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Estradiol Protects Cognition Against Stress

Estradiol Therapy After Menopause Mitigates Effects of Stress on Cortisol and Working Memory

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Context. Postmenopausal estradiol therapy (ET) can reduce the stress response. However, it remains unclear whether such reductions can mitigate effects of stress on cognition.

Objective. Investigate effects of ET on cortisol response to a physical stressor, cold pressor test (CPT), and whether ET attenuates stress effects on working memory.

Design. Women completed the CPT or control condition across two sessions and subsequently completed a sentence span task.

Setting. General community; participants were recruited from the Early versus Late Intervention Trial with Estradiol (ELITE).

Patients or Other Participants. ELITE participants (Mage=66, SDage=6.8) in this study did not suffer from any major chronic illness or use medications known to affect the stress response or cognition.

Interventions. Participants had received a median of randomized 4.7 years of estradiol (n = 21) or placebo (n = 21) treatment at time of participation in this study.

Main Outcome Measures. Salivary cortisol and sentence span task performance.

Results. Women assigned to estradiol exhibited blunted cortisol responses to CPT compared to placebo (p = .017), and lesser negative effects of stress on working memory (p = .048).

Conclusions. We present novel evidence suggesting ET may protect certain types of cognition in the presence of stress. Such estrogenic protection against stress hormone exposure may prove beneficial to both cognition and the neural circuitry that maintains and propagates cognitive faculties.

An average of nearly 5 years of post-menopause estradiol treatment reduced cortisol response to the cold pressor and mitigated effects of stress on sentence span task performance.

1. Introduction

Estradiol therapy (ET) after menopause may protect women from the deleterious effects of stress exposure (1), via the hormone’s ability to reduce hypothalamic-pituitary-adrenal (HPA) responses to mental and immune stressors (2-4). One such deleterious effect of stress is interference with prefrontal cognitive processes such as working memory (5). Since ET can reduce the HPA response to stress, the hormone may also mitigate the effects of stress on working memory by limiting the cortisol response to the stressor.
Evidence from the double-blinded, placebo-controlled randomized Early versus Late Intervention Trial with Estradiol (ELITE; ClinicalTrials.gov Identifier: NCT00114517) suggests that estradiol treatment, in the absence of stress threat, does not exert an effect on cognition (6). However, the ability of ET to reduce the stress response might benefit some types of cognitive processes when under stress. In particular, cortisol impairs working memory (7-9). Thus, the ability of ET to dampen the cortisol response to acute stressors may mitigate the impairing effect of stress on working memory due to the attenuated cortisol release. If this is the case, then maintenance of estradiol levels after menopause with ET may improve working memory performance under stress.

In this study, we examined how ET affected the stress response and modulated the effects of acute stress on working memory. We hypothesized that ET would decrease the bioavailable cortisol response to stress, thereby limiting the effects of stress on working memory performance. In addition, we examined whether there were differences according to whether ET was initiated within 6 years of menopause (early initiation) or beyond 10 years of menopause (late initiation). A possible critical window for hormone therapy (10,11), related to the theory of a healthy cell bias for estradiol (E2) action (12), may result in different effects of ET when treatment is initiated years later past menopause, such as abolishment of the attenuating effect of HPA reactivity, or even potentiation of a stress response.

2. Materials and Methods

2.1 Participants

This study was approved by the University of Southern California Institutional Review Board. Forty-nine post-menopausal women were recruited from ELITE, where participants received either 1 mg oral micronized 17β-estradiol (E2) daily, or placebo (PL), for a median of 5 years. Participants were enrolled within 6 years of menopause (early initiation) or beyond 10 years of menopause (late initiation), creating 4 groups: early initiation-E2, early initiation-PL, late initiation-E2, and late initiation-PL. The primary outcome of ELITE was the rate of change in intima-media thickness of the right carotid artery. Secondary outcomes were 1) cognitive measures, including verbal memory, executive function, and global cognition, and 2) development/progression of coronary atherosclerosis measured by cardiac computed tomography (13).

ELITE participants in this study had received E2 or placebo for a median of 4.7 years at time of participation (see Table 1 for exclusionary criteria). Women with an intact uterus received sequential progesterone (45 mg delivered via 4% vaginal gel), or a placebo-matched gel is assigned to PL, daily for 10 days in each 30-day cycle. Women were instructed to take their medication upon awakening with brushing teeth or breakfast. Our study sample included three women with unilateral oophorectomies (one with accompanying hysterectomy) and two women with a hysterectomy without oophorectomy.

2.2 Sessions

Participants completed one stress and one control session, order counterbalanced. All women provided written informed consent at the beginning of their first session. Of forty-nine women enrolled in the study, forty-two (n=21 E2; n=21 PL) completed both sessions and were included in the analyses (participant attrition/withdrawal explained in Table 1). At both sessions, participants provided saliva samples, completed questionnaires, and completed a working memory task (see Figure 1A for session timing protocol).
Sessions for this study took place when women attended their ELITE research clinic sessions between the hours 7:00 to 14:00. Although desirable to conduct stress studies in the afternoons to control for the diurnal cortisol rhythm, it has been shown that while baseline free cortisol levels are affected by time of day, the magnitude of the cortisol response to laboratory stress does not differ in the morning or afternoon (14).

2.3 **Hormone Sampling**

Bioavailable levels of free cortisol, estradiol, and progesterone were measured in saliva (15,16). Participants refrained from exercise and food/drink (except water) within one hour, sleep within two hours, and caffeine and alcohol within three hours of their session start time.

Baseline cortisol levels follow a diurnal cycle (17), with peak baseline levels occurring 30 minutes after waking (18). To avoid seeing women during the acrophase, women were told to awaken at least 2 hours prior to their appointment start time. To verify participants had been awake for the minimum amount of time, at the beginning of each session they were asked what time they woke up.

The baseline sample was collected via passive drool and was processed for free cortisol and sex hormone levels. The post-stress stress sample was processed for free cortisol only and was collected using two sponge sorbettes (bvi Visitec, Wallham, MA). Sorbettes were placed in participants’ mouths, one at a time, and remained in the mouth until the sponge was adequately moist. Participants were told to not chew or suck on the sponge. Sorbettes were then placed in a tube for storage and frozen. Samples were packaged and transported frozen in dry ice to CLIA-certified analytical laboratories (Salimetrics, LLC, State College, PA) where samples were processed using enzyme-linked immunosorbent assay (ELISA). Lower limits of detection were <0.007 µg/dl for cortisol, 0.1 pg/ml for estradiol, and 5 pg/ml for progesterone. All samples were processed in duplicate. Inter- and intra-assay variations were 4.4% and 5.0% for cortisol, 3.9% and 6.9% for estradiol, and 5.0% and 9.1% for progesterone.

2.4 **Stress Manipulation**

Participants completed the Cold Pressor Test (CPT) or control across the two sessions. The CPT is a physical stressor that has been shown to induce cortisol secretion (19). Participants submerged their non-dominant hand, up to the wrist, in ice-water (0-5°C immediately before hand immersion) for as long as possible up to three minutes. The control condition used warm water (37-40°C immediately before hand immersion). Order of the CPT and control sessions was randomly counterbalanced.

2.5 **Subjective Measures of Stress and Pain Pre- and Post-CPT**

Participants completed pre- and post-hand-immersion pain and stress ratings using visual analog scales. Immediately before immersing their hand in water, participants rated how much pain they were currently feeling and how much stress they were currently feeling, from none to most possible. Immediately after removing their hand from the water, participants completed two additional ratings for the peak amount of stress and pain felt while their hand was in the water, again from none to most possible.

2.6 **Working Memory: Sentence Span Task**

The working memory task began approximately 21.5 minutes after stress onset. Sentences were presented one at a time on a computer screen, via PsyScope (20). Participants were told to remember the last word of each sentence. After presentation of a sentence, participants reported whether the sentence made semantic and syntactic sense. At the end of each load, participants were asked to recall the last word of each sentence in that load. “Makes sense” and “Nonsense”
judgments were recorded by key press on a computer keyboard, while word recall was recorded on a paper scoring sheet by the experimenter (see Figure 1B for sentence span timing and protocol). Loads spanned from two to six sentences.

A lenient scoring criterion was used; women were given 1 point for each word they remembered whether or not they recalled the words in the order presented. This task took approximately 11 minutes to complete. Participants saw different sentences at each session.

2.7 Statistical Analysis
To examine subjective effects of CPT, we conducted separate 2 (stress: CPT vs. control) x 2 (treatment: estradiol vs. placebo) x 2 (initiation: early vs. late) mixed-model analyses of variance (ANOVAs) on post-hand-immersion minus pre-hand-immersion difference scores for stress and pain ratings. To examine cortisol response, we conducted a 2 (stress: CPT vs. control) x 2 (time: baseline vs. 15-minutes post-stress onset) x 2 (treatment: estradiol vs. placebo) x 2 (initiation: early vs. late) mixed-model ANOVA on free cortisol response to stress. Sentence span loads were divided into Low and High loads: the average proportion of words recalled in 2- and 3-sentence loads (Low Load) and in the 4-, 5-, and 6-sentence loads (High Load) were dependent variables in a 2 (stress: CPT vs. control) x 2 (load: low load vs. high load) x 2 (treatment: estradiol vs. placebo) x 2 (initiation: early vs. late) mixed-model ANOVA. Additional post hoc independent and paired t-tests were conducted where appropriate. Boxplots did uncover outliers; however, removal of these data did not change the overall presented results. For this reason, all data have been included in the analyses. Outliers have been depicted in the figures and any differences in results have been described in the respective figure caption.

3. Results
3.1 Hormone levels and demographics
As expected, women receiving ET had significantly higher E2 levels than women receiving PL, \( M_{\text{diff}} = 46.52 \, \text{pg/mL}, 95\% \, \text{CI}[9.37, 83.66] \). Groups did not differ in their progesterone levels, \( M_{\text{diff}} = -12.85 \, \text{pg/mL}, 95\% \, \text{CI}[-77.63, 51.92] \). ET and PL did not differ on any demographic information, verbal intelligence, negative affect, positive affect, or depression scores during the sessions (Table 2).

3.2 Pre- and Post-CPT Stress and Pain Ratings
Participants found the CPT more stressful than the control task as shown by higher stress rating difference scores (post-hand-immersion rating minus pre-hand-immersion rating) in the CPT than the control condition, \( F(1,38) = 53.255, p < .001, \eta_p^2 = .584, M_{\text{diff}}(\text{CPT-control}) = 42.6, 95\% \, \text{CI}[30.8, 54.4] \). With this same measure, ET participants reported larger increases in subjective stress than PL participants, \( F(1,38) = 6.170, p = .018, \eta_p^2 = .140, M_{\text{diff}} = 11.6, 95\% \, \text{CI}[2.1, 21.1] \), Timing of initiation of randomized treatment relative to menopause had no effect on the stress ratings difference scores in the control or CPT sessions (p > .05; see Figure 2A).

Compared with control, CPT also led to significantly higher pain rating difference scores (post-hand-immersion rating minus pre-hand-immersion rating), \( F(1,38) = 94.852, p < .001, \eta_p^2 = .714, M_{\text{diff}}(\text{CPT-control}) = 51.9, 95\% \, \text{CI}[41.1, 62.6] \) with increases during the CPT session and decreases in the control session. Randomized treatment and time of initiation relative to menopause had no effect on the pain ratings in either the control or CPT sessions (p > .05; Figure 2B).

3.3 Cortisol response to CPT
The working memory task occurred approximately 21 minutes after stress onset, when free cortisol levels typically begin to peak (21). Saliva was collected immediately prior to behavioral tasks. Cortisol analyses focused on this time frame to test differences in approximate peak levels.

Free cortisol levels increased in the CPT session, not the control session, $F(1,38)=4.358, p=.044, \eta_p^2=.103, M_{diff}=0.035 \mu g/dl, 95\% CI[0.001, 0.070]$. A time by stress interaction, $F(1,38)=11.486, p=.002, \eta_p^2=.232$, revealed cortisol levels increased in response to CPT. There was a significant interaction of time and treatment, $F(1,38)=6.266, p=.017, \eta_p^2=.142$, with an increase in cortisol over time only observed in PL. Neither treatment nor initiation factors significantly affected free cortisol levels during the CPT or control sessions.

Analysis of the CPT session alone, collapsed across initiation groups, confirmed the above patterns. Free cortisol levels increased from pre- to post-CPT, $F(1,40)=8.960, p=.005, \eta_p^2=.183, M_{diff}=0.058 \mu g/dl, 95\% CI[0.019, 0.097]$. A time by treatment interaction, $F(1,40)=4.368, p=.043, \eta_p^2=.098$, was driven by PL participants experiencing significant increases in free cortisol, $t(20)=2.921, p=.008, M_{diff}=0.098 \mu g/dl, 95\% CI[0.028, 0.169]$, and ET participants experiencing no change in cortisol levels, $t(20)=91.6, p>.05, M_{diff}=0.018 \mu g/dl, 95\% CI[-0.022, 0.057]$, (Figure 3). The same analysis testing the control session revealed no main effects or interactions (Figure 3).

### 3.4 Working Memory

CPT decreased word recall working memory performance compared with control, $F(1,38)=7.083, p=.011, \eta_p^2=.157, M_{diff}=-0.043, 95\% CI[-0.075, -0.010]$, as did increasing loads, $F(1,38)=231.648, p<.001, \eta_p^2=.859, M_{diff}=-204.95\% CI[-231, -177]$, (see Figure 4). An initiation by load interaction, $F(1,38)=4.981, p=.032, \eta_p^2=.116$, revealed a pattern of larger decreases in performance as load increased in late initiation than early initiation groups. A three-way interaction of stress, load, and treatment, $F(1,38)=4.168, p=.048, \eta_p^2=.099$, showed that while both ET and PL had poorer performance on High Loads than Low Loads, ET participants did not differ in their performance after CPT or the control condition in either load, whereas PL participants showed poorer performance on Low Loads and after CPT compared with the control condition (Figure 4). Neither treatment nor initiation exerted main effects.

When ET participants were tested alone, decreased performance as load increased was confirmed in both CPT and control sessions, $F(1,20)=66.046, p<.001, \eta_p^2=.768, M_{diff}=-203.95\% CI[-255, -151]$. However, CPT did not significantly affect performance, $F(1,20)=2.581, p>.05, \eta_p^2=.114, M_{diff}=-0.032, 95\% CI[-0.074, .010]$. In contrast, when PL participants were tested alone, CPT impaired performance, $F(1,20)=4.856, p=.039, \eta_p^2=.195, M_{diff}=-0.054, 95\% CI[-.105, -0.033], as did increases in load, $F(1,20)=233.605, p<.001, \eta_p^2=.921, M_{diff}=-203, 95\% CI[-231, -176]$. A stress by load interaction, $F(1,20)=5.770, p=.026, \eta_p^2=.224$, indicated that CPT exerted its effect on the Low Load blocks, with performance on Low Load blocks worse after CPT, $t(20)=-3.219, p=.004, M_{diff}=-0.09, 95\% CI[-15, -0.03]$, while performance on High Load blocks remained the same across CPT and control sessions, $t(20)=.595, p>.05, M_{diff}=-0.02, 95\% CI[-.08, .04]$.

T-tests showed ET and PL groups performed similarly on Low Load blocks during the control session, but that ET performed significantly better than PL participants during the CPT session, $t(40)=2.350, p=.024, M_{diff}=-11.95\% CI[0.02, .20]$ (Figure 4). Similar analyses comparing performance on High Load blocks revealed no differences between ET and PL participants during the control sessions, $t(40)=1.514, p>.05, M_{diff}=0.09, 95\% CI[-.03, .21]$, or CPT, $t(40)=1.232, p>.05, M_{diff}=0.06, 95\% CI[-.04, .17]$. 
4. Discussion

Previous research among women has shown that short-term random assignment to estrogen therapy decreases cortisol responses to stressors (22, 23). Our study extends these findings, providing evidence that long-term ET after menopause can reduce the free cortisol response to a physical stressor, with similar effects regardless as to whether treatment is initiated within or beyond six years of menopause. Our findings also extend the cognitive findings reported from ELITE (6). In ELITE, when women were tested in the absence of threat, ET failed to influence cognitive performance regardless of when ET was initiated. Results in the control session of our study support this finding. However, our study also shows that ET can exert a beneficial effect on cognition after an episode of acute stress.

While our primary hypotheses were supported, an unexpected finding was the larger increase in subjective stress ratings in the ET as compared to the PL condition, as reflected in the post-minus pre-hand-immersion difference scores (Figure 2A). This finding may speak to the robust nature of the E2 effect on the free cortisol response. Despite ET women reporting higher levels of subjective stress, these women still showed significantly lower free cortisol responses to the physical stressor than did their PL counterparts.

We also tested effects of ET on working memory during stress. Both hydrocortisone administration (8) and psychological stressors (5) impair working memory performance. We hypothesized that by decreasing free cortisol responses to stress, ET should also protect working memory from stress-induced impairment. Indeed, random assignment to ET reduced the stress response, and prevented stress-induced decrements in working memory performance, while assignment to PL did not prevent stress-induced decrements. This ET protection was limited to the Low Load working memory blocks, which may have been due to a floor effect reducing our ability to see stress impairments in the High Load blocks, as women generally performed worse on the High Load blocks regardless of treatment group, initiation group, or CPT vs control session.

One interpretation for the pattern of higher pre- to post-hand-immersion stress ratings with lower cortisol responses to CPT could be that ET women were experiencing chronic stress, which has been shown to blunt cortisol responses to acute stressors (24). Chronic stress leading to blunted cortisol responses also is associated with elevated baseline cortisol levels (24), which may contribute to the blunted stress responses through a ceiling effect. However, since ET and PL women did not differ in their baseline levels and PL women reached numerically higher levels of cortisol post-hand-immersion in the CPT session, we do not believe our observation is driven by this mechanism. Further, ET and PL women did not differ in their subjective rating for stress level that day, both groups reported stress levels on the lower end at each session, with average ratings for each group at each session ranging from 2.4 to 3.6 (maximum of 9).

Instead, it is possible that corticosteroid binding globulin (CBG) can explain the blunting effect of E2 on the stress response and the subsequent prevention of stress-induced decrements in working memory. Cortisol primarily binds to CBG leaving only 5%-10% available to act on tissue (25). Estradiol has been shown to increase CBG in plasma (26), which could account for the lower free cortisol levels in response to stress in ET, despite reporting larger changes in subjective stress from immediately before to immediately after CPT exposure. Further, as E2-induced CBG upregulation binds more of the released cortisol, less cortisol is left available to act on tissue, including prefrontal cortical regions integral to working memory (27).
The ability of ET to reduce the HPA response to a variety of stressors also has important implications beyond attenuating acute-stress-induced decrements in cognitive performance. Aging has been associated with hyperactive dysfunction of the HPA axis in response to stress (28). The dysfunction is attributed to a reduced ability of the primary negative feedback source in the brain, the hippocampus, to dampen the HPA response to acute stressors. This failure leads to prolonged glucocorticoid secretion and exposure, causing additional receptor loss in the hippocampus and further inability to effectively dampen the HPA response to future stressors. In addition to prolonged exposure of glucocorticoids leading to receptor loss, prolonged stress exposure leads to hippocampal degeneration (29), as well as prefrontal cortical degeneration (30). Importantly, due to the heavy involvement of these regions in cognition, it is possible that degradation resulting from age-related hyperactivation of the HPA axis may impair cognitive processes.

However, the ability of ET to reduce cortisol response to acute stressors may prevent this age-related hyperactivation of the HPA axis (31), by preventing bouts of prolonged HPA responses to acute stressors. Protection of the brain via ET may be two-fold, as E2 can directly protect neuronal tissue from various neurotoxic insults, including glucocorticoids (32). Thus, whether via upregulation of CBG, protecting brain regions in the face of HPA dysregulation, or other mechanisms, ET-induced reductions to cortisol exposure may delay or minimize the age-related dysfunction of the axis and any potential cognitive effects related to hyperactivation-induced neuronal damage. On the other hand, the rapid and dramatic decline in E2 levels during menopause may leave women more vulnerable to the detrimental effects of stress hormone exposure on HPA, neural, and cognitive integrity.

While our hypotheses regarding effects of treatment on stress response and stress effects on cognition were supported, we found time of treatment initiation relative to menopause only affected performance on High Load blocks. Since we found no interaction between E2 and time of initiation on load it appears that the effect of initiation on load simply reflected the age difference between groups (Table 2) rather than an effect of time of ET initiation. Such failure to find any treatment by initiation interactions could indicate that a critical window for ET administration does not apply to all physiological and cognitive domains; however, the lack of interaction may also result from one of the limitations of this study. Eligibility criteria for this study were quite stringent, requiring women recruited from ELITE to be free from cardiovascular diseases and cognitive impairment, and not using beta-adrenergic, corticosteroid, or psychoactive (e.g., anti-depressants, anti-anziolitics, Adderall, etc.) medications. Thus, despite older age in the late initiation treatment group, these women lacked serious illness, including cardiovascular conditions associated with aging, such as prior myocardial infarction. Based on the premise of the healthy cell bias theory (12), the good health of our sample may have translated to reduced age-related brain changes and placed them in a category above the threshold of decline or damage, protecting them from the potentially deleterious effects of E2 on cognitive function observed in at-risk populations (33).

Replication with a larger sample is important to confirm these effects. It also is unclear if other executive functions, or cognitive domains not encompassing executive function, are offered the same protection under stress. A prior study examined a set of cognitive functions such as vigilance and episodic memory and found that older women (average age 65) who had three months of E2 supplementation as well as social stress before testing showed worse performance than those who experienced social stress without prior E2 supplementation. It is possible that performance on these tasks benefits from stress, and by reducing the stress
response, E2 attenuated those benefits. However, there was no stress-free comparison group which limits conclusions (34). We also included no measures of total (bound and unbound) cortisol levels, which would be beneficial in elucidating which mechanisms might be involved in E2-induced reductions of free cortisol levels after stress exposure.

Despite these limitations, this study suggests there are other roles of ET besides relief from menopause-related symptoms, including limiting effects of stress on working memory and perhaps aiding in maintenance of proper HPA reactivity. With the growing interest in estrogens that limit the negative effects of ET, such as selective estrogen receptor modulators (35) and the tissue-selective estrogen complex (36), ET after menopause may become a more feasible treatment for symptoms and as a preventative strategy against a host of other health-related declines observed to increase after menopause, including age-related dysfunction of the HPA axis and the associated neural damage caused by such dysfunction.

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**Figure 1A. General protocol and timing of all sessions, relative to Cold Pressor Test (CPT) onset.** Order of first CPT condition (cold or warm water) counterbalanced. A subset of negative, positive, and neutral pictures from the International Affective Picture System (IAPS; 43) was used in the emotional picture slideshow. Emotional picture slideshow occurred between the CPT and the working memory task. It was expected that the emotional picture slideshow would not affect the magnitude of the free cortisol response to CPT, as viewing IAPS does not elicit increases in salivary cortisol levels (44).

**Figure 1B. Sentence span protocol and timing.** Sentences were collected from various sources and have been used in similar tasks (45-47). Nonsense sentences were created by inverting the four words just before the final word of a sentence as done in Turner, Engle (48). Sentences were presented on the center of the screen with the last word in all capital letters (e.g. The boy said HELLO), for 5 seconds. Immediately after sentence presentation, participants made “Makes Sense” and “Nonsense” judgments before seeing the next sentence. Participants completed 13 blocks. Blocks 1 and 2 were practice and consisted of 1-sentence and 2-sentence loads, respectively. At the end of each block, the participant was prompted to tell the experimenter the last word of the 1 or 2 sentences just viewed, in the order they were presented. The remainder of the task proceeded in the same fashion and included 4 blocks of 2-sentence load, 3 blocks of 3-sentence load, and 2 blocks each of 4-, 5-, and 6-sentence loads.

**Figure 2A. Difference scores for subjective stress ratings immediately before and immediately after hand immersion in the control and CPT sessions.** Women completed visual analog scales for subjective stress ratings immediately before and immediately after immersing their hand in water in both sessions. Difference scores were calculated by subtracting pre-hand-immersion ratings from post-hand-immersion ratings, thus, positive values indicate an increase in stress and negative values indicate a decrease in stress. There were main effects of stress condition and treatment, with no other main effects or interactions. Five outliers were detected for stress rating differences scores during the control session. When these outliers were removed, there was no longer a main effect of treatment. CPT: cold-pressor test. “O”: outlier with accompanying data point. **p < .001, *p < .05.**
Figure 2B. Difference scores for subjective pain ratings immediately before and immediately after CPT exposure in the control and CPT sessions. Women completed visual analog scales for subjective pain ratings immediately before and immediately after immersing their hand in water in both sessions. Difference scores were calculated in the same manner as stress difference scores. There was a main effect of stress condition, but no other main effects or interactions. Two outliers were detected for pain rating differences scores during the control session. Results remained the same when these outliers were removed. CPT: cold-pressor test. “O”: outlier with accompanying data point. ***p < .001.

Figure 3. Free cortisol response during control and CPT sessions in women assigned to daily estradiol (ET) or placebo (PL). There was a main effect of stress condition and a significant time x stress condition interaction, with an increase over time in the CPT session, and no change in the control session. When the CPT session was analyzed alone (right panel), there was a main effect of time and a time x treatment interaction, where PL experienced significant increases in free cortisol after CPT and ET experienced no significant change in free cortisol levels after CPT. One outlier was detected for baseline cortisol during the control session. Results remained the same when this outlier was removed. BL: baseline, 15M: 15-minutes post-stress onset, CPT: cold-pressor test. “O”: outlier with accompanying data point. **p < .01, *p < .05.

Figure 4. Average proportion of words recalled in the sentence span task for Low Load blocks (2- and 3-sentence blocks) and High Load blocks (4-, 5-, and 6-sentence blocks) during the control and CPT sessions by women assigned to either estradiol (ET) or placebo (PL). There were main effects of stress condition and load, as well as a load x initiation interaction and stress condition x load x treatment interaction. All women performed worse on High Load blocks than Low Load blocks regardless of session. ET and PL performed similarly on Low Load blocks during the control session, however ET performed significantly better than PL during the CPT session. ET also performed similarly in both the control and CPT sessions, while PL performed worse in the CPT session than the control session. ET and PL performed similarly on High Load blocks during both the control and CPT sessions. Four outliers were detected for working memory performance during the stress session; one in the low load and three in the high load. Removal of these outliers resulted in the following differences: 1) in the overall ANOVA the load by time of initiation interaction is no longer significant, 2) the main effect of stress was no longer significant in the Placebo-only analyses, although all other interactions and follow-up analyses remained the same, and 3) the significant follow-up independent t-test showing differences between estradiol and placebo women in the low load during the stress session was no longer significant. “O”: outlier with accompanying data point. CPT: cold-pressor test. ***p < .001, *p < .05.

Table 1. Exclusionary criteria for participation in this study and dropped/withdrawn participants. All participants were recruited from the Early versus Late Intervention Trial with Estradiol (ELITE). This table lists the exclusionary criteria for participation as well explanations of participant loss.
Table 1. Exclusionary criteria for participation in this study and dropped participants.

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<td>diabetes</td>
<td></td>
</tr>
<tr>
<td>Reynaud’s phenomenon</td>
<td></td>
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<tr>
<td>cryoglobulinemia</td>
<td></td>
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<tr>
<td>vasculitis</td>
<td></td>
</tr>
<tr>
<td>lupus</td>
<td></td>
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<tr>
<td>tingling or numbness in the hands and/or feet</td>
<td></td>
</tr>
<tr>
<td>or any other serious chronic illness contraindicated for exposure to our stressor</td>
<td></td>
</tr>
<tr>
<td>Medications</td>
<td></td>
</tr>
<tr>
<td>beta-blockers</td>
<td></td>
</tr>
<tr>
<td>corticosteroid-based medications</td>
<td></td>
</tr>
<tr>
<td>psychoactive medications or drugs (e.g., antidepressants, anxiolytics, Adderall, marijuana)</td>
<td></td>
</tr>
<tr>
<td>Smoking Status</td>
<td></td>
</tr>
<tr>
<td>current smoker</td>
<td></td>
</tr>
<tr>
<td>Vision and Hearing</td>
<td></td>
</tr>
<tr>
<td>non-corrected vision or hearing</td>
<td></td>
</tr>
<tr>
<td>Language</td>
<td></td>
</tr>
<tr>
<td>lacking fluency in English</td>
<td></td>
</tr>
<tr>
<td>Cognitive Status</td>
<td></td>
</tr>
<tr>
<td>score ≤ 29 on the TICS-m</td>
<td></td>
</tr>
</tbody>
</table>

Reason for participant attrition/withdrawal (number of participants):
- Discomfort providing saliva samples (1)
- Computer failure during session (1)
- Failure to complete the Cold Pressor Task (2)
- Failure to return for second session (3)

TICS-m: Telephone Interview for Cognitive Status – modified (37,38).

Table 2. Hormone, demographic, and psychological measures by randomized treatment and time of initiation relative to menopause. Participants completed several questionnaires, including a health, demographies, and daily event form (e.g., amount of sleep the night before, time food or caffeine was last consumed, etc.); the Daily Stress Inventory (DSI; 39); the Positive and Negative Affective Scale (PANAS; 40); the Center for Epidemiological Studies Depression Scale (CES-D; 41); the Wechsler Test of Adult Reading (WTAR; 42) as a measure of verbal intelligence; and subjective ratings for how stressed women felt at the beginning of each session. ET: Estradiol-condition women, PL: Placebo-condition women, Early: Early-initiation women (within 6 years of menopause), Late: Late-initiation women (beyond 10 years since menopause), CPT: Cold-pressor test.

Table 2. Hormone, demographic, and psychological measures for randomized treatment and initiation groups.

<table>
<thead>
<tr>
<th></th>
<th>ET (n=21) mean ± SD</th>
<th>PL (n=21) mean ± SD</th>
<th>p-value</th>
<th>Early (n=21) mean ± SD</th>
<th>Late (n=21) mean ± SD</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Salivary estradiol (pg/mL)</td>
<td>48.9 ± 84.2</td>
<td>2.4 ± 1.6</td>
<td>.015</td>
<td>31.0 ± 76.4</td>
<td>20.4 ± 48.4</td>
<td>.59</td>
</tr>
<tr>
<td>Salivary progesterone (pg/mL)</td>
<td>41.5 ± 43.6</td>
<td>54.3 ± 140.2</td>
<td>.69</td>
<td>34.5 ± 45.6</td>
<td>20.4 ± 138.6</td>
<td>.41</td>
</tr>
<tr>
<td>Age at menopause</td>
<td>52 ± 4.3</td>
<td>51 ± 4.1</td>
<td>.61</td>
<td>52.9 ± 3.5</td>
<td>50.2 ± 4.6</td>
<td>.047</td>
</tr>
<tr>
<td>Age at this study</td>
<td>66 ± 7.5</td>
<td>65 ± 6.3</td>
<td>.89</td>
<td>61.3 ± 4.2</td>
<td>70.6 ± 5.6</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Age at randomization in ELITE</td>
<td>61 ± 7.5</td>
<td>60 ± 6.6</td>
<td>.80</td>
<td>56.4 ± 4.3</td>
<td>65.9 ± 5.7</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Years of education</td>
<td>17 ± 1.8</td>
<td>16 ± 2.1</td>
<td>.10</td>
<td>17 ± 1.5</td>
<td>16 ± 2.4</td>
<td>.10</td>
</tr>
<tr>
<td>WTAR</td>
<td>45 ± 5.0</td>
<td>42 ± 6.5</td>
<td>.12</td>
<td>45 ± 5.7</td>
<td>43 ± 6.0</td>
<td>.20</td>
</tr>
<tr>
<td>Positive affect (PANAS)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control session</td>
<td>31.8 ± 5.3</td>
<td>36.1 ± 8.4</td>
<td>.052</td>
<td>35.2 ± 8.4</td>
<td>32.8 ± 5.8</td>
<td>.28</td>
</tr>
<tr>
<td>CPT session</td>
<td>31.9 ± 5.6</td>
<td>35.9 ± 8.4</td>
<td>.08</td>
<td>35.0 ± 8.2</td>
<td>32.8 ± 6.3</td>
<td>.35</td>
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<tr>
<td>Negative affect (PANAS)</td>
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<tr>
<td>Control session</td>
<td>11.0 ± 1.5</td>
<td>11.3 ± 2.4</td>
<td>.55</td>
<td>11.1 ± 1.5</td>
<td>11.2 ± 2.4</td>
<td>.88</td>
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<tr>
<td>CPT session</td>
<td>11.3 ± 1.7</td>
<td>11.2 ± 1.5</td>
<td>.85</td>
<td>11.1 ± 1.7</td>
<td>11.3 ± 1.5</td>
<td>.70</td>
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<tr>
<td>Depression (CES-D)</td>
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<tr>
<td></td>
<td>Control session</td>
<td>CPT session</td>
<td></td>
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<td>--------------------------</td>
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<td></td>
<td></td>
</tr>
<tr>
<td>Control session</td>
<td>5.8 ± 8.6</td>
<td>7.1 ± 5.0</td>
<td>.54</td>
<td>9.0 ± 9.8</td>
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<tr>
<td></td>
<td>7.1 ± 3.8</td>
<td>7.4 ± 5.2</td>
<td>.17</td>
<td>5.2 ± 3.9</td>
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<tr>
<td>Subjective stress level today (1=very low, 9=very high)</td>
<td>9.1 ± 10.1</td>
<td>5.4 ± 3.6</td>
<td>.12</td>
<td>9.1 ± 9.8</td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>5.4 ± 3.6</td>
<td>5.4 ± 3.6</td>
<td>.13</td>
<td>5.5 ± 4.2</td>
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<tr>
<td>Control session</td>
<td>2.4 ± 1.6</td>
<td>2.9 ± 1.8</td>
<td>.33</td>
<td>2.4 ± 1.4</td>
<td></td>
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<tr>
<td></td>
<td>2.6 ± 1.9</td>
<td>3.6 ± 2.2</td>
<td>.14</td>
<td>3.0 ± 2.9</td>
<td></td>
<td></td>
</tr>
<tr>
<td>CPT session</td>
<td>2.4 ± 1.6</td>
<td>2.9 ± 1.8</td>
<td>.33</td>
<td>2.9 ± 2.0</td>
<td></td>
<td></td>
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<td>.14</td>
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<td>2.4 ± 1.6</td>
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<td>.33</td>
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<td>3.6 ± 2.2</td>
<td>.14</td>
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<tr>
<td></td>
<td>2.6 ± 1.9</td>
<td>3.6 ± 2.2</td>
<td>.14</td>
<td>3.2 ± 2.1</td>
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</tbody>
</table>
### Figure 1A
Session Procedure

<table>
<thead>
<tr>
<th>Time Frame</th>
<th>Event</th>
</tr>
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<tbody>
<tr>
<td>0-3 minutes</td>
<td>CPT</td>
</tr>
<tr>
<td>25 - 0 minutes</td>
<td>Questionnaires</td>
</tr>
<tr>
<td></td>
<td>Baseline saliva sample</td>
</tr>
<tr>
<td></td>
<td>Pre-stress and -pain</td>
</tr>
<tr>
<td></td>
<td>ratings</td>
</tr>
<tr>
<td></td>
<td>Post-stress and -pain</td>
</tr>
<tr>
<td></td>
<td>ratings</td>
</tr>
<tr>
<td></td>
<td>Questionnaires</td>
</tr>
<tr>
<td>15-20 minutes post-CPT-onset</td>
<td>Emotional picture slideshow</td>
</tr>
<tr>
<td>21-32 minutes post-CPT-onset</td>
<td>Sentence Span Task</td>
</tr>
</tbody>
</table>

### Figure 1B
Sentence Span Procedure

- The boy said **HELLO**
- Makes Sense, Nonsense
- 5,000 ms, Self-timed key press
- Please tell the experimenter the last word for the 2 sentences just presented.
- Verbal response recorded by experimenter.
Figure 3
Cortisol response during the control and CPT sessions in women taking Estradiol or Placebo

BL •BL •15m

Estradiol
Placebo

Control Session

CPT Session

Estradiol
Placebo
Figure 4
Word Recall in the Sentence Span task during the control and CPT sessions in women taking Estradiol or Placebo

- Control Session
- CPT Session

![Graph showing word recall in the Sentence Span task during control and CPT sessions in women taking Estradiol or Placebo.](image-url)