The Effect of Hormone Replacement Therapy on Cardiac Autonomic Response to Laboratory Stressors

by

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A Thesis Presented in Partial Fulfillment
of the Requirements for the Degree
Master of Science

Approved April 2013 by the
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ARIZONA STATE UNIVERSITY

May 2013

ABSTRACT

The objective of this study was to examine the potential effects of long term hormone replacement therapy on cardiovascular autonomic nervous system responses to laboratory social stressors. The participants were 38 postmenopausal women, 18 using estrogen and progesterone hormone replacement therapy for at least 2 years and 20 control participants without hormone replacement therapy. All women completed orthostasis (standing and sitting), then speech and math tasks (speech and math were counterbalanced). Cardiovascular measures of sympathetic nervous system (pre-ejection period, PEP) and parasympathetic nervous system (respiratory sinus arrhythmia, RSA) along with heart rate were collected throughout all periods (baseline, orthostasis, and stressors). For orthostasis, results of mixed analyses of variance (ANOVAs) showed expected period effects for heart rate, RSA and PEP, but no group or group by period interaction was significant. For the psychological stressors, period main effects were significant for all three variables, suggesting that the tasks were effective at inducing stress. Also, there was a significant interaction between group and period for RSA, demonstrated by greater decrease during the psychological stressor period in the group using HRT. The interactions between group and period for heart rate and PEP were non-significant. These findings support the notion that HRT may slow age-related decreases in parasympathetic responsiveness. Furthermore, changes in vagal reactivity in relation to use of HRT appear to occur within mechanisms involving response and coping with psychological stressors, rather than mechanisms that accommodate basic physiological task such as orthostasis.

ACKNOWLEDGMENTS

I cannot find the words to express my gratitude to Dr. Mary Burleson without whom I would have never been able to finish this project. Through her guidance and support, she provided me with an exceptional research experience, and reinforced my love for psychology, human body and mind.

I would like to thank Edward Acevedo, the best husband in the world, my only love, who patiently stood by me, in good and bad times throughout this entire project.

Finally, I would like to thank my parents and my sister, who despite being thousands of miles away from me were the best cheerleading squad one could ever ask for.

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LIST OF USED ABBREVIATIONS

ANS – Autonomic Nervous System

ECG - Electrocardiograph

ERT – Estrogen Replacement Therapy

HPA – Hypothalamus-Pituitary-Adrenal axis

HRT – Hormone Replacement Therapy

HRV – Heart Rate Variability

PEP – Cardiac Pre-ejection Period

PNS – Parasympathetic Nervous System

RSA – Respiratory Sinus Arrhythmia

SAM axis – Sympathetic-Adrenal-Medullary axis

SNS – Sympathetic Nervous System

The Effect of Hormone Replacement Therapy on Cardiac Autonomic Response to Laboratory Stressors

Negative effects of stress on health have been studied from many different angles in the last few decades. It is now well established that response to stress, also labeled as stress reactivity, occurs in response to merely mild levels of either acute or chronic stressors. Studies haverepeatedly linked increased stress reactivity to a substantial range of health problems, such as insomnia (Wheatley, 1998), increased pain sensitivity to headaches(Cathcart, Petkov, & Pritchard, 2008), obesity (Dallman, Pecoraro & La Fleur, 2005), suppression of the immune system (Calcagni & Elenkov, 2006), reproductive system setbacks (Negro-Vilar, 1993), or chronic illnesses such as diabetes (Räikkönen, Keltikangas-Järvinen, Adlercreutz, & Hautanen,1996) and irritable bowel syndrome (Whitehead, Crowell, Robinson, Heller, & Schuster, 1992). Elevated levels of thehormone cortisol, anestablished indicator of stress response of the neuroendocrine system, have been linked to a variety of somatic and emotional issues, as well as potential irreversible changes in the hypothalamus-pituitary-adrenal axis (HPA axis) and the sympathetic-adrenal-medullary axis (SAM axis) (Kudielka & Kirchbaum, 2005; Lovallo, Farag, Vincent, Thomas, & Wilson, 2006; Keitel et al., 2011). Perhaps the most alarming health consequences of stress are on the cardiovascular system.An extensive body of research has shown that elevated cardiovascular stress reactivity is a potential contributor to the development of cardiovascular disease (Bosma, Peter, Siegrist, & Marmot, 1998; Trieber et al., 2003).Furthermore, a low level of estrogen, the primary female sex steroid hormone, has been previously linked to increased risk of development of cardiovascular disease (Christodoulakos, Lambrinoudaki, & Botsis, 2007).

The purpose of this study is to investigate whether hormone replacement therapy (HRT), a supplement that mimics endogenous hormones (estrogen and progesterone),is related tostress responses in postmenopausal women, primarily by examiningcardiovascular indicators ofautonomic nervous system (ANS) activity. In addition, this paper reviews current research findings in the area of stress on health, specifically the cardiovascular system; reviews some commonly used indicators of cardiac autonomic functioning; and discusses outcomes of studies evaluating the benefits and risks of HRT.

Stress and the Autonomic Nervous System

The term “stress” has been operationalized in countless ways, but broadly, it can be described as the body’s biological reaction to change in the environment. This change in the environment canbe also referred to as the stressor. The stressor is typically perceived as a physical, psychological or social threat that generates a stress response. Depending on the character of the stressor such as intensity and length in time, it can be further classified as mild (low intensity) to severe (high intensity), and acute (short term) to chronic (long term). Stress responsesalter autonomic functions, which in turn initiate further biological, cognitive, and emotional reactions, with the purpose ofpreparing the body for a sudden burst of energy demand (Greenberg, 2008; Sapolsky, 2004).

The autonomic nervous system (ANS) stimulates changes in most of the body’s internal organs. In response to psychological stress, activation of the ANS is mediated by the cortex and limbic system, while responses to more basic, biological stressors are initiated by signals from the brainstem (Fitzgerald, Gruener, & Mtui, 2012). The ANS is further divided into two branches – the sympathetic and parasympathetic nervous

systems. The sympathetic nervous system (SNS) produces the fight-or-flight response which mediates mobilization and vigilance, primarily by a release of hormones such as cortisol and epinephrine (adrenaline) from the HPA and SAM axes. These chemical messengers then elicit increases in heart rate, respiration, and blood flow in skeletal muscles, pupil constriction, and reduction in digestion and reproduction. The parasympathetic branch (PNS) facilitates rest by slowing the heart rate and respiration, dilating pupils, and promoting digestion and reproduction. (Greenberg, 2008). The vagus nerve(cranial nerve X) is the primary mediator of parasympathetic messages to the heart. Previous studies have proposed that parasympathetic responsiveness, also known as vagal tone, should be used as an indicator of "cardiac age"(Hrushesky, Fader, Schmitt, & Gilbertsen, 1984). In addition, it has been shown that vagal tone negatively correlates with age, increasing the dependency on sympathetic control of the heart (Fukusaki, Kawakubo, & Yamamoto, 2000). Extended states of "fight-or-flight" under SNS, SAM and HPA axis control can lead to allostatic overload, and therefore instigate abovementioned health results (McEwen, 2000).Because vagal activation or withdrawal is not driven by the release of epinephrine from the adrenal medulla, it is believed to be less damaging to the body. Furthermore, it has been shown that its effects are shorter in duration than the ones of sympathetic activation (Saul & Cohen, 1994).

Studies have shown that the SNS and PNS work together as one, in some situations both being co-activated, in others being activated reciprocally (Berntson, Norman, Hawkley, & Cacioppo, 2008). However, a wide –range of individual factors influencing stress reactivity make it problematic to uncover the fundamental mechanisms by which the SNS and PNS interact.

Cardiovascular Indicators of Autonomic Nervous System Activity

Cardiovascular system activity can be assessed by a number of noninvasive methods. Biological indicators such as heart rate and changes in respiration rate are the most basic. More advanced measures, such as respiratory sinus arrhythmia and cardiac pre-ejection period are more specialized ways by which autonomic cardiac function is tested. In research on cardiovascular reactivity to psychological stressors, an increase in heart rate and respiration generally signifies sympathetic activity whereas a decrease in heart rate and respiration signifies parasympathetic activity. However, as described earlier, physiological responses to stress may vary among individuals and situations, and therefore understanding the underlying mechanisms that culminate in a given response represents a challenge. For example, Ritvanen, Louhevaara, Helin, Väisänen, and Hänninen (2006) found that perceived high stress periods led to elevated heart rate in young female teachers yet did not influence the blood pressure, whereas in older female teachers, it did not elevate heart rate, yet influenced blood pressure. Because the means by which these individual differences in stress responsesoccur are not yet well-defined, further investigation is required.

The method of impedance cardiography, first presented by Kubicek, Karnegis, Patterson, Witsoe, and Mattson in 1966, involves measuring ongoing changes in the resistance (impedance) to a small alternating current passed through the thorax. Since then, the method has been repeatedly used and refined in controlled experimental studies, and is now utilized to monitor and measure multiple different aspects of cardiac activity. Using the first derivative of raw impedance, which is the amount of change in the

impedance divided by the change in time, impedance cardiography allows the determination of aortic valve opening and thereby measurement of the cardiac pre-ejection period (PEP) (Sherwood et al., 1990). PEP is defined as the difference in time between the initiation of leftventricular contraction (marked by the R wave of the electrocardiogram) and opening of the aortic valve (Woltjer, Bogaard, & deVries, 1997). It has been determined that the length of this time period is almost entirely determined by sympathetic activation, and therefore, PEP can be described as a biological indicator of sympathetic activation on the heart. Short PEP represents higher SNS activation, whereas longer PEP means decrease in SNS activity.

Heart rate variability (HRV) is described as the oscillation of heart beats in time (Stein, Bosner, Kleiger, & Conger, 1994). Some predictable variations of inter-beat intervals are primarily due to respiration and are a part of normal heart function. By recording the heart's continuous beat-by-beat measurements and evaluating deviations from the mean, estimates of autonomic cardiac activity can be made. The information on HRV is primarily collected through electrocardiography (ECG or EKG), which measures the electrical output of the heart. Respiratory sinus arrhythmia (RSA) is a measure of HRV and an indicator of vagal activity on the heart. Berntson, Cacioppo, and Quigley (1993a) describe RSA as a rhythmical fluctuationin heart periods associated with respiration, causing acceleration of heart rate at inhalation and deceleration of heart rate at exhalation. Increases in RSA can be interpreted as indicating increasedvagal and therefore parasympathetic activity; whereas decreases in RSA can be understood as a decrease in vagal and therefore parasympathetic activity. Reduced vagal activity has been shown to diminish the ability to recover after stress (Fukusaki et al., 2000). Because RSA

is closely linked to respiration, it is important to consider respiratory function as a potential confound. Ritz and Dahme (2006) have concluded that most studies on RSA have failed to implement respiration controls and underline the necessity to consider both respiration rate and tidal volume for purer measure of RSA. Other researchers suggest that correction of RSA values for respiration may be unnecessary under many conditions (Grossman, Wilhelm, & Spoerle, 2004).In general, previously conducted research lacks standardized operationalization of RSA and PEP, which may in turn affect the outcomes (Grossman et al., 2004; Sherwood et al., 1990)

Heart rate is frequently used to assess cardiovascular performance. In general, it represents the quantity of ventricular contractions, and therefore it is defined as the number of beats per minute (Graham, 1978). In the past, heart rate increase was primarily viewed as an indicator of SNS activation, but more recent studies have shown that it is also influenced by other aspects of the ANS (Berntson, Cacioppo, & Quigley, 1993b). Vagal activation, characterized by increase in RSA, leads to cardiac slowing, consequently decreasing the number of beats per minute. On the other hand sympathetic activation, and shorteningin PEP, leads to increase of the number of beats per minute (Drew & Sinoway, 2011). These relationships therefore are often characterized by negative correlation between heart rate and both PEP and RSA (Berntson et al., 1993b).

Both researchers and clinicians commonly use orthostatic stress tasks to assess basic homeostatic ANS activity on the heart. Orthostasis can be described as a body's natural response to a change in body posture. Upon standing, a spontaneous response of ANS adjustments, such as increase in heart rate and blood pressure is required to prevent the body from orthostatic hypotension which is commonly manifested by fainting

(Perlmuter & Greenberg, 1996). As mentioned previously, brain regions responsible for initiation of stress responses differ based on the type of stressor (psychological vs. homeostatic), with orthostatic responses being controlled by the brainstem.

Stress, Cardiovascular Disease and Sex Steroids

Stress-related cardiovascular disease is the number one killer in the United States(Sapolsky, 2004). It is characterized by a variety of symptoms, such as hypertension, heart beat arrhythmia, atherosclerotic plaque, thrombus formation, increased respiration, or heart muscle thickening, each having the potential of fatal consequences. Major risk factors, such as obesity, tobacco use, unhealthy diets, lack of physical exercise,and aging have been well established. The reviewed literature agrees that the prevalence of cardiovascular disease is greater in men, but also acknowledges that some of the abovementioned risk factors represent a greater threat for women than men (Roger et al., 2012; Sapolsky, 2004). For example, brain imaging studies suggest that greater prevalence of cardiovascular disease among men is a result of gender differences in limbic region activation, such as lateralization of amygdala activation as a response to emotional stressors (Cahill et al., 2001).On the other hand, Sapolsky (2004) argues that diabetes, increasing obesity, and smoking confer a greater risk of acquiring cardiovascular disease for women than men. Traustadóttir, Bosch, and Matt (2003) propose that difference in prevalence of cardiovascular disease between the sexes may exist due to prevailing sex hormone levels.In fact, it has been hypothesized that female sex hormones are likely to be protective effect against hypertension. Additionally, women have been shown to have better SNS regulation, but whether female sex

hormones play a role in that is not yet known (Hinojosa-Laborde, Chapa, Lange & Haywood, 1999).

To further investigate the idea that prevailing sex hormone levels can influence changes in cardiovascular activity, this study analyzes women in postmenopausal status and their response to short-term laboratory stressors, while measuring cardiovascular responses. Additionally, the widespread use and prescription of HRT necessitates further, more comprehensive understanding of its effect on health to help women going through the menopause transition to determine whether HRT is the best choice for their well-being.

Hormone Replacement Therapy and Health

Healthy women show the first signs of menopause at 50 years of age on average (Lignieres, & MacGregor, 2000). During this transitional period, the body undergoes biological changes, affecting women's physical, and possibly their psychological state. Menopause is the cessation of ovarian cycling. Lack of follicular development leads to decreased production of the ovarian hormones estrogen and progesterone. Reduced levels of estrogen are believed to be the primary reason for the most commonly experienced symptoms, such as hot flashes, and night sweats as well as the greater risk of development of bone disorders such as osteoporosis (Cauley et al., 2003). Other commonly reported symptoms include sleep problems, headaches, heart palpitations, weight and mood changes, fatigue, and sexual dysfunctions, but there is less agreement about the etiology of these phenomena (Nappi & Lachowsky, 2009). All of these symptoms commonly fluctuate in severity, varying from one individual to another, implying multiple mechanisms which are not yet understood by researchers. Furthermore,

estrogen is known to modulate autonomic tone and therefore cardiac function. Several studies have associated low levels of estrogen and postmenopausal status with greater stress reactivity to laboratory stressors, potentially increasing the risk of development of cardiovascular disease (Saab, Matthews, Stoney, & McDonald, 1989; Rubinow & Girdler, 2011).

The most commonly prescribed treatment for alleviation of the abovementioned symptoms related to menopause is estrogen replacement therapy (ERT). ERT supplements are available in many forms, differingin the type of active ingredient (e.g. estriol, or estradiol), and forms of administration (e.g. oral, or transdermal patches) (Canderelli, Leccesse, Miller, & Uhrun Davidson, 2007).Progesterone replacement therapy is commonly recommended as a supplementary treatment to ERT, for women in menopause who have not undergone hysterectomy and still have their uterus. It serves as a balancer to estrogen against proliferation of the endometrium, consequently being a preventative measure against endometrial cancer (Douglas, Litman, White & Leslie, 2002). This combined regimen is referred to as hormone replacement therapy (HRT).

The underlying principle that HRT restores estrogen and progesterone levels in postmenopausal women can create a general view that HRT is purely beneficial. In contrast to this perception, collected evidence suggests both negative and positive health consequences, making the use of HRT a very controversial topic. ERT has been repeatedly shown to alleviate many of the abovementioned symptoms accompanying menopause; (Canderelli et al., 2007;Manson & Martin, 2001; Stearns et al., 2002). The use of HRT has also been associated with elevated mood (Carranza-Lira & Valentino-Figueroa, 1999), improved glycemic control in type II diabetes (Ferrara, Karter,

Ackerson, Liu, & Selby, 2001), or negating effects on osteoporosis (Cauley et al., 2003), colorectal cancer (Chlebowski et al., 2004) and Alzheimer's Disease (Mulnard et al. 2000). As for disadvantages, evidence of many studies has shown that women using HRT have an increased chance of developing an ovarian or breast cancer when compared to non-users (Clemons & Goss, 2001; Beral, 2007). HRT users may also be at increased risk of developing cardiovascular disorders (Rossouw et al., 2002), although this may depend on the mode of administration, the hormones used, the duration of use, and other factors.

Hormone Replacement Therapy and Cardiovascular System

Unquestionably, the greatest diversity in the results of experimental studies is on clinical impacts of HRT on the cardiovascular system. Stampfer and colleagues (1991) found that postmenopausal women using HRT appeared to have lower incidence of coronary heart disease than women who did not use HRT. Moreover, use of ERT was shown to reduce low-density lipoprotein cholesterol and increase high-density lipoprotein cholesterol, essentially decreasing the risk of developing heart disease (Espeland et al., 1998). Alternatively, Hulley and colleagues (1998) found that the use of HRT did not affect the occurrence rate of coronary heart disease after an average of 4.1 years follow up, when compared to postmenopausal women in the placebo group. Opposing results were found in a longitudinal experiment conducted by Women's Health Initiative (WHI). This study concluded that HRT increases the risk of cardiovascular disease and stroke, warning that damaging effects of HRT prevail over the helpful ones (Rossouw et al., 2002).

Results of studies on HRT effects on autonomic cardiovascular control are somewhat conflicting. Perhaps the most comprehensive study on HRT and the stress

response, carried out by Burleson and colleagues (1998), assessed the effects of both HRT and ERT alone on neuroendocrine, immune, and cardiovascular system reactions to brief psychological stressors. The outcome revealed that long-term use of ERT was related to significantly greater increase in heart rate and greater parasympathetic response, demonstrated by a greater decrease in RSA in response to the stress tasks. In contrast, in a subsequent report, Matthews, Flory, Owens, Harris, & Berga (2001) did not find an effect of HRT on heart rate response to stress, nor did they find an effect on PEP. In an experimental study by Farag and colleagues (2002), postmenopausal women were randomly assigned to three months of treatment (HRT, ERT alone, no hormones), and ANS regulation of cardiovascular responses to acute psychological stressors was examined by measuring heart rate, PEP, and HRV. Neither heart rate, nor PEP responses showed treatment effects, but high-frequency HRV, understood as a measure of vagal activity (Pomeranz et al., 1985), decreased more in response to stress only in the HRT group. Finally results of a study by Vongpatanasin, Tuncel, Mansour, Arbique, & Victor (2001) indicated that transdermal use of ERT decreased sympathetic activity, as indicated by microneurography of the peroneal nerves, in postmenopausal women. Potential explanations for the inconsistencies among this group of findings include different drug administrations (e.g. transdermal vs. oral; variations in type of estrogen, and progesterone), or duration of administration.

The current study used data collected by impedance cardiography and electrocardiograph to explore whether long-term use of HRT among postmenopausal women was related to cardiac stress reactivity to orthostatic stressors, and to further explore its effects on psychological stress responses by including an indicator of

sympathetic responding. Analyses compared heart rate, RSA, and PEP responses to the stressors between women using both estrogen and progesterone replacement, and women not using any kind of replacement therapy. Considering the reviewed literature, it was hypothesized that women using HRT would have higher stress reactivity during the psychological stress tasks, represented by greater decrease in RSA and a greater increase in heart rate in comparison to women without HRT. PEP was also studied, although a directional prediction was not made due to previous conflicting results. As noted above, an additional interest was how HRT might affect basic homeostatic responses of the ANS and how these effects may differ from effects on psychological stress responses. Therefore, heart rate, RSA and PEP responses to orthostatic stressors were compared between the two HRT groups, but no specific directional predictions were made.

Method

Participants

Thirty-eight women between the ages of 50 and 60 (*M* age=54.92, *SD*=2.76) were recruited from the community by flyers and newspaper advertisement. Criteria for participation in the study were as follows: 1) no menstrual periods for at least two consecutive years; 2) body mass index (BMI) (calculated as weight divided by squared height) not exceeding 34 (*M*=24.52, *SD*=3.34); 3) no intake of cardiovascular medication; 4) no history of cardiovascular disease (e.g. hypertension); 5) no chronic diseases (e.g. cancer, diabetes, or kidney disease); and 6) low to moderate exercise; 7) no smoking or any other forms of tobacco use; 8) averaging less than ten caffeine and ten alcoholic beverages consumed per week; 9) no speech or math phobia; 10) willingness and capability of completing the tasks. Participants were compensated $50.00 for

participation. The criteria for membership in treatment groups were then further specified as follows: for the women in no HRT group (N=20), no use of any form of hormone replacement therapy for at least two consecutive years; for women in HRT group (N=18), use of both Premarin (an estrogen supplement containing estradiol-17β, estrone, and estriol, along with three additional equine estrogens, .625 mg/day) and Provera (a supplement comprising medroxyprogesterone acetate, a synthetic progesterone, 2.5 mg/day[one participant took 5 mg/day]) on a daily or nearly daily basis for at least two consecutive years (*M* of HRT duration=3.86 years, *SD*=2.16).

Procedure

All participants were asked to refrain from use of any non- prescription drugs, alcohol, and caffeine one day prior to their scheduled laboratory session. In addition, they were asked not to eat or drink anything other than water for four hours before their appointment.To reduce variability due to daily cycles, all laboratory sessions were scheduled for approximately 4:00 PM. Upon arrival, participants were given verbal description of tasks and measurements performed during the lab session. After answering additional questions and obtaining informed consent, weight, height, initial heart rate and blood pressure were recorded. Next, band sensors and spot sensors were attached around the trunk and connected to an impedance cardiograph, and a respirometer belt was placed. After about 30 minutes of adaptation and equipment calibration, the recording was started. For the baseline, participants were asked to remain as still as possible and to refrain from talking and moving around for a period of 5 minutes. After this baseline, participants performed an orthostasis task. In this task, participants stood up for a period of 3 minutes and then immediately sat down for 3 minutes.

Following the baseline and orthostatic task, participants completed two stress tasks: math and speech[1]. To control for sequence effects, these tasks were administered in a counterbalanced order, as illustrated in Figure 1 (see Appendix A). Similar to the procedures used by Cacioppo and colleagues (1995), the second of the math or speech stressors immediately followed completion of the first, since the primary purpose of these mild brief psychological stressors was to be representative of those encountered in daily lives rather than to study individual differences between types of stressors. For the math task, participants performed a 5 minute mental arithmetic task. They were instructed to subtract as quickly and accurately as possible for five 1-minute increments. Participants were given a different number to work from each minute. Making an arithmetic error was noted and the participant was corrected. The method used for the speech task, was similar to that by Saab and colleagues (1989). It consisted of two parts: speech preparation, of 3 minutes in duration, followed by actual speech delivery, also 3 minutes in length. To introduce the speech task, participants were asked to visualize a scenario that they were falsely accused of shoplifting a belt. After being taken to the store manager by the security guard, they had a chance to defend themselves. Participants were instructed to prepare to present specific points in their defense, such as 1) telling their side of the story; 2) telling the manager what the security guard did wrong; 3)telling the manager why the security guard might have thought they stole the belt; 4) explaining how they could prove

[1]A third task (illusion task, which involves attention instead of stress) was also administered. Preliminary analyses indicated that this task could be omitted without compromising the results regarding the stress tasks. Therefore, in order to focus this report on the stress effects, results from the illusion task was omitted. However, the timing of the illusion task is illustrated in Figure 1 in order to better explain the events in the study.

that they did not steal the belt;5) describing what should happen to the security guard for the mistake;6) summarizing all of their points. During speech task instructions, the importance of using of the entire 3 minute speech delivery and making a well thought-out and intelligent speech was emphasized. To potentially increase the degree of evaluation anxiety, participants were told that the speech would be recorded and compared with the speeches of others. Recovery time of 7 minutes followed the completion of the math and speech tasks, regardless of task order. Cardiovascular measures of sympathetic and parasympathetic activity, heart rate and respiration were collected throughout all stress tasks, baseline, and recovery period. Recovery period was not analyzed for the current study.

Measures

A Minnesota Impedance Cardiograph (Model 304B; Instrumentation for Medicine, Greenwich, CT) was used to measure the ECG, basal thoracic impedance (Z_0), and the first derivative of the change in the impedance signal (dZ/dt). Disposable ECG spot electrodes were placed in the Lead II configuration for external ECG recording with the impedance cardiograph. Disposable band electrodes (Instrumentation for Medicine) were placed around the neck and chest in a tetrapolar configuration for impedance data collection (Sherwood et al., 1999). A 4-mA alternating current at 100 kHz was passed through the outer 2 electrodes, and Z_0 and dZ/dt were recorded from the 2 inner electrodes. Respiration was monitored using an EZ-AMP amplifier and strain gauge (EPM Systems, Midlothian, VA), also placed around the trunk.

The impedance cardiograph and respirometer were interfaced with a microcomputer, and the Z_0, dZ/dt, and ECG signals wereconverted to digital signals (12-

bit A/D converter, 500 Hz for dZ/dt and ECG, 250 Hz for Z_0) which were edited, reduced, and analyzed off-line. To minimize artifacts in recording, the ECG, dZ/dt, and respiration waveforms were monitored during collection. ECG was bandpass filtered (1 Hz to 10,000 Hz) prior to digitization. Minutes were rejected if artifacts comprised more than 10% of the beats. The respiration signal was bandpass filtered (.12 to .40 Hz) with an interpolated finite impulse response filter, digitized, and edited to eliminate movement artifacts.

For respiratory sinus arrhythmia (RSA), beat-by-beat heart period data were transformed to a 500-ms interval time series. RSA was derived with a Porges-Bohrer filter and confirmed using spectral analysis (valid range .12 to .40 Hz).The impedance data were ensemble averaged within 1-min epochs, and each waveform was verified or edited prior to analyses using interactive software (Kelsey & Guethlein, 1990). The PEP was quantified as the time interval in milliseconds from the onset of the ECG Q-wave to the B-point of the dZ/dt wave. Mean heart rate, RSA, PEP, and respiration rate were calculated across each 1-minute period.

For the orthostasis task, the minute-by-minute means were averaged over the last 2 minutes of the initial baseline, the last 2 minutes of standing, and the last 2 minutes of sitting, to produce means for initial baseline, standing, and sitting.For the stress task, the minute-by-minute means were averaged over the appropriate 2-minute task baseline and the 11 minutes of speech preparation, speech delivery, and math, to produce means for task baseline and aggregated stress.The time period used for the 2-minute task baseline differed depending on the sequence (see Figures 2 and 3).For sequences where the stress tasks occurred before the illusion task, the last 2 minutes of the sitting period served as

the task baseline.For sequences where the illusion task occurred before the stress tasks, the task baseline comprised the last 2 minutes of the post-illusion recovery period.

Data Analysis

Mixed analyses of variance (ANOVAs), with period as the within-subject variable and HRT group as the between-subject variable, were used to analyze the data. Follow-up single-degree-of-freedom contrasts were used to explore significant 3-level main effects and significant interactions.

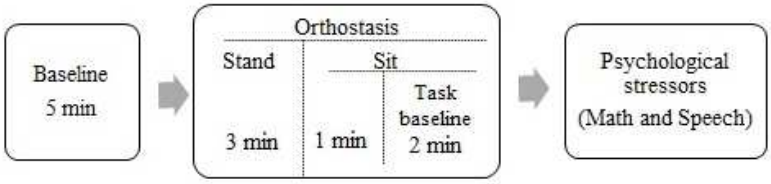

*Figure 2.*Diagram of minute order for task baseline for sequences A and B

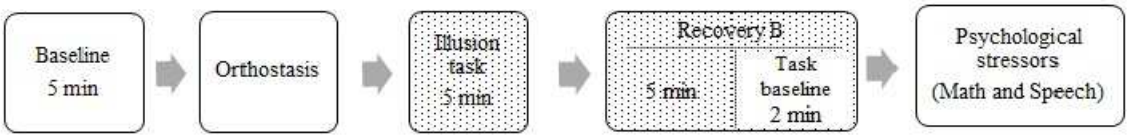

*Figure 3.*Diagram of minute order for task baseline for sequences C and D

Results

Preliminary Analyses

Order effects. As noted above, tasks were administered in counterbalanced order (Figure 1).Mixed analyses of variance (ANOVAs) were conducted to explore the effects of task sequence (speech first vs. math first) on the dependent measures. No order effects were significant, nor were there any significant interactions between order and

group.However, for PEP, both the order effect and the interaction between order and period (task baseline vs. stressor) were marginally significant. Therefore task sequence was included in the initial analysis of stress effects on PEP. For the other analyses, task sequence was not included.

Targeted Analyses

Psychological stress effects. To test the hypotheses that HRT was associated with greater heart rate and RSA reactivity, and examine potential effects on PEP, separate mixed ANOVAs were conducted[2]. For heart rate and RSA, group (No HRT vs. HRT) was the between-subjects variable and period (task baseline vs. stressor) was the within-subjects variable. Means are illustrated in Figures 4, 5, and 6. For heart rate, the main effect of period was significant, $F(1,36)=84.60$, $p<.001$, but neither the main effect of group nor the group by period interaction was significant (Fs< 1.5). For RSA, the main effect of period was significant, $F(1,36)=21.57$, $p<.001$, as was the group by period interaction, $F(1,36)=9.40$, $p=.004$, whereas the main effect of group was not significant ($F< 1$). For PEP, task sequence and the interactions between task sequence and the other variables were included in the initial analysis. The interaction between task sequence and group was not significant, so task sequence was dropped from the analysis. The main effect of period was significant, $F(1,35)=34.23$, $p<.001$, but neither the main effect of group nor the group by period interaction was significant (Fs< 1).

The significant main effects of period for heart rate, RSA, and PEP suggest that the stress tasks were effective in stimulating physiological stress responses. The

[2]As noted above, some researchers maintain that respiratory amplitude and rate should be statistically controlled in order to obtain the most valid RSA values. Inclusion of these variables in the analyses did not alter the results, so they were omitted for simplicity.

significant interaction between group and period for RSA indicates that the group differed in their RSA reactivity to the stressors. Examination of the means for the interaction shows that the HRT group had a larger decrease in RSA (greater RSA reactivity) than the No HRT group. However, post-hoc probes of the interaction show that although task baseline RSA appeared higher and stressor RSA appeared lower in the HRT group than the No HRT group, these differences were not significant.

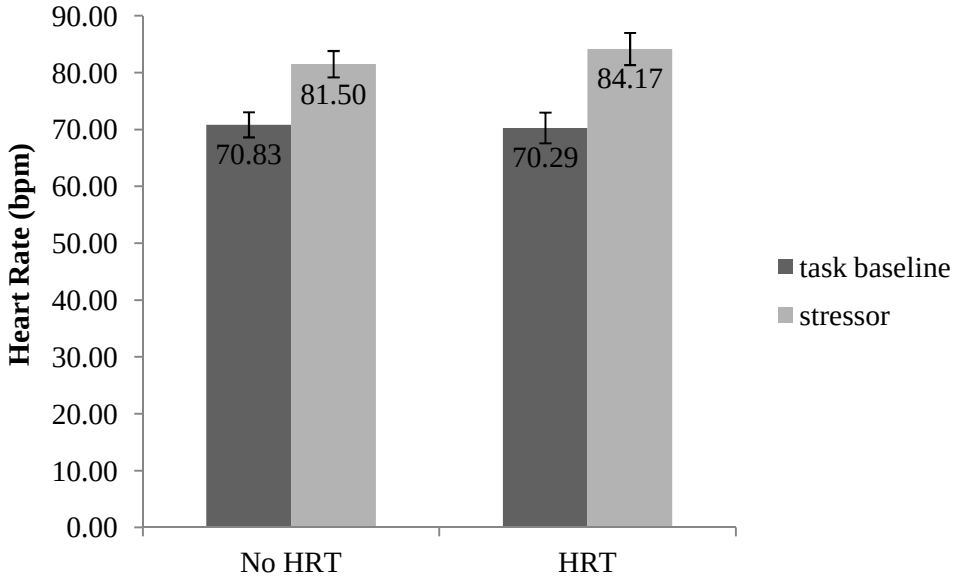

*Figure 4.*Heart rate differences between groups in response to psychological stressors

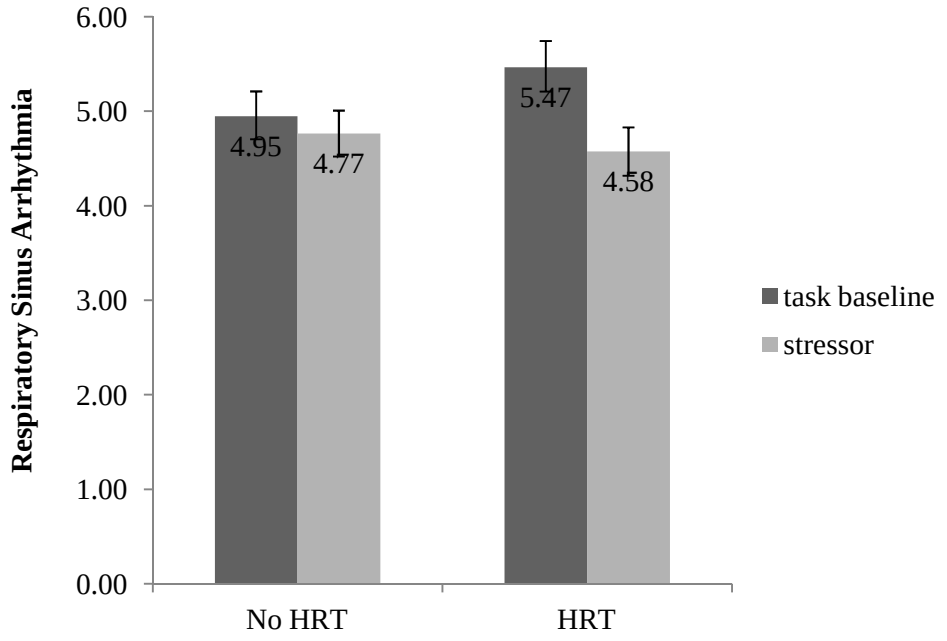

*Figure 5.*Respiratory sinus arrhythmia differences between groups in response to psychological stressors

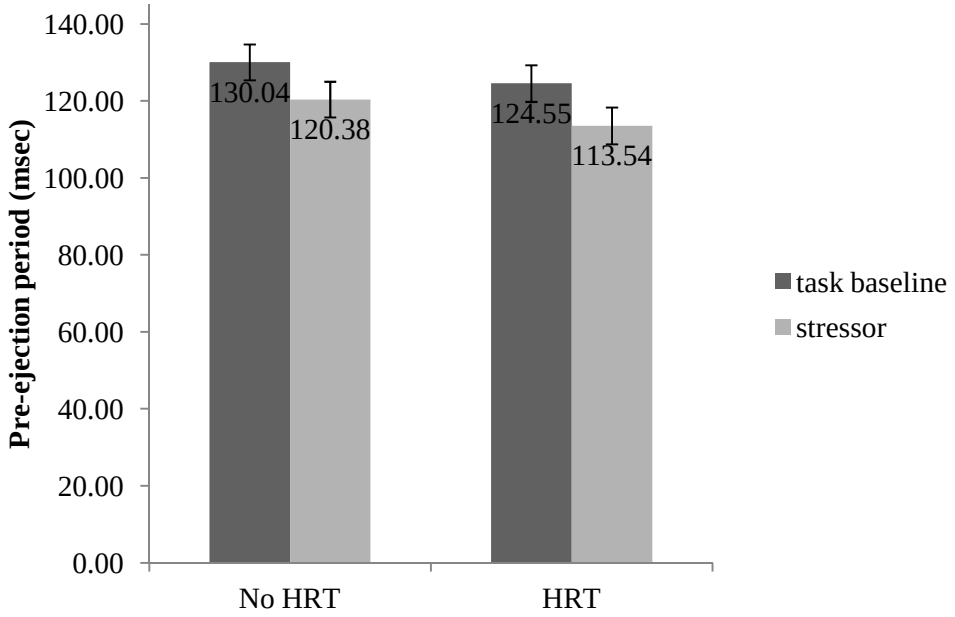

*Figure 6.*Pre-ejection period differences between groups in response to psychological stressors

Orthostasis effects. To investigate the possibility that HRT influenced basic homeostatic functions of the ANS, separate mixed ANOVAs were conducted for heart rate, RSA, and PEP. Group (No HRT vs. HRT) was the between-subjects variable and period (baseline vs. standing vs. sitting) was the within-subjects variable. Means are illustrated in Figures 7, 8 and 9. For heart rate, the main effect of period was significant, $F(2,35)=54.67$, $p<.001$, but neither the main effect of group nor the interaction was significant (Fs< 1). Post-hoc examination of the period main effect revealed that baseline was lower than standing, $t = 8.51$, $p< .001$, baseline was higher than sitting, $t = 5.55$, $p< .001$, and standing was higher than sitting, $t = 10.32$, $p< .001$. For RSA, the main effect of period was significant, $F(2,35)= 12.51$, $p<.001$, but neither the main effect of group nor the interaction was significant (Fs< 1). Post-hoc examination of the period main effect revealed that baseline was higher than standing, $t = 4.36$, $p< .001$, standing was lower than sitting, $t = 5.07$, $p< .001$, and baseline and sitting did not differ. For PEP, the main effect of period was significant, $F(2,34)= 8.25$, $p<.001$, but neither the main effect of group nor the interaction was significant (Fs< 1). Post-hoc examination of the period main effect revealed that baseline was higher than standing, $t = 3.62$, $p = .003$, standing was lower than sitting, $t = 3.91$, $p = .001$, and baseline and sitting did not differ.

The significant period effects for heart rate, RSA, and PEP reveal the expected patterns of homeostatic changes to compensate for changes in posture. The lack of significant group main effects or interactions between period and group indicate that HRT did not influence orthostatic responses.

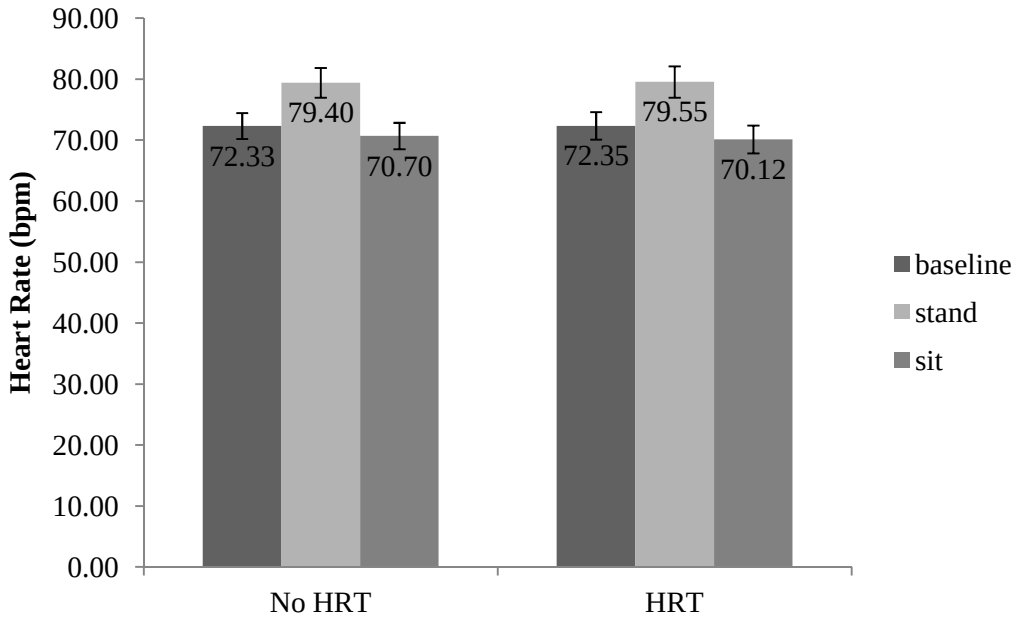

*Figure 7.*Heart rate differences between groups in response to orthostasis

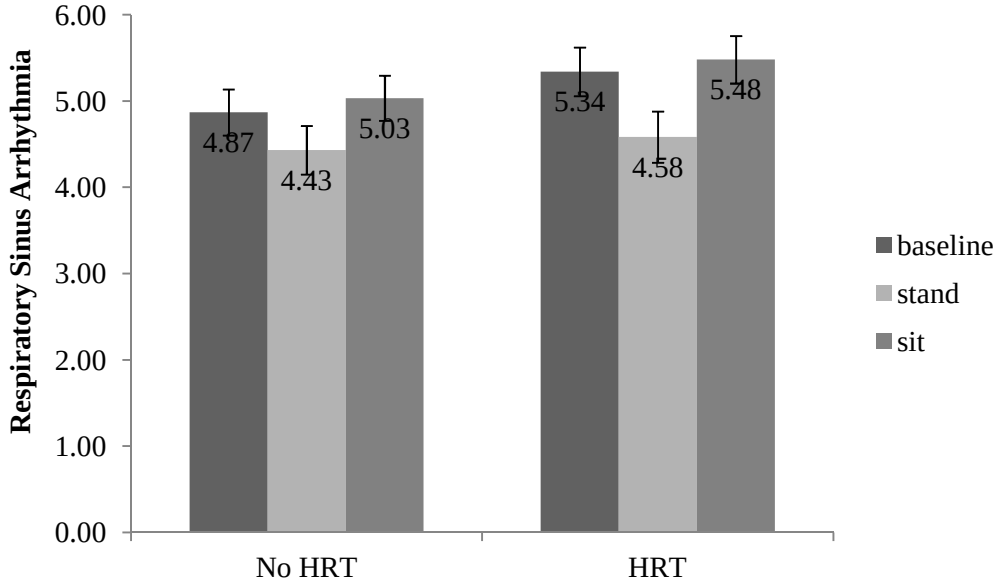

*Figure 8.*Respiratory sinus arrhythmia differences between groups in response to orthostasis

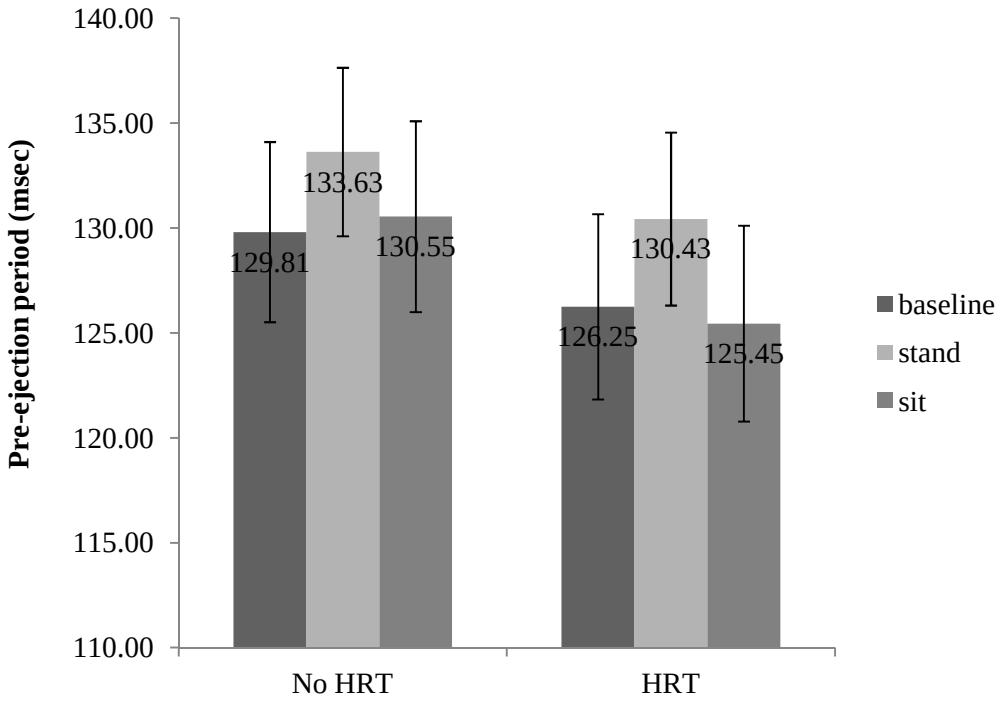

*Figure 9.*Pre-ejection period differences between groups in response to orthostasis

Discussion

The primary objective of the presented study was to investigate the influence of HRT on cardiac sympathetic and parasympathetic reactivity to orthostatic and psychological stressors in postmenopausal women. Biological markers of autonomic cardiac function, includingheart rate, RSA, and PEP were collected during all periods of the study. The research questions of this study were primarily guided by the conflicting results from previous studies on HRT and cardiovascular autonomic responses.

Response to Psychological Stressors

Results from the analyses showed significant heart rate, RSA, and PEP changes between task baseline and stressor periods. These findings suggest that the psychological tasks were effective in inducing a stress response.

Based on previous findings by Burleson and colleagues, it was hypothesized that use of HRT would result in higher heart rate reactivity to psychological stressors. In contrast to the hypothesis, heart rate increase did not vary significantly across groups. This result supports a number of previous research findings on HRT and heart rate (Matthews et al., 2001, Farag et al., 2002). It is noteworthy that the experiment by Farag and colleagues (2002), which tested the effects of short-term HRT on stress responses, also did not find heart rate differences between groups. Taken together, these results suggest that neither short- nor long-term HRT influences heart rate responses to brief psychological stressors in postmenopausal women. As noted above, Burleson and colleagues (1998), found significantly higher heart rate reactivity in women using long-term ERT. Hypothetically, the differences in findings may be accounted for by differences in the supplements used (ERT vs. HRT), suggesting that the type of supplement may affect cardiovascular functioning post menopause. In fact, Omar, Ramirez and Gibson (1995) found that progesterone may attenuate the stress response in premenopausal women, especially during pregnancy, but whether this relationship exists in postmenopausal women is not known.

The results for RSA, the cardiovascular indicator of parasympathetic cardiac activity were concurrent with the hypothesis. The significant interaction between group and period revealed that women using HRT had a greater decrease in RSA in response to the stressors, thereby exhibiting greater vagal responsiveness than women without HRT. As mentioned previously, aging is accompanied by reduced parasympathetic responsiveness (Hrushesky et al., 1984). Consequently, long term use of HRT may in

theory produce more favorable aging of autonomic cardiovascular function in comparison with non-users, preserving the parasympathetic system and therefore promoting health.

Previous studies of HRT influences on PEP, the impedance-derived measure of sympathetic cardiac reactivity, are very limited. Consequently, this study made no directional predictions on PEP between groups. Similar to results by Farag and colleagues (2002), there was not a significant interaction between period and HRT group in PEP. Based on these findings, it is reasonable to propose that long term use of oral HRT does not alter sympathetic cardiac responses. A study by Vongpatanasin and colleagues (2001) indicated that a decrease of sympathetic response resulted from use of transdermal ERT. Because the current study used oral supplements of both estrogen and progesterone, however, the results cannot be directly compared.

Findings on RSA and PEP support the notion presented by Berntson and colleagues (2008) that the SNS and PNS do not always work together in a reciprocal manner. Women using HRT showed greater parasympathetic responsiveness than women not using HRT, while their sympathetic responsiveness did not significantly differ. These findings suggest that HRT may promote beneficial altering of cardiac stress response. Moreover, based on these results, HRT may represent a potential solution to the distressing idea noted by Fukusaki and colleagues (2000) that aging increases the dependency on sympathetic control by decrease in vagal tone.

Response to Orthostasis

The significant period effects for heart rate, RSA, and PEP reveal the expected patterns of homeostatic adjustments to compensate for changes in posture. As noted above, these responses to more basic, biological stressors are initiated by signals from the

brainstem, whereas activation of the ANS in response to a psychological stressor is mediated by the cortex and limbic system (Fitzgerald et al., 2012). Thus, the lack of HRT effects in the orthostasis tasks suggests that if HRT influences vagal responses, its effects occur above the brainstem.

Limitations

Perhaps the most important methodological limitation of this study is that it was not an experiment, and therefore the results may have been influenced by a number of factors in addition to the difference in HRT. Even though this study controlled for age, BMI, and general health, selection bias cannot be discounted. Women, who choose to take HRT, as compared to women who choose not to take HRT, may have characteristics that this study failed to recognize, and that may account for the difference in RSA reactivity. In other words, these women may have higher parasympathetic responsiveness to stress prior to using HRT. Future studies should further investigate individual differences between these two groups of women. Examples of such factors may include diet, activity level, self-reported stress level, socioeconomic status, and social support.

Although the current study enhances what is known about the use of long-term oral HRT and cardiovascular autonomic functioning, further explorations are still necessary. Future research should investigate whether the use of transdermal administration of HRT would replicate the results of the present study. Such an approach would rule out any potential role of first-pass metabolism (occurring with oral HRT) in HRT effects. Additionally, it is fundamental to consider that new, diverse supplements are produced and available on the market very frequently. These products may not only vary in types, amounts, and combinations of active ingredients (estradiol, estriol, estrone

or synthetic hormones), but can also have a very particular effect (beneficial or detrimental) on the cardiac autonomic responses and body in general.

Conclusions

This study sought to acquire a better understanding of how long term use of HRT may impact cardiovascular health. Analyses of cardiovascular indicators of sympathetic and parasympathetic cardiovascular stress reactivity between groups concluded that the use of HRT does not alter heart rate or sympathetic responses. In addition, vagal responsiveness was more favorable in the HRT group, which may indicate that long term use of HRT enhances parasympathetic cardiac control.Further, if HRT does influence vagal responsiveness, its effects appear to occur in limbic or cortical regions of the nervous system. However, due to quasi-experimental design, causal relationships between the use of HRT and cardiovascular responses cannot be assumed.

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APPENDIX A

DIAGRAM OF BLOCK SEQUENCES

<table>
<tr><td rowspan="2">Sequence A</td><td colspan="8">Cardiovascular measurement period</td></tr>
<tr><td>Baseline
5 min</td><td colspan="2">Orthostasis</td><td>Math</td><td colspan="2">Speech</td><td>Recovery A
7 min</td><td>Illusion task
5 min</td><td>Recovery B
7 min</td></tr>
<tr><td></td><td></td><td>Stand
3 min</td><td>Sit
3 min</td><td>5 * 1 min</td><td>Prep
3 min</td><td>Delivery
3 min</td><td></td><td></td><td></td></tr>
<tr><td rowspan="2">Sequence B</td><td rowspan="2">Baseline
5 min</td><td colspan="2">Orthostasis</td><td colspan="2">Speech</td><td>Math</td><td rowspan="2">Recovery A
7 min</td><td rowspan="2">Illusion task
5 min</td><td rowspan="2">Recovery B
7 min</td></tr>
<tr><td>Stand
3 min</td><td>Sit
3 min</td><td>Prep
3 min</td><td>Delivery
3 min</td><td>5 * 1 min</td></tr>
<tr><td rowspan="2">Sequence C</td><td rowspan="2">Baseline
5 min</td><td colspan="2">Orthostasis</td><td>Illusion task
5 min</td><td>Recovery B
7 min</td><td>Math</td><td colspan="2">Speech</td><td rowspan="2">Recovery A
7 min</td></tr>
<tr><td>Stand
3 min</td><td>Sit
3 min</td><td>5 * 1 min</td><td>Prep
3 min</td><td>Delivery
3 min</td></tr>
<tr><td rowspan="2">Sequence D</td><td rowspan="2">Baseline
5 min</td><td colspan="2">Orthostasis</td><td>Illusion task
5 min</td><td>Recovery B
7 min</td><td colspan="2">Speech</td><td>Math</td><td rowspan="2">Recovery A
7 min</td></tr>
<tr><td>Stand
3 min</td><td>Sit
3 min</td><td>Prep
3 min</td><td>Delivery
3 min</td><td>5 * 1 min</td></tr>
</table>

Figure 1.Diagram of block sequencesacutally administered

APPENDIX B

HUMAN SUBJECTS INSTITUTIONAL REVIEW BOARD (IRB) APPROVA

Office of Human Research Administration
Vice Provost for Research
Arizona State University

Box 878206
Tempe, AZ 85287-8206
602/965-6788 FAX: 602/965-7772

M E M O R A N D U M

August 20, 1999

TO: Mary Burleson
Social and Behavioral Sciences

FROM: Charles Claiborn, Chair
Human Subjects IRB

SUBJECT: "Postmenopausal Hormone Replacement: Effects on ~~Automatic~~ Autonomic Response to Mild Stress" HS #05426-00

The Human Subjects Institutional Review Board has approved the above-referenced application for the conduct of research involving human subjects on August 18, 1999 contingent upon stipulations that have been fulfilled.

The IRB would like to remind you that Federal regulations require investigators to immediately report to the board any complaints, incidents, or injuries that may occur as part of the project.

Please sign below indicating your willingness to comply with these procedures, and return one copy with original signature to Karol Householder at the Office of Human Research Administration (mail code 8206) for our files.

kh

Mary N. Burleson
Signature

8/24/99
Date