Postmenopausal Hormone Replacement: Effects on Autonomic, Neuroendocrine, and Immune Reactivity to Brief Psychological Stressors

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Objective: Postmenopausal status increases some aspects of women's physiological responses to psychological stress; however, the influences of chronic hormone replacement with estrogen and progestogen on these responses are not known. We investigated possible effects of long-term estrogen replacement therapy (ERT), both with and without progestogen, on physiological reactivity to brief laboratory stressors. *Method:* We studied three groups of postmenopausal women: 16 on estrogen alone, 14 on estrogen and progestogen, and 25 control participants receiving no replacement therapy. Cardiovascular, neuroendocrine, and immune data were collected at baseline and after speech and math tasks. *Results:* In all groups, the stressors reduced vagal cardiac control (indexed by respiratory sinus arrhythmia); increased heart rate and plasma epinephrine, adrenocorticotropic hormone, and cortisol levels; and altered T lymphocyte response (measured by mitogen-induced cell proliferation), natural killer cell lysis, and circulating leukocyte subsets. Women on either type of ERT had higher total cortisol levels (reflecting an estrogen effect on cortisol binding globulin) and greater mitogen-induced blastogenesis across measurement periods than controls. They also showed greater vagal withdrawal and less decline in mitogen-stimulated blastogenesis in response to the stressors. Combined estrogen and progestogen was associated with higher epinephrine and lower circulating total lymphocytes, T cells, and CD4+ T cells across measurement periods, and with intermediate levels of vagal withdrawal in response to the stressors. *Conclusions:* Long-term ERT was associated with enhanced parasympathetic responsiveness to stress, suggesting possible reduced demand for potentially detrimental sympathetic activation; and with higher overall levels and smaller stress-induced reductions of mitogen-stimulated blastogenesis, suggesting up-regulated T cell function. **Key words:** Psychological stress, postmenopausal hormone replacement, estrogen, cardiovascular reactivity, respiratory sinus arrhythmia; cellular immune response.

ERT = estrogen replacement therapy; SAM = sympathetic adrenal medullary; HPA = hypothalamic pituitary adrenal; PBL = peripheral blood lymphocytes; NK = natural killer; ConA = concanavalin A; PHA = phytohemagglutinin; BMI = body mass index; ECG = electrocardiography; RSA = respiratory sinus arrhythmia; HR = heart rate; CORT = cortisol; ACTH = adrenocorticotropic hormone; EPI = epinephrine; NEPI = norepinephrine; E-only = estrogen alone; E+*p* = estrogen plus progestin

INTRODUCTION

Research suggests that postmenopausal status increases some aspects of women's physiological responses to psychological stress. For example, several studies using laboratory stressors, such as speech or serial subtraction, have reported higher cardiovascular or catecholamine stress reactivity in postmenopausal women (eg, Refs. 1–4). It has been assumed that this increase in reactivity is associated with reduced estrogen levels, raising the possibility that estrogen replacement might be ameliorative. Two experiments with estradiol treatment supported this view, as the pattern of reactivity for women given estradiol has been similar to that found in premenopausal women. Using a randomized, placebo-controlled design, Lindheim et al. (1) showed that transdermal estradiol treatment eliminated significant cardiovascular (systolic and diastolic blood pressure, heart rate) and neuroendocrine (adrenocorticotropic hormone, cortisol, norepinephrine, and androstenedione) responses to laboratory stressors. A randomized, double-blind, placebo-controlled, crossover study in young men also found reduced heart rate, systolic blood pressure, epinephrine, and norepinephrine reactivity after transdermal estradiol treatment (4). Thus, evidence for an estradiol-mediated reduction in sympathetic-adrenal medullary (SAM) responses to laboratory stressors is limited but consistent, and there is some evidence for a reduction in the response of the hypothalamic-pituitary-adrenal (HPA) axis. Heightened stress reactivity has been proposed to contribute to cardiovascular disease (eg, Refs. 5, 6); thus an estrogen-related reduction in stress reactivity may account for some health-protective effects of postmenopausal hormone replacement therapy.

Previous studies of estrogen effects on stress reactivity have been performed within a short time (eg, days or weeks) after the onset of estrogen therapy, early in the process of physiological adaptation to treatment. Furthermore, they have used 17-β estradiol, either oral or transdermal, as the hormone medication. Clinically, however, postmenopausal hormone replacement is often prescribed for several years. In addition, the most commonly prescribed estrogen replacement drug is Premarin (Wyeth-Ayerst, Princeton, NJ), a mixture of six estrogenic substances, only one of which is 17-β estradiol. To maximize the clinical relevance of this research, we assessed whether long-term treatment with this oral estrogen would affect cardiovascular and neuroendocrine stress responses in the same way as reported for short-term treatment with estradiol.

In addition, because unopposed estrogen replacement has been linked to endometrial cancer, the use of a progestogen is now recommended for women without hysterectomies. The effects of progestogens on the response to acute stress have not been studied. Progesterone can antagonize many of the documented effects of estrogen (eg, Refs. 7, 8). Furthermore, some of the improvements in cardiovascular indicators associated with hormone replacement therapy are smaller when combination treatments are used (eg, Ref. 9). In light of these data, and of the widespread use of combined estrogen and progestogen replacement in postmenopausal women, our sec-

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ond objective was to compare cardiovascular and neuroendocrine responses to brief stress in women taking combination therapy with those in women taking estrogen alone.

Our third goal was to investigate the effects of hormone replacement therapy on immune parameters in postmenopausal women. Gonadal steroids influence the immune system both in animals and in humans (for reviews, see Refs. 10–12). Differences in plasma levels of these hormones probably contribute to the many documented sex differences in immune function and immune-related disorders. In general, immune function in females is up-regulated compared with that in males. Many animal studies have documented changes in acquired and innate immune responses resulting from gonadectomy, and sex steroid replacement typically restores presurgical function (see Ref. 13). Similar results have been found in women undergoing surgical menopause and hormone replacement (eg, Ref. 14). Sex steroids have complex and wide-ranging effects on immune function in vitro. For example, the addition of physiological levels of estradiol to pokeweed mitogen-stimulated human peripheral blood lymphocytes (PBL) enhanced the number of B cells secreting immunoglobulin M (IgM) antibody; the effect was apparently mediated by inhibition of CD8+ T lymphocytes (15), which possess estrogen receptors (16). On the other hand, progestogens can be immunosuppressive (17, 18). These and other data suggest that estrogens and progestogens may modulate both baseline and stress-reactive immune function.

To address these issues, we studied three groups of postmenopausal women: Women who did not use hormone replacement, women who were taking Premarin alone, and women who were taking Premarin and Provera (Upjohn, Kalamazoo, MI). They performed verbal subtraction and speech preparation and delivery, two mildly stressful psychological tasks. To assess their SAM and HPA activity and aspects of their cellular immune function, we measured heart rate, respiratory sinus arrhythmia (an index of cardiac parasympathetic control [19, 20]), respiration rate and amplitude; plasma catecholamine, adrenocorticotropic hormone, and cortisol levels; numbers and percentages of circulating lymphocyte subsets; natural killer (NK) cell cytotoxicity; and lymphocyte responsiveness to the mitogens concanavalin A (ConA) and phytohemagglutinin (PHA). Measures were taken before and after the stressors, providing estimates of baseline function and stress reactivity.

METHOD

Participants

Fifty-five women between 50 and 80 years old were recruited from the community by advertisement, and were paid $75.00. Participants met these criteria: a) no menstrual periods for at least 2 years; b) body mass index (BMI) (calculated as weight in kilograms divided by squared height in meters) less than 34 (ie, not severely obese); c) no chronic disease; d) no diagnosed hypertension; e) no use of cardiovascular medication; f) no tobacco use; g) on average, less than 10 caffeine and 10 alcohol drinks consumed per week; h) on average, less than 5 hours of exercise per week; i) adequate nutrition (as measured by serum albumin and ferritin; j) no speech, math, or needle phobia; and k) no current illness. The criteria for membership in the three hormone replacement therapy groups were as follows: for the women taking no estrogen replacement (No ERT; $N = 25$), no hormone replacement therapy for at least 2 years; for the group taking only an estrogen supplement (E-only; $N = 16$), use of Premarin alone on a daily or approximately daily basis for at least 2 years; and for the women taking both estrogen and progestin (E+P; $N = 14$), use of Premarin and Provera on a daily or approximately daily basis for at least 2 years. Estrogen replacement is considered essential when the ovaries are removed. Side-effects of progestin treatment (eg, uterine bleeding) lead many to reject it unless necessary; however, increased risk of uterine cancer associated with unopposed estrogen effectively mandates progestin in women without hysterectomies. Our sample reflected current medical practice and the population at large: 73% of the No ERT group and 100% of the E+P group had no reproductive surgery, and 94% of the E-only group had hysterectomies. Mean time since hysterectomy was 19.6 years (range 5.0 to 37.0 years). Table 1 displays other demographic and lifestyle characteristics of the three groups of participants.

TABLE 1. Participant Characteristics

Measure	No Estrogen Replacement		Estrogen Only		Estrogen Plus Progestogen	
	N	Mean[a] ± SEM	*N*	Mean ± SEM	*N*	Mean ± SEM
Age	25	$67.92_a \pm 1.60$	16	$59.06_b \pm 1.90$	14	$60.93_b \pm 1.24$
Body mass index[a]	25	$26.07_{ab} \pm 0.77$	16	$26.69_a \pm 1.03$	14	$23.85_b \pm 1.02$
Weekly physical activity[b]	25	$2.64_a \pm 0.45$	16	$2.44_a \pm 0.58$	14	$3.07_a \pm 0.62$
Hours slept previous night	22	$6.55_a \pm 0.29$	16	$6.13_a \pm 0.35$	14	$6.86_a \pm 0.38$
Alcohol consumed						
Past 48 hours[c]	25	$0.40_{ab} \pm 0.15$	16	$0.13_a \pm 0.13$	14	$0.79_b \pm 0.32$
Past 12 hours[c]	25	$0.08_a \pm 0.06$		$0.00_a \pm 0.00$		$0.00_a \pm 0.00$
Caffeine consumed past 48 hours[d]	25	$2.68_a \pm 0.75$	16	$3.19_a \pm 0.78$	14	$3.64_a \pm 0.71$
Years postmenopause[e]	25	$19.48_a \pm 1.94$	16	$16.69_{ab} \pm 2.28$	14	$10.29_b \pm 1.76$
Years ERT[f]			13	$15.08_a \pm 2.91$	13	$6.15_b \pm 1.31$
Estrogen dosage[g]			15	$0.43_a \pm 0.03$	12	$0.41_a \pm 0.03$

Means with different subscripts are significantly different at $p < .05$ or less.

[a] Body mass index = weight (kg)/[height (m)]^2.

[b] Self-reported number of times per week of 20 or more minutes of activity vigorous enough to cause perspiration.

[c] Expressed as number of equivalents to one "drink" (eg, 12 oz beer or 6 oz wine).

[d] Expressed as number of equivalents to one "cup" (eg, 8 oz coffee or 12 oz soda).

[e] Number of years since last menses, whether natural or surgical.

[f] Number of years of continuous estrogen replacement up until present.

[g] Expressed as [Premarin (mg)/day/weight (lb)] × 10^2.

Procedure

Each participant was reminded not to exercise or use alcohol or nonprescription drugs during the day before the study, and not to eat or drink anything other than water from midnight until her appointment at either 7:45 AM or 10:15 AM (counterbalanced). Upon her arrival, informed consent was obtained; height, weight, initial blood pressure, and pulse were measured; spot electrodes for electrocardiography (ECG) were attached; and a 20-gauge indwelling catheter was inserted into an antecubital vein. The participant then spent 20 to 25 minutes completing a set of questionnaires, and rested in a supine position for 15 additional minutes to allow adaptation to the setting. After adaptation, she was seated upright and relaxed quietly while ECG was recorded for 6 minutes. A 50-ml blood sample was then collected for endocrine and immune assays.

After these baseline measures, the participants received instructions for both the math and speech stress tasks, which were then administered in counterbalanced order with the second stressor immediately following the first. ECG was recorded throughout the 3-minute speech preparation, the 3-minute speech delivery, and the 6-minute serial subtraction task. Blood (55 ml) was drawn immediately after both stressors were finished.

Speech Stressor. The speech task was similar to that used by Saab et al. (3). The participant was asked to imagine that she was in a department store when a security guard falsely accused her of shoplifting. She was given 3 minutes to prepare a 3-minute talk covering a set of specific points, and asked to give an intelligent and well-thought-out speech because it would be recorded and compared with the speeches of other participants.

Math Stressor. The participants performed a 6-minute mental arithmetic task similar to that used by Cacioppo et al. (21), which entails six 1-minute serial subtraction problems performed continuously. Participants were told to work as quickly and accurately as possible and prompted to speed up their responses at Minutes 2, 4, and 6. Errors were corrected by the experimenter. To maintain moderate task difficulty and maximal involvement, the number that was subtracted during each minute was contingent on the participant's performance during the preceding minute (ie, accurate performance led to more difficult subtraction problems).

Measures

Cardiovascular Assessment. ECG was measured with a Minnesota Impedance Cardiograph (Model 304B) and converted to digital signals (12-bit A/D converter, 500 Hz) that were edited, reduced, and analyzed off-line (22). The ECG data were monitored during collection and bandpass filtered (1 Hz to 10,000 Hz) before digitization. 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 (.12 to .40 Hz). Respiration was monitored using an EZ-AMP amplifier and strain gauge (EPM Systems, Midlothian, VA). The signal was bandpass filtered (.12 to .40 Hz) with an interpolated finite impulse response filter, digitized, and then edited to eliminate movement artifacts. Mean heart rate (HR), RSA, and respiration rate and amplitude were calculated across each 1-minute period, and these minute-by-minute means were averaged over the baseline and stressor periods.

Neuroendocrine Measurement. Cortisol (CORT) and adrenocorticotropic hormone (ACTH) were tested in heparinized plasma stored at −70° until assay. CORT was measured with a fluorescent polarization technique (TDX; Abbott Laboratories, Chicago, IL) with intra- and interassay coefficients of variation (CVs) of 10% or less. ACTH was measured using an immunoradiometric assay (Allegro HS-ACTH kit, Nichols Institute) with a sensitivity of 1 pg/ml, an intraassay CV of 3%, and an interassay CV of 8% or lower.

Levels of epinephrine (EPI) and norepinephrine (NEPI) in plasma treated with ethylenediaminetetraacetic acid (EDTA) were determined by high-performance liquid chromatography using a Waters system (Millipore; Waters Division, Marlborough, MA) with electrochemical detection. Alumina was used for extraction; extraction efficiency, evaluated with a dihydroxybenzylamine (DHBA) standard, is 60% to 90%. A Waters catecholamine eluent was used for the mobile phase. For EPI, the sensitivity of this system is 10 pg/ml, and the intra- and interassay CVs are 12% or less. For NEPI, the sensitivity is 20 pg/ml and the CVs are 9% or less.

Immune Measurement. Complete blood counts and differentials were performed on each blood sample, using 5 ml of EDTA-treated blood, by the Clinical Immunology Laboratory at the Ohio State University Hospital. Mononuclear cells were obtained from heparinized blood and the percentages of T lymphocytes (CD3+), T helper cells (CD4+), T cytotoxic cells (CD8+), and NK cells (CD56+) were determined using monoclonal antibodies (Coulter, Miami, FL) by fluorescence activated cell sorter (FACS) analysis using routine procedures as described previously (23).

NK cell cytotoxicity was measured by incubation of various concentrations of PBLs with ^{51}Cr-labeled K-562 target cells as previously described (24). Values were standardized at the 37.5:1 E:T cell ratio with a logistic regression (25).

Mitogen-stimulated PBL activity was assessed using the Cell Titer 96 AQ_{ueous} Non-Radioactive Cell Proliferation Assay (Promega, Madison, WI), which determines the number of viable proliferating cells by colorimetry. The assay is based on the conversion of the tetrazolium salt, 3-(4,5-dimethylthiazol-2-yl)-5-(3-carboxymethoxyphenyl)-2-(4-sulfophenyl)-2H-tetrazolium (MTS), into a formazan that is soluble in tissue culture medium. MTS is converted into the formazan by dehydrogenase enzymes found in metabolically active cells. The quantity of formazan product, and thus the amount of 490 nm absorbance, is directly proportional to the number of living cells in culture. Optical density (OD) is measured directly from a 96-well plate.

Samples were set up in triplicate on 96-well plates, with ConA (Sigma, St. Louis MO) and PHA (Sigma) at final concentrations of 10.0 μg/ml, 5.0 μg/ml, and 2.5 μg/ml. Fifty microliters of sample cells from a stock solution of a concentration of 1×10^6 cells/ml in RPMI-1640 medium, supplemented with 5% fetal bovine serum (FBS) was added to 50 μl of each mitogen dilution and the media control. The plates were incubated in an atmosphere of 5% CO_2 at 37°C, with humidity, for approximately 68 hours. After incubation, 20 μl of a 20:1 solution of MTS:phenazine methosulfate was added to the plates. The plates were then incubated for an additional 4 hours, after which the OD was recorded using a Titertek Multiscan MCC plate reader (Helsinki, Finland). The background absorbance of the plate was removed by using a reference wavelength of 650 nm, per the manufacturer's suggestion.

Data Analysis

Several questions guided our data analyses. First, we wished to examine whether the psychological stress tasks led to physiological changes typically associated with mild acute psychological stress in previous studies, so we evaluated baseline and poststress scores in a repeated-measures framework. Second, we wanted to determine whether long-term use of an estrogen supplement (in this case, Premarin) was associated with differences in physiological reactivity to brief stressors. Thus, our analyses included a priori single degree of freedom contrasts between the group of women taking no estrogen replacement (No ERT) and the group taking either estrogen alone or estrogen and progestogen (ERT). Third, we wanted to determine whether the inclusion of a progestogen supplement (in this case, Provera) would alter the relationship between long-term estrogen replacement and stress responses. To examine this possibility, we used a priori single degree of freedom contrasts between the group of women taking estrogen alone (E-only), and the group taking estrogen and progestogen (E+P). Finally, our general goal of exploring the influence of long-term ERT on immune function was addressed by examining both baseline and reactivity levels of immune parameters using these sets of comparisons. The planned contrasts were orthogonal, and were performed simultaneously in a repeated-measures

TABLE 2. Mean (± SE) Baseline and Reactivity Levels of Physiological Measures for All Participants

Measure	N	Baseline	Reactivity
Cardiovascular parameters			
Heart rate (bpm)[a]	53	66.16 ± 1.12	10.62 ± 0.93
Respiration rate (breaths/min)[a]	50	14.26 ± 0.29	1.72 ± 0.26
Respiration amplitude (units)[a]	50	375 ± 24	−83 ± 16
Respiratory sinus arrhythmia (ln[msec^2])[a]	53	5.44 ± 0.16	−0.54 ± 0.13
Neuroendocrine parameters			
Epinephrine (pg/ml)[b]	52	24.25 ± 1.54	3.71 ± 1.11
Norepinephrine (pg/ml)	54	524.50 ± 23.08	10.35 ± 13.67
Adrenocorticotropic hormone (pg/ml)[b]	51	9.20 ± 0.61	2.49 ± 0.99
Cortisol (μg/dl)[a]	54	11.75 ± 0.64	1.59 ± 0.46
Immune parameters			
Concanavalin A blastogenesis (OD units)	53	0.26 ± 0.02	−0.01 ± 0.01
Phytohemagglutinin blastogenesis (OD units)[c]	53	0.39 ± 0.02	−0.02 ± 0.01
Natural killer cell cytotoxicity (per cent lysis)[a]	52	56.54 ± 2.37	6.30 ± 1.01
Natural killer cell per cent[b]	53	12.22 ± 0.81	3.93 ± 0.67
Natural killer cell number (per ml blood)[a]	48	214 ± 22	119 ± 17
Lymphocyte number (per ml blood)[a]	49	1720 ± 80	370 ± 80
CD3+ cell number (per ml blood)[c]	53	1308 ± 58	134 ± 58
CD4+ cell number (per ml blood)	48	948 ± 44	131 ± 41
CD4+ cell per cent[a]	54	55.83 ± 1.19	−4.12 ± 0.59
CD8+ cell number (per ml blood)[a]	48	370 ± 35	138 ± 25
CD8+ cell per cent[a]	54	21.60 ± 1.05	2.23 ± 0.38
CD4+/CD8+ ratio[a]	54	3.06 ± 0.21	−0.59 ± 0.10

OD = optical density.
[a] F ratio for stressor, $p < .01$.
[b] F ratio for stressor, $p < .05$.
[c] F ratio for stressor, $p < .10$.

framework using baseline and poststress scores. Previous research (eg, Refs. 1–4) suggested that our cell sizes of 13 to 16 would provide sufficient power to detect the expected effects.

RESULTS

Preliminary Analyses

To control for possible confounds with the hormone replacement groups, we first examined potential differences among the groups in several demographic and lifestyle characteristics (see Table 1). Significant differences between the No ERT and ERT groups were found for age, $F(1,52) = 16.65$, $p < .001$, $\eta^2 = .24$, and years since menopause, $F(1,52) = 6.35$, $p = .02$, $\eta^2 = .11$. A priori contrasts also revealed significant differences between the E-only and the E+P groups in BMI ($F(1,52) = 3.93$, $p < .05$, $\eta^2 = .07$), alcohol consumed within the past 48 hours ($F(1,52) = 4.69$, $p < .04$, $\eta^2 = .08$), and years of hormone replacement therapy ($F(1,48) = 15.76$, $p < .002$, $\eta^2 = .25$). Therefore, these variables were tested as covariates in the next set of preliminary analyses. After accounting for group membership, years past menopause was a significant predictor of respiration rate and CORT, and alcohol consumed in the past 48 hours was a significant predictor of NK cell number. Therefore, years past menopause and alcohol consumption were retained as covariates in additional analyses of the corresponding variables. This is a conservative procedure, as it tends to remove experimental effects.

Stress and Hormone Replacement Effects

We next performed the a priori contrasts described above on baseline and poststress values of the physiological parameters in a repeated-measures framework. Our first goal was to determine whether the stress tasks resulted in changes similar to those that have been found after acute stress in previous research.

Stress Effects. Posttask changes in physiological variables were largely similar to those found in previous research using brief psychological stressors. Cell means for the effects of the stress tasks on cardiovascular, neuroendocrine, and immune activity, across all participants, are shown in Table 2. As expected, the tasks led to an increase in HR ($F(1,49) = 153.05$, $p < .0001$, $\eta^2 = .76$) and respiratory rate ($F(1,46) = 23.77$, $p < .0001$, $\eta^2 = .34$), and to a decrease in RSA ($F(1,51) = 25.19$, $p < .0001$, $\eta^2 = .33$) and respiratory amplitude ($F(1,48) = 25.37$, $p < .0001$, $\eta^2 = .35$). Repeated-measures analysis also revealed task-induced increases in EPI ($F(1,49) = 10.16$, $p < .002$, $\eta^2 = .17$) and ACTH ($F(1,45) = 5.32$, $p < .03$, $\eta^2 = .11$). In addition, controlling for years postmenopause, there was a significant task-induced increase in CORT ($F(1,48) = 3.79$, $p < .05$, $\eta^2 = .07$). The task-induced increase in NEPI was not significant, despite the fact that the power to detect the expected effect was at least .90. We have no complete explanation for this finding; however, we suspect that it may be because of an artificially high baseline in some participants due to postural realignment immediately before baseline blood sampling.

NK cell cytotoxicity[1] increased significantly after the stress tasks ($F(1,47) = 34.54$, $p < .0001$, $\eta^2 = .42$), as did both

[1] Repeated-measures analyses including both measurement period and either concentration of mitogen (for ConA and PHA) or E:T ratio (for NK cell lysis) were used to evaluate proliferative responses to both mitogens and NK cell cytotoxicity. No interactions were significant; therefore, only the mitogen concentrations and E:T cell ratio resulting in maximal proliferative response or percent lysis across measurement periods was retained in the analyses. For ConA, this concentration was 2.5 μg/ml; for PHA, it was 10.0 μg/ml. For NK cell cytotoxicity, it was an E:T cell ratio of 75.1.

number and percentage of NK cells ($F(1,41) = 42.31$, $p < .0001$, $\eta^2 = .51$ and $F(1,50) = 31.15$, $p < .0001$, $\eta^2 = .38$, respectively). PHA-induced blastogenesis showed a marginally significant decrease ($F(1,50) = 2.99$, $p < .09$, $\eta^2 = .06$). There was no overall difference from baseline to poststress for ConA-induced blastogenesis ($F < 1$, $\eta^2 = .07$).

The stress tasks resulted in significantly more circulating total lymphocytes ($F(1,45) = 8.53$, $p < .01$, $\eta^2 = .16$), and there was a marginal increase in circulating T lymphocytes ($F(1,45) = 3.13$, $p < .09$, $\eta^2 = .07$). Analyses of T cell subset percentages showed that the percentage of cytotoxic T cells (CD8+) increased significantly poststress ($F(1,49) = 32.51$, $p < .0001$, $\eta^2 = .40$), whereas the percentage of helper T cells (CD4+) decreased significantly ($F(1,49) = 47.74$, $p < .0001$, $\eta^2 = .49$), as did the ratio of circulating helper to cytotoxic T cells (CD4+/CD8+) ($F(1,49) = 36.11$, $p < .0001$, $\eta^2 = .42$). Analyses of absolute numbers of T cell subsets produced comparable results with the exception of the number of T helper cells (CD4+), which was unaffected by the stress tasks.

Estrogen Replacement vs. Control. The use of estrogen replacement therapy, either alone or in combination with progestogen, moderated the short-term stress effect on four of the physiological variables. In addition, six variables displayed significant or marginal differences in either baseline or overall levels as a function of estrogen replacement. Baseline and reactivity means for all of these variables, as a function of use of estrogen therapy, are shown in Table 3.

There was a significant overall difference between the ERT and No ERT groups for HR ($F(1,49) = 6.40$, $p < .02$, $\eta^2 = .12$). Women in the ERT group had higher HR across both measurement periods than women in the No ERT group. In addition, a significant interaction between measurement period (baseline vs. poststress) and the contrast between No ERT and ERT groups ($F(1,49) = 8.65$, $p < .01$, $\eta^2 = .15$) was detected for HR. Although both group increases were highly significant, women who were using ERT had a larger increase in HR after the stressor than did women not using ERT ($F(1,50) = 120.73$, $p < .0001$, $\eta^2 = .71$, and $F(1,50) = 34.10$, $p < .0001$, $\eta^2 = .41$, respectively). RSA also demonstrated a significant interaction between measurement period and the contrast between No ERT and ERT groups ($F(1,51) = 14.20$, $p < .001$, $\eta^2 = .22$). Women who were using ERT had a significant decrease in RSA after the stressor ($F(1,52) = 30.77$, $p < .0001$, $\eta^2 = .37$), whereas in women who were not using ERT, poststressor levels of RSA were not significantly different from prestress levels ($F < 1$, $\eta^2 = .00$). Baseline RSA did not differ between groups ($F < 1$, $\eta^2 = .00$). An overall difference in respiration amplitude was found between the No ERT and ERT groups ($F(1,48) = 8.05$, $p < .007$), in which the hormone users had higher overall respiration amplitude than the nonusers. RSA amplitude is influenced by both respiration rate and volume, making it important to note that there was no interaction between hormone use and measurement period for either respiration rate (controlling for years postmenopause) or respiration amplitude. Therefore, the difference in RSA reactivity could not be explained by a difference in respiration reactivity. Because tachycardia can result from either sympathetic activation or parasympathetic withdrawal, or both, we compared HR responses with the stressors while controlling for RSA responses. With RSA as a covariate, the HR reactivity difference between the No ERT and ERT groups was not significant ($F(1,48) = 1.88$, $p < .20$, $\eta^2 = .04$). Because RSA is a measure of vagal cardiac control, this suggests that the HR increase may have been largely related to vagal withdrawal.

Controlling for years postmenopause, significant main effects also were found for the a priori contrast on both baseline and overall CORT levels ($F(1,49) = 8.09$, $p < .007$, $\eta^2 = .14$, and $F(1,48) = 7.30$, $p < .01$, $\eta^2 = .13$, respectively). Plasma levels of CORT were higher at baseline and across measurement periods in the ERT group than in the No ERT group.

Significant differences between women taking either form of estrogen replacement and women not taking estrogen replacement were found for both baseline and overall levels of ConA- and PHA-induced blastogenesis. The blastogenic response to ConA was higher in the ERT group than in the No ERT group both at baseline ($F(1,51) = 12.02$, $p < .002$, $\eta^2 = .19$), and across measurement periods ($F(1,50) = 17.06$, $p < .0001$, $\eta^2 = .25$), respectively. Similar results were found for the blastogenic response to PHA: baseline, ($F(1,51) = 18.90$, $p < .0001$, $\eta^2 = .27$); and overall, ($F(1,50) = 21.24$, $p < .0001$, $\eta^2 = .30$). In addition to the main effects described above, analysis of ConA-stimulated blastogenesis revealed a significant interaction between measurement period and the contrast between No ERT and ERT ($F(1,50) = 8.96$, $p < .01$,

TABLE 3. Group Mean (± SE) Baseline and Reactivity Levels of Physiological Measures That Differ as a Function of Estrogen Replacement Therapy

Measure	No Estrogen Replacement			Estrogen Replacement		
	N	Baseline	Reactivity	*N*	Baseline	Reactivity
Heart rate (bpm)[a]	23	63.93 ± 1.43	7.55 ± 1.07	29	67.43 ± 1.59	12.66 ± 1.29
Respiration amplitude (units)[b]	24	316 ± 39	−64 ± 25	26	430 ± 24	−99 ± 20
Respiratory sinus arrhythmia (ln[msec^2])[a]	24	5.37 ± 0.25	−0.09 ± 0.16	29	5.49 ± 0.21	−0.91 ± 0.17
Cortisol (μg/dl)[b]	23	9.32 ± 0.48	2.07 ± 0.66	30	13.70 ± 0.95	1.22 ± 0.64
ConA blastogenesis (OD units)[a,b]	23	0.20 ± 0.02	−0.04 ± 0.01	30	0.31 ± 0.02	0.02 ± 0.01
PHA blastogenesis (OD units)[b,c]	23	0.31 ± 0.03	−0 04 ± 0.01	30	0.46 ± 0.03	−0.01 ± 0.02
Natural killer cell per cent[b]	24	13.57 ± 1.38	4.33 ± 0.81	30	10.98 ± 0.89	3.60 ± 0.99

ConA = concanavalin A; OD = optical density; PHA = phytohemagglutinin; ERT = estrogen replacement therapy.
[a] F value for interaction between stressor and ERT group, $p < .05$.
[b] F value for main effect of ERT group on baseline or overall values, $p < .05$.
[c] F value for interaction between stressor period and ERT group, $p < .15$.

$\eta^2 = .15$). The No ERT group declined significantly after the stressor ($F(1,51) = 7.81, p < .01, \eta^2 = .13$), whereas the ERT group did not change ($F(1,51) = 1.98, p < .20, \eta^2 = .04$). The pattern of results for PHA-stimulated blastogenesis was similar. Values in the No ERT group decreased significantly after the stressor ($F(1,51) = 6.28, p < .02, \eta^2 = .11$), whereas the ERT group did not change ($F < 1, \eta^2 = .01$), although the interaction between measurement period and the contrast between No ERT and ERT was not significant ($F(1,50) = 2.21, p < .15, \eta^2 = .04$). Thus, in the women using estrogen therapy, levels of mitogen-induced blastogenesis were initially higher, and they declined less in response to the stress tasks. Because mitogen-stimulated blastogenesis is an indicator of T cell responsiveness to antigens, this suggests up-regulated cellular immune function in the ERT group.

Percentage of NK cells was marginally lower in the ERT group than in the No ERT group at baseline ($F(1,51) = 3.84, p < .07, \eta^2 = .07$), and across measurement periods ($F(1,50) = 3.55, p < .07, \eta^2 = .07$), although there was no difference between these groups in NK cell cytotoxicity ($F < 1, \eta^2 = .00$).

Estrogen Alone vs. Estrogen and Progestogen. Fewer differences were found between the E-only and E+P groups. The baseline and reactivity means are shown in Table 4. There was a significant interaction between measurement period and the contrast between E-only and E+P for RSA ($F(1,51) = 4.30, p < .04, \eta^2 = .08$). Although significant in both groups, the stress-induced decline in RSA was greater in the E-only group than in the E+P group ($F(1,52) = 31.46. p < .0001, \eta^2 = .38$ and $F(1,51) = 6.29, p < .02, \eta^2 = .11$, respectively).

Levels of EPI were higher at baseline and across measurement periods in the E+P group than in the E-only group ($F(1,49) = 13.79, p < .001, \eta^2 = .22$ and $F(1,49) = 10.46, p < .002, \eta^2 = .18$, respectively). Total number of circulating lymphocytes and number of T cells (CD3+) at baseline were higher in the E-only group than in the E+P group ($F(1,49) = 8.49, p < .006, \eta^2 = .15$ and $F(1,49) = 8.16, p < .007, \eta^2 = .14$, respectively). Finally, the number of T helper cells (CD4+) was higher in the E-only group at both baseline ($F(1,49) = 12.50, p < .001, \eta^2 = .20$), and across measurement periods ($F(1,45) = 5.03, p < .03, \eta^2 = .10$).

DISCUSSION

In the older postmenopausal women evaluated for this study, the brief psychological stress tasks led to heightened autonomic activation (as indicated by increased HR and a reduction of RSA), increased adrenomedullary activity (as indexed by increased plasma EPI concentration), and increased activation of the HPA axis (as evidenced by increased plasma ACTH and CORT levels). These findings are largely consistent with previous research on the effects of brief laboratory stressors (eg, Refs. 1, 21, 26, 27). Across all three of our groups, the tasks also resulted in increased NK cell cytotoxicity and percentage, increased CD8+ percentage, and decreased CD4+ percentage and CD4+/CD8+ ratio. These stress-related changes in cellular immune parameters also are in agreement with effects commonly reported in previous studies (see Ref. 27 for review; also see Refs. 21, 28–30).

Enhanced stress responsiveness in both the HPA and SAM axes has been linked with negative health consequences (eg, Refs. 5, 31). Lower stress reactivity in the SAM system has been reported for premenopausal women in previous reports (eg, Refs. 1, 3), and short-term estradiol treatment led to reduced reactivity in both axes (1, 4). One of our primary interests in the current study was to investigate whether long-term postmenopausal estrogen replacement with conjugated estrogens would be associated with similar potentially health-protective changes in stress reactivity. We found differences between hormone users and non-users in two major areas. First, the No ERT group had smaller HR increases and RSA decreases in response to the stress tasks than did the ERT group. Second, the No ERT group showed reduced mitogen-stimulated blastogenesis after the stressors, whereas the ERT group had no such decrease in mitogen responsiveness. We also found one difference in stress reactivity between women taking unopposed estrogen and those using both conjugated estrogens and a progestogen—the E+P group had a smaller decrease in RSA poststress than did the E-only group.

Tachycardia can arise from sympathetic activation, hence higher HR reactivity has often been interpreted as one marker of increased sympathetic reactivity (eg, Ref. 32). Viewed in this light, our finding that women in the ERT group displayed higher HR reactivity to the stressors than did women in the No ERT group could be seen as inconsistent with previous studies in which premenopausal status or exogenous estrogen has been associated with reduced, not enhanced, stress reactivity in the SAM system. Examination of the RSA data, however, provided another explanation. In the current sample, long-term use of either type of ERT was associated with greater vagal responsiveness, and this group difference in RSA reactivity could not be explained by group differences in respiratory reactivity. Even when controlling for age, women taking either type of hormone replacement showed greater parasympathetic withdrawal in response to the stressor than

TABLE 4. Group Mean (± SE) Baseline and Reactivity Levels of Physiological Measures That Differ as a Function of Progestogen Therapy

Measure	Estrogen Only			Estrogen and Progestogen		
	N	Baseline	Reactivity	*N*	Baseline	Reactivity
Respiratory sinus arrhythmia (ln[msec^2])[a]	15	5.84 ± 0.30	−1.23 ± 0.27	14	5.12 ± 0.28	−0.57 ± 0.15
Epinephrine (pg/ml)[b]	15	17.87 ± 1.42	3.93 ± 2.36	14	31.72 ± 4.18	3.29 ± 2.29
Lymphocyte number (per ml blood)[b]	13	2000 ± 160	40 ± 180	13	1470 ± 110	300 ± 100
CD3+ cell number (per ml blood)[b]	13	1497 ± 108	−71 ± 128	13	1111 ± 107	179 ± 79
CD4+ cell number (per ml blood)[b]	13	1148 ± 89	−104 ± 102	13	799 ± 66	115 ± 60

ERT = estrogen replacement therapy.
[a] *F* value for interaction between stressor and ERT group, $p < .05$.
[b] *F* value for main effect of ERT group on baseline or overall values, $p < .05$.

did women not using hormones, possibly accounting for their greater poststress tachycardia. Furthermore, duration of hormone therapy was positively correlated with amount of vagal withdrawal. Increased vagal responsiveness to stress may have important implications. Ordinarily, aging is associated with reduced vagal tone and responsiveness and an increasing dependency on sympathetic control of cardiac response (33, 34). Vagal control of tachycardia may be less deleterious than that arising from sympathetic influences—it is typically more specific (35), and its effects are of shorter duration, stemming in part from events at the cardiac synapses and in part from the fact that it does not stimulate the release of EPI from the adrenal medulla. In fact, Hrushesky et al. (33) proposed that vagal tone (indexed by RSA responsiveness) be used as an index of "cardiac age." Thus, in this sample it seems that ERT, particularly unopposed estrogen (E-only), was associated with enhanced parasympathetic withdrawal in response to stress, as opposed to more potentially detrimental sympathetic activation.

In contrast to several previous studies (eg, Refs. 21, 28), we did not find significant main effects of the stress tasks on ConA- or PHA-stimulated lymphocyte proliferation. However, these studies were performed in populations not using estrogen replacement. Closer examination of our data revealed that among the women in the No ERT group, the expected stress-related declines in blastogenesis did occur. Only in the women using ERT was mitogen-stimulated blastogenesis unaffected by the stress tasks. If stress-related changes in cellular immune function result from activation of the SAM system, as proposed by Manuck et al. (32), then these findings would be consistent with reduced sympathetic reactivity in the women using estrogen replacement. In previous studies, premenopausal status or exogenous estrogen has been associated with reduced stress reactivity in the SAM system, as indexed by HR, blood pressure, or catecholamine levels. Our data provide no direct evidence of reduced SAM activation with estrogen therapy, as we found no difference in catecholamine reactivity or overall levels between the No ERT and ERT groups, and the difference in HR reactivity may have been accounted for by a difference in parasympathetic withdrawal. Use of progestogen, however, was associated with increased EPI at both baseline and poststress, and also with reduced numbers of circulating lymphocytes, T cells, and T helper cells across both time points. Schedlowski et al. (36) found reduced CD3+ and CD4+ percentages after injection of EPI. Thus, in our sample, higher catecholamine levels may be mediating a tonic immunosuppressive effect of progestogen.

Both groups of hormone users had significantly higher baseline and overall levels of both ConA- and PHA-induced blastogenesis than women who did not use hormones. Because the level of mitogen-stimulated blastogenesis is considered an index of the ability of lymphocytes to respond to a pathogen, higher levels may be beneficial, for example, in the face of infectious disease. In the current case, however, the picture may be more complicated. In general, immune function in females tends to be higher than that in males. Females have higher levels of immunoglobulins, a stronger in vitro response to mitogens, and better resistance to the induction of immune tolerance (reviewed in Ref. 10). This higher level of immune function can be beneficial, as in the case of better resistance in females to a variety of infections. On the other hand, females are far more prone to autoimmune diseases, and sex hormones seem to be a factor in this susceptibility (10). If tonically up-regulated T cell function leads to increased humoral responsiveness, it could increase the risk for autoimmunity. Indeed, postmenopausal estrogen replacement therapy recently has been associated with a higher relative risk of systemic lupus erythematosus (37).

CORT levels were significantly higher in the women using ERT, at both baseline and poststress measurement periods. At first glance, this result would seem inconsistent with the idea that ERT reduces stress responses, as increased CORT levels have been associated with chronic stress (38, 39). We believe, however, that the higher CORT levels in the ERT group may be accounted for by the fact that exogenous estrogen stimulates increased production of cortisol-binding globulin (CBG). Higher CBG levels lead to higher levels of circulating bound CORT, whereas the unbound or free (biologically active) fraction remains essentially unchanged (39). Thus, the higher level of total CORT that we detected in ERT users is probably a result of increased CBG consequent to long-term use of oral estrogen, and may not be biologically relevant. Assuming normal feedback regulation of ACTH exerted by the free CORT fraction, the similar levels of plasma ACTH in the No ERT and ERT groups is consistent with this formulation.

Because of the potential negative health effects of heightened stress responses, clinicians have looked for ways to reduce these responses. In the only previous study of estrogen therapy effects on stress reactivity in postmenopausal women (1), estradiol treatment either eliminated or significantly attenuated the stress-related increase in HR, EPI, NEPI, ACTH, and CORT, which supported the idea that estrogen therapy might be useful to reduce both SAM and HPA reactivity to psychological stressors. At first glance, our findings seem contradictory to these earlier results. For example, in our sample, the HR outcome was different—the HR increase in response to the stress tasks was higher, rather than lower, in postmenopausal women taking ERT. However, our RSA data suggest that this higher HR reactivity in the ERT users may have been accounted for by higher vagal responsiveness, rather than by increased SAM reactivity. Furthermore, our finding of stronger ConA- and PHA-induced blastogenesis in response to the stressors is consistent with reduced SAM reactivity, even though no difference between the No ERT and ERT groups was observed on our catecholamine measures.

Finally, there were several differences between our study and previous work that we believe may account for the apparent differences in our results. Our study was cross-sectional, thus it is possible that unknown factors may have contributed to our findings. Other important methodological differences in our study may have implications for clinical practice. First, as described above, the type of estrogen used was different—it may be that the five estrogenic substances in Premarin other than estradiol have physiological effects that are different in nature or extent from those of estradiol (40–42). The current study is the first to examine stress responses in the context of long-term estrogen replacement therapy with Premarin and a progestogen, the ERT regimen used by most postmenopausal women.

Second, the manner of hormone administration was different. Previous researchers (1) used a transdermal estradiol patch, which has the effects of bypassing first-pass metabolism in the liver, allowing estradiol to be delivered directly to

tissue, and maintaining a more consistent blood level than does oral administration (43). Previous studies of estrogen influences on several physiological systems have documented widely differing effects of oral and transdermal administration (eg, Refs. 44, 45). Nevertheless, oral ERT currently is the method of choice for most women.

A third possibility is that the long duration of estrogen therapy in the women from the current study resulted in physiological adaptation. In other words, it may be that oral Premarin would have effects similar to transdermal estradiol if stress reactivity were tested within the first few weeks of treatment, but that these effects are attenuated as the body adjusts to the medication. Because ERT typically continues for several years, it may be important to monitor changes in its effects as the duration of use increases. Additional investigation of these issues may prove helpful for clinical decision-making regarding the mode of estrogen therapy for postmenopausal women.

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ANNOUNCEMENT

APS Scholars Award Program

In recognition of the importance of attracting and retaining promising young members to the American Psychosomatic Society, the APS Board of Directors has recently decided to initiate the APS Scholars Award program. The aim of the program is to generate more student and trainee submissions and participation in the Annual Meeting; to stimulate individuals to pursue careers in fields relevant to psychosomatic medicine; and to encourage and support future members and leaders in the Society.

The APS Scholars Awards will recognize promising, very early career members (including graduate, medical, and postdoctoral students, interns, residents and fellows), who are first authors on accepted abstracts, with a $500 travel stipend to help defray the costs of lodging at the Annual Meeting. For this first year, we are offering 8 awards. We are expecting to increase the number of awards in the second year of the program.

Students and trainees who have submitted abstracts for the upcoming national meeting will automatically be considered for the APS Scholars Award. Notification of awards will be made in writing before the meeting. This year's meeting will be held at the Doubletree Surfside Hotel in Clearwater Beach, Florida.