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Heart rate variability and endogenous sex hormones during the menstrual cycle in young women

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To our knowledge, the relationship between all four endogenous female sex hormones and resting cardiac autonomic function has not been studied. The aim of the current study was to examine the association between the normal endogenous levels of oestrogen (17β-oestradiol), progesterone, luteinising hormone and follicle-stimulating hormone and heart rate variability (HRV) during the menstrual cycle in young eumenorrheic women. Ten healthy, young, female subjects volunteered for this study. HRV and endogenous hormone levels were recorded at three phases of the menstrual cycle: menses (day 3.8 ± 0.5), ovulation (day 15.8 ± 0.7) and luteal (day 22.1 ± 0.4) to ensure HRV recordings at times of low (menses) and high (ovulation and luteal) hormonal influence. Heart rate recordings were obtained from supine resting subjects and analysed on a Holter analysis system. Total power (TP, 0–1.0 Hz), low frequency (LF, 0.041–0.15 Hz), high frequency (HF, 0.15–0.80 Hz) and LF/HF components of HRV were examined. Despite a significantly greater HR at ovulation and normal cyclic variations in all endogenous sex hormone levels, no measure of HRV was significantly different between menstrual cycle phases. Significant correlations between oestrogen levels and absolute measures of HRV at ovulation were identified. The results of the current study demonstrated that the normal cyclic variations in endogenous sex hormone levels during the menstrual cycle were not significantly associated with changes in cardiac autonomic control as measured by HRV. Significant correlation between peak oestrogen levels and HRV measures at ovulation provided further support for the reported cardioprotective effects of oestrogen in healthy females. *Experimental Physiology* (2003) **88.3**, 441–446.

Our knowledge of the autonomic nervous system has increased with an understanding of heart rate variability (HRV), a non-invasive measure of cardiac autonomic control (Pagani *et al.* 1986; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Two main frequency components of HRV have been demonstrated, low frequency (LF, 0.04–0.15 Hz) reflecting the interaction of both the sympathetic and parasympathetic (vagal) nervous systems, and high frequency (HF, > 0.15 Hz) reflecting solely the activity of the parasympathetic nervous system (Pagani *et al.* 1986; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). The most prominent aspect of this new measure (HRV) has been its use as a predictor of mortality in patients with cardiovascular disease (Kleiger *et al.* 1987) and in the general population (Tsuji *et al.* 1996). Tsuji and colleagues (1996), who were part of the Framingham Heart Study, reported that low HRV measures were significantly associated with the risk of cardiac events in healthy adults examined regularly over a 36 year period.

The extent to which HRV is influenced by the sex of the subject has been contentious, with studies reporting significantly greater (Gregoire *et al.* 1996; Huikuri *et al.* 1996), significantly lower (Bigger *et al.* 1995; Sinnreich *et al.* 1998) or similar (Stein *et al.* 1997; Yeragani *et al.* 1997) HRV for females compared to males. To our knowledge, studies examining HRV differences between the sexes have not considered the menstrual cycle phase at which HRV recordings were obtained for females. Discrepancies in studies reporting male/female differences in HRV may be a result of the time of the menstrual cycle for females at which the HRV comparison was made with males. Sato *et al.* (1995) reported greater sympathetic activity (LF/HF) during mental challenge in the luteal phase compared with the follicular phase. Others have reported autonomic changes during the normal menstrual cycle (Saeki *et al.* 1997; Ettinger *et al.* 1998). Saeki *et al.* (1997) reported

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significantly greater parasympathetic activity during the follicular phase compared to other phases of the ovarian cycle in healthy women and concluded that parasympathetic activity appeared to be influenced by oestrogen while sympathetic activity was modulated by progesterone. The relationship between endogenous female sex hormones, HRV and the autonomic nervous system still remains to be clarified. Therefore, the aim of the current study was to elucidate further the association between HRV and endogenous female sex hormone fluctuations during the menstrual cycle and its possible contribution to male/female differences in HRV.

As previously stated, cardiac autonomic fluctuations have been reported at various times during the menstrual cycle. However, there have been few studies to examine any possible association between HRV and the endogenous levels of pituitary gonadotrophins, luteinising hormone (LH) and follicle-stimulating hormone (FSH), or their interaction with oestrogen and progesterone during the menstrual cycle. Therefore, in the current study we examined the relationship between HRV and the four endogenous sex hormones at times of low and high hormonal influence. It was theorised that HRV fluctuations during the menstrual cycle may result from endogenous female sex hormone interactions. Further, it was hypothesised that if a relationship between oestrogen levels and parasympathetic activity existed, then HRV would exhibit cyclic variations in synchrony with oestrogen levels. Such a relationship would further clarify the mechanism for the reported cardioprotective effects of oestrogen (Mercuro *et al.* 2000; Saleh *et al.* 2000).

METHODS

Subjects

Ten healthy female subjects with a mean (± S.E.M.) age of 24.7 ± 2.4 years and body mass of 63.8 ± 3.9 kg volunteered for this study. Subjects had not exercised regularly for at least 3 months prior to commencement of the study and all completed questionnaires to confirm their healthy status. Subjects were not taking any medications including oestrogen/progesterone contraceptives and all were confirmed as non-smokers. All subjects had regular menstrual cycles prior to HRV determination as indicated by 3 months of daily supine basal temperature recordings using an oral digital thermometer (Becton Dickinson, Ontario, Canada). Each subject was familiarised with the testing equipment and procedures used in the laboratory and then provided written informed consent prior to participation. The study was approved by the University of Southern Queensland Human Ethics Committee.

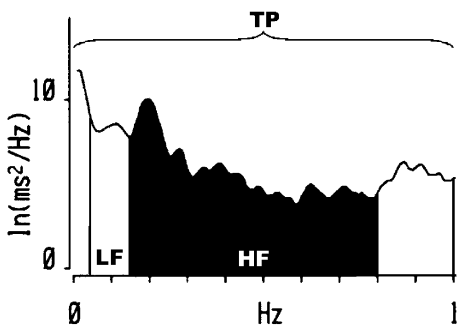

Figure 1

An example of the three heart rate variability components examined during each phase of the menstrual cycle. LF, low frequency (0.041–0.15 Hz); HF, high frequency (0.15–0.80 Hz); TP, total power (0–1.0 Hz).

Protocol

Subjects reported to the laboratory at each of the following phases of the menstrual cycle: menses (day 3.8 ± 0.5; range, 1–5); ovulation (day 15.8 ± 0.7; range, 11–21) and luteal (day 22.1 ± 0.4; range, 21–24). These phases were based upon the cyclic variations in gonadotrophic and ovarian hormone levels during the menstrual cycle (Guyton & Hall, 2000) and were utilised to ensure that HRV recordings were obtained at times of low (menses) and high (ovulation and luteal) hormonal influence. Recordings of the onset of menstrual bleeding and daily supine basal temperature enabled each subject to identify each menstrual cycle phase for HRV determination. For each menstrual cycle phase, subjects arrived at the laboratory (room temperature 20–24 °C) at the same time of day between 06.00 and 12.00 h, 12 h post-prandial (Widerlov *et al.* 1999) and at least 24–36 h post-exercise (Furlan *et al.* 1993). Body mass was recorded and skin was prepared for the application of heart rate monitoring electrodes (3M Australia Pty Ltd, Brisbane). A Marquette Holter monitor 8500 (Marquette Electronics Inc., Milwaukee, WI, USA) was connected to the electrodes using modified V_1 and V_5 leads to record HR. Each subject rested in the supine position for 40 min, and resting HRV was determined during the final 20 min. An initial 20 min period was utilised to ensure the attainment and stabilisation of resting HR. Throughout the resting period subjects lay awake on a bench in a quiet environment with minimal noise and body movement. Breathing rate and depth were spontaneous for all subjects and not controlled as prior studies have demonstrated that controlled (metronome) breathing was a stressor and altered HR (Patwardhan *et al.* 1995). Recently, others have demonstrated minimal changes in HRV and vagal modulations during spontaneous and controlled breathing over the typical resting breathing rates (Bloomfield *et al.* 2001; Patwardhan *et al.* 2001). At the completion of the heart rate recording and with the subject free of all testing equipment, 10 ml of blood was removed by antecubital venipuncture. Blood was allowed to clot normally and then centrifuged with the serum removed and stored at −80 °C for later analysis. Serum LH, FSH, oestrogen (17β-oestradiol) and progesterone levels were determined by an independent laboratory using a Ciba Corning Automated Chemiluminescence System 180 or microparticle enzyme immunoassay.

Data and statistical analysis

Heart rate (HR) recordings were analysed for HRV on a commercially available Holter analysis system (Marquette series 8000). Frequency domain measurements of HRV were determined by spectral analysis using fast Fourier transformation (FFT) as previously described (Leicht *et al.* 2003). Briefly, R–R intervals over a 2 min period were sampled every 469 ms to produce a 256-point Radix 2 FFT without overlap. A Hanning window was

applied to each power spectrum to minimise spectral leakage and the following three frequency components examined for each power spectrum (Fig. 1): (1) low frequency (LF, 0.041–0.15 Hz) which reflects sympathetic and parasympathetic modulation; (2) high frequency (HF, 0.15–0.80 Hz), which reflects parasympathetic modulation; and (3) total power (TP, 0–1.0 Hz), which reflects primarily parasympathetic modulation (Pagani *et al.* 1986; Task Force of the European Society of Cardiology and the North American Society of Pacing and Electrophysiology, 1996). Each component was expressed in absolute units ($ms^2\,Hz^{-1}$) and where possible in normalised units (nu). Normalised units were calculated by dividing the absolute power of a given component by the TP minus frequencies below 0.041 Hz (Pagani *et al.* 1986). The LH/HF ratio was also determined for each spectrum and, along with the measures of HRV in nu, was used as an index of sympathovagal balance (Pagani *et al.* 1986).

All data are presented as means ± S.E.M. Statistical comparisons were made using the statistical package SPSS (SPSS Inc. Chicago, IL, USA). Significant differences for HRV, HR and hormone levels during the menstrual cycle were determined by one-way repeated measures ANOVA and Fisher's least significant difference test (Howell, 1992). Data not fitting the assumptions of these parametric parameters were analysed using Friedman's χ^2 test and Nemenyi's procedure (Hatch & Lazaraton, 1991). Relationships between variables were determined by Spearman's rank-order correlation (ρ). Only correlation coefficients > ±0.5 were considered. $P < 0.05$ was taken as statistically significant.

RESULTS

Cyclic variations in all endogenous female sex hormones were evident during the menstrual cycle and included significantly greater oestrogen (17β-oestradiol) and LH levels at ovulation, and significantly greater oestrogen (17β-oestradiol) and progesterone levels at the luteal phase (Fig. 2).

Despite these normal cyclic hormonal variations and a significantly greater resting HR at ovulation (menses, 62.6 ± 2.5 beats min^{-1}; ovulation, 66.3 ± 2.3 beats min^{-1}; luteal, 62.8 ± 1.8 beats min^{-1}; $P < 0.05$), no measure of HRV was significantly different between menstrual cycle phases (Fig. 3).

Correlations between oestrogen (17β-oestradiol) levels and absolute measures of HRV (LF, HF and TP) at ovulation were observed (Fig. 4). No other significant correlations were identified.

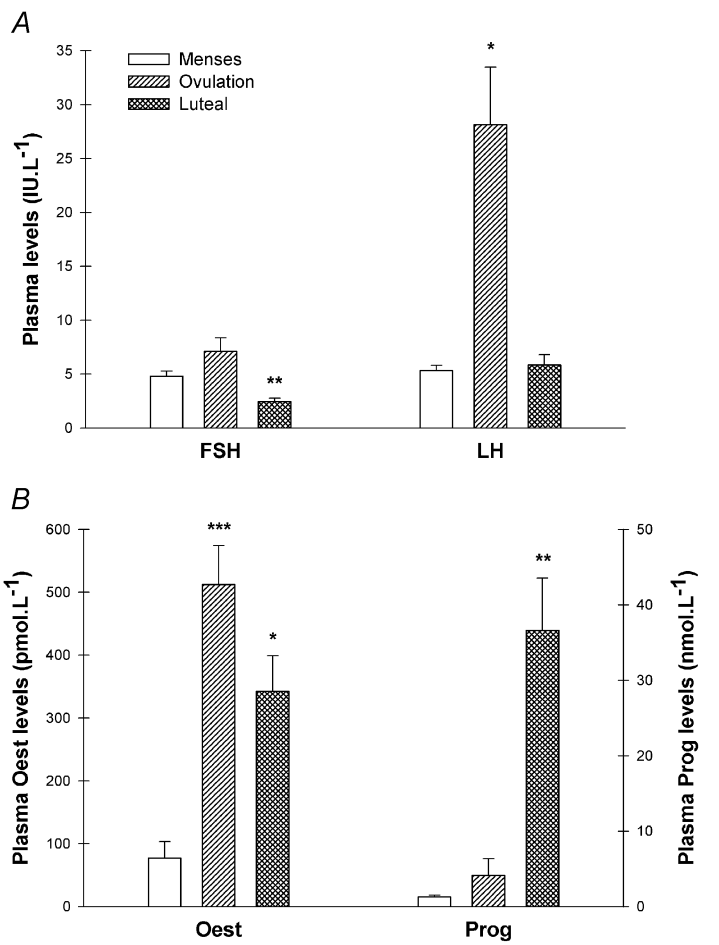

Figure 2

Plasma concentrations of follicle-stimulating hormone (FSH) and luteinising hormone (LH) (*A*), and 17β-oestradiol (Oest) and progesterone (Prog) (*B*) during the three main phases of the menstrual cycle. Values shown are mean + S.E.M. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$, significantly different to menses.

DISCUSSION

Significantly greater HRV and vagal activity in the follicular phase (Sato *et al.* 1995; Saeki *et al.* 1997) and greater sympathetic activity during the luteal phase (Guasti *et al.* 1999; Yildirir *et al.* 2002) compared with other phases of the menstrual cycle have been reported. The enhanced vagal activity at ovulation has been attributed to the increased endogenous oestrogen levels, while the reduced HRV and greater sympathetic activity during the luteal phase has been attributed to the greater endogenous progesterone levels (Sato *et al.* 1995; Saeki *et al.* 1997; Yildirir *et al.* 2002). However, such fluctuations in HRV during the menstrual cycle could reflect the interaction of several endogenous sex hormones since FSH and LH peak around ovulation and oestrogen peaks again around day 21 (luteal) of the menstrual cycle. In the current study we have demonstrated similar HRV regardless of menstrual cycle phase and endogenous female sex hormone levels, in agreement with previous studies (Kondo *et al.* 1989; Yildirir *et al.* 2002). Further, no significant correlations between HRV and FSH or LH were evident in the current study, suggesting that FSH and LH were not associated with modification of cardiac autonomic control as measured by HRV. In the current study we examined HRV only at times of low and high sex hormone levels during the menstrual cycle as it was anticipated that during these times the possible interaction between the levels of endogenous female sex hormones and HRV would be more apparent. However, the lack of HRV fluctuations during the menstrual cycle in the current study may be associated with the timing of HRV recordings as previous studies have reported increased vagal activity (lower LF nu and LF/HF ratio, greater HF nu) only during the follicular phase (days 3–11) compared with other phases of the menstrual cycle (Sato *et al.* 1995; Saeki *et al.* 1997). Collectively, the results of the current and previous studies indicate that vagal activity may be greater during the early follicular phase of the menstrual cycle and subsequently lower at other phases of the menstrual cycle, possibly due to the influences of increasing levels of FSH and LH (ovulation) and progesterone (ovulation and luteal phases).

In the current study, significant correlations between oestrogen levels and all absolute measures of HRV at ovulation were demonstrated reflecting a positive relationship between oestrogen and vagal activity as only the parasympathetic nervous system regulates HR control at all frequencies (Akselrod *et al.* 1985). This significant relationship between oestrogen levels and HRV in the

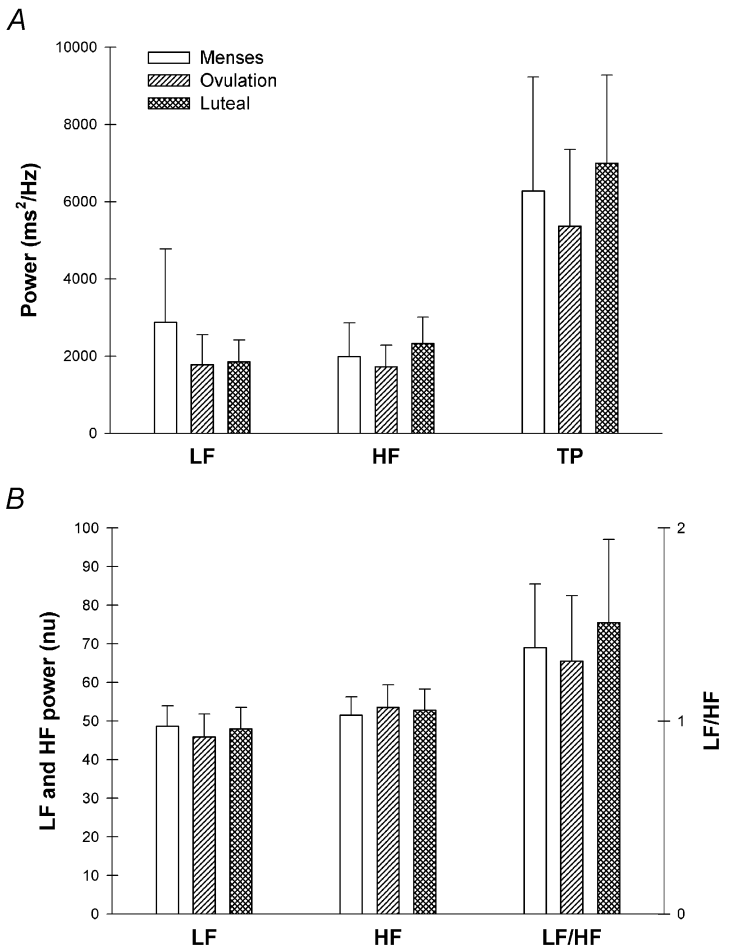

Figure 3

Absolute (*A*) and normalised (*B*) components of HRV during the three main phases of the menstrual cycle. LF, low frequency; HF, high frequency; TP, total power. Values shown are mean + S.E.M.

current study, and reports of oestrogen-induced enhancement of vagal activity (Saleh & Connell, 2000; Saleh *et al.* 2001) and reduction of sympathetic activity (Ettinger *et al.* 1998; Mercuro *et al.* 2000) suggest that vagal dominance during the early follicular phase of the menstrual cycle may result from increasing levels of endogenous oestrogen. Subsequent increases in endogenous levels of FSH, LH and progesterone may then inhibit the influence of oestrogen on cardiac autonomic control as demonstrated by the similar HRV during the ovulation and luteal phases in the current and previous studies (Kondo *et al.* 1989; Saeki *et al.* 1997). Previous reports of greater sympathetic activity during peak progesterone levels of the luteal phase (Sato *et al.* 1995; Guasti *et al.* 1999; Yildirir *et al.* 2002) and increased HR and lower HRV in post-menopausal women following combined oestrogen/progesterone hormone replacement therapy (HRT) (Christ *et al.* 2002) provide further support of the proposed vagal inhibitory nature of progesterone. The possible existence and extent of the inhibitory influences of FSH and LH on cardiac autonomic control is presently unknown. Further studies are needed to explore the possible vagal inhibitory actions of FSH and LH and whether there is a period during the menstrual cycle of reduced cardioprotection and increased risk of cardiac arrhythmias/events (Kleiger *et al.* 1987; Tsuji *et al.* 1996).

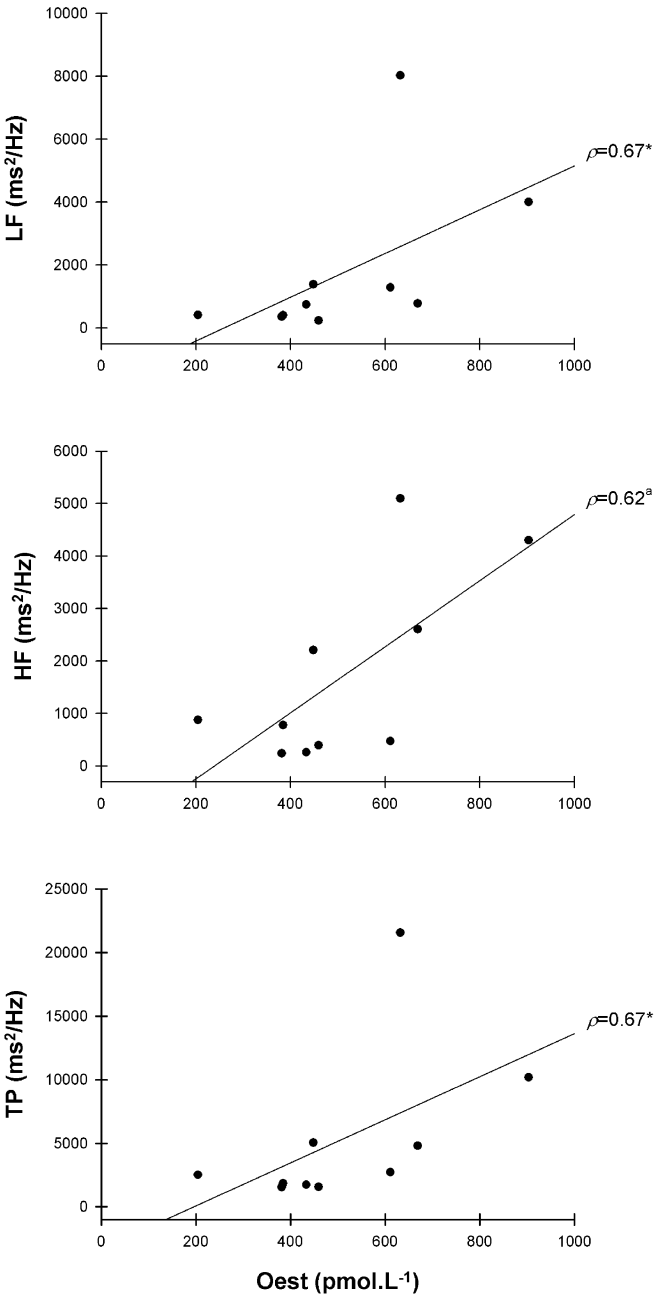

Figure 4

Correlation between absolute measures of HRV and 17β-oestradiol (Oest) at ovulation. LF, low frequency; HF, high frequency; TP, total power. * $P < 0.05$, [a] $P < 0.054$.

To date, the reported differences in HRV between the sexes have been equivocal (Bigger *et al.* 1995; Gregoire *et al.* 1996; Huikuri *et al.* 1996; Yeragani *et al.* 1997; Sinnreich *et al.* 1998), with several studies reporting greater parasympathetic activity for females compared to males (Gregoire *et al.* 1996; Huikuri *et al.* 1996). Reported male/female differences in HRV could be the result of comparing HRV in females at a time of the menstrual cycle when HRV is enhanced due to ovarian hormonal influences, that is, during the early follicular phase (Sato *et al.* 1995; Saeki *et al.* 1997). To our knowledge, studies examining male/female differences in HRV have not considered the menstrual cycle phase at which HRV recordings were obtained for females. Therefore, it is suggested that HRV measurements in females, for comparisons between females and between the sexes, be conducted at a similar phase of the menstrual cycle to account for the effects of endogenous female sex hormones. For practical reasons it is suggested that HRV recordings be obtained at times other than the early follicular phase (days 5–12) of the menstrual cycle when vagal activity is enhanced compared to other phases of the cycle (Sato *et al.* 1995; Saeki *et al.* 1997).

In the present study we have demonstrated no synchronistic changes in HRV with endogenous female sex hormone levels at the three main phases of the menstrual cycle. Possible inhibitory influences of FSH, LH and progesterone on oestrogen could account for the lack of oestrogen-induced increased HRV and vagal activity during the menstrual cycle in the current study. Correlations between oestrogen and HRV at ovulation in the current study provided further support of the reported cardioprotective effects of oestrogen. Possible male/female differences in HRV may result from recording HRV at a time of minimal female endogenous sex hormone interaction (i.e. early follicular phase only).

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