Effects of Hormone Replacement Therapy on Heart Rate Variability in Postmenopausal Women

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Background: Hormone replacement therapy (HRT) is associated with reduced cardiovascular risk, but the underlying mechanism(s) are not fully understood. This study investigated the effects of a 6-month course of HRT on cardiac autonomic function parameters assessed by heart rate variability (HRV) in postmenopausal women.

Methods: Forty-six healthy postmenopausal women (age 48 ± 5 , range 40-60) with normal baseline electrocardiogram and negative exercise testing were enrolled. HRT, which was either 0.625 mg/day conjugated equine estrogen (CEE) plus 2.5 mg/day medroxyprogesterone acetate or 0.625 mg/day CEE alone were administered depending on hysterectomy status. Power spectral analysis of HRV was performed to calculate the low frequency component in absolute (LF) and normalized units (LF nu), high frequency component in absolute (HF), and normalized units (HF nu), and the LF/HF ratio. The standard deviation of RR intervals (SDNN) was calculated from the time series of RR intervals.

Results: A 6-month course of HRT did not significantly alter resting heart rate ($P > 0.05$). The LF/HF ratio and LF nu significantly decreased after HRT ($P = 0.022$ and $P = 0.032$), whereas a significant increase was noted in the HF component of HRV ($P = 0.043$), indicating an improvement in cardiac autonomic function. The SDNN value, which was 28.8 ± 11.8 ms before HRT significantly increased to 35.4 ± 16.7 ms after 6 months ($P = 0.011$).

Conclusion: Our results indicate that a 6-month course of HRT may significantly improve cardiac autonomic function parameters, a finding that could at least partly explain the potential cardioprotective effect(s) of HRT.

A.N.E. 2001;6(4):280-284

hormone replacement therapy; heart rate variability; menopause

Hormone replacement therapy (HRT) is associated with decreased cardiovascular risk in postmenopausal women.¹ The underlying mechanism(s) of HRT-related cardioprotection are not fully understood, but most likely to be multifactorial including the favorable effects on lipid profile and metabolic factors, as well as, direct hemodynamic effects.²

Heart rate variability (HRV) measurement from electrocardiographic recordings has been shown to

be useful to assess cardiac autonomic function. The frequency specific fluctuations in heart rate behavior and the sympathetic/parasympathetic integrity can be evaluated non-invasively with power spectrum analysis of HRV.^{3,4} An increased sympathetic tone or a reduced vagal activity has been shown to be an independent predictor of cardiovascular mortality after acute myocardial infarction.^{5,6}

There is a paucity of data related to the effect(s) of HRT on cardiac autonomic function with con-

tradictory results.⁷⁻⁹ The aim of the present study was to investigate the impact of HRT on cardiac autonomic control assessed by HRV in healthy postmenopausal women.

METHODS

Study Population

Forty-six consecutive postmenopausal women presenting as outpatients to the Department of Obstetrics and Gynecology at our institution were enrolled. All participants were between 40 and 60 years old, smoked less than 5 cigarettes/day and were normotensive (less than 140/90 mmHg). None of the women had used HRT before or taken any cardiovascular medications before or during the study period. Patients with a history or symptoms relevant to diseases known to modify autonomic control (such as diabetes) or current use of drug that may alter autonomic functions were excluded. Natural menopause was considered if the menses had ceased for at least 6 months. Surgically castrated women were enrolled into the surgical menopause group. All patients in the surgical menopause group had undergone bilateral salpingo-oophorectomy with hysterectomy at least 3 months before they were included into the study. Postmenopausal status was confirmed by serum follicular stimulating hormone > 35 IU/L and estradiol < 30 pg/ml. Baseline electrocardiogram (ECG) and treadmill exercise testing were performed in all patients. Patients with abnormal baseline ECG or positive exercise testing were excluded.

Hormone regimens were continuous 0.625 mg/day conjugated equine estrogen (CEE) alone or 0.625 mg/day CEE plus 2.5 mg/day medroxyprogesterone acetate (MPA). Women with a history of hysterectomy received the former regimen, while those with uterus had MPA added. Compliance with the HRT regimen was supervised with bi-monthly phone calls and a pre-term visit at the third month.

All subjects were questioned about symptoms of autonomic nervous system (ANS) dysfunction and underwent complete cardiac and neurologic examination.

Heart Rate Variability Analysis

Recordings for HRV analysis were made with a PC-based high resolution ECG system (Kardiosis,

Ankara, Turkey) after 30 minutes resting in a silent environment in supine position. Bipolar X deviation (0.5-340 Hz) was recorded and sampled at a rate of 1000 samples per second and digitized using an 8-bit A/D converter. Each recording lasted 8 minutes, and raw data were stored to disk for post-processing. A segment of 5 minutes of the recording with no artifacts and premature depolarizations was processed to provide the results of HRV analysis. The R-R tachograms were extracted from the data. The detection of R waves was done with a thresholding algorithm. The detected R waves were visually confirmed and any undetected regular R wave was marked either manually or by interpolation. Similarly, any point which is not an R wave but found as one, was unmarked manually. By this method, raw R-R tachograms was extracted. Raw tachogram was then interpolated at 1-second intervals by linear interpolation. From the interpolated R-R tachogram, power spectral densities were calculated by autoregressive modeling. Power spectrums for heart rate variations were calculated with autoregressive modeling. In power spectrums of R-R intervals, three major peaks, one around 0.03 Hz (very low frequency—VLF peak), one around 0.03-0.15 Hz (low frequency—LF peak) and the other around 0.15-0.40 Hz (high frequency—HF peak) were seen. The peak around < 0.03Hz was attributed to several physiologic variables and was filtered out from the tachograms before modeling. For spectral analysis, the following variables were calculated: 1) The low frequency component in absolute (LF) and normalized units (LF nu); 2) The high frequency component in absolute (HF) and normalized units (HF nu); 3) The ratio of the absolute low and high frequency component (LF/HF). In addition, the standard deviation of R-R intervals (SDNN) was also calculated from the time series of R-R intervals. The absolute values of the results were in ms for the time domain and ms² for power spectral analysis.

The power spectral analysis method of HRV from short-term recordings has been accepted as a reliable technique to evaluate the ANS functions¹⁰ and we used the same method in our previously published studies.¹¹

Statistical Analysis

The Statistical Package for the Social Science (SPSS 9.1 version for Windows) was used for statistical analyses. The Kolmogorov-Smirnov good-

Table 1. Effects of a 6-Month Course of Hormone Replacement Therapy on Heart Rate Variability Parameters

	Before HRT	After HRT	P
Heart rate	73 ± 10	72 ± 12	0.812
LF (ms ² /Hz)*	250 ± 186	293 ± 269	0.726
LF nu	58.7 ± 15.8	52.2 ± 20.1	0.032
HF (ms ² /Hz)*	153 ± 135	296 ± 381	0.043
HF nu	33.7 ± 15.1	37.4 ± 15.8	0.106
LF/HF*	2.7 ± 2.7	2.1 ± 2.0	0.022
SDNN (ms)	28.8 ± 11.8	35.4 ± 16.7	0.011

HRT = hormone replacement therapy; LF = low frequency; HF = high frequency; LF nu = low frequency in normalized unit; HF nu = high frequency in normalized unit; LF/HF = low frequency/high frequency ratio; SDNN = standard deviation of R-R intervals. * Statistics were calculated on log-transformed data.

ness of fit test was used to test normality. Because of skewed distribution, LF power, HF power, and LF/HF ratio were transformed by calculating their natural logarithm. Values were expressed as mean ± SD. Paired sample *t*-test was used to compare HRV parameters before and after therapy. Pearson correlation test was used to analyze the association between variables. Statistical significance was set at $P < 0.05$.

RESULTS

Of the 46 women included in the study, 16 received CEE and the remaining 30 received CEE + MPA. The mean age and menopausal age of the study group were 48 ± 5 years (range 40-60) and 46 ± 4 years (range 38-53), respectively. The duration of menopause was 34 ± 39 months (range

3-165). Cardiac and neurological examination of the subjects did not reveal any abnormal findings.

The effects of HRT on various HRV parameters are given in Table 1. HRT did not alter resting heart rate ($P > 0.05$). At the end of 6 months, significant decreases were noted in LF nu ($P = 0.032$) and LF/HF ratio ($P = 0.022$), whereas the power of HF significantly increased with HRT ($P = 0.043$), indicating an improvement in cardiac autonomic functions. The SDNN value which was 28.8 ± 11.8 ms before HRT, significantly increased to 35.4 ± 16.7 ms with HRT ($P = 0.011$).

When the subjects were subdivided into two, based on the drug regimen as CEE and CEE + MPA groups, we observed the same trend of change in all of the HRV parameters studied with HRT (Table 2). However, all but one of the comparisons between pre- and posttreatment HRV parameters were insignificant due to the small size of the subgroups ($P > 0.05$). The only significant change in the subgroup analysis was an increase in SDNN value noted in the CEE + MPA group ($P = 0.022$).

Percentage change in LF/HF ratio, LF nu, and HF were not associated with the age of the women and the duration of estrogen deprivation after menopause ($P > 0.05$).

DISCUSSION

Hot flashes and sweating are the ANS-mediated menopausal symptoms. They are thought to be the consequences of the decreased production of estrogen after menopause and are successfully treated with estrogen.¹² This observation suggests the hypothesis that sex hormones may alter cardiac autonomic modulation.

Table 2. Effects of Hormone Regimens (CEE + MPA vs CEE alone) on Heart Rate Variability Parameters

	CEE + MPA (n = 30)		CEE (n = 16)	
	Pre	Post	Pre	Post
Heart rate	73 ± 10	72 ± 12	71 ± 9	72 ± 10
LF (ms ² /Hz)*	241 ± 159	298 ± 280	268 ± 232	282 ± 254
LF nu	59 ± 16	53 ± 19	58 ± 15	51 ± 23
HF (ms ² /Hz)*	153 ± 140	318 ± 421	154 ± 129	255 ± 296
HF nu	35 ± 16	39 ± 16	32 ± 13	34 ± 14
LF/HF*	2.7 ± 3.0	2.0 ± 1.9	2.5 ± 2.3	2.2 ± 2.2
SDNN (ms)	27.1 ± 11.8	35.0 ± 18.4#	32.1 ± 11.6	36.2 ± 13.6

CEE = conjugated equine estrogen; MPA = medroxyprogesterone acetate; LF = low frequency; HF = high frequency; LF nu = low frequency in normalized unit; HF nu = high frequency in normalized unit; LF/HF = low frequency/high frequency ratio; SDNN = standard deviation of R-R intervals. * Statistics were calculated on log-transformed data. (# $P = 0.022$, all other comparisons between pre- and post-treatment values were nonsignificant.)

As supportive evidence to this hypothesis, Ramaekers et al.¹³ investigated the gender-specific differences in autonomic modulation in female and male healthy subjects by HRV. In this study, female subjects had significantly lower LF/HF ratio and LF power compared to males, indicating a more favorable autonomic modulation. This difference in cardiac autonomic functions between both sexes seemed to narrow or disappear between the ages of 40 and 50 years as the female became menopausal, suggesting that the protective factor might be the female hormone estrogen.

Experimental studies have provided further evidence to the alteration of the cardiac autonomic modulation by estrogen.¹⁴ In a rat model, ovariectomy reduced baroreflex sensitivity and suppressed the respiratory component of HRV, both indices of vagal cardiac control. However, estrogen replacement therapy restored the HRV parameters to normality.¹⁵

Despite these observations suggesting the alteration of cardiac autonomic innervation by estrogen, there are very limited clinical data on the characteristics of cardiac autonomic function parameters in menopause and the effects of HRT on these parameters.

Rosano et al.,⁷ in a prospective controlled study, investigated the effects of HRT on HRV parameters in 30 patients. Of the 30 patients, 18 were assigned to estrogen replacement therapy and the remaining 12 who refused HRT served as controls. Subjects and controls underwent 24-hour Holter monitoring to investigate the time domain, as well as frequency domain characteristics of HRV at baseline and after 1 and 4 months. No significant alteration was noted in the HRV parameters in the control group during the study period. However, estrogen users had significantly higher HF power and significantly lower LF/HF ratio compared to baseline and with controls at the first and fourth months, suggesting an improvement in autonomic control of the cardiovascular system. In addition, all subjects showed significant improvement of their menopausal symptoms. This study provided the first evidence of favorable effects of HRT on HRV in postmenopausal women, but was restricted with the small number of patients both in the study and control groups. In the present study we had relatively higher number of patients and longer duration of follow-up. Furthermore, we investigated and compared two different HRT regimens, estrogen alone or combined with progesterone. Our re-

sults are consistent with the findings of Rosano et al, indicating significant reductions in LF nu and LF/HF ratio and a significant increase in HF component of HRV with both regimens. Similarly, we also noted a significant increase in SDNN, a measure indicative of vagal tone after 6 months of HRT.

However, Virtanen et al.⁸ who evaluated the effects of estradiol replacement therapy on ANS tests, baroreflex sensitivity, and HRV in 14 healthy postmenopausal women indicated no changes in the frequency or time-domain analysis of HRV parameters after 3 months of HRT.

In contrast to the previously mentioned studies and our study, which indicated either a favorable or neutral effect, unfavorable results of HRT on HRV parameters has also been reported. Christ et al.,⁹ in a cross-sectional study, evaluated the HRV parameters in 45 healthy postmenopausal women with time and frequency-domain analyses calculated from short-term recordings. Of the 45 women, 21 used HRT for more than 3 months. Of the 21 women using HRT, 13 used estrogen plus progestin replacement and the remaining 8 used estrogen only. A significant depression of HRV parameters related to parasympathetic tone was noted in HRT-users. Subgroup analysis showed that the depression in HRV factors was observed only in women on the combined regimen, whereas these measures were similar in pure estrogen users and controls. The authors concluded that attenuation of HRV was restricted to HRT containing progestins. This study, although it was not statistically powerful enough because of the small number of women studied, is an important one because it investigated the addition of progesterone to the regimen. However, this is a cross-sectional study, therefore does not address the changes in HRV parameters before and after HRT.

Most studies on the impact of HRT on cardiovascular risk has mainly focused on the beneficial actions of estrogen, however, progestagen-containing HRT is the choice of treatment for the women with uterus. We administered both regimens in our study leaving the dose and the route of estrogen the same and observed that the alteration of cardiac autonomic function was irrespective of the progesterone use.

Several mechanism(s) may be responsible for the HRT-related improvement in HRV parameters. The favorable effects of HRT on heart has mainly been attributed to the systemic effects, however it

is not possible to eliminate the direct actions of sex hormones on the cardiovascular system. The presence of nuclear and cytosolic receptors for sex hormones has been shown throughout cells within the cardiovascular system and its neuroregulatory tissues,¹⁶ suggesting the idea that sex hormones could exert direct or indirect effects on the cardiovascular system by these receptors and may regulate gene expression in the myocardium. However, the exact mechanism of the HRT-related changes in HRV parameters is unclear.

Small sample size and the lack of a control group are the limitations of our study. However, the only study with a control group⁷ did not indicate any alteration in HRV parameters with time in subjects not receiving HRT. Furthermore, in our study the mean duration was about 3 years after menopause, and we do not expect much alteration in the autonomic symptoms during our follow-up period of 6 months.

CONCLUSIONS

In conclusion, HRT may significantly improve cardiac autonomic function. This improvement was irrespective of the use of progesterone and may be one of the mechanism(s) of the favorable effect(s) of HRT on heart. Further powerful prospective randomized studies are required to support such a cardioprotective mechanism of action of HRT especially in women with cardiovascular disease in whom HRV has higher prognostic significance.

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