Abstract  The purpose of the present study was to determine the fluctuation in cardiovascular reactivity to mental stress during the menstrual cycle by comparing heart rate variability (HRV), and other physiological and psychological data in females with those in males. Cardiovascular reactivity to two mental tasks was measured in 14 females during the follicular and luteal phase of menstruation over two menstrual cycles. The same tasks were subsequently given to a matched pair of males (N=14), at the same intervals as their corresponding females. Heart rate, blood pressure and HRV were used as indices of cardiovascular reactivity. Subjective mental workload was measured at the end of each task. Power spectral analysis of HRV showed that the high frequency (HF) component in HRV decreased more during the luteal phase than the follicular phase. The low frequency (LF) component in HRV and the LF/HF ratio in the luteal phase were significantly higher than that in the follicular phase. The LF component and the LF/HF ratio were significantly lower in females than in males; conversely, the HF component was significantly higher in females than in males. Neither significant effects of menstrual cycle, gender and mental stress nor any significant interactions were found for mental workload. These findings indicate that sympathetic nervous activity in the luteal phase is significantly greater than in the follicular phase whereas parasympathetic nervous activity is predominant in the follicular phase. The results also suggest that predominance of sympathetic nervous activity in males compared with a dominant parasympathetic nervous activity in females. J Physiol Anthropol Appl Human Sci 23(6): 215–223, 2004 http://www.jstage.jst.go.jp/browse/jpa

Keywords:  mental stress, menstrual cycle, gender differences, heart rate variability

Introduction  Excessive cardiovascular reactivity to mental stress has been hypothesized to be a risk factor for coronary heart disease (CHD) (Krantz and Manuck, 1984). Research on CHD has often excluded females or included them in relatively small numbers (Beery, 1995; Lundberg, 1998), since females have several advantages over males with respect to CHD development. For example, the incidence of CHD is more frequent in males than in premenopausal females (Litschauer et al., 1998), and the progression of CHD is delayed roughly 10 years in females relative to males (Stoney, 1998). However, with the rapid increase in females in the workforce, females are being exposed to mental stress to the same level as males, and CHD is the leading cause of death for females in industrialized countries (Czajkowski, 1998). Consequently, investigators have begun examining cardiovascular reactivity to mental stress in females.

Another reason of the preference of males as participants in CHD studies is the variability in females’ physiology caused by reproductive hormones (Kaplan et al., 1990). It was long before researchers began to set about examining the effects of reproductive hormones on cardiovascular reactivity. Of the approaches available, examination of the fluctuation in cardiovascular reactivity during the menstrual cycle has been methodologically easy and relatively noninvasive (Mills et al., 1996). While several investigators have reported greater reactivity to mental stress in heart rate and/or blood pressure during the luteal phase than during the follicular phase (e.g., Greenberg et al., 1985; Manhem et al., 1991), others have found no significant phase differences in those parameters (e.g., Weidner and Helmig, 1990; Beek et al., 1996). These discrepancies across findings may partly be due to the experimental designs employed. Findings from early studies using a between-subjects design have tended to be more inconsistent than those from a within-subject design (Mills et al., 1996).

Differences in the sensitivity of measurement of the various cardiovascular parameters may also be responsible for inconsistent findings. Stoney (1992) suggested that at least as far as heart rate and blood pressure are concerned, it is likely that the relatively small fluctuations in reproductive hormones during the normal menstrual cycle are not large enough to
exert any significant influence on stress responses. Instead of traditional cardiovascular measurements such as heart rate and blood pressure, some researchers have used power spectral analysis of heart rate variability (HRV) to examine the fluctuations in autonomic nervous activity during the menstrual cycle (Saeki et al., 1997; Guasti et al., 1999). In humans, power spectral analysis of R–R interval variability has revealed that there are two major spectral analysis components: the high-frequency (HF) component at the respiratory frequency and the low-frequency (LF) component at 0.03–0.15 Hz. The HF component corresponds to the respiratory sinus arrhythmia and is modulated solely by the parasympathetic nervous system (Pagani et al., 1986; Eckberg, 1983; Akselrod et al., 1985), whereas the LF component corresponds to blood pressure oscillations occurring around 0.1 Hz, (i.e., the Mayer waves), and is jointly modulated by the sympathetic and parasympathetic nervous systems (Baselli et al., 1986; Baselli et al., 1988). In addition, the LF/HF ratio is also a useful parameter that reflects the balance of autonomic nervous activities (Pagani et al., 1986; Lombardi et al., 1987; Rimoldi et al., 1990).

Studies have revealed that HF component of HRV are significantly higher in the follicular phase than in the menstrual phase in a supine or sitting position (Saeki et al., 1997) and the luteal phase of the menstrual cycle are associated with a greater increase in the LF component and a greater decrease in the HF component, resulting in a higher LF/HF ratio (Sato et al., 1995). These results suggest that sympathetic nervous activity is predominant in the luteal phase during the menstrual cycle. However, little attention has been paid to HRV to mental stress during the menstrual cycle. Thus, the purpose of the present study was to determine the fluctuation in cardiovascular reactivity to the mental stress during the menstrual cycle by comparing HRV, and other physiological and psychological data in females with those in males.

Methods

Participants

Twenty-two undergraduate females who responded to an advertisement on a bulletin board at a university underwent an initial screening interview. The participants were requested to confirm that they had had regular menstrual cycle lengths between 26 and 36 days for at least 6 months prior to participation, had not taken hormonal contraceptives, were not pregnant, were not smokers and had no history of cardiovascular disease were recruited after collection of all data from all females, and sessions were scheduled to match the intervals between laboratory sessions of the females.

The above procedures resulted in 14 female participants (mean=20.2 years, SD=1.2) with a normal menstrual cycle (mean=29.9 days, SD=1.8) and 14 male participants (mean=22.6 years; SD=1.5). Before enrollment in the study, written informed consent was obtained from all participants.

Experimental tasks

The experimental tasks consisted of a modified mirror tracing task and a mental arithmetic task that is frequently used to examine cardiovascular reactivity in the laboratory setting. In the modified mirror tracing task, a complex pathway was presented to participants on a computer screen for 5 minutes. Participants traced the pathway with a mouse as accurately and as rapidly as they could. The horizontal and/or vertical axis controls of the mouse were reversed. The mental arithmetic task consisted of serial subtraction of 17 from a random 4-digit number presented on a computer screen for 5 minutes. Participants were required to enter the answer by clicking the 10 numeric (from 0 to 9) buttons displayed on the screen with a mouse. Instructions emphasized the need for accuracy and speed.

Physiological measures

Electrocardiogram (ECG, CM5 lead) and respiration signals were monitored with a polygraph system (Synafit 2200 NEC Sanei) and continuously recorded on a digital data recorder (RD-135T TEAC). Off-line analysis was performed using a personal computer. The ECG data were digitized at a sampling frequency of 1 kHz by an analog/digital converter (Canopus ADXM-98L), interpolated by the cubic-spline method and re-sampled at 2 Hz for spectral analysis. To move trends, these equidistant R–R interval data were passed through a high-pass digital filter with a 0.05 Hz cut-off frequency. Next, the autoregressive model (AR) power spectrum from trendgram was calculated by the fixed tenth-order AR model. The order was determined empirically. The power spectral density was divided into the sum of spectral components by means of a spectral decomposition method, and the center frequency and the power of every spectral component were obtained. Only components larger than 5% of the total power were considered significant. In this study, a spectral component with a center frequency of between 0.05 and 0.15 Hz was considered as the LF component, and a spectral component with the same cycle as the respiratory one and a center frequency of around 0.25 Hz was considered as the HF component. Each spectral component was normalized by dividing it by the total power less the first-order component, if present (Pagani et al., 1986). LF/HF was also calculated to assess the sympathovagal balance. Heart rate (HR) was derived from reciprocal of R–R interval by 60,000. Respiration activity was transduced by a strain-gauge placed around the participant’s abdomen halfway
between the rib cage and navel. Systolic blood pressure (SBP) and diastolic blood pressure (DBP) were measured using an automatic blood pressure monitoring system (Omron HEM-739) via a cuff placed on the left upper arm.

Questionnaires
Subjective mental workload was evaluated using the Japanese version of National Aeronautics and Space Administration Task Load Index (NASA-TLX) ratings (Miyake and Kumashiro, 1993). The NASA-TLX consists of six component scales: mental demand, physical demand, temporal demand, own performance, effort and frustration level. After completion of each task, participants compared the six components of the NASA-TLX in all possible pair-wise combinations to identify which of the pair contributes more to the subjective workload. Next, participants rated their subjective workload for each component scale on a 12 cm visual-analog scale.

The Menstrual Distress Questionnaire (MDQ) (Moos, 1968) was translated into Japanese by the authors and used to assess the self-reports of mood and physical symptoms associated with the menstrual cycle. The MDQ consists of 47 items providing 8 subcategories of symptoms: pain, concentration, behavioral change, autonomic reactions, water retention, negative effect, arousal and control. Females completed the MDQ using a 6-point scale (1=not at all, 6=extremely) in both the follicular and luteal phases.

Experimental procedure
On the basis of BBT data and self-reported menstrual cycle duration, the experimental days for females were determined; the time schedule for the luteal phase was from days 3 to 7 before the first day of bleeding, and for the follicular phase was from days 7 to 11 after the first day of bleeding. Females participated in the experiments across two menstrual cycles. Males were tested four times, matched for the time interval between laboratory sessions for the females. All participants were requested to refrain from eating and drinking for at least 2 hours prior to the experiment and to avoid heavy exercise and intake of alcoholic beverages after midnight. On arriving at the laboratory, participants were attached to electrodes and instructed to seat quietly for 10 minutes. The last 5-minutes of this period was defined as the baseline block (PRE). After PRE, blood pressure was measured. Following a 5-minute modified mirror tracing task period (MT), blood pressure was measured again. After 15 minutes inter-task resting, blood pressure was measured, and participants then performed the mental arithmetic task for 5 minutes (MA). Blood pressure was then measured. Finally, a 5-minute ECG reading was conducted for collection of recovery data (POST). Participants controlled their respiratory frequency during the last 5 minutes of the resting period before the modified mirror tracing task, both tasks, and recovery period after the mental arithmetic task because the HRV component is affected by breathing pattern (Hirsh and Bishop, 1981; Grossman et al., 1991). They paced their breathing with an auditory tone that signaled inhalation and exhalation. The pace of respiration was determined individually by counting the first 5-minute resting period before the modified mirror tracing task.

Statistical analysis
The averaged data during the follicular and luteal phases were used to examine the menstrual cycle effects on each measurement. Menstrual cycle and block effects on heart rate and HRV measurements in females were assessed with a 2(phase)×(block) repeated measures of analysis of variance (ANOVA). Changes in SBP and DBP in females were assessed with a 2(phase)×2(task)×2(pre-post task) repeated measures of ANOVAs. As for NASA-TLX score in females, a 2(phase)×2(task) repeated measures of ANOVAs was performed. A paired t-test was conducted for MDQ scores.

To examine the gender differences on each measurement, the values were averaged over four sessions in males and females. Gender differences in heart rate and HRV measurements were assessed with a 2(gender)×4(block) repeated measures of ANOVAs. As for SBP and DBP, a 2(gender)×2(task)×2(pre-post task) repeated measures of ANOVAs were conducted. NASA-TLX score in both groups was assessed with a 2(gender)×2(task) repeated measures of ANOVAs. Where ANOVAs revealed significant effects, post hoc comparisons were made using Tukey’s Honestly Significant Difference (HSD) procedure. For within-subject analysis, the Greenhouse-Geisser correction was used where appropriate. Data are expressed as mean±S.E.

Results
Menstrual cycle effects
Figure 1 presents the changes in heart rate for the follicular and luteal phases in females. ANOVA revealed a significant main effect for block \[F(3, 39)=13.74, p<0.01, \varepsilon=0.71\]. Post hoc comparisons indicated that heart rate during the mental arithmetic task (MA) was significantly greater than those during the baseline (PRE) and recovery (POST) periods and modified mirror tracing task (MT) \((p<0.01)\). Neither significant phase effects nor phase-by-block interactions were found for heart rate in females.

Figure 2 shows the changes in LF component for the follicular and luteal phases in females. ANOVA for LF component revealed significant main effects for phase \[F(1, 13)=6.49, p<0.05\] and block \[F(3, 39)=15.14, p<0.01, \varepsilon=0.84\]. The LF component was significantly higher during the luteal phases than during the follicular phases. Post hoc comparisons showed that the LF component was significantly higher during the two tasks (MT, MA) than during the baseline (PRE) and recovery (POST) periods \((p<0.01)\). There was no significant phase-by-block interaction.

The changes in HF component for the follicular and luteal phases in females are shown in Fig. 3. Significant main effects were obtained for phase \[F(1, 13)=14.37, p<0.01\] and block...
The values in the follicular phase were significant higher than those in the luteal phase. Post hoc comparison on the HF values indicated that the HF component during the two tasks (MT, MA) was lower than during the baseline (PRE) (\(p<0.01\)) and recovery (POST) (MT-POST: \(p<0.01\); MA-POST: \(p<0.05\)). There was no significant phase-by-block interaction.

To correct the skewness in distribution of variance, a natural logarithmic transformation was adopted for LF/HF ratio. ANOVA yielded significant main effects for phase \([F(1, 13)=15.25, p<0.01, \eta^2=0.75]\). The value of the LF/HF ratio during the luteal phase was significantly higher than during the follicular phase. Post hoc comparisons indicated that the LF/HF ratio was higher during the two tasks (MT, MA) than during the baseline (PRE) and recovery (POST) periods (\(p<0.01\)). No significant interaction was found for LF/HF ratio.

The changes in SBP and DBP before and after the tasks in females are shown in Figs. 5 and 6. ANOVA revealed that there were significant differences in SBP and DBP between the pre and post periods, indicating SBP and DBP after task were higher than those before task [SBP: \(F(1, 13)=31.50, p<0.01\); DBP: \(F(1, 13)=50.34, p<0.01\)]. Neither significant phase effects nor interactions were found for either SBP or DBP.

Figure 7 presents the subjective mental workload for each task in females. No significant main effects of phase, or task were observed, and there was no phase-by-task interaction.

Table 1 gives the MDQ scores for females. No significant differences in MDQ scores were observed between the luteal
and follicular phases.

**Gender effects**

Figure 8 demonstrates the changes in heart rate in males and females. ANOVA revealed a significant main effect for block \([F(3,78) = 28.21, p < 0.01, \varepsilon = 0.78]\). Post hoc comparisons indicated that heart rate during the mental arithmetic task (MA) was significantly greater than those during the baseline (PRE) and recovery (POST) periods, as well as the modified mirror tracing task (MT) \((p < 0.01)\). Neither significant gender effects nor gender by block interactions were found for heart rate.

The changes in LF component in males and females are displayed in Fig. 9. ANOVA for LF component revealed significant main effects for gender \([F(1, 26) = 22.59, p < 0.01]\) and block \([F(3,78) = 24.30, p < 0.01, \varepsilon = 0.86]\). The LF component was significantly higher in males than in females. Post hoc comparisons showed that the LF component was significantly higher during the two tasks (MT, MA) than during
the baseline (PRE) and recovery (POST) periods \((p<0.01)\), and were greater during the recovery period (POST) than during the baseline period \((p<0.05)\). There was significant gender-by-block interaction \([F(3,78)=2.89, p<0.05]\). Females had greater changes in the LF component than males during tasks.

Figure 10 presents the changes in HF component in both groups. Significant main effects were obtained for gender \([F(1, 26)=21.38, p<0.01]\) and block \([F(3,78)=19.99, p<0.01, \varepsilon=0.82]\). The HF component in females was significantly higher than in males. The HF component during the two tasks (MT, MA) was lower than during the baseline (PRE) and recovery (POST) periods \((p<0.01)\). Significant gender-by-block interaction was found in the HF component \([F(3,78)=3.37, p<0.05]\). Females were more reactive with the HF components during tasks than males.

The changes in LF/HF ratio for both groups are shown in Fig. 11. There were significant main effects for gender \([F(1, 26)=23.04, p<0.01]\) and block \([F(3,78)=26.80, p<0.01, \varepsilon=0.82]\). Males showed a significantly higher LF/HF ratio than females. Post hoc comparisons indicated that the LF/HF ratio was higher during the modified mirror tracing task (MT) than during the baseline (PRE) and recovery (POST) periods (PRE-MT: \(p<0.01\); POST-MT: \(p<0.05\), respectively) and was greater during the mental arithmetic task (MA) than during the baseline (PRE) \((p<0.05)\). Significant gender-by-block interaction was found for LF/HF ratio \([F(3,78)=2.92, p<0.05]\). Females exhibited greater changes in the LF/HF ratio than males during tasks.

Figures 12 and 13 show the changes in SBP and DBP before and after the tasks in both groups. A significant main effect was found for gender. SBP for males was higher than that for females \([F(1, 26)=7.65, p<0.05]\). No significant difference in DBP was observed between males and females. ANOVA revealed that SBP and DBP after tasks were significantly higher than those before tasks \([SBP; F(1, 26)=55.66, p<0.01, \ DBP; F(1, 26)=76.96, p<0.01]\). No significant interactions were found for either SBP or DBP.

Figure 14 displays the subjective mental workload for both groups. No significant main effects of task, or gender were observed, and there was no gender-by-task interaction.

**Discussion**

Significant differences in autonomic nervous activity were found to exist between the follicular and luteal phases of the menstrual cycle. The fluctuation in the HRV index reported in the present study was mostly consistent with the results of previous studies, in that LF and LF/HF ratio increased during the luteal phase whereas HF showed a significant reduction...
The HF component in HRV is modulated solely by the parasympathetic nervous system (Eckberg, 1983; Akselrod et al., 1985; Pagani et al., 1986), while the LF/HF ratio reflects the balance between autonomic nervous activities (Pagani et al., 1986; Lombardi et al., 1987; Rimoldi et al., 1990). Accordingly, the fluctuation in the HRV index reported in the present study suggests that sympathetic nervous activity in the luteal phase is more dominant than in the follicular phase. Although the mechanism by which the menstrual cycle affects the autonomic nervous system is unclear, estrogen may contribute by activating the parasympathetic nervous system, while progesterone influences the sympathetic nervous system (Saeki et al., 1997).

As for gender differences, our findings demonstrated that males exhibit a lower HF component, and a higher LF component and a LF/HF ratio than females. In addition, SBP in males was higher than that in females. Therefore, the sympathetic nervous activity of males appears more dominant than that of females, in the present study. Previous studies have found a lower LF component and higher HF/LF ratio in females than males (Liao et al., 1995) and a greater respiratory frequency power and HF/LF ratio in females than in males (Ryan et al., 1994). Furthermore, females reportedly have a higher HF component, whereas males exhibit a higher LF/HF and LF component (Kuo et al., 1999). Overall, these studies as well as the present findings demonstrate a similar trend in autonomic nervous activity in males and in females, namely higher sympathetic nervous activity and lower parasympathetic nervous activity in males than females. It is not clear why autonomic nervous activity differs between females and males. One possible explanation for this gender difference is that female reproductive hormones like estrogen may inhibit sympathetic activation (Ryan et al., 1994). The results of gender-by-block interaction in HRV indices suggested that the magnitude of reactivity in HRV to the tasks were significantly different between males and females. The reasons why females exhibited the greater reactivity in HRV during two tasks are unclear. The previous studies using total peripheral resistance and cardiac output as physiological indices reported that mechanism in cardiovascular control to the mental tasks were different between males and females (Girdler et al., 1990). Monitoring those hemodynamic changes in addition to HRV indices could lead to the better understanding of gender differences in the mechanism of cardiovascular control to mental stress.

Heart rate during the modified mirror tracing task did not significantly change, although HRV and the blood pressure index showed activation of the sympathetic nervous system during this task. Of a large body of literature on cardiovascular response to behavioral stress, recent studies have demonstrated that different types of behavioral stress induce different types of autonomic nervous activity. That is, certain stressors such as
mental mathematics and the aversive reaction time task are more likely than others to increase heart rate whereas other stressors such as a cold pressor and the mirror tracing task are more likely to decrease or not change heart rate (Saab and Scheneiderman, 1993). The former stressors have been shown to induce beta-adrenergic activity with elevation in cardiac output, blood pressure increase and tachycardia while the latter stressors induce alpha-adrenergic activity with elevations in total peripheral resistance, blood pressure increase, and bradycardia (Schneiderman and McCabe, 1989). Although speculative, differences in hemodynamic mechanism induced by different types of tasks (i.e. mirror tracing and mental arithmetic) may have reflected on fluctuations in the heart rate.

Subjective mental workload was not affected by menstrual cycle. It is possible that the NASA-TLX was insensitive to slight fluctuations in mental workload with menstrual cycle. Future studies using other subjective indices are required to verify this issue.

Consistent with our previous study (Sato et al., 1995), no significant differences in MDQ scores were observed. Large individual differences in rating scores were found across the phases. This may partly depend on individual awareness of the menstrual phases (Olasov and Jackson, 1987). Another possibility is a daily mood change may conceal the slight effect of menstrual cycle on mood, at least for non-premenstrual syndrome females.

There are two limitations in this study. First, we adopted young females without premenstrual syndrome as participants. Females with premenstrual syndrome or post menopause females have different hormonal profiles. Further research should focus on the cardiovascular reactivity to mental stress for these females. Secondly, the controversy in interpreting the HRV index is still in existence (Berntson et al., 1998). Therefore, the updated interpretations of HRV must be considered when the HRV index is used as a tool to evaluate autonomic nervous activity during the menstrual cycle.

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