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Influence of hormone therapy on the cardiovascular responses to stress of postmenopausal women

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Abstract

Epidemiological and psychophysiological data suggest that groups that differ in reproductive hormones and stress responses also differ in risk for cardiovascular disease. To evaluate the effects of hormone therapy on women's cardiovascular responses to laboratory stressors, 89 healthy postmenopausal women were tested twice, before and after exposure for about 8 weeks to one of the five conditions: placebo, Estratab (primarily estrone), Estratab plus Prometrium (micronized progesterone), Estratab plus Provera (synthetic progestin), and Estratest (same estrogen as in Estratab plus methyltestosterone). Results showed that women assigned to Estratab plus Prometrium and Estratest had diminished systolic blood pressure responses to stress upon retesting, whereas the other groups did not change in the level of their responses. Women assigned to Estratab plus Prometrium had diminished diastolic blood pressure responses during a speech stressor upon retesting, whereas women assigned to Estratab plus Provera increased. Our findings show that hormone therapy does affect women's stress responses, but they do not provide a simple explanation as to why groups at high and low risk for cardiovascular disease differ in reproductive hormones and stress responses.

Keywords: Reproductive hormones; Hormone therapy; Cardiovascular responses; Stress; Women

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1. Introduction

Frequent and large cardiovascular responses to psychosocial stress are posited to be a marker of or risk factor for progression of atherosclerosis (Matthew et al., 1986; Treiber et al., 2003). Available prospective data demonstrate an association between the magnitude of cardiovascular responses to stress, termed cardiovascular reactivity, and carotid atherosclerosis in men and women (Barnett et al., 1997; Everson et al., 1997; Lynch et al., 1998; Matthews et al., 1998b) and coronary atherosclerosis in a cynamolgus monkey model (Manuck et al., 1989). Other relevant findings are prospective associations between blood pressure reactivity and the development of hypertension, a risk factor for atherosclerosis (Matthews et al., 2004; Treiber et al., 2003).

Groups that differ in reproductive hormone status differ in the magnitude of their cardiovascular responses to psychosocial stress as well as their risk for atherosclerosis. Men are at higher risk for coronary heart disease (CHD) than are premenopausal women and have elevated blood pressure and neuroendocrine responses to laboratory stressors (Matthews and Stoney, 1988; Stoney et al., 1987). Furthermore, men have greater total peripheral resistance responses, whereas women have greater cardiac output responses (Allen and Matthews, 1997; Girdler et al., 1990). Postmenopausal women are at higher risk for CHD than are premenopausal women, although statistical controls for risk factors and age reduce the association substantially (Colditz et al., 1987; Gordon et al., 1978). Postmenopausal women have elevated cardiovascular and neuroendocrine responses to stress relative to premenopausal women, especially to an interpersonal stressor (Blumenthal et al., 1991; Owens et al., 1993; Saab et al., 1989). Pregnant women exhibit decreases in stress-induced diastolic blood pressure responses compared to their prepregnancy levels or to matched nonpregnant controls (Matthews and Rodin, 1992). Women who undergo bilateral oophorectomy or ovariectomized cynamologus monkeys are at elevated risk for CHD or extensive atherosclerosis (Adams et al., 1985). Women who undergo bilateral salpingo oophorectomy tend to show larger increases in blood pressure responses to stress after surgery relative to those exhibited by women who underwent hysterectomy with ovarian conservation (Stoney et al., 1997). Taken together, these findings support the possibility the reproductive hormones influence cardiovascular responses to stress, which, in turn, affect CHD risk.

One approach to test this possibility is to compare the stress responses of postmenopausal women using estrogen therapy with those who do not or who are on placebo. Table 1 describes studies that compare women using various hormone preparations, usually estrogen therapy. Two randomized trials exposed women to 1 day of high dose transdermal estrogen and reported declines in blood pressure reactivity, with one study also showing a decline in epinephrine reactivity (Del Rio et al., 1998; Manlem et al., 2002). Of the 11 randomized trials exposing women to more standard duration and types of regimens, three reported lowering of blood pressure or vascular resistance during stress with estrogen therapy as compared to placebo (Ceresini et al., 2000; Komesaroff et al., 1999; Lindheim et al., 1992, 1994) and two reported the reduced blood pressure response in subgroups (nonsmokers in Girdler et al. (2000); diabetics in Manwaring et al. (2002); c.f. McCubbin et al. (2002)). In contrast, three reports using overlapping participants found a lowering of blood pressure with estrogen that was not specific to stress

Table 1
Summary of studies relating hormones to stress responses

First author/year	Design/Ss	HT type/duration	Stress tests/measures	Significant findings
Burleson et al. (1998)	Cross-sectional/30 HT, 25 no HT, 50–80 years, groups differ by age, hysterectomy	16 Premarin; 14 Premarin + continuous Provera/≥2 years	Speech, Math/HR, RSA, Epi, NE, K	HT > no HT: ↑HR, ↓RSA, K level: E + P > E, Epi level: E > E + P, ↓RSA
Ceresini et al. (2000)	RCT crossover/10, *M* = 54 years, natural menopause	Transdermal 17-beta estradiol 50 mg, placebo/3 weeks	Digit span, Math/HR, HF HRV, LF HRV, BP, Epi, NE	HT > no HT: ↑DBP, ↓Epi, ↓LF/HF HRV
Del Rio et al. (1998)	RCT cross over/15	Transdermal estradiol 100 μg, estradiol + progesterone, placebo/1 day	Stroop/HR, BP, NE, E, K, AUC	E > Placebo: ↓Epi, E, E + P > placebo, ↓SBP
Farag et al. (2002)	RCT/40, 45–65 years, normotensive nonsmokers	Estradiol 2 mg, estradiol + Provera 5 mg, placebo/3 months	Speech/HR, HF HRV, LF HRV, BP, impedance measures	E + P > E or placebo: ↑SV, ↑CO, ↓HF HRV, ↓TPR
Girdler et al. (2000)	RCT/41 HT (9 smokers), 20 no HT (2 smokers)	Premarin 0.625 mg, Premarin + Provera 10 mg, depending on hysterectomy, placebo/6 months	Math/HR, BP, Epi, NE, K	Nonsmokers HT > placebo: ↓TPR, ↓MAP, smokers: ns
Girdler et al. (2004)	RCT/82 smokers, 39–72 years, healthy	Premarin 0.625 mg + Provera 2.5 mg, Climara 0.05 mg/day + Provera 2.5 mg, placebo/6 months	Speech, stroop, forehead cold pressor/HR, BP, NE, impedance measures	Climara > placebo: levels ↓MAP, TPR, NE ↑SV, CO, Premarin > placebo: levels ↓TPR, ↑CO
Komesaroff et al. (1999)	RCT/12, 49 years	Progynova 2 mg, placebo/2 months	Math/HR, BP, Epi, NE, K, forearm blood flow	HT > placebo: ↓SBP, DBP, K, Epi, NE
Light et al. (2001)	RCT/69, 40–69 years, 20 hypertensives, groups differ by hysterectomy	Premarin 0.625 mg, Premarin + Cycrin 10 mg, placebo/6 months	Speech, stroop, cold pressor/HR, BP, impedance measures	E & E + P > placebo: levels ↓BP, TPR, NE
Lindheim et al. (1992)	RCT/16	Estraderm 0.1 mg, placebo/6 weeks	Math, stroop, speech, cold pressor/HR, BP, NE, K	E > placebo: ↓SBP, K, NE
Lindheim et al. (1994)	RCT crossover/14	Estraderm 0.1 mg, Estraderm + Provera 10 days, 13 placebo women from Lindheim et al. (1992)/6 weeks	Math, stroop, speech, cold pressor/HR, BP, NE, K	E > E + P and placebo: ↓SBP, NE, K

Table 1 (*Continued*)

First author/year	Design/Ss	HT type/duration	Stress tests/measures	Significant findings
Manlem et al. (2002)	RCT crossover/11	Transdermal estradiol 100 μg, placebo/1 day	Math/BP, HR, Epi, NE	E > placebo: ↓DBP levels
Manwaring et al. (2002)	RCT/20 Type II diabetics, 20 nondiabetics	Premarin 0.625 mg, Premarin + Provera 5 mg, continuous, placebo/4 weeks	Math, isometric exercise/BP beat by beat	E > placebo or E + P: ↓SBP for diabetics only
Matthews et al. (1998a,b)	RCT/36 menstruating	Cycle: 4 monthly Lupron 7.5 injections, 3 months follow-up; patch: 7 monthly, Lupron injections with 3 months Estraderm; control: like cycle, except first testing after 4 months Lupron injections	Mirror image tracing, stroop, speech/HR, BP, E, NE	Nonsignificant
Matthews et al. (2001)	Study 1: cross-sectional/29 HT, 29 no HT	Various/≥4 months	Mirror image tracing, math, speech/HR, BP, impedance measure	HT > no HT, decline in SBP in math and speech; ↓PP in speech and mirror image tracing
	Study 2: RCT/38	Climara 0.1 mg, placebo patches/6 weeks	Mirror image tracing, speech/HR, BP, impedance measures	Nonsignificant
McCubbin et al. (2002)	Cross-sectional/39 HT (17 no CHD family history), 40 no HT (14 no CHD family history)	20 E + P, 19 E/≥6 months	Math/HR, BP	HT > no HT: ↓BP with CHD family history
West et al. (2001)	Cross-sectional RCT/10 compared to 32 women from Light et al. (2001) without hysterectomy	Estraderm 0.05 mg + Cycrin for 10 days a month/6 months/compared to 23 Premarin + Cycrin, 9 placebo/6 months	Speech, stroop, cold pressor/HR, BP, impedance measures/NE level	HT > placebo levels ↓BP, vascular resistance, NE ↑SV level

AUC: area under the curve; HT: hormone therapy; E: estrogen; P: progestin; RCT: randomized clinical trial; Epi: epinephrine; NE: norepinephrine; SV: stroke volume; CO: cardiac output; TPR: total peripheral resistance; HF HRV: high frequency heart rate variability; LF HRV: low frequency heart rate variability; BP: blood pressure; HR: heart rate; K: cortisol; ↑, ↓: direction of reactivity to stress.

(Girdler et al., 2004; Light et al., 2001; West et al., 2001) and several reported no differences between estrogen therapy and placebo conditions in blood pressure or vascular reactivity (Farag et al., 2002; Matthews et al., 2001; c.f. Komesaroff et al., 1999; Matthews et al., 1998a). Taken together, these findings suggest that estrogen might have a beneficial impact on blood pressure regulation, but its effect may not be unique to stress responses. This hypothesis is consistent with data showing that estrogen stimulates release of nitric oxide from the endothelium, which, in turn, leads to vasodilation (Schwertz and Penckofer, 2001; Virdis et al., 2000) and that postmenopausal women using hormone therapy have greater brachial artery flow-mediated dilation than postmenopausal women not using hormone therapy (Bush et al., 1998; McCrohon et al., 1996; c.f. Girdler et al., 2004).

Estrogen is not the only reproductive hormone that differs by sex, menopausal status, or pregnancy status. Perhaps the effects of estrogens on cardiovascular responses to stress are modified by their interaction with progesterone and androgens, which also differ by these groups. Some data suggest that progesterone, a specific steroid made by the placenta and corpus luteum, and progestins, synthetic molecules that have progestational effects, may attenuate the beneficial effects of estrogens on cardiovascular risk factors. For example, in the Postmenopausal Estrogen/Progestin Interventions Trial (PEPI, 1995), postmenopausal women randomized to Premarin (i.e., conjugated equine estrogens), plus Provera, a progestin (specifically, medroxyprogesterone acetate or MPA), had lower high-density lipoprotein cholesterol and higher total cholesterol than women randomized to placebo, Premarin alone, or Premarin plus micronized progesterone. Two hour fasting glucose levels were also elevated in the Provera group.

The influence of progestins has been tested in several randomized studies (see Table 1). Several suggest that adding MPA attenuates the beneficial effect of estrogen therapy on blood pressure responses to stress (Lindheim et al., 1994; Manwaring et al., 2002), but two studies show no differences between those on MPA versus not (Light et al., 2001; West et al., 2001) and one study showed a favorable lowering of resistance and increase in cardiac output during stress with the addition of Provera, relative to estradiol alone or placebo (Farag et al., 2002). To our knowledge, no study has evaluated the effects of progesterone (as opposed to progestin) therapy on stress responses.

Androgens added to estrogen lead to a reduction in sex hormone binding globulin, thereby increasing the bioavailability of endogenous and exogenous androgens (Simon et al., 1999). There are no studies of the influence of the addition of androgen to estrogen therapy on stress responses, which is of interest, given sex differences in androgen exposure and the relative differences in exposure in postmenopausal women. Studies indirectly relevant to the role of androgens have examined the stress responses of women with central adiposity, which is associated with high androgen levels and increased risk of coronary disease (Despres et al., 1990). Women with central adiposity show elevated blood pressure and vascular resistance responses to stress (Davis et al., 1999; Waldstein et al., 1999), with a mediating role thought to be related to hyperinsulinemia. On the other hand, some data suggest that androgen actions on the arterial wall appear to support the vasodilator and antiatherosclerotic effects of estrogens (Adams et al., 1995; Sarrel and Witta, 1997). Perhaps these latter beneficial effects are apparent only in the presence of normal levels of insulin.

The purpose of the present study was to evaluate the effects of four different hormone regimens compared to placebo on cardiovascular responses to psychological stressors of healthy postmenopausal women. Placebo and estrogen alone (Estratab, primarily estrone) conditions were included for comparison with conditions of estrogen plus the following: continuous Provera, micronized progesterone (Prometrium), and testosterone (Estratest). Women were randomized to these five conditions and tested on two occasions, one prior to randomization and one after 8 weeks of treatment. Their menopausal-related quality of life was measured to determine whether any obtained stress effects might be due to or accompanied by changes in physical or psychological function (c.f. Hammarback et al., 1985; Sarrel, 1999). We anticipated that estrogen alone would have the most favorable effect on stress-induced changes in blood pressure and vascular function, i.e. lead to a smaller response to stress after treatment, and that the addition of Provera would lessen the beneficial effect of estrogen.

2. Methods

2.1. Subjects

Participants were 97 women between the ages of 48 and 65 recruited through local advertisements. Exclusion criteria were body weight >30% than ideal body weight as determined by Metropolitan Life Tables; history of medication-dependent diabetes, heart disease, pulmonary embolism or deep vein thrombosis, liver or pancreatic disease, and hypertension; use of lipid lowering drugs, and daily use of steroids, current use of medications that would affect cardiovascular function; and unwillingness to not smoke for 12 h prior to testing. Women had not had a menstrual period or any unexplained vaginal bleeding for 12 months prior to the study, or used HT in the 3 months before study entry. Women were excluded from participation if HT use was contraindicated, i.e., history of gynecological cancer, an abnormal pap smear or mammogram, fasting serum triglyceride levels >250 mg/dl (measured for verification), known allergy to progesterone or peanuts, or an adverse previous response to HRT. If menopausal status was questionable, ovarian hormone levels were ascertained prior to participation to confirm menopausal status. The Institutional Review Board at the University of Pittsburgh approved the protocol and all participants provided written informed consent. After the first laboratory session, women were randomly assigned to one of five groups and were followed for 8 weeks until the second laboratory session. Eight women withdrew after the first session and 89 women completed the study.

2.2. Hormone treatment intervention

The treatment groups included: (a) Estratab (1.25 mg/day) and placebo pill; (b) Estratab (1.25 mg/day) and Provera continuous (5 mg); (c) Estratab (1.25 mg/day) and Prometrium, micronized progesterone (100 mg/day); (d) Estratest, combination estrogen and androgen, and placebo pill; and (e) two placebo pills. All women took the same number of pills per day for approximately 8 weeks. Following participation in the study, women assigned to the Estratab alone and Estratest groups were administered 5 mg Provera daily for 12 days to

restore the endometrium to basal levels and to counteract any potential risk of endometrial hyperplasia. Estratab is prepared from plant estrol precursors and contains primarily conjugated estrone, the major postmenopausal estrogen. Estratest is a combination of the same estrogen as in Estratab plus methyl testosterone. Provera is medroxyprogesterone acetate or MPA, a synthetic progestin. Prometrium contains micronized progesterone from plant sources and is chemically identical to progesterone of human origin.

2.3. Measures of cardiovascular function

Heart rate was monitored with EKG via electrodes placed on either side of the upper portion of the chest and the ground electrode on the left lower portion of the chest, below the heart. The EKG was filtered through a Coulbourn Instruments high gain bioamplifier with bandpass filter and 60 Hz notch filter to reduce interference. R-waves were detected electronically, and interbeat intervals were calculated in milliseconds. Heart rate was calculated from the interbeat intervals. Blood pressure was monitored with the IBS Model SD-700A automated monitor (IBS Corporation, Waltham, MA). A standard cuff was placed over the brachial artery of the dominant arm. Cuff inflation levels for the laboratory stressors were based on baseline values. Blood pressures taken by the automated device were compared with blood pressures taken with a mercury column and were within 4 mmHg. Voltages associated with systolic and diastolic blood pressure were passed through a 12-bit analog-to-digital converter displayed, and subsequently stored on an IBM-PC.

Forearm blood flow was determined using a Hokanson model EC-5R strain-gauge plethysmograph, a rapid cuff inflator (Hokanson model E20) and air source (Hokanson model AG101). Two cuffs were placed on the woman's nondominant arm on the wrist and the upper arm. An Ag-in-silastic strain-gauge 2–4 cm smaller than the woman's forearm circumference was placed around the forearm at the widest part (approximately 1–2 in. below the elbow) and lightly taped in place. With the forearm elevated at a 35° angle, the wrist cuff was inflated to 220 mmHg for at least 1 min before the upper arm cuff was inflated to 50 mmHg to occlude venous return. The upper arm cuff was rapidly inflated for 7 s during which blood flow signals were collected. The procedure was repeated every 15 s, resulting in four measurements per minute. Blood flow signals were digitized and stored on an IBM PC using the Non-Invasive Vascular Program version 5.24 (NIVP3; D. E. Hokanson) and later edited using the NIVP3 software package. Blood flow slopes were computed using diastolic troughs for the first three beats following upper arm cuff inflation. Measurements were excluded if the signals did not allow for a valid estimate of a positive slope. Based on gauge size, sensitivity settings, and slope, the NIVP3 software calculated % cc blood flow/100 cc tissue/min for each measurement. Measures were averaged across minutes. Minutes not including at least two valid inflow readings were excluded. Vascular resistance measures were calculated by dividing each mean arterial pressure taken at the time of the blood flow reading by blood flow minute average.

2.4. Measurement of menopausal quality of life

The Menopausal Quality of Life Questionnaire was developed to measure quality of life in the perimenopausal and postmenopausal years through assessing symptoms in four

domains: physical (16 items), psychosocial (7 items), vasomotor (3 items), and sexual (3 items). Examples of items in each domain are, respectively, involuntary urination when coughing or laughing; experiencing poor memory; hot flushes or flashes; and change in sexual desire. Each item was rated on a 7-point scale ranging from ''not at all bothersome'' to ''extremely bothersome''. Test–retest reliability coefficients across 1 month as well as Cronbach alpha coefficients were greater than 0.80 in the original validation study (Hilditch et al., 1996).

2.5. Experimental tasks

The experimental tasks included math, speech, star-tracing, and cold pressor tasks. The math task required participants to serially subtract the number 7 (or 13 at the second session) from an initial 4-digit number for a period of 3 min. Participants were instructed to speak out loud, and the experimenter corrected them each time they made an error. The speech task required participants to imagine that they were shopping in a mall and had stopped to examine a scarf. After they picked up the scarf, a security guard accused them of trying to shoplift it. Participants were asked to prepare and deliver a speech in which they defended their actions to a judge in court. They were given 2 min to prepare, and 3 min to deliver the speech. The star-tracing task required participants to trace the outline of a star with the nondominant hand while viewing the image in a mirror. Participants were required to trace the outline as many times as possible without sacrificing accuracy during a 3-min period, and they were told that the task would be scored for accuracy. For the cold pressor task, an icebag was placed on the participant's forehead for 60 s. Order of presentation of the tasks alternated between women and was either speech, math, star, or math, speech, star. The tasks were presented in the same order for Sessions 1 and 2. The cold pressor task was administered only at the end of the second laboratory session. The content of the speech varied at the second session. At the second session, participants were told that they had received a ticket for failing to stop at an obscured stop sign and they were asked to argue in traffic court that they should not receive the ticket because the sign was hidden from view. In addition, to counteract the effects of habituation to the testing procedures that we have obtained in our previous studies (e.g., Matthews et al., 1998a), an experimenter remained in the room during the speech at the second session, and whenever possible, the first and second sessions were conducted by different experimenters.

2.6. Procedure

All participants attended an initial session in the morning after an overnight fast. After obtaining informed consent, a blood sample was obtained to determine serum triglyceride levels. Participants also completed several baseline psychosocial and demographic questionnaires, including the menopausal quality of life questionnaire. Both laboratory sessions were conducted in the afternoon and all participants agreed to refrain from exercise and smoking on the day of the testing session, and from caffeine consumption for the 4 h prior to the session. They were also instructed to eat a light lunch 3 h prior to the laboratory sessions, and were given a list of possible meals when they attended the initial session. Height, weight, waist/hip ratio and skinfold thickness for the calculation of

percentage body fat were assessed at the first laboratory session. The laboratory protocol commenced with the research assistant placing EKG electrodes on the chest and a blood pressure cuff on the dominant arm. Then participants rested quietly for 20 min and completed several psychosocial questionnaires. Participants then completed the standardized laboratory protocol that included a 15-min baseline, the three experimental tasks separated by 10-min inter-task rest periods, and a final 15-min rest period. During the baseline and the inter-task periods, participants listened to soothing music through headphones.

Blood pressure was assessed at minutes 9, 11, and 13 of the baseline period, minutes 11 and 13 of the final rest period, and at minutes 4, 6, and 8 of the inter-task rest periods. Blood pressure was assessed after 30 and 90 s of the speech delivery period, the math task and the star task. After the final 15-min rest period at Session 2, the forearm blood flow equipment was attached and blood flow measurements were obtained during the 1 min prior to the cold pressor task, during the 1 min of the cold pressor task, and then during a 2 min recovery period, with blood pressure measured at the beginning of the 1 min baseline, at the beginning of the 1 min task period, and twice during the 2 min recovery period.

Women were asked to record on a calendar if they forgot to take their medication and to return any unused medication in the original containers. We did not verify levels of hormones in blood because there are no assays for some of these compounds. In between the two laboratory sessions, the women were contacted by phone and asked about the experience of any side effects. After the second experimental session, participants were thanked for their participation and paid $100.00.

2.7. Data reduction and analytic strategy

Blood pressures were averaged for the baseline and task periods separately. Heart rate was averaged for the last 6 min of the baseline period, for the entire task period and for the last 3 min of the final baseline. For the forearm blood flow measurement, values were averaged with the baseline, task, and recovery periods separately. Change scores were computed by subtracting average baseline levels from average task levels. Chi-square analyses were used to compare groups on demographic categorical variables and on questionnaires where response categories were categorical. A series of one-way analyses of variance (ANOVA's) with group (five treatment groups) as between factors were conducted to assess group differences on continuous variables prior to randomization to medication group. Analyses for forearm blood flow were based on women with complete data during all periods at Session 2. Comparisons of those with complete data versus incomplete data showed no significant differences in other baseline or reactivity measures. Repeated measures analyses of variance were conducted on the women with session (1, 2) and task (star, speech and math) as a within factors and group as a between factor. For blood flow measures, repeated measures analyses of variance were conducted with trial period (baseline, cold pressor, and recovery period) as the within factor and group as the between factor. A one-way ANOVA was also conducted on the change in quality of life scores between sessions with group as the between factor. Change scores were used because they were normally distributed whereas the scores at each session were not. Greenhouse-Geisser degree of freedom corrections for repeated measures were used to control for possible

violations of the homogeneity of variance assumption. Post-hoc analyses were conducted with simple effects analyses. All tables show unadjusted means and standard errors, and alpha level of <0.05 was considered significant.

3. Results

3.1. Demographic and anthropometric characteristics

The characteristics of the entire sample at the first laboratory session are shown in Table 2. There were no significant group differences in age, years of education, race, marital status, highest educational degree attained, current occupational status, family income, or family history of high blood pressure, diabetes, angina, myocardial infarction, other heart disease, stroke or cancer. Eight women were smokers and 1–3 smokers were in each group. There were no group differences in baseline SBP, DBP, or HR at either Session 1 or Session 2. However, baseline SBP ($F(1,84) = 4.82$, $P = 0.03$) and DBP levels ($F(1,84) = 12.41$, $P = 0.001$) were overall greater at Session 1 than Session 2. HR was similar at both sessions.

3.2. Cardiovascular reactivity to speech, math, and star-tracing tasks

Change scores for blood pressure and heart rate during the speech, math and star-tracing tasks at Sessions 1 and 2 are shown in Tables 3–5. HR reactivity during stress declined at Session 2 relative to Session 1 ($F(1,75) = 6.60$, $P = 0.01$, respectively), whereas BP reactivity remained the same. During the speech task, participants had greater increases in SBP relative to the mirror image tracing task ($F(1,82) = 49.73$, $P < 0.001$); greater increases in DBP relative to the math ($F(1,82) = 9.51$, $P = 0.003$) and mirror image tracing tasks ($F(1,82) = 5.29$, $P = 0.03$); and greater increases in HR relative to the math ($F(1,75) = 9.91$, $P = 0.002$) and mirror image tracing tasks ($F(1,75) = 67.0$, $P < 0.001$). DBP reactivity was greater during the mirror image tracing task than during the math task ($F(1,82) = 9.51$, $P = 0.003$), while HR increases were greater during the math task relative to the mirror image tracing task ($F(1,75) = 9.01$, $P = 0.004$).

Table 2
Mean (S.E.M.) of sample characteristics at Session 1 (prior to randomization) by treatment group

Characteristic	Placebo ($n = 16$)	Estratab ($n = 18$)	Estratab/Provera ($n = 18$)	Estratab/Prometrium ($n = 17$)	Estratest ($n = 20$)
Age (years)	57.0 (0.94)	57.0 (1.1)	57.5 (0.90)	56.0 (0.89)	56.7 (0.79)
Height (in.)	62.9 (0.65)	64.0 (0.42)	64.5 (0.65)	64.1 (0.57)	63.8 (0.62)
Weight (lbs)	152.6 (6.2)	150.7 (4.3)	157.2 (5.0)	151.2 (4.6)	149.5 (6.5)
Waist/hip ratio (in.)	77 (0.01)	82 (0.03)	80 (0.02)	78 (0.02)	79 (0.01)
Education (years)	14.1 (0.60)	14.1 (0.52)	16.0 (0.87)	14.9 (0.62)	15.9 (0.57)
Baseline					
SBP (mmHg)	119.3 (3.6)	116.7 (4.7)	119.2 (5.4)	112.0 (4.6)	108.7 (3.6)
DBP (mmHg)	75.9 (2.5)	74.0 (1.9)	73.5 (2.6)	73.0 (1.7)	71.9 (2.0)
HR (bpm)	71.1 (1.6)	66.8 (1.9)	63.9 (2.1)	68.1 (1.5)	67.5 (1.8)

Table 3
Mean (S.E.M.) increase in systolic blood pressure (mmHg) during tasks from baseline by treatment group at Sessions 1 and 2

Group	Speech		Math		Star	
	Session 1	Session 2	Session 1	Session 2	Session 1	Session 2
Placebo	27.5 (4.0)	27.0 (3.9)	16.5 (3.4)	17.5 (3.3)	15.4 (3.3)	16.7 (3.0)
Estratab	28.6 (3.6)	28.8 (3.5)	20.2 (3.1)	22.3 (3.0)	19.1 (3.0)	20.0 (2.8)
Estratab/Provera	23.7 (3.7)	27.6 (3.6)	19.1 (3.2)	21.0 (3.1)	21.1 (3.1)	20.6 (2.8)
Estratab/Prometrium	26.7 (3.7)	22.9 (3.6)	16.9 (3.2)	13.3 (3.1)	22.6 (3.1)	15.2 (2.8)
Estratest	29.8 (3.4)	22.2 (3.3)	19.9 (3.0)	11.7 (2.8)	19.2 (2.8)	16.6 (2.6)

Table 4
Mean (S.E.M.) increase in diastolic blood pressure (mmHg) during tasks from baseline by treatment group at Sessions 1 and 2

Group	Speech		Math		Star	
	Session 1	Session 2	Session 1	Session 2	Session 1	Session 2
Placebo	10.7 (2.6)	8.0 (2.3)	8.0 (1.8)	6.9 (1.5)	9.3 (2.0)	11.3 (2.1)
Estratab	14.9 (2.3)	13.3 (2.1)	11.4 (1.6)	9.7 (1.4)	10.9 (1.8)	11.1 (1.9)
Estratab/Provera	9.2 (2.4)	16.2 (2.1)	7.8 (1.7)	8.3 (1.4)	10.9 (1.9)	10.3 (1.9)
Estratab/Prometrium	16.3 (2.4)	10.7 (2.1)	8.4 (1.7)	9.0 (1.4)	13.3 (1.9)	9.0 (1.9)
Estratest	12.6 (2.2)	11.9 (2.0)	9.3 (1.5)	6.4 (1.3)	9.7 (1.7)	9.5 (1.8)

The primary study hypothesis was that type of hormone therapy would affect the change in women's cardiovascular responses from Session 1 to 2 over and above the effect of repeated testing observed in women on placebo, which would be confirmed by significant Session by Group or Session by Group by Task interaction terms, with appropriate follow-up contrasts. There was a significant Session by Group interaction for SBP change during stress ($F(4,328) = 2.87$, $P = 0.03$). Simple comparisons showed that women prescribed Estratest declined in SBP reactivity from Session 1 to Session 2, $P = 0.007$, and women prescribed Estratab/Prometrium tended to decline in SBP reactivity from Session 1 to 2, $P = 0.095$. The other groups did not change in SBP reactivity across sessions (see Fig. 1).

Table 5
Mean (S.E.M.) increase in heart rate (bpm) during tasks baseline by treatment group at Sessions 1 and 2

Group	Speech		Math		Star	
	Session 1	Session 2	Session 1	Session 2	Session 1	Session 2
Placebo	15.9 (2.1)	14.2 (1.8)	10.6 (1.5)	9.4 (1.2)	7.2 (1.5)	7.1 (1.4)
Estratab	9.7 (1.9)	9.2 (1.6)	7.0 (1.3)	7.1 (1.0)	4.9 (1.3)	5.0 (1.2)
Estratab/Provera	13.9 (1.9)	10.7 (1.6)	10.2 (1.4)	8.0 (1.0)	8.6 (1.3)	6.8 (1.2)
Estratab/Prometrium	11.4 (1.9)	11.3 (1.6)	6.3 (1.4)	7.9 (1.0)	7.6 (1.3)	6.4 (1.2)
Estratest	13.3 (1.8)	10.6 (1.5)	8.2 (1.3)	6.4 (1.0)	6.5 (1.2)	4.5 (1.1)

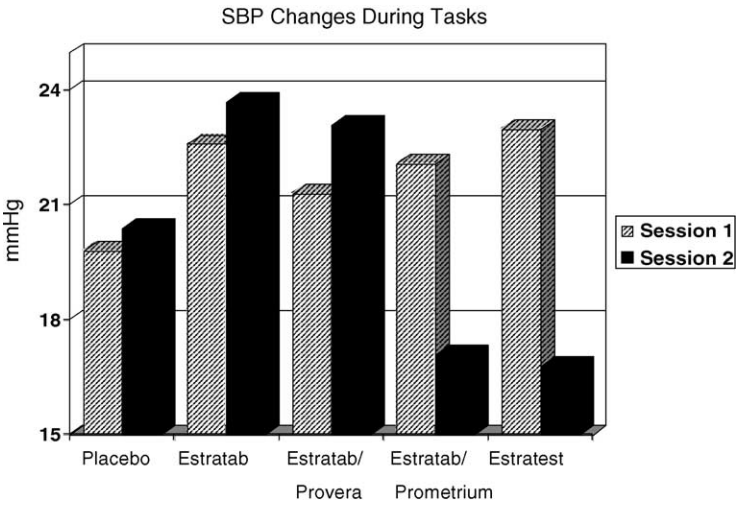

Fig. 1. Mean systolic blood pressure increase (mmHg) averaged across all tasks from baseline at Sessions 1 and 2 for women assigned to five treatment groups.

For DBP, there was a significant Session by Task by Group interaction ($F(8,256) = 2.58$, $P = 0.01$) (see Table 4). Analyses conducted separately by task revealed a significant Session by Group interaction for the speech task only ($F(4,332) = 3.05$, $P = 0.02$). Women taking Estratab/Provera increased in DBP reactivity from Session 1 to 2 ($P = 0.002$), whereas women taking Estratab/Prometrium tended to decline in DBP reactivity ($P = 0.06$) from Session 1 to 2. The other groups showed no change between sessions. There were no significant group interactions in HR reactivity with session or treatment group (Table 5).

3.3. Forearm blood flow and vascular resistance responses to cold pressor at Session 2

Table 6 shows mean rates of forearm blood flow and vascular resistance by period. Analyses revealed a marginally significant effect of period on forearm blood flow ($F(2,100) = 2.66$, $P = 0.08$) and a significant effect on forearm vascular resistance ($F(2,100) = 7.34$, $P = 0.002$). Simple comparisons showed that forearm blood flow declined somewhat from baseline to the cold pressor task ($P = 0.08$) and increased from the

Table 6
Mean (S.E.M.) of forearm blood flow (ml/100 ml forearm volume per min) and forearm vascular resistance (mean arterial pressure/forearm blood flow) at baseline, task and recovery by treatment group

Group	Forearm blood flow			Forearm vascular resistance		
	Baseline	Cold pressor	Recovery	Baseline	Cold pressor	Recovery
Placebo	2.0 (0.26)	1.7 (0.21)	1.9 (0.25)	51.1 (5.5)	71.3 (5.6)	56.9 (4.6)
Estratab	2.1 (0.23)	2.1 (0.18)	2.3 (0.22)	45.8 (4.8)	50.9 (5.0)	47.8 (4.0)
Estratab/Provera	2.2 (0.21)	2.1 (0.17)	2.5 (0.21)	45.7 (4.8)	52.0 (5.0)	45.0 (4.0)
Estratab/Prometrium	2.2 (0.31)	2.1 (0.25)	2.0 (0.30)	39.9 (7.1)	40.0 (7.3)	46.8 (5.9)
Estratest	2.0 (0.21)	1.7 (0.17)	1.9 (0.21)	46.5 (4.8)	57.6 (5.0)	50.4 (4.0)

cold task to the recovery period ($P = 0.03$), which was similar to the blood flow levels at the baseline levels. Forearm vascular resistance increased from baseline to the cold pressor task ($P = 0.002$) *and* remained elevated throughout the recovery period, compared to baseline ($P = 0.05$). There were no significant Group or Group by Period interactions for forearm blood flow or forearm vascular resistance, although women assigned to placebo ($M = 59.8$) had higher resistance across all periods than Estratab alone ($M = 48.2$, $P = 0.05$), Estratab/Provera ($M = 47.6$, $P = 0.04$), and Estratab/Prometrium ($M = 42.2$, $P = 0.02$) groups. The mean of Estratest group was 51.5 and was similar to the mean of women assigned to placebo.

3.4. Changes in menopause-related quality of life

The mean symptom scores across all groups at the initial evaluation were 2.6 for vasomotor, psychological, and physical function indices and 2.2 for the sexual function index. None of the groups differed. Groups varied in the change in menopause-related quality of life for the vasomotor ($F(4,82) = 3.32$, $P = 0.01$) and physical indices ($F(4,79) = 2.51$, $P = 0.05$). Regarding the vasomotor index, compared to women assigned to placebo ($M = -0.38$), women assigned to Estratab only ($M = -2.57$, $P = 0.03$) or Estratest ($M = -2.02$, $P = 0.05$) groups improved in the vasomotor index with treatment. Women taking placebo improved in physical function ($M = -0.70$), relative to women taking Estratab and Prometrium ($M = -0.01$, $P = 0.05$). The analyses for group differences in the psychosocial and sexual function indices were nonsignifcant.

4. Discussion

The overarching aim of our work has been to understand why groups that differ in reproductive hormone levels also differ in the magnitude of their cardiovascular and neuroendocrine responses to stress. We initially focused on conducting naturalistic experiments by examining changes in stress responses during and after pregnancy (Matthews and Rodin, 1992), and before and after hysterectomy/bilateral oophorectomy (Stoney et al., 1997), to evaluate the possible role of changes in reproductive hormones. Encouraged by our findings, we then conducted several randomized experiments. In one study of healthy premenopausal women (Matthews et al., 1998a), we suppressed ovarian hormones to early postmenopausal levels by a GnRH agonist for 3 months and then evaluated the effects of a temporary suppression of ovarian hormones on the women's cardiovascular and neuroendocrine responses compared to their responses during the follicular stage of the menstrual cycle. We found no changes in the magnitude of stress responses with changes in ovarian hormone levels. However, the absolute differences in hormones between groups at the time of testing were small by design and the effects of habituation on stress responses were substantial. Thus, in our next experiment, we evaluated the stress effects of exogenous hormones, which can result in a more substantial change in circulating hormone levels, and randomized postmenopausal women to transdermal estradiol or placebo for 6–8 weeks. Circulating estradiol levels increased five-fold from pretreatment in women assigned to active treatment whereas women on

placebo declined slightly. There were no effects of estrogen treatment on blood pressure, heart rate, or impedance-derived measures of cardiac output and total peripheral resistance.

Our next step was to investigate the influence of reproductive hormones other than estrogen on stress responses, hence our present investigation. Our results showed some support for hormone therapy impacting stress responses. Women assigned to Estratab/Prometrium and Estratest diminished in systolic blood pressure reactivity across tasks across sessions, whereas women assigned to placebo, Estratab alone or Estratab/Provera did not change. Women assigned to Estratab/Prometrium diminished somewhat in diastolic reactivity to the speech task across sessions, whereas those assigned to Estratab/Provera increased in diastolic reactivity to the speech task from across sessions; other groups remained the same. No effects were obtained for heart rate reactivity. Although not statistically significant in the full group analyses, it is also noteworthy that women assigned to the Estratab/Prometrium as well as Estratest had lower vascular resistance prior to, during, and following the cold pressor test than women assigned to placebo. The Estratab/Prometrium women did not improve in menopause-related quality of life more than other groups. In fact, women assigned to Estratab alone or Estratest showed the greatest improvement in vasomotor symptoms. Taken together, these findings suggest that the addition of Prometrium to Estratab has the most beneficial effect on blood pressure reactivity and the benefit is not secondary to improvement in psychological and physical well being.

Why does Prometrium have a different effect on blood pressure reactivity compared to Provera? Evidence suggests that progesterone causes smooth muscle cell relaxation as well as vasodilation in the presence of physiologic dose of estradiol (Jiang et al., 1992). In contrast, progestins lead to tonic contraction of smooth muscle cells, with some suggestion that they induce a vasospastic response (Sarrel, 1999). Other data suggest that progesterone can result in endothelium-independent relaxation of the coronary arteries at high concentrations and natural progesterone, unlike MPA, does not antagonize the effect of estrogen on coronary atheroma or coronary vasospasm (Bellinger et al., 1998; Miyagawa et al., 1997). Progesterone enhances the beneficial effect of estrogen on exercise-induced myocardial ischemia in female coronary patients, whereas MPA does not (Rosano et al., 2000).

Two other features of our data are noteworthy. First, women assigned to Estratab alone did not differ from women assigned to placebo in blood pressure reactivity, although they did show less vascular resistance overall. These data suggest that a weak estrogen, like estrone, when administered alone does not improve stress responses, a finding consistent with a substantial number, but not the majority of prior hormone studies. Vongpatanasin et al. (2001) reported that Premarin did not decrease sympathetic activity and ambulatory blood pressure in postmenopausal women, whereas transdermal estradiol did reduce sympathetic nerve discharge and modestly reduce blood pressure, raising that hormone effects may vary by whether first-pass hepatic metabolism is involved. Second, women assigned to Estratest declined in systolic blood pressure responses to stress across sessions and concomitantly experienced a reduction in the discomfort of vasomotor symptoms. Perhaps the decline in systolic reactivity is secondary to the improvement in symptoms. However, analyses adjusting for the effect of improvement in symptoms did not alter that

pattern of results (results not shown). Thus, our findings suggest that addition of androgen may have benefits on blood pressure responses to psychological stress. As suggested earlier, the effects of androgens may be quite different in the absence or presence of central adiposity and its associated hyperinsulinemia. Clearly our finding needs further confirmation.

4.1. Implications for the cardiovascular reactivity hypothesis and risk for heart disease

The impetus for this line of research was to understand the role of stress responses in the differential risk for coronary disease of men versus women, postmenopausal versus premenopausal women, and women who have a bilateral oophorectomy versus hysterectomy or natural postmenopausal status. Our current and prior results in Table 1 show that the effects of hormones vary by type of hormone therapy, mode of delivery, and type of stressor. Thus, our understanding of the role of reproductive hormones on stress responses is becoming more sophisticated but also more complex. In the context of understanding the role of stress responses on coronary risk, however, reproductive hormones' impact on stress responses probably does not account in large part for the differential risk of the above groups.

It is also noteworthy that a greater understanding of the complex role of hormone therapy is also occurring in the scientific community interested in prevention of and treatment for heart disease. Observational data suggest that women who use hormone therapy have lower risk of heart disease than women who do not (Stampfer and Colditz, 1991) and short-term clinical trials have shown that hormone therapy has beneficial effects on some cardiovascular risk factors (The Writing Group for PEPI, 1995). Many observers have noted that hormone users are healthier prior to use of the hormones and have suggested that part of apparent benefit of hormones is due to the characteristics of hormone users as opposed to a direct pharmacologic effect (Matthews et al., 1996). Recent clinical trials show that estrogen does not protect against coronary disease morbidity, stroke, and mortality and may in fact increase risk for cardiovascular events (Hulley et al., 1998; The Writing Group for the Women's Health Initiative Investigators, 2002, 2004). There are many suggestions offered why this might have occurred, e.g. the specific estrogen preparation was not a good choice, progestins had a negative effect, hormone therapy was started after the critical period of development of coronary disease, crossover effects of those randomized to placebo or active treatment, and route of administration, oral versus nonoral. Nonetheless, our findings, like those of the randomized clinical trials, show the utility of experimental methods in identifying the precise effects of reproductive hormones on risk for women's coronary disease.

In summary, our experimental study showed that estrogen therapy alone did not have a beneficial effect on the magnitude of women's cardiovascular stress responses. Women who were administered a progesterone or an androgen in addition to estrogen experienced a reduction in blood pressure responses during stress. Provera did not provide any benefit and by some indicators increased stress responses. Based on our own data as well as others, we conclude that the influence of estrogen therapy on stress responses is dependent on multiple factors, including interactions with progestins and androgens.

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