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Short-term Hormone Therapy with Transdermal Estradiol Improves Cognition for Postmenopausal Women with Alzheimer's Disease: Results of a Randomized Controlled Trial

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Abstract

We aimed to conduct a placebo-controlled, double-blind, parallel-group design intervention study to evaluate the therapeutic efficacy of hormone therapy (HT) in postmenopausal women with mild to moderate Alzheimer's disease (AD). The trial was designed to evaluate the dose-dependent effects of transdermal 17-β estradiol, unopposed and opposed with medroxyprogesterone (MPA, Provera©), for 12 months in 43 postmenopausal women with AD. Participants were assessed using cognitive measures at baseline, months 1, 3, 6, and 12 of treatment and eight weeks post treatment (month 15). The dropout rate was 49% across 12 months. As a result of theWomen's Health Initiative (WHI) and anticipated increased attrition, the protocolwas modified to examine data only at time points where attrition was less than 30%. The results of sensitivity analyses indicated robust and reliable data collected in the first three months of the trial. Data collected in the first three months of the trial for forty-three participants were analyzed. HT had favorable cognitive effects across multiple cognitive domains, including visual memory (p-values < 0.030) and semantic memory (p-values < 0.037) in postmenopausal women with AD. Moreover, treatment-related changes in plasma estradiol were positively correlated with improvements in visual memory. Short-term HT that includes the use of estradiol has favorable effects on cognition in women with AD.

Keywords

Alzheimer's disease; clinical trial; cognition; estradiol; estrogen; hormone therapy; medroxyprogesterone; memory

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INTRODUCTION

Findings from basic science [1], observational [2, 3], and clinical studies [4, 5] suggest that hormone therapy (HT) administered during the menopausal transition could potentially reduce the risk of developing Alzheimer's disease (AD) [6–8]. After diagnosis, use of estrogen as an alternative therapy for AD is controversial [4, 5, 9]. TheWomen's Health Initiative [10] (WHI) and the WHI Memory Study [11] (WHIMS), characterized the cognitive efficacy and adverse effects profile of conjugated equine estrogen (CEE) with and without medroxyprogesterone acetate (MPA) in older postmenopausal women [12]. WHIMS found an increased risk for dementia in postmenopausal women aged 65 and older, treated with CEE and MPA. In contrast, other studies have demonstrated HT-related improvements in cognition including reduced risk of dementia [6, 13, 14].

Evidence that fails to support a cognitive benefit of HT in AD emanates from controlled and uncontrolled clinical studies [15–17] that rely on global cognitive measures limited in sensitivity, and have predominately employed CEE instead of estradiol. CEE, the most widely used form of estrogen replacement therapy among postmenopausal women in the United States, is comprised of estrone sulfate and at least ten other steroid hormones with unknown neurobiological effects [4]. Estradiol forms of HT, an alternative to CEE, are comprised of 17-β estradiol, the most potent and natural human form of estrogen [9]. Transdermal estradiol formulations likely confer additional benefits, because oral formulations have been linked to increased thrombotic risk and cognitive deficits due to microthrombi [18–20].

To date, ten placebo-controlled randomized clinical trials have examined the cognitive effects of HT in postmenopausal women with AD [4, 5, 17, 21–27]. A meta-analysis of seven of these trials concluded that estradiol but not estrone forms of HT may confer short term cognitive benefits in women with AD [28]. The limited number of randomized trials conducted thus far, and methodological discrepancies including differences in HT formulation and the cognitive tests employed likely account, at least in part, for conflicting results regarding the cognition-enhancing effects of HT for postmenopausal women.

Our previous randomized clinical trials have shown that treatment with 17β-estradiol for eight weeks resulted in significant improvements in attention, verbal memory, visual memory, and semantic memory in postmenopausal women with AD. The improvements in cognition were correlated with plasma estradiol concentrations and were observed at doses commonly used in clinical practice [4, 29]. Our findings are consistent with the results of studies surveyed in a meta analysis [30] and with the findings of other uncontrolled estradiol studies [6, 13, 31], lending additional support for a potential beneficial effect of estradiol on cognition in postmenopausal women with AD [32, 33].

The current randomized, placebo-controlled, double-blind, parallel-group design intervention study, aimed to evaluate the potential dose-dependent, cognition-enhancing efficacy of opposed and unopposed transdermal estradiol administration for postmenopausal women with AD.

METHODS

Participants

Participants included 43 outpatient, postmenopausal women (ages 55–85 years) with mild to moderate AD, recruited through AD research programs at the University of Washington and Veterans Affairs Puget Sound Health Care System in Seattle, and at the University of Wisconsin-Madison.

Alzheimer's dementia was diagnosed by consensus through Memory Disorders Clinics using information gathered via medical records, clinical interview, and cognitive testing (Mini-Mental Status Examination, MMSE; Blessed Memory and Information Concentration Test, BMICT). Inclusion criteria included normal gynecological and breast examination within 3 months, normal mammogram and papanicolaou test (pap smear) within the last year, baseline endometrial thickness, as measured by transvaginal ultrasound of less than 5 mm, education level of 9 years or equivalent, Hatchinski score of 4 or less, and a Hamilton Depression Scale score of 14 or below. Both hysterectomized and non-hysterectomized women were recruited.

Exclusion criteria included medical disorders and conditions that contraindicate use of estrogen. Participants were free of any medical or neurological illness apart from AD, and underwent a detailed history, screening blood tests and medical, neurological, and gynecologic examination. HT was discontinued, when applicable, at least 8 weeks before study enrollment.

Study procedures

We conducted a randomized, placebo-controlled, double-blind, parallel-group design intervention study to evaluate the dose-dependent effects of unopposed and opposed (with medroxyprogesterone, MPA) transdermal 17β-estradiol on cognition in postmenopausal women with AD. Participants were assessed on the cognitive outcome measures at baseline, and at months 1, 3, 6, and 12 in the treatment phase of the study. Treatment was discontinued after 12 months and outcome measures were reassessed at month 15 to characterize the effects of HT withdrawal.

Women were assigned to 1 of 5 treatment arms: 1) Low dose unopposed HT: 50μg transdermal 17β-estradiol and a placebo tablet daily, 2) Low dose opposed HT: 50μg transdermal 17β-estradiol and 2.5 mg of MPA daily, 3) High dose unopposed HT: 100μg transdermal 17β-estradiol and a placebo tablet, 4) High dose opposed HT: 100μg transdermal 17β-estradiol and 2.5 mg of MPA daily, or 5) Placebo skin patch and placebo tablet daily.

During each clinic visit, participants were evaluated by the study physician for adverse effects. Under the supervision of a neuropsychologist, cognitive tests were administered by trained psychometrist. To maintain the blind, the study physician did not share any participant information with either the neuropsychologist or psychometrist, and the psychometrist did not discuss adverse events with the participant.

Two years into the study, the results from the WHI and the WHIMS indicated that CEE formulations of HT might be associated with increased risk of cardiovascular disease and dementia. In response, a protocol modification was implemented to inform all participants of the WHI findings and alter the randomization protocol for participants who elected to reconsent and continue in the study. Before the WHI, women were randomized to the 5 treatment arms equally, regardless of hysterectomy status. Post-WHI, randomization to progesterone was stratified by hysterectomy status such that hysterectomized women were assigned equally to unopposed HT or placebo and non-hysterectomized women received opposed HT or placebo.

Cognitive tests

Cognitive function was evaluated using a comprehensive battery of nine neuropsychological tests assessing change in cognitive domains reportedly affected by HT and AD [4]. Different but comparable versions of the battery were administered at each visit to avoid practice effects. The battery included measures of semantic memory (Boston Naming Test [34]),

visual memory (Figural Memory Test [35], Complex Figure Test [36] and Visual Paired Associates [35, 37]), verbal fluency [38], and verbal memory (Paragraph Recall [35, 37], list learning [39]), attention (Trail-Making Test B), and the Stroop Color-Word Interference test [40, 41]), described in detail elsewhere [4, 5]. Similar batteries have been utilized in our previous HT studies, are well tolerated by persons with AD, and are used to evaluate aspects of cognition selectively impaired in early AD [4].

Profile of mood states questionnaire

The effects of HT on mood were measured using the Profile of Mood States (POMS) [42]. The POMS queries mood changes over the preceding week on six categories (anger, anxiety, confusion, depression, fatigue, and vigor) comprised of 65 adjectives (such as friendly or listless) using a 5-point Likert scale. The POMS has been extensively validated to evaluate mood effects of estrogen treatment and is commonly used in AD populations [43–46].

Laboratory tests

Participants underwent an extensive diagnostic work-up including laboratory blood tests, a urinalysis, and an electrocardiogram to assess overall health and exclude treatable medical disorders with cognitive symptoms similar to AD. If not available within the past 12 months, a mammography and a pap smear were conducted during the gynecologic examination. In addition, a trans-vaginal ultrasound (TVUS) measurement of endometrial thickness was performed at baseline, 6 months and 12 months. Participants with an endometrial thickness of 5mm or more at baseline were excluded.

Adherence and correlational analyses were performed on estradiol, estrone, testosterone (T), and luteinizing hormone (LH) levels in blood collected at baseline and at month 3 using an enzyme immunoassay (EIA kit, Alpco Diagnostics, Salem, NH, USA).

Estrogen (estradiol and placebo skin patches) was provided by Berlex Pharmaceuticals (now Bayer HealthCare Pharmaceuticals). Provera© and matching placebo tablets were provided by the Pharmaceutical Research Centers at the University of Washington and the University of Wisconsin-Madison, per FDA regulations.

Analyses

Primary outcomes were selected in light of our studies [5, 29] and earlier reports [47–50], and included scores on tests of semantic and visual memory. The remaining cognitive variables were examined as secondary outcomes given prior reports of HT-related improvements in these measures. Covariates included baseline age, education, and MMSE scores. As a result of the WHI and WHIMS, we anticipated high attrition rates and modified our protocols accordingly [51].

To minimize the potential impact on power or sample selection, we conducted analyses for time points when attrition in treatment arms was less than 30%. Sensitivity analyses evaluate potential bias caused by missing values in follow-up measures to ensure peak sensitivity at all time points [52, 53] and reliability at time points where attrition is less than 30%. Sensitivity analyses are typically conducted to assess the effect of dropouts on inferences about the target parameters, and are particularly important when the treatment arms are unequal [53]. Although there are no clear guidelines regarding the acceptable amount of missing data in a clinical trial (or any longitudinal study), values ranging from 60%–80% as minimum acceptable follow-up rates have been proposed [54–56].

To maximize power in light of high attrition rates, we conducted analyses on all participants taking any form of HT (‘any HT’ group) versus placebo, collapsing across dose. Next, for

those cognitive tests that showed a significant difference between treatment and placebo, we examined potential differences between opposed versus unopposed treatment arms, once again, collapsing across dose. All analyses were conducted using linear mixed-effects (LME) models. The LME approach has the advantage of incorporating fixed-effects parameters and random effects, are more suitable for unbalanced data, and take full advantage of all available variables [57].

Cognitive outcomes collected at baseline and months 1 and 3 were included as repeated outcome measures. In all models, the parameter estimate of primary interest was the treatment by time interaction. Estimation of regression parameters as well as the variance-covariance matrix of random effects was conducted using restricted maximum likelihood (REML) algorithms and robust standard errors. In all models, we assumed an unstructured covariance structure for the two repeated measures. REML methods are generally more appropriate than standard maximum likelihood, particularly in small samples [58]. All models were estimated using SAS, version 9.2.

For all treatment groups (collapsed across dose), we also examined relationships between cognition and hormone levels when 1) hormone levels changed over 3 months of treatment and 2) cognitive performance was altered by treatment. Correlations were calculated using Spearman's rank method.

RESULTS

Participants

Figure 1 illustrates participant enrollment, randomization, and adverse events by the five treatment arms using a CONSORT-style diagram. A total of 43 subjects were randomized across five treatment groups. Prior to the publication of the WHI results, nine of the 43 women had been randomized. Of this group of nine participants, five withdrew early (self-reported worsening of dementia symptoms, $n = 4$; vaginal bleeding, $n = 1$). Post WHI, 34 women were randomized to the study. Sixteen of the 34 (47%) withdrew early. The main reason for discontinuation was vaginal bleeding ($n = 8$) resulting in unblinding of participants. Unlike many HT studies, including the WHI and WHIMS, no incidence of venous thromboembolism, stroke or cardiac events were observed.

Table 1 lists attrition rates for the five treatment arms by study visit. Participants who completed the month 12 visit were considered to have completed the study regardless of whether they returned for the month 15 follow-up visit. The overall withdrawal rate was 49%.

Sensitivity

Imputation through month 3 added 18 to 25 more observations to the analyses, depending on the outcome measure, representing a 22–25% increase in available data. Analyses of 3-month imputed values yielded similar results to analyses of the original dataset that included only non-missing values, indicating that the month 3 results are robust. Analyses through month 6 resulted in an additional 44 records (a 35% increase in available data). Parameter estimates and *p*-values showed high variability between the original and imputed data for the any HT versus placebo comparisons. These findings suggest that modeling results up to, and beyond month 6 are unreliable, and thus were not subjected to analysis in this study.

In light of the results of the sensitivity analyses indicating reliable and robust data in the first 3 months of the trial, we examined the effects of HT treatment on cognitive performance at baseline, month 1 and month 3. While participants had complete data at baseline for certain cognitive tests (e.g., Boston Naming Test), some tests had a higher rate of non-completion

due to issues associated with advanced stages of AD (e.g., fine motor tremors and executive function problems). Thus, tests such as Trails B had higher rates of missing data, even at baseline (23% missing). At baseline, there were no differences between any of the treatment groups in age, education, MMSE, GDS, BMICT, or ApoE4 status (Table 2). As expected, there was a significant difference between opposed and unopposed treatment groups by hysterectomy status ($p < 0.001$, data not shown).

HT and cognitive performance

At baseline, there were no group differences (HT versus placebo) for any of the cognitive measures. Table 3 provides a list of the cognitive tests administered and indicates significant treatment effects when appropriate. The black box highlights the primary outcomes of interest in light of our previous findings. Three months of HT had significant favorable effects on semantic memory (Boston Naming Test, $p = 0.036$), an effect that did not differ across the opposed and unopposed HT groups ($p = 0.85$). Three months of HT had favorable effects on episodic visual memory (Figural Memory Test, $p = 0.015$), and this effect was more pronounced for women who received opposed rather than unopposed HT ($p = 0.08$). A similar pattern of results, though not reaching statistical significance, was also observed on a second test of visual memory, the Complex Figure Test ($p = 0.09$). No significant difference in mood as measured by the total POMS score was observed between the HT and placebo groups ($p = 0.22$)

Treatment effects on hormone levels

Table 4 shows change in hormone levels by treatment group over time. As expected, plasma levels of estradiol and estrone increased for the treated groups (p-values < 0.01). Although plasma T levels were not significantly affected by treatment for any of the groups, LH levels decreased for women receiving unopposed or opposed HT (p-values < 0.015). Hormone levels remained stable over time for women in the placebo group.

Change in estradiol and the estradiol-to-estrone ratio were positively correlated with scores obtained on the Boston Naming task (estradiol: $r = 0.80$, $p = 0.002$; ratio: $r = 0.81$, $p = 0.001$) in the any treatment group. In addition, change in estrone was positively correlated with immediate recall on the Complex Figure Test ($r = 0.64$, $p = 0.048$) in the opposed HT group. There were no significant correlations between estrogen levels and cognition for the unopposed HT group.

DISCUSSION

Our findings indicate that three months of HT administration with transdermal 17β-estradiol had significant favorable effects on semantic memory (Boston Naming Test) and visual memory (Figural Memory Test) in postmenopausal women with AD. These findings, consistent with our earlier reports and the reports of others [4, 5, 30], indicate that short-term HT that includes transdermal 17 β-estradiol may augment some cognitive abilities in older postmenopausal women with AD. Given the small sample size and short duration of treatment, the clinical relevance of the present and other similar studies needs to be confirmed in larger clinical trials of HT over extended periods of time.

Presently, drugs designed to treat AD mainly include cholinesterase inhibitors, which work by preventing the synaptic breakdown of acetylcholine in the brain. However, cholinesterase inhibitors attenuate only some AD symptoms and a positive treatment response is seen in a considerably small subset of the affected population. An ideal pharmacologic treatment for AD should be directed towards multiple pathophysiological mechanisms, have the potential to favorably alter disease neurobiology, be associated with minimum toxicity, and result in

clinically significant improvements in AD symptoms. Short-term use of HT with estradiol may represent one such alternative treatment to improve cognitive symptoms associated with AD in older postmenopausal women. Unlike cholinergic drugs that primarily enhance cholinergic neurotransmission [59], estrogen exerts multiple salutary effects on the brain that have the potential to both enhance cognition and favorably alter AD pathology (for a comprehensive review, see [60]). Among others, some of these salutary effects include enhanced serotonergic, cholinergic and dopaminergic neurotransmission, anti-inflammatory effects, antioxidative efficacy, an ability to favorably alter amyloid-β protein precursor metabolism, multiple neurotrophic effects (e.g., increased synaptogenesis and dendritic spine density), and an ability to enhance glucose metabolism in areas known to be afflicted by AD pathology and involved in memory (e.g., the hippocampus) [61–63]. Findings from clinical studies suggest that, in addition to improving cognition, HT enhances cerebral blood flow and glucose utilization [64–67].

The results of the current study not only replicate our previous findings, but also are consistent with other reports demonstrating cognitive improvement associated with increased endogenous estradiol levels in younger women and exogenous levels via HT in healthy older women [68, 69]. Cognitive assessment was obtained using a comprehensive battery of well-established tests, across multiple cognitive domains pertinent to HT and AD. Together, these findings provide further support for a favorable effect of estradiol for healthy and pathological aging. This conclusion is not without controversy, as others have failed to show a beneficial effect on cognitive function [17, 22]. The lack of a clinical consensus likely relates to a variety of complex financial, social, psychological, and scientific issues, including several important methodological inconsistencies between studies, such as formulation and route of estrogen administration, hysterectomy status, age at time of exposure, exposure duration, sensitivity of cognitive tests administered, and inter-individual differences in hormone levels achieved following HT.

Our findings provide preliminary evidence to suggest that opposed 17 β-estradiol administration may confer greater benefits for visual memory than unopposed therapy. This finding is surprising, given recent evidence that MPA is neurotoxic and could further worsen cognitive performance [70]. When the current study was designed, MPA was the most commonly used progesterone to oppose estrogen. Unlike natural progesterone, MPA binds to glucocorticoid receptors with a much higher affinity and may have a greater impact on the hypothalamic–pituitary–adrenal axis, basal forebrain and limbic areas such as the amygdala and hippocampus, areas of the brain that are particularly stress-sensitive [71]. It is possible that the MPA-induced androgenic and progestogenic actions may result in short-term improvements on visuospatial ability, which may also explain reports that CEE + MPA was associated with a trend toward beneficial effects on figural memory in the Women's Health Initiative Study of Cognitive Aging (WHISCA) study [72]. Taken together, these results suggest that the potential negative cognitive effects associated with MPA administration likely take longer than 3 months to manifest.

The primary limitation of the present study relates to attrition. Published risks from the WHI and WHIMS significantly increased attrition of participants in our study relative to attrition rates observed in our previous HT trials and adversely affected the overall recruitment resulting in a smaller than anticipated sample size. In a retrospective analysis of 29,718 new HT users, 54.4% were non-adherent after one year [73]. Moreover in women over 65 years of age, 62% discontinued HT within 12 months compared to 48% of younger women 50 to 55 years [74]. To minimize the potential impact on power or bias caused by such limitations, we performed analyses only on data collected up to month 3 for comparison groups collapsed across HT dose (i.e., any HT, versus placebo; opposed versus unopposed). A second important limitation relates to the mandated IRB modification to the randomization

scheme in the wake of the WHI and WHIMS reports. That is, hysterectomized women were assigned equally to unopposed HT or placebo and non-hysterectomized women received opposed HT or placebo. As a result, the potential effects of hysterectomy status on cognitive response to HT could not be evaluated. Type of hysterectomy and duration since surgery is now recognized as an important factor and has been associated with an increased risk of cognitive impairment [75]. Finally, we were not able to address whether variables such as type of hysterectomy, duration and type of HT for prior users, cardiovascular history, and smoking history modulated response to HT as this information was not collected in our study.

In summary, our findings indicate favorable effects of short-term HT that includes estradiol on cognitive function in older postmenopausal women with AD. Future, larger trials to examine the cognitive effects of HT for older women with AD will be essential to further investigate the short-term therapeutic benefit of estradiol HT.

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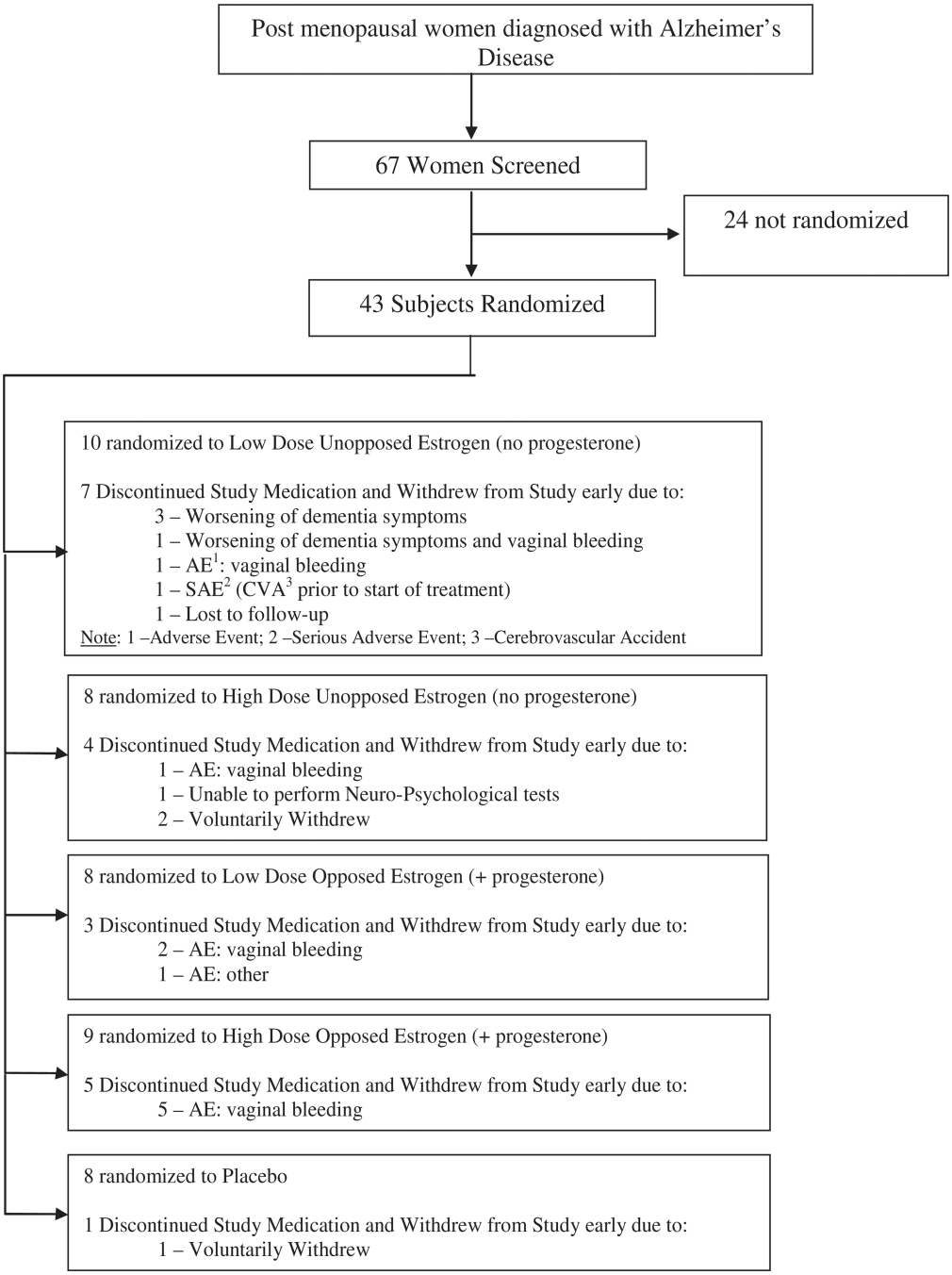

Fig. 1.
Diagram describing study enrollment, randomization, and follow-up protocol.

Table 1

Attrition by treatment arm

	Sample Size at Month – Subjects with Any Cognitive Data					
Group	**Baseline**	**1**	**3**	**6**	**12**	**15**
Placebo	8	8 (100%)	8 (100%)	8 (100%)	7 (87.5%)	7 (87.5%)
Low Dose Estrogen Unopposed	10	7 (70%)	6 (60%)	5 (50%)	3 (30%)	2 (20%)
High Dose Estrogen Unopposed	8	7 (87.5%)	7 (87.5%)	6 (75%)	4 (50%)	4 (50%)
Low Dose Estrogen Opposed	8	8 (100%)	6 (75%)	5 (62.5%)	5 (62.5%)	5 (62.5%)
High Dose Estrogen Opposed	9	9 (100%)	7 (78%)	4 (44%)	4 (44%)	3 (33%)
Estrogen Unopposed	18	14 (78%)	13 (72%)	11 (61%)	7 (39%)	6 (33%)
Estrogen Opposed	17	17 (100%)	13 (76%)	9 (53%)	9 (53%)	8 (47%)
Any HT	35	31 (89%)	26 (74%)	20 (57%)	16 (46%)	14 (40%)
Total	43	39 (91%)	34 (79%)	28 (65%)	23 (53%)	21 (49%)

Table 2

Comparison of treatment groups according to demographic, mood, ApoE4 status and cognitive variables at baseline

Variable	Treatment Group	n	Mean	SD	Contrast	KW* *p*-value
Age (y)	1. Placebo	8	74.4	5.2		
	2. Estrogen Unopposed	18	78.1	8.4	2, 1	0.131
	3. Estrogen Opposed	17	76.5	7.8	3, 1	0.431
	4. Any Estrogen	35	77.3	8	4, 1	0.205
Blessed Memory	1. Placebo	8	28	6.8		
	2. Estrogen Unopposed	14	26.7	4.8	2, 1	0.321
	3. Estrogen Opposed	16	27.3	6.3	3, 1	0.781
	4. Any Estrogen	30	27	5.6	4, 1	0.495
Education (y)	1. Placebo	8	13.9	1.5		
	2. Estrogen Unopposed	17	13.2	2.1	2, 1	0.239
	3. Estrogen Opposed	16	13.3	1.9	3, 1	0.398
	4. Any Estrogen	33	13.3	1.9	4, 1	0.255
GDS	1. Placebo	8	3.5	1.9		
	2. Estrogen Unopposed	18	2.4	2.1	2, 1	0.114
	3. Estrogen Opposed	17	2.5	2.7	3, 1	0.121
	4. Any Estrogen	35	2.5	2.4	4, 1	0.087
MMSE	1. Placebo	8	21.8	6.4		
	2. Estrogen Unopposed	15	22.4	3.8	2, 1	1
	3. Estrogen Opposed	17	24.5	3.8	3, 1	0.365
	4. Any Estrogen	32	23.5	3.9	4, 1	0.599
Variable	Treatment Group	n	N	%	Contrast	Chisq
ApoE ε4+	1. Placebo	5	7	71.43		
	2. Estrogen Unopposed	7	12	58.33	2, 1	0.568
	3. Estrogen Opposed	12	15	80	3, 1	0.655
	4. Any Estrogen	19	27	70.37	4, 1	0.956

*The nonparametric Wilcoxon scores one-way ANOVA approach of the Kruskal Wallis test was used to assess differences between the treatment groups.

Table 3

Treatment effects on cognitive performance scores

Cognitive Tests	Any HT vs. Placebo				Opposed vs. Unopposed *p*-value
	Estimate	SE	*t*-ratio	*p*-value	
Boston Naming (Total)	1.48	0.66	2.24	0.032	0.853
Boston Naming (Spontaneous)	1.13	0.52	2.19	0.036	0.889
Figure Memory-Total	0.63	0.24	2.60	0.015	0.082
CFT-Immediate Recall	2.08	1.19	1.75	0.090	0.029
CFT-Total Recall	4.66	2.57	1.81	0.081	0.279
CFT Delayed Recall	2.04	1.62	1.26	0.218	0.664
VPA: Immediate	−0.89	0.88	−1.01	0.319	
VPA: Delayed Recall	0.43	0.30	1.42	0.166	
VPA: Total	−0.43	0.97	−0.44	0.663	
Fluency	0.87	1.65	0.53	0.602	
Paragraph-Delayed Recall	−0.07	1.43	−0.05	0.960	
Paragraph: Immediate Recall	−0.47	1.67	−0.28	0.780	
Paragraph: Total Recall (Imm. + Del)	−0.63	2.49	0.25	0.800	
List Learning-Total Recall	−1.78	1.49	−1.19	0.244	
List Learning-Delayed Recall	−0.23	0.44	−0.51	0.614	
List Learning-Immediate	−1.43	1.27	−1.13	0.268	
Trails B (time)	−11.58	17.75	−0.65	0.519	
Stroop-Interference (time)	28.75	18.57	1.55	0.132	

All linear mixed effects models controlled for age, education level and Mini-Mental State Examination (MMSE) scores. Models were estimated using restricted maximum likelihood and robust standard errors.

Table 4

Hormone levels across time and treatment group

		Estradiol BL pg/mL	Estradiol M3 pg/mL	Estrone BL pg/mL	Estrone M3 pg/mL	Estradiol Estrone Ratio BL pg/mL	Estradiol Estrone Ratio M3 pg/mL	T BL pg/mL	T M3 pg/mL	LH BL pg/mL	LH M3 pg/mL
Unopposed	Mean	16.56	96.33	32.61	76.83	0.59	1.23	269.94	211.25	12.33	7.69
	n	18	12	18	12	18	12	18	12	18	12
	SD	7.16	98.01	17.00	56.47	0.26	0.64	320.53	139.68	4.57	3.62
	pvalue		0.005*		0.008*		0.041		0.433		0.012*
Opposed	Mean	14.00	107.38	28.76	59.15	0.56	1.76	302.29	174.08	14.87	8.06
	n	17	13	17	13	17	13	17	13	17	13
	SD	0.00	93.29	12.08	34.58	0.21	0.92	274.46	127.96	6.35	4.46
	pvalue		0.002*		0.004*		0.001*		0.055		0.001*
Any HT	Mean	15.31	102.08	30.74	67.64	0.58	1.50	285.66	191.92	13.56	7.88
	n	35	25	35	25	35	25	35	25	35	25
	SD	5.23	93.73	14.73	46.27	0.23	0.83	295.10	132.24	5.57	4.00
	pvalue		0.000*		0.000*		0.000*		0.045		0.000*
Placebo	Mean	14.00	19.75	35.75	25.00	0.54	0.81	282.50	145.13	13.12	13.75
	n	8	8	8	8	8	8	8	8	8	8
	SD	0.00	16.26	25.18	14.31	0.25	0.28	237.67	114.40	6.82	8.77
	pvalue		0.317		0.183		0.017		0.036		0.401

*P-values reflect the results from pair-wise Wilcoxon rank sum tests.