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Differences in verbal memory performance in postmenopausal women receiving hormone therapy: 17 β -estradiol versus conjugated equine estrogens

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Abstract

Objective—Much controversy exists and many questions remain unanswered about the effects of hormone therapy (HT) on cognition in postmenopausal women. There is growing evidence suggesting that HT compounds containing conjugated equine estrogen (CEE) have negative effects on cognition whereas 17 β -estradiol (17 β -E) either has positive or neutral effects. The present study sought to further examine this issue in a sample of postmenopausal women with risk factors for Alzheimer's disease (AD).

Design—Cross-sectional neuropsychological evaluation, as part of a larger longitudinal study.

Setting—Academic research clinic.

Participants—68 healthy postmenopausal women (aged 49–68) receiving either 17 β -E or CEE for at least one year and at increased risk for Alzheimer's disease (AD).

Measurements—Neuropsychological test battery of the cognitive domains of attention/working memory/processing speed, verbal memory, visual memory, and executive functioning.

Results—Women receiving 17 β -E showed significantly better verbal memory performance compared to women receiving CEE, regardless of age, IQ, years of education, risk factors for AD (including APOE- ϵ 4 carriership), duration of endogenous and exogenous estrogen exposure, concurrent progesterone use, or natural or surgical menopause status.

Conclusions—Verbal memory performance was better in menopausal women receiving 17 β -E compared to CEE in a sample population of women with risk factors for AD. Genetic risk for AD as well as other confounds did not affect this finding. The results suggest that differential effect of the type of HT on verbal memory, with 17 β -E being a preferential compound. Further evaluation of HT types, regimens and duration of use on cognitive performance in postmenopausal women in a controlled longitudinal design is warranted.

Keywords

memory; cognition; menopause; postmenopausal women; hormone therapy; 17-Beta estradiol; conjugated equine estrogens; risk for Alzheimer's disease

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Introduction

Many questions remain unanswered regarding the utility of estrogen hormone therapy (HT) to slow the progression of age related cognitive decline or impact pathological aging such as Alzheimer's disease (AD) in women. Overall, findings from meta-analyses of clinical trials do not support the use of HT to improve or preserve cognition in older postmenopausal women ¹. Similar conclusions are yet to be made with respect to potential benefits of HT for specific subgroups of women, such as younger postmenopausal and perimenopausal women and those with significant risk factors for neurodegenerative disorders. Further, type of HT and a route of administration (oral vs transdermal) were also suggested to affect cognitive performance ^{1, 2}. The current study aimed to address some of these specific questions.

Observational studies suggest long-term benefits to cognition with HT use in midlife ^{3–7}. However, observational studies carry inherent biases since women who opt to use HT tend to be more educated, have better cardiovascular risk factor profiles, and live healthier lifestyles ⁸, factors that influence cognitive decline and dementia development. In addition, when past HT use is collected retrospectively, women with dementia are shown to under-report use compared to women who do not have dementia ⁹.

Until the negative findings from the Women's Health Initiative (WHI) Study were released, historically the most common type of hormone therapy prescribed in the United States contained conjugated equine estrogen (CEE) whereas many other countries more commonly prescribed estrogens containing 17 β -estradiol (17 β -E) ^{10–12}. Our own findings from the Swedish Twin Registry revealed positive association with HT use and cognitive performance ¹³, with the majority of that sample receiving 17 β -E preparations. Differences between HT preparations and the route of administration (e.g. oral vs. transdermal) are also thought to be significant factors confounding the results of many prospective studies and much work remains to differentiate between the risks and benefits between treatment protocols.

Although some studies do not show differences between women who use or ever used HT on verbal memory measures ^{12, 14, 15}, there is growing evidence that some, but not all, estrogens may affect cognition positively at least in the short run ^{16–19}. In particular, a positive effect on verbal memory has been seen in several clinical trials using short term (2–4 months) 17 β -E in women $\leq$ age 65 ^{20–22}. In contrast, a negative effect on verbal memory was shown in a similar trial when CEEs were used ²³. (See meta-analysis of treatment trials by Hogervorst and Bandelow ²⁴.)

Declines in verbal memory are thought to be one of the early indicators of AD ^{25, 26}, particularly when delayed word list and story recall measures are combined in verbal memory assessment ²⁶, as in the case of the current study. Incidence rates of AD are expected to more than double from 1995 to the year 2050 as baby boomers age ²⁷, producing a devastating global burden. Epidemiological studies estimate that an increase of cognitive reserve of only 5% would substantially reduce the incidence rate of AD by one third ²⁸. Interventions that precede the manifestation of AD could provide significant reductions in incident rates and potentially slow functional declines for many women.

Below we describe results from the baseline neuropsychological assessment in a longitudinal study of a community sample of healthy postmenopausal women with specific putative risk factors for AD, currently receiving estrogen HT for at least one year. The current analysis is part of a larger NIA-funded longitudinal study assessing the impact of HT on brain structure and function and cognition in women with putative risk factors for AD. Data were analyzed to determine whether differences in cognitive performance (attention/working memory/processing speed, verbal memory, visual memory, and executive function)

existed between the women who were receiving 17 β -E compared to women who were receiving CEE.

Subjects and Methods

Participants

The study was approved by the Stanford University Human Research Institutional Review Board. Informed consent forms were signed by all study participants. Sixty-eight postmenopausal women with specific putative risk factors for AD, currently on HT for at least one year, and between the ages of 49 and 68 were recruited through the flyers and advertisements from the local community surrounding the School of Medicine at Stanford University. All subjects were Caucasian, with the exception of one Asian -American (17 β -E user). Verification of postmenopausal status was confirmed by plasma levels of follicle stimulating hormone (≥ 40 mIU/ml) and current estrogen usage was substantiated by pharmacy records. All study participants were cognitively intact with at least one or more of the following putative risk factors for AD: first-degree family history of AD^{29, 30}, presence of the apolipoprotein (APOE) $\epsilon 4$ allele, history of a major mood disorder (depression or bipolar disorder)^{31–35}, and/or history of hypothyroidism^{36–39}. However, only women with a history of hypothyroidism were included in the study only if the hypothyroidism was treated and medically stable. Similarly, only women with a history of depression were included if they were not currently depressed (see exclusion criteria). Other than the having at least one putative risk factor, overall only healthy, educated, euthymic, middle-aged women were recruited into the study.

By virtue of the overall study design, all women were users of estrogen-containing HT for at least one year prior to screening. HT included 17 β -E or CEE that was either opposed or unopposed by progesterone through any route of administration (i.e. oral, transdermal). All participants were required to have at least 8 years of education and adequate visual and auditory acuity to allow neuropsychological testing. All participants were assessed for age at menarche and menopause, parity, use of hormonal contraception during reproductive years, type of menopause (natural or surgical), and duration of menopausal transition in relation to time of start of HT use, and type of menopausal symptoms.

As part of the screening process, all participants underwent psychiatric, physical and neurological examination, and laboratory blood safety measures. Because cognitive decline may be caused by a wide variety of conditions having different cerebral metabolic signatures, we excluded participants with impairment from numerous causes (e.g., vascular disease, Parkinson's disease). This increased the likelihood of recruiting a population at specific risk for AD. Volunteers with a history of transient ischemic attacks, carotid bruits, or lacunes on MRI scan were excluded. Other exclusion criteria included evidence of current depression as determined by a score of >8 on the 17-item Hamilton Depression Rating Scale, history of drug or alcohol abuse, contraindication for MRI scan (e.g., metal in body, claustrophobia), history of mental illness (excluding mood disorders), or significant cognitive impairment, as evidenced by impairment in daily functions and/or Mini-Mental Status Exam score < 27 , history of myocardial infarction within the previous year or unstable cardiac disease, significant cerebrovascular disease, uncontrolled hypertension (systolic BP > 170 mmHg or diastolic BP > 100 mmHg), history of significant liver disease, clinically significant pulmonary disease, or cancer. Participants were excluded if they already had possible or probable AD or any other dementia (e.g., vascular, Lewy body, frontotemporal), or evidence of neurological or other physical illness that could be expected to imminently produce cognitive deterioration. Participants were also excluded if they used drugs with the potential to significantly affect psychometric test results, including centrally active beta-blockers, narcotics, clonidine, anti-Parkinsonian medications, antipsychotics,

systemic corticosteroids, medications with significant cholinergic or anticholinergic effects, anticonvulsants, warfarin, or sporadic use of phytoestrogen-containing products, which may produce estrogen-like agonist and antagonist effects.

MRI scans were obtained as screening tests for mass lesions or infarcts as well as for image analysis and hypothesis testing predictor(to be reported elsewhere). If a mass lesion or infarct was identified a subject was excluded from the study and an appropriate referral was made.

Procedures

Neuropsychological assessment—Baseline neuropsychological data was collected on 68 women by personnel well trained in the administration of neuropsychological tests. A neuropsychological battery was administered to the cognitive domains of attention, verbal memory, visual memory, and executive functioning. The following measures were utilized.

Auditory Consonant Trigrams (ACT ⁴⁰)—In this attention and memory test, the subject attempts to recall three consonants followed by a delay (either 0, 3, 9, or 18 seconds), during which the subject is required to count backwards.

Benton Visual Retention Test (BVRT ⁴¹)—The BVRT is a measure of visual spatial memory that is a sensitive indicator of short-term and secondary memory impairment. Geometric designs are presented for 10-seconds and the subject is then required to draw the design from memory.

Buschke-Fuld Selective Reminding Test (SRT ⁴²)—This multi-trial verbal free-recall test requires subjects to learn a list of words over several presentation trials. For each successive trial, only the words not recalled are presented.

Color Trail Making Test (Color Trails 1 & 2⁴³)—This test assesses attention, shifting of conceptual set, and motor speed. The subject is asked to connect a series of numbers in consecutive order from 1 to 25 (Color Trails 1), and then to connect the numbers and alternate between two colors (Color Trails 2). Time to completion and several types of errors can be scored.

Rey-Osterrieth Complex Figure Test (CFT ^{44, 45})—This test entails direct copying, a 3-minute immediate, and a 30 minute-delayed recall of a complex geometric design.

Delis Kaplan Executive Function System (DKEFS ⁴⁶) Color-Word and Verbal Fluency Tests—The Color-Word Test consists of four tasks, naming colored squares as quickly as possible, reading words as quickly as possible, naming the color of color-words as quickly as possible and switching between naming color-words and reading words as quickly as possible. The Verbal Fluency Test a test of verbal fluency consisting of three tasks; naming as many words as possible beginning with three specific letters (letter/phonemic fluency) in one minute, naming as many items from specific categories in one minute (category/semantic fluency), and alternate between two categories, naming as many items as possible in one minute.

Wechsler Abbreviated Scale of Intelligence (WASI ⁴⁷)—The WASI is a short, reliable measure of general intellectual functioning. A Full Scale Intellectual Quotient (FSIQ) can be obtained using two the subtests Vocabulary and Matrix Reasoning. The Vocabulary subtest requires subjects to provide definitions for a list of words and reflects expressive vocabulary, verbal knowledge, and fund of knowledge. The Matrix Reasoning

subtest is a series of incomplete gridded patterns requiring an individual to complete the pattern from five choices. It measures nonverbal fluid reasoning.

Wechsler Adult Intelligence Scale-Third Edition (WAIS -III ⁴⁸)—The WAIS is comprised of thirteen subtests that assess a broad range of cognitive abilities. Three subtests (Digit Span, Letter Number-Sequencing, Digit Symbol Coding) were used. Digit Span requires individuals to repeat back strings of digits either forwards or backwards that increase in length. Letter Number-Sequencing requires an individual to rearrange a group of letters and numbers in chronological and alphabetical order. Digit Symbol Coding requires an individual to copy symbols that are associated with the numbers 1–9 as quickly as possible. It is a measure of visual processing speed and particularly sensitive to brain dysfunction.

Wechsler Memory Scale-third edition (WMS -III ⁴⁹)—Logical Memory I and II. Two short stories are presented for which the subject needs to recall both immediately and after a 30 minute delay. Details and thematic recall of story content is scored.

Because the quantity of data was so extensive and to reduce Type 1 errors due to multiple comparisons, data from individual measures were combined *a priori* into groupings that reflected different cognitive domains using Z-score transformations (see Table 1). The attention/working memory/processing speed domain was formed to assess early encoding processes. Both verbal and visual memory domains were formed to assess later (rehearsal, recall) learning and memory.

Statistical Analysis

Statistical analyses were performed using SPSS software version 18.0 (SPSS Inc., Chicago, IL). Significance was set at the $p < .05$ level without Bonferroni correction, given the small sample size. The two subject groups (17 β -E and CEE; Table 2) were first tested for any differences in clinical or demographic variables using two-group t-tests and chi-square tests. Next, two-group t-tests tested for unadjusted differences in domain scores by type of estrogen. Lastly, multivariate analysis of variance (MANOVA) was applied to the 4 cognitive domains (attention/working memory/processing speed, verbal memory, visual memory, and executive function) with type of estrogen as the independent variable and age, FSIQ, presence of progesterone, and presence of APOE- $\epsilon 4$ as covariates. Given that all subjects were postmenopausal women receiving estrogen hormone therapy, a second MANOVA model was applied to all four cognitive domains with type of estrogen as the independent variable and duration of endogenous estrogen exposure (e.g. length of reproductive life, or age at menopause minus age at menarche), duration of exogenous estrogen exposure (duration of HT), and type of menopause (natural or surgical) as covariates. A third MANOVA model was applied to all 4 cognitive domains with type of estrogen as the independent variable and presence or absence of each of the 4 risk factors as covariates. Partial eta-squares were calculated for each domain within each MANOVA model.

Results

No significant differences were found among demographic or clinical variables between women receiving 17 β -E compared to those receiving CEE. Characteristics of the subjects are summarized in Table 2. Two-group t-test results showed significantly worse verbal memory in women receiving 17 β -E compared to women receiving CEE ($t(66)=2.737$, $p=.009$), while differences were not significant for visual memory ($t(66)=0.959$, $p=.343$), executive function ($t(66)=1.732$, $p=.091$), or attention/working memory/processing speed

($t(66)=1.945$, $p=.059$). Unadjusted means and standard errors of each cognitive domain for women receiving 17 β -E versus CEE are displayed in Figure 1.

The three MANOVA model results are summarized in Table 3. In each of the MANOVA models, women taking CEE consistently showed significantly worse performance in the verbal memory domain compared to women taking 17 β -E, while performance in the visual memory, executive function, and attention/working memory/processing speed domains did not differ between groups.

A significant difference was found between estrogen types in the rate of use of medroxyprogesterone acetate (MPA) versus micronized progesterone within those subjects who were receiving concurrent progesterone. However, two-group mean comparison of MPA users vs. micronized progesterone did not show any significant differences in the cognitive domains (verbal memory $t(44)=0.462$, $p=.646$; visual memory $t(44)=0.026$, $p=.979$; executive function $t(44)=1.204$, $p=.235$; attention/working memory/processing speed $t(44)=0.463$, $p=.646$).

Discussion

As part of a larger longitudinal study of younger (≤ 68 years) postmenopausal women with specific putative risk factors for AD and receiving HT for at least one year, the current analysis of baseline neuropsychological data tested for differences in the cognitive domains of attention/working memory/processing speed, verbal memory, visual memory, and executive functioning between women receiving either HT containing 17 β -E or CEE. Findings from this analysis consistently showed significantly better verbal memory performance in women who receiving 17 β -E compared to women receiving CEE, regardless of demographic and clinical variables. The results also showed significantly better attention/working memory/processing speed and executive function performance among women receiving 17 β -E, but only after controlling for menopause -related variables (length of endogenous and exogenous estrogen exposure, type of menopause). Our findings of better verbal memory in women receiving 17 β -E compared to women receiving CEE are consistent with several studies that show differential effects on verbal memory with different estrogen compounds.

For example, Gleason et al.¹⁶ found better verbal memory in middle -aged women with a parental history of AD (APOE- $\epsilon 4$ carriership was limited) who were using opposed 17 β -E compared to those using opposed CEE use or who were HT -naïve. The HT-naïve group also significantly outperformed the CEE group, as well¹⁶. In the current study we evaluated type of estrogen in relation to APOE- $\epsilon 4$ carriership, as well as other putative risk factors for AD. We found no main affect for any risk factors, including APOE- $\epsilon 4$, on verbal memory, strengthening our hypothesis that type of estrogen influences cognition. Consistent with the WHIMS report, these findings suggest that not only are no benefits derived from the CEE compound, there may be detrimental effects.

Some studies have not found differences in verbal memory performance between use of past HT, current HT or no HT use^{12, 14}. Differences between our findings and other studies might be explained by differences in the assessment of verbal memory. Tests that have low ceiling effects may not be sensitive enough to detect differences between groups of otherwise normal women. In addition, single measures are used or distinctions between immediate and delayed memory are not always provided. Because we studied a sample population of high functioning women, we expected difficulty detecting differences and hence chose to use more challenging cognitive tasks. We also used multiple measures to derive the construct of verbal memory. When multiple measures of verbal memory were

used by Wharton et al.¹⁸, better verbal memory was detected in HT users compared to non-HT users after controlling for demographic and clinical variables (e.g. cardiovascular risk). Hence, differences in the definition and measurement of verbal memory are highly variable between studies and further complicate the understanding of the effects of HT on selective memory domains.

Extrapolation of results from prospective studies is partly hampered by inconsistency in the type of HT preparations examined, with type of HT either not reported or differences in type of HT not analyzed. Low et al.¹⁴ found no benefits on cognition between women with current or former HT use compared to non-users¹⁴, yet 37.7% of current HT users were receiving CEE, 30.5% were receiving 17 β -E, and the rest were receiving various other preparations. Among former HT users, 48.9% had received CEE, 37.0% had received 17 β -E, and the remainder received various other estrogen preparations. Over one-third of the women (37.6%) studied were HT-naïve¹⁴. Thus any differences between types of estrogen were likely diluted simply by study population. In addition, a recent meta-analysis of 24 randomized clinical trials on the effects of HT use versus placebo on cognition concluded that HT does not prevent cognitive decline¹. However, these HT trials studied variable populations of women using a wide variety HT types, dosages, modes of administration, and time spans (2 weeks to 5 years). None of these studies compared types of estrogens (17 β -E versus CEE).

Although HT containing either 17 β -E or CEE are shown to have comparable effects in the treatment of menopause-related symptoms⁵⁰, CNS effects may be different. CEE is a complex formulation consisting of several estrogens, only one of which is 17 β -E. Similarly, type of menopause may underlie differences in HT effects in the CNS. In the case of surgical menopause, precipitous withdrawal from estrogen may hasten verbal memory decline and there is evidence, albeit limited, that type of estrogen differentially affects cognition as well in women who undergo hysterectomy with some suggesting positive effects on verbal memory in women given 17 β -E compared to women given placebo^{22, 51}. At the same time, CEE alone arm of the WHI Memory Study (WHIMS) in postmenopausal women with prior hysterectomy found no effects on verbal memory over time, as well as lower spatial rotational ability that diminished over time⁵². Taken together these studies suggest that, even in surgically menopausal women, verbal memory performance is better in women receiving 17 β -E versus placebo compared those receiving CEE. Because of the sample size of the current study, we were unable to evaluate differences between type of estrogen in surgically versus naturally menopausal women. However, we did not observe any significant differences between women receiving 17 β -E or CEE with respect to type of menopause (natural or surgical).

Another confounding variable in interpreting the effects of HT on cognition is the use of a progesterone component in HT. In a more recent review of randomized clinical trials, Maki and Sundermann¹⁵ found evidence to support that estrogen alone may provide specific benefits to verbal memory in younger naturally and surgically-induced menopausal women. They also described even stronger evidence to support a detrimental effect on verbal memory with combined CEE and MPA in both younger and older women. The most notable randomized clinical trial, the WHIMS, found that the CEE with MPA had a negative impact on verbal memory^{53, 54} and verbal learning¹⁵, compared to women on placebo in their sample of older (>65 years of age) postmenopausal women. However, declines in verbal learning and memory were not significantly different with CEE alone compared to placebo⁵².

The sample size in the current study did not permit us to evaluate differences between types of estrogen with types of progesterone. Because CEE is often combined with MPA

(Prempro®), we did have a higher proportion of women receiving CEE and MPA (18/25) than women on 17 β -E and MPA (7/43) which could have affected our findings. Verbal memory differences remained significant however, when menopause related variables (progesterone use and type of menopause) were used as covariates in the second MANOVA model. A 5-year observational study⁵⁵ as well as the WHIMS both showed no long-term benefits to verbal memory over time with CEE use either with or without MPA.

With respect to HT dosing, data from Zhao and Brinton⁵⁶ demonstrated that low concentrations of estrogens (i.e. low dose) may not provide clinically significant neuroprotection. Further, the predominant estrogen compound in CEE (estrone) has been shown to be one-to two-thirds less potent than 17 β -E in terms of binding affinity to estrogen receptors⁵⁷. When medium-and high-doses (24 or 36 μ g daily, respectively) of CEE were given to middle-aged rats enhancement of memory was found, whereas low-dose CEE (12 μ g daily) was shown to impair both learning and memory⁵⁸. Short term treatment with higher levels of 17 β -E (1 mg for one month, then 2 mg for 2 months) compared to placebo has been shown to attenuate anticholinergic drug-induced challenge to verbal memory measured with the BSRT in younger women (aged 50–62) but impair verbal memory to this challenge in older women aged 70–81⁵⁹. The mode of delivery also affects plasma levels of estradiol, with transdermal administration being the preferred route⁶⁰. The sample size in the current study did not allow a comparison between doses and/or mode of estrogen delivery. However, it is likely that the group of women receiving 17 β -E naturally got a higher dose of this type of estrogen than the women receiving CEE, which only partially contained 17 β -E.

Animal studies support observations of differential effects of timing of HT initiation on cognitive processing. In vitro models suggest that exposure to 17 β -E is beneficial to healthy hippocampal neurons but leads to poorer neuronal function and death when hippocampal neurons are less healthy, such as what would be expected with increased age⁶¹. When medium-and high-doses (24 or 36 μ g daily, respectively) of CEE were given to middle-aged rats enhancement of memory was found, whereas low-dose CEE (12 μ g daily) was shown to impair both learning and memory⁵⁸. Short term treatment with higher levels of 17 β -E (1 mg for one month, then 2 mg for 2 months) compared to placebo has been shown to attenuate anticholinergic drug-induced challenge to verbal memory measured with the BSRT in younger women (aged 50–62) but impair verbal memory to this challenge in older women aged 70–81⁵⁹. These findings are consistent with the proposed window of opportunity theory that suggests HT use may be neuroprotective when used by younger peri- and postmenopausal women but not by older postmenopausal women, at least for a limited time period^{62–65}.

Differential effects of HT initiation timing are posited to be a contributing factor to the risk/benefit profile of HT on cognition and neurodegeneration. The window of opportunity theory suggests HT use may be neuroprotective when used by younger peri- and postmenopausal women but not by older postmenopausal women, at least for a limited time period^{62–65}. Epidemiological and observational studies also show reduced risk of AD^{5, 66} and cognitive decline¹³ with HT use when HT use is initiated prior to 65 years of age (i.e. closer to menopause transition) in contrast to the negative findings from WHIMS where CEE was initiated after the age of 65 years. In vitro models demonstrate that exposure to 17 β -E is beneficial to healthy hippocampal neurons. However, poorer neuronal function and death occur with 17 β -E exposure when hippocampal neurons are less healthy, such as what would be expected with increased age⁶¹. In a randomized clinical trial, Tierney et al.¹⁷ also found that women on 17 β -E had better verbal recall compared to women not on 17 β -E, but only with women who scored average or above average at baseline suggesting these women had “healthier” brains at the beginning of the study. Given that the women in the current

study were younger and that their HT was initiated in perimenopause or in early postmenopause, the current findings (at least for the women receiving 17 β -E) were consistent with the window of opportunity theory.

Finally, a primary difference between our study and many other studies is the presence of at least one putative risk factor for AD. A recent observational study in France followed 3,130 naturally postmenopausal women and found that current HT use attenuated risk of dementia development in women with APOE- ϵ 4 carriership compared to APOE- ϵ 4 positive women not currently on HT¹². Interestingly, the women in this study primarily received 17 β -E rather than CEE, but potential differential effects by type of estrogen were not specifically analyzed. In the present study, we investigated a sample of women at higher risk for AD than the general population and showed differential effects on verbal memory with type of estrogen regardless of APOE- ϵ 4 carriership. These results are especially notable because overall this was a group of healthy, well-educated middle-aged high functioning women.

There are several limitations to the present study. The type of HT could not be controlled for in terms of dosage and use of progestins since inclusion into the study required only that women were currently on prescribed estrogen-containing HT, limiting conclusions about specific dosing and preparations. Because the HT groups (17 β -E vs. CEE) were small, potential confounding effects of the various types of progestins could not be determined, although inclusion of progestin use in the analysis did not alter the finding of worse verbal memory in women receiving CEE compared to those receiving 17 β -E. There are also general limitations due to the small sample size as well as limitations that make generalization difficult (i.e. the use of a highly educated convenience sample surrounding Stanford University). Lastly, as originally conceptualized in our funded research proposal (R01 AG22008), the study included depression as one of the putative risk factors for AD, and as such there was a high proportion of women with a lifetime history of a major mood disorder (76.5% across the sample), which itself may be associated with verbal memory problems⁶⁷. However, we note that t-test comparison of women with and without a lifetime history of a major mood disorder showed no difference between groups for verbal memory ($t(66)=1.306$, $p=.196$). We note that recent data from the Framingham Heart Study offers continued support for depression as a risk factor for AD⁶⁸.

The current study reduced some but not all problematic confounds in other observational studies. For instance, women who use HT tend to be healthier, do have a higher SES, are more educated and expected to perform better on cognitive tasks. Women recruited for this study tended to be typical of HT users and no differences were found between estrogen type groups in terms of educational level, intellectual functioning, and overall health. The women from this study were also rigorously screened, including structural brain imaging for evidence of neurological problems that could have confounded the results. Participants had specific putative risk factors for AD, and APOE- ϵ 4 carriership was tested on all participants, allowing the study to focus on HT use in a particularly vulnerable population. Finally, rather than generalizing cognitive abilities from one or two cognitive tasks and in order to reduce the possibility of Type I errors, the current study combined several measures into specific *a priori* determined cognitive domains. This served to broaden the characterization of each cognitive domain and provide a more consistent representation of these domains.

The findings from the current study support the hypothesis that a particular type of estrogen in HT compounds (17 β -E) may influence selective memory domains and may help to explain some of the controversy that has taken place over the past several years in regards to the role of HT in the protection against neurodegeneration. Clearly more studies are needed to determine whether HT has a role in the treatment of postmenopausal women beyond treating menopausal symptoms. Given the increasing age of the population, it is imperative

for treatments that might prevent or slow the progress of neurodegenerative disorders, such as AD, be rigorously and thoroughly examined.

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Figure 1. Unadjusted means and standard errors of each of the cognitive domains for women receiving 17β-E versus CEE are displayed in Figure 1.

Table 1

Neuropsychological Measures

Cognitive Domain	Measure	Outcome Variable
General Intellectual Ability	WASI	FSIQ (derived from Vocabulary and Matrix Reasoning subtests)
Verbal Memory	BSRT	Trials 1–8
		Delayed recall
	WMS-III	Logical Memory I
		Logical Memory I-Thematic score
		Logical Memory II
		Logical Memory II-Thematic score
		Logical Memory Recognition
Visual Memory	BVRT	Total score
		Error score
	RCFT	Immediate recall
		Delayed recall
Executive Functions	DKEFS	Color Word Inhibition
		Color Word Inhibition/Switching
		Verbal Fluency Category Switching
		Accuracy
		Verbal Fluency Letter
	Color Trails 2	Total time
Attention/Working Memory/Processing Speed	WAIS-III	Digit Span
		Letter Number-Sequencing
		Digit Symbol Coding
	ACT	Total score
	BSRT	1 st trial total score
	WMS-III	Logical Memory 1 st trial total score
	Color Trails 1	Total time
	DKEFS	Word Reading

ACT = Auditory Consonant Trigrams; BVRT = Benton Visual Retention Test; BVRT = Buschke-Fuld Selective Reminding Test ⁴²; Color Trail Making Test ⁴³; DKEFS = Delis Kaplan Executive Function System ⁴⁶; RCFT = Rey-Osterrieth Complex Figure Test ^{44,45}; WAIS-III = Wechsler Adult Intelligence Scale-Third Edition ⁴⁸; WASI = Wechsler Abbreviated Scale of Intelligence ⁴⁷; WMS-III = Wechsler Memory Scale-Third Edition ⁴⁹

Table 2

Sample Characteristics

	17 β -E (n=43)	CEE (n=25)
	Mean (SD)	
Age	57.5 (4.2)	58.8 (5.2)
Years of Education	16.4 (1.9)	15.9 (2.3)
WASI-FSIQ	122.9 (8.0)	120.5 (11.6)
MMSE	29.3 (0.9)	29.2 (1.0)
Length of HT Use	10.1 (7.4)	12.2 (6.6)
Years of Endogenous Estrogen	33.8 (7.0)	31.2 (8.2)
Age at Menopause	47 (7.0)	45.3 (7.4)
BMI	25.0 (3.8)	26.6 (3.6)
	Within-Group Ratios	
Natural vs. Surgical Menopause	26/43 (60%)	15/25 (60%)
Surgical Menopause	17/43 (40%)	10/25 (40%)
Concurrent Progesterone	29/43 (67%)	15/25 (60%)
No Progesterone	14/43 (33%)	10/25 (40%)
Type of progesterone (<i>where applicable</i>)		
Medroxyprogesterone Acetate (MPA) vs. Micronized Progesterone	7/29 (24%)	11/15 (73%)
	22/29 (76%)	4/15 (26%)
	% Per Group	
APOE- ϵ 4 Carriership	37%	40%
Family History of AD	42%	48%
History of Major Mood Disorder	74%	80%
History of Hypothyroidism	35%	40%

MMSE = Mini Mental State Exam

Table 3

MANOVA Results

	First MANOVA Model		Second MANOVA Model		Third MANOVA Model	
	F result	Partial Eta ²	F result	Partial Eta ²	F result	Partial Eta ²
Multivariate test	F[4,58]=1.812, p=.154	.200	F[4,60]=2.294, p=.071	.241	F[4,59]=2.048, p=.101	.223
<u>Between-Subjects</u>						
Verbal Memory	F[1,54]=6.419, p=.016	.167	F[1,56]=9.253, p=.005	.219	F[1,55]=8.763, p=.005	.154
Visual Memory	F[1,54]=0.054, p=.818	.002	F[1,56]=0.748, p=.393	.022	F[1,55]=0.561, p=.457	.016
Attention	F[1,54]=3.485, p=.071	.098	F[1,56]=6.017, p=.020	.167	F[1,55]=3.685, p=.060	.063
Executive Function	F[1,54]=1.772, p=.192	.052	F[1,56]=6.249, p=.018	.159	F[1,55]=0.759, p=.387	.014

Each MANOVA model was conducted with type of estrogen as the independent variable and all 4 cognitive domains entered together as the dependent variables, while the covariates differed across models. Covariates in the first MANOVA were age, WASI-FSIQ, years of education, presence of progesterone, and presence of APOE-ε4. Covariates in the second MANOVA were duration of endogenous estrogen exposure (e.g. length of reproductive life, or age at menopause minus age at menarche), duration of exogenous estrogen exposure (duration of HT), and type of menopause (natural or surgical). Covariates in the third MANOVA were each of the 4 risk factors (APOE-ε4, family history of AD, personal history of mood disorder, and personal history of hypothyroidism).