Effects of estrogen on gender-related autonomic differences in humans

C. C. Liu,1* Terry B. J. Kuo,2,4* and Cheryl C. H. Yang3
1Shou-Feng Health Station, Hualien 974; 2Institute of Neuroscience and
3Department of Physiology, Tzu Chi University, Hualien 970; and 4Department
of Neurology, Tzu Chi Buddhist General Hospital, Hualien 970, Taiwan

Submitted 20 March 2003; accepted in final form 18 July 2003

Liu, C. C., Terry B. J. Kuo, and Cheryl C. H. Yang. Effects of estrogen on gender-related autonomic differences in humans. Am J Physiol Heart Circ Physiol 285: H2188–H2193, 2003. First published July 24, 2003; 10.1152/ajpheart.00256.2003.—Our previous studies demonstrated that premenopausal women have dominant vagal and subordinate sympathetic activity compared with age-matched men. This study was designed to investigate the role of estrogen in gender-related autonomic differences. We evaluated the heart rate variability of four healthy groups: age-matched postmenopausal women without hormone replacement therapy (PM), postmenopausal women on conjugated estrogen replacement therapy (PME), men, and non-age-matched premenopausal women (PreM). Frequency-domain analysis of short-term and stationary R-R intervals was performed to evaluate low-frequency power (LF; 0.04–0.15 Hz), high-frequency power (HF; 0.15–0.40 Hz), the ratio of LF to HF (LF/HF), and LF in normalized units (LF%). No gender-related autonomic differences existed between the PM and men groups, but they did exist between the PME and men group. Compared with the PreM group, the PM group had a lower HF and higher LF% and LF/HF. Compared with the PM group, the PME group had a higher HF but lower LF% and LF/HF. These results suggest that conjugated estrogen replacement therapy may facilitate vagal and attenuate sympathetic regulation of heart rate in postmenopausal women. In addition, estrogen may play an important role in gender-related autonomic differences.

Our previous study (19), which evaluated heart rate (HR) variability (HRV), demonstrated that women younger than 50 yr old compared with age-matched men have dominant vagal and subordinate sympathetic activity. However, gender-related differences were not detected in older subjects. In that report, the mean age when menopause occurred was 48 yr old, and most of the postmenopausal women were not receiving hormone replacement therapy (HRT). Thus we doubted that estrogen played an important role in such gender-related autonomic differences. Meanwhile, epidemiological studies have revealed that the incidence of cardiovascular disease is lower in premenopausal women than in age-matched men (21) and postmenopausal women (17).

Estrogen replacement therapy (ERT) is associated with a reduction in the incidence of coronary heart disease (CHD) as well as in mortality from cardiovascular disease (32). Human and/or animal studies have shown that ERT or 17β-estradiol may affect lipid and lipoprotein metabolism (26), atherosclerotic plaque formation (2), and vasomotion (8, 38). Some human studies have reported that women on ERT have lower blood pressure (9, 35), decreased HR (35), increased baroreceptor sensitivity (9, 16), and elevated HRV (16). As for the autonomic nervous system (ANS), animal studies (11, 28) under general anesthesia have revealed significant effects of 17β-estradiol on evoked vagal or sympathetic activity. Most human studies (12, 14, 36), however, have not demonstrated a significant difference in spontaneous autonomic activity between postmenopausal women with ERT and those without HRT.

Until now, the role of estrogen in the autonomic control of gender-related differences or menopausal autonomic changes has remained ambiguous.

Frequency-domain analysis of HRV is a sophisticated and noninvasive tool for detection of ANS regulation of the heart. It has been well established that HRV can be categorized into high-frequency power (HF; 0.15–0.40 Hz) and low-frequency power (LF; 0.04–0.15 Hz) components according to its oscillating frequency and development mechanism (34). The HF component is equivalent to the well-known respiratory sinus arrhythmia (RSA) and is considered to represent vagal control of HR (13). LF% and the ratio of LF to HF (LF/HF) are considered by some investigators to mirror sympathovagal balance or to reflect sympathetic modulations (22, 24, 34). The standard procedure and interpretation of HRV analyses were first reported in 1996 (34). With a small modification in the analytic technique, our previous study (19) demonstrated significant effects of gender and age on the autonomic control of HR. On the basis of a similar methodology, the present study tests the hypothesis that estrogen plays an important role in gender-related differences in cardiac autonomic control.

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C. C. Liu and T. B. J. Kuo contributed equally to this study.

Address for reprint requests and other correspondence: C. C. H. Yang, Dept. of Physiology, Tzu Chi Univ., 701 Chung Yang Rd., Sect. 3, Hualien 970, Taiwan (E-mail: cchyang@mail.tcu.edu.tw).
METHODS

Study sample and experimental setup. The participants were recruited from community residents who came to our department for health examination and patients of our clinics. A total of 229 subjects was recruited. After detailed questionnaire screening and chart review, subjects with diabetic neuropathy, cardiac arrhythmia, or other cardiovascular diseases that affect HRV were excluded. Subjects who used drugs that have been reported to affect cardiovascular fluctuations or menstrual cycle, such as hypnotics, autonomic blockers, contraceptive oral pill, HRT with continuous combined estrogen and progestin regimen, and smoking, were also excluded. In total, 67 women and 14 men were enrolled in this study. 15 women were premenopausal (PreM), 33 women were postmenopausal without HRT (PM), 19 women were postmenopausal and had received regular conjugated estrogen (Premarin at 0.625 mg/day) replacement therapy (PME) for at least 2 mo, and another 14 men were age-matched with the postmenopausal women. Postmenopausal women included in this study had all experienced menopause naturally at least 2 yr previously. In the PME group, nine subjects were on Premarin for 2 mo, five subject were on Premarin for 4 mo, and five subjects were on Premarin for 1 yr. Informed written consent was obtained from all participants, and the experiment protocol was approved by the Ethics Committee of Tzu-Chi Buddhist General Hospital.

Processing of electrocardiogram signals. The detailed procedures for HRV analysis have been previously reported (19, 39). In brief, a pericardiac ECG was taken for 5 min in the daytime while each subject lay quietly and breathed normally. ECG signals were recorded using an analog-to-digital converter with a sampling rate of 256 Hz. The digitized ECG signals were analyzed on-line and were simultaneously stored on a hard disk for off-line verification. Signal acquisition, storage, and processing were performed on an IBM-compatible portable personal computer. Our computer algorithm then identified each QRS complex and rejected each ventricular premature complex or noise according to its likelihood in a standard QRS template. Stationary R-R values were resampled and interpolated at a rate of 7.11 Hz to produce the continuity in the time domain.

Frequency-domain analysis of HRV. Frequency-domain analysis was performed using a nonparametric method of fast Fourier transformation (FFT). The direct current component was deleted, and a Hamming window was used to attenuate the leakage effect (18). For each time segment (288 s; 2,048 data points), our algorithm estimated the power spectrum density based on FFT. The resulting power spectrum was corrected for attenuation resulting from the sampling and the Hamming window. The power spectrum was subsequently quantified into standard frequency-domain measurements as defined previously (34), including total variance, HF (0.15–0.40 Hz), LF (0.04–0.15 Hz), LF%, and LF/HF. Variance, HF, LF, and LF/HF were logarithmically transformed to correct for the skewness of the distribution (19).

Statistical methods. Values are expressed as means ± SE. Data between groups were compared using one-way ANOVA, followed by Fisher’s least significant difference test. Comparisons between two sets of data were performed with the unpaired Student’s t-test. Differences were considered statistically significant at P < 0.05.

RESULTS

The mean ages of the PM, PME, and men groups did not significantly differ; however, they were all significantly higher than that of the PreM group due to the selection criteria (Table 1). Body height and body weight of the PreM, PM, and PME groups did not significantly differ, but those of the three groups compared with the men group were significantly lower. The body mass index and diastolic blood pressure of the four groups did not significantly differ. The systolic blood pressure of the PreM group was significant lower than that of the other groups. The HR of the PM group was significant lower than that of the PreM group. The menopausal ages of the PM and PME groups did not statistically differ. Typical examples are illustrated in Fig. 1. Time-domain analysis of successive R-R values from a woman in the PreM group revealed a prominent RSA fluctuating in a 5-min time window (Fig. 1A). Below 0.5 Hz, the frequency-domain analysis of the R-R series showed a more detailed observation of HRV. The dominant HF, which is equivalent to RSA in the time domain, and LF were clearly identified at 0.2–0.4 and 0.04–0.1 Hz, respectively. The PM group had a lower fluctuation of RSA in time-domain measurements (Fig. 1B), and HF and LF of a PM subject observed from frequency-domain measurements could not be clearly identified. A dramatic increase in HRV was observed in the case of a PME subject (Fig. 1C). Obvious fluctuations of RSA were seen with almost the same amplitude as that of the PreM group in the time domain. Frequency-domain analysis showed a prominent HF of over 0.2–0.3 Hz. Patterns of the time- and frequency-domains in the men group were similar with those of the PM group (Fig. 1D). Quantitation of HRV from the four groups is demonstrated in Fig. 2. The men group compared with the age-matched PM group showed no significant differ-

Table 1. Description data of the study groups

<table>
<thead>
<tr>
<th>Groups</th>
<th>n</th>
<th>Age, yr</th>
<th>Height, cm</th>
<th>Weight, kg</th>
<th>BMI, kg/m²</th>
<th>SBP, mmHg</th>
<th>DBP, mmHg</th>
<th>HR, beats/ min</th>
<th>Age of Menopause, yr</th>
</tr>
</thead>
<tbody>
<tr>
<td>PreM</td>
<td>15</td>
<td>43.1 ± 1.2</td>
<td>153.7 ± 1.2</td>
<td>53.5 ± 1.9</td>
<td>22.7 ± 0.7</td>
<td>112.7 ± 2.8</td>
<td>71.1 ± 2.6</td>
<td>73.0 ± 2.4</td>
<td></td>
</tr>
<tr>
<td>PM</td>
<td>33</td>
<td>56.2 ± 0.8*</td>
<td>151.5 ± 1.1</td>
<td>53.4 ± 1.0</td>
<td>22.5 ± 0.8</td>
<td>121.3 ± 2.2*</td>
<td>74.2 ± 1.4</td>
<td>69.4 ± 1.4</td>
<td>50.2 ± 0.6</td>
</tr>
<tr>
<td>PME</td>
<td>19</td>
<td>57.3 ± 1.2*</td>
<td>151.6 ± 1.0</td>
<td>52.0 ± 1.0</td>
<td>22.7 ± 0.5</td>
<td>118.9 ± 3.2*</td>
<td>73.6 ± 1.4</td>
<td>66.0 ± 1.1*</td>
<td>50.2 ± 1.0</td>
</tr>
<tr>
<td>Men</td>
<td>14</td>
<td>59.6 ± 2.1*</td>
<td>162.3 ± 1.6**</td>
<td>64.0 ± 1.7**</td>
<td>23.8 ± 0.5</td>
<td>122.4 ± 2.1*</td>
<td>76.7 ± 1.6</td>
<td>71.4 ± 3.4</td>
<td></td>
</tr>
</tbody>
</table>

Values are presented as means ± SE; n, no. of subjects per group. BMI, body mass index; SBP, systolic blood pressure; DBP, diastolic blood pressure; HR, heart rate; PreM, premenopausal women; PM, postmenopausal women without hormone replacement therapy; PME, postmenopausal women on conjugated estrogen replacement therapy. *P < 0.05 vs. PreM; †P < 0.05 vs. PM, ‡P < 0.05 vs. PME (Fisher’s least significant difference test).
ences; however, the men group compared with the PreM group had significantly higher LF/HF and lower HF values. Compared with the PME group, the men group had significantly higher LF% and LF/HF and lower HF values. In addition, we found that the PM group had lower HF and higher LF% and LF/HF values compared with those of the PreM group and that the PME group had significantly higher HF and lower LF% and LF/HF values compared with those of the PM group. Table 2 demonstrates HRV measurements of three postmenopausal women before and 2 mo after conjugated estrogen supplementation. Increased HF and decreased LF/HF were seen in all three of these women without a consistent change of blood pressure.

DISCUSSION

On the basis of the noninvasive technique of HRV analysis (19, 34), our previous study (19) demonstrated that women who are younger than 50 yr old had higher vagal but lower sympathetic modulations of HR than the age-matched men did, whereas these gender-related autonomic differences disappeared in the elderly. After we further divided women into PM and PME groups, the present study demonstrated that no gender-related autonomic differences existed between the PM group and age-matched men but that they did exist between the PME group and age-matched men. In addition, the vagal and sympathetic activities of the PM group were lower and higher, respectively, than those of the PreM group. The postmenopausal women with conjugated ERT, however, had significantly increased vagal and reduced sympathetic modulations of HR. In three 2-mo prospective observations of postmenopausal women, we found that in all cases conjugated ERT effectively increased HF and decreased LF/HF. This evidence suggests that estrogen plays an important role in gender-related differences of HRV. According to our present knowledge of HRV, our results also indicate that physiological levels of estrogen may increase vagal but decrease sympathetic modulation of HR in women.

We found that the PM group had a significantly reduced vagal index of HRV and a higher sympathetic index of HRV compared with those of the PreM group. Our findings support the results of a prior study by Rosano et al. (25). Such physiological changes may be
confounded by age or blood pressure (4, 19, 20). Merceru et al. (23) studied the effects of autonomic changes before and after oophorectomy in premenopausal women and proved the definite role of the ovaries in higher vagal and lower sympathetic functions. Our results were also supported by Saeki et al. (27), who focused on the changes of ANS functions during the menstrual cycle in premenopausal women and found that parasympathetic activity is predominant in the follicular phase. Therefore, they suggested that this phenomenon was influenced by estrogen (27). In the present study, we demonstrate that conjugated ERT may effectively reverse the vagal deficit and sympathetic overactivity in postmenopausal women. Sudhir et al. (33) demonstrated that estrogen administration in premenopausal women attenuated vasoconstrictor responses to norepinephrine and reduced total body norepinephrine spillover, which is an index of sympathetic neural activity. With the use of the HRV method, Rosano et al. (25) suggested that ERT reduced the intense sympathetic drive in symptomatic menopausal women. With the use of direct recording of muscle sympathetic nerve activity, Weitz et al. (37) and Vongpatanasin et al. (35) demonstrated that postmenopausal women with transdermal ERT introduced sympathoinhibitory effects but that postmenopausal women with conjugated ERT did not have similar effects (35). Meanwhile, evidence from Mercero et al. (23) proved that estrogen replacement may reverse vagal tone and decrease sympathetic tone in premenopausal women after oophorectomy. Our results are also compatible with those of some animal studies (11, 28) carried out under general anesthesia. Nevertheless, many studies (12, 14, 36) have shown insignificant autonomic changes between the PME and PM groups. These different findings may have been due to different methods, environments, and physiological interpretations. As for the sex-related autonomic differences, some previous studies (1, 15, 16) have demonstrated sympathovagal representations with various technologies accordant as in our study. Our results further indicated that estrogen might play an important role in it.

Because blood pressure is lower in the PreM group, it may be considered whether blood pressure was a confounding factor. This did not seem to be the case because the response of HRV to lower blood pressure shows a decrease of HF and an increase of LF/HF (22). These are opposite to our findings. Thus we consider that the change of HRV is not due to the change of blood pressure. The three postmenopausal women with 2-mo ERT provide other evidence because their HF increased without a consistent change of blood pressure.

In studying the ANS, various techniques and maneuvers have been developed to detect the function or integrity of the sympathetic and parasympathetic systems. Most techniques have focused on the evoked response of the ANS, such as the cold pressor test, Valsalva maneuver, the tilting table test, etc. The evoked activities, however, might not reflect the spontaneous or tonic state of the body. Besides being a noninvasive study procedure, an important advantage of frequency-domain analysis of HRV is that it utilizes spontaneous fluctuations in HR to estimate tonic ANS functions. However, it should be noted that a well-controlled condition is required for spontaneous ANS

### Table 2. Changes of blood pressure and HR variability measurements of 3 postmenopausal women after 2 mo of conjugated estrogen replacement therapy

<table>
<thead>
<tr>
<th>Subject</th>
<th>Age, yr</th>
<th>SBP, mmHg</th>
<th>DBP, mmHg</th>
<th>Mean R-R Interval, ms</th>
<th>Variance of R-R Interval, ln(ms²)</th>
<th>HF, ln(ms²)</th>
<th>LF, ln(ms²)</th>
<th>LF%, nu</th>
<th>LF/HF, ln(ratio)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>PreE</td>
<td>PostE</td>
<td>PreE</td>
<td>PostE</td>
<td>PreE</td>
<td>PostE</td>
<td>PreE</td>
<td>PostE</td>
</tr>
<tr>
<td>1</td>
<td>47</td>
<td>105.0</td>
<td>102.0</td>
<td>71.0</td>
<td>81.0</td>
<td>764.6</td>
<td>877.0</td>
<td>5.289</td>
<td>6.979</td>
</tr>
<tr>
<td>2</td>
<td>56</td>
<td>100.0</td>
<td>106.0</td>
<td>71.0</td>
<td>72.0</td>
<td>789.2</td>
<td>967.8</td>
<td>7.355</td>
<td>7.351</td>
</tr>
<tr>
<td>3</td>
<td>54</td>
<td>135.0</td>
<td>130.0</td>
<td>81.0</td>
<td>71.0</td>
<td>754.2</td>
<td>901.4</td>
<td>5.405</td>
<td>6.176</td>
</tr>
</tbody>
</table>

HF, high-frequency power; LF, low-frequency power; LF%, normalized LF; nu, normalized units; LF/HF, ratio of LF to HF; PreE, before conjugated estrogen replacement therapy; PostE, 2 mo after conjugated estrogen replacement therapy.
functional recordings. In the present study, we used spectral analysis of HRV while subjects were in supine rest in a quiet and relaxing atmosphere for 5-min recordings. In addition, our previous studies with widely aged women and men demonstrated a severe skewness in variance, HF, LF, and LF/HF; thus nature logarithm transformation was recommended to correct for the skewness before statistical analysis is performed (19). The recordings of spontaneous HR signals, the control of the experimental environment, and the correction for skewness of the HRV indexes in our study may offer a stable and precise estimation of tonic ANS functions, from which the beneficial effects of ERT on cardiac autonomic function can be successfully demonstrated. Lacking a strict control of Premarin therapy duration and of daily activity levels may be limitations of this study. Therefore, the findings need to be interpreted with caution given the potential confounding effects of these factors.

CHD is a leading cause of death in many developed countries. The relation between estrogen and CHD has been discussed in many epidemiological studies. Evidence shows that the incidence of CHD increases after menopause in women (17) and that the cardiovascular mortality rate of postmenopausal women with ERT is lower than that of women without ERT (5). To explain the mechanism of this phenomenon, the effects of estrogen on the lipid profile and vascular activity have been discussed and established in many studies (30).

The relation between HRV and CHD was recently explored after the development of HRV techniques. Lower HRV was proven to be associated with a greater risk for developing hypertension among normotensive men (31), and hypertension is one of the major risk factors of CHD. Acute myocardial infarction is accompanied by a decreased HRV, which is due to reduced vagal or increased sympathetic outflow to the heart (6). In a prior study using anesthetized animals, Saleh et al. (29) demonstrated that 17β-estradiol administration prevented or reversed acute stroke-induced autonomic dysfunction, suggesting a neuroprotective effect of estrogen. The cardiac vagotonic and sympatholytic effects of estrogen can explain, at least in part, why premenopausal women compared with postmenopausal women have a lower CHD incidence and mortality rate (5) and why ERT may decrease the risk of CHD events in postmenopausal women (3, 32).

Many HRT regimens are used clinically. Combined estrogen and progesterone regimens, either continuously or sequentially, are used world wide. Although some studies have found that progesterone may decrease the cardioprotective effects of estrogen (7), the clinical effect of combined HRT on autonomic HR control of postmenopausal women is still unclear. HRV measurements would be a good methodology to resolve this question due to its noninvasiveness and convenience. Detailed mechanisms linking estrogen and HRV warrant further exploration. For example, it has been reported that estrogen has a facilitating effect on cardiac vagal function (10). On the other hand, the loss of cardiac protection after menopause in women concerns women, men, and their doctors. The vagotonic and sympatholytic effects of estrogen demonstrated in this study may offer some idea for future therapeutic and preventive medicine. Although the potential carcinogenic effects of sex hormones must still be determined, the promising protective effects of estrogen on the lives of women and men should be pursued.

DISCLOSURES

This study was supported by National Science Council (Taiwan) Grant NSC-91-2320-B-320-007.

REFERENCES


