progesterone in the luteal phase causes parasympathetic withdrawal resulting in increases in metabolic rate, ventilation, and body temperature; however, the main parasympathetic neurotransmitter, acetylcholine, has been shown to stimulate the secretion of estrogen and progesterone from human granulosa cells (Bodis, Koppan, Kornya, Tinneberg, & Török, 2002; Kornya, Bodis, Koppan, Tinneberg, & Török, 2001), an effect not seen by other catecholamines (Bodis et al., 2002). Obviously, the cause-and-effect interplay of the autonomic nervous system and the endocrine system is an intertwined ecosystem. As this interplay may substantially impact clinical research using HRV, further study is needed in this area.

Study Limitations

The results of this study cannot be generalized to postmenopausal or amenorrheic women as well as women taking hormonal birth control, lowering the population to which this particular study can be applied. However, the Centers for Disease Control reported that only 21.6% of reproductive-age women use hormonal birth control in the United States (Jones, Mosher, & Daniels, 2012), indicating a substantial population benefit from research on eumenorrheic women. The time-intensive nature of well-controlled studies across the menstrual cycle commonly result in a low sample size (Bai et al., 2009; Hirschoren et al., 2002; Leicht et al., 2003; Sato et al., 1995), and this study is no exception. Given the sample size, our inclusion of three participants exhibiting slight menstrual cycle deviations may also have a confounding effect. Further studies with larger samples and hormonal data are needed confirm the present findings. Our preliminary use of the piecewise function is promising, though further research should perform a complete validation of the method and seek to fully understand the impact on interpreting HRV results.

Conclusions

This study examined the changes in HRV across the menstrual cycle at five observational time points. In addition to collecting data at more points in the menstrual cycle than previous research, this study introduced and demonstrated the utility of a novel piecewise function to account for the effect of breathing rate on HRV spectral analysis. This study demonstrates that heart rate variability decreases across the menstrual cycle and that this decrease is mediated by parasympathetic withdrawal. Future research should continue to validate and refine the use of the piecewise function to remove the ventilatory effects from HRV analyses. Future studies examining changes in HRV across the menstrual cycle should utilize a larger study sample and assess various hormones and sex hormone-binding globulin using a data series time lag commonly found in econometrics research. The time-lagged data series analysis may reveal that the changes in HRV across the cycle do result from a predictable temporal lag with hormone oscillations.

References


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sympathetic tone but may be a measure of modulation of cardiac autonomic outflows by baroreflexes. Experimental Physiology, 96, 1255–1261.


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