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Early Postmenopausal Transdermal 17β-Estradiol Therapy and Amyloid-β Deposition

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Abstract

Background—It remains controversial whether hormone therapy in recently postmenopausal women modifies the risk of Alzheimer's disease (AD).

Objective—To investigate the effects of hormone therapy on amyloid-β deposition in recently postmenopausal women.

Methods—Participants within 5–36 months past menopause in the Kronos Early Estrogen Prevention Study, a randomized, double blinded placebo-controlled clinical trial, were randomized to: 1) 0.45 mg/day oral conjugated equine estrogens (CEE); 2) 50-μg/day transdermal 17-β-estradiol; or 3) placebo pills and patch for four years. Oral progesterone (200 mg/day) was given to active treatment groups for 12 days each month. ^{11}C Pittsburgh compound B (PiB) PET imaging was performed in 68 of the 118 participants at Mayo Clinic approximately seven years post randomization and three years after stopping randomized treatment. PiB Standard unit value ratio (SUVR) was calculated.

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Results—Women (age = 52–65) randomized to transdermal 17-β-estradiol (n = 21) had lower PiB SUVR compared to placebo (n = 30) after adjusting for age [odds ratio (95%CI) = 0.31(0.11–0.83)]. In the *APOE* ε4 carriers, transdermal 17β-estradiol treated women (n = 10) had lower PiB SUVR compared to either placebo (n = 5) [odds ratio (95%CI) = 0.04(0.004–0.44)], or the oral CEE treated group (n = 3) [odds ratio (95%CI) = 0.01(0.0006–0.23)] after adjusting for age. Hormone therapy was not associated with PiB SUVR in the *APOE* ε4 non-carriers.

Conclusion—In this pilot study, transdermal 17β-estradiol therapy in recently postmenopausal women was associated with a reduced amyloid-β deposition, particularly in *APOE* ε4 carriers. This finding may have important implications for the prevention of AD in postmenopausal women, and needs to be confirmed in a larger sample.

Keywords

Alzheimer's disease; amyloid-β; cognitive function; estrogen; hormone therapy; menopause; PET; prevention

INTRODUCTION

Hormone therapy consisting of conjugated equine estrogens (CEE) along with medroxyprogesterone acetate initiated in the late postmenopause stage (≥ 65 years) increased the risk of dementia in the Women's Health Initiative Memory Study (WHIMS) [1]. However, there is controversy on whether estrogen with or without progesterone can preserve neurological function and decrease the risk of dementia when administered early in menopause, i.e., during a "window of opportunity" phase [2–8]. Although determining the effects of hormone treatment shortly after menopause on the risk of dementia would require decades of follow-up, non-invasive imaging markers of Alzheimer's disease (AD) pathophysiology can potentially assess the efficacy of preventive interventions in the short term.

The Kronos Early Estrogen Prevention Study (KEEPS) was a multi-center, randomized, placebo-controlled, double-blinded trial of hormone treatment in recently menopausal women who were in good cardiovascular health. KEEPS tested the hypothesis that hormone therapies administered soon after the onset of menopause would slow progression of atherosclerosis; [9] however, no effect was observed on several imaging markers of progression of atherosclerosis during the four year trial [10], or cognitive function [11]. Although estrogens, in particular, are thought to modify the risk of AD, estrogen effects on amyloid-β (Aβ) pathology have not been investigated in a hormone treatment trial. Examining data obtained at a one KEEPS enrollment site, we report the effects of two forms of hormone therapy, oral CEE and transdermal 17γ estradiol therapy on Aβ deposition measured by Pittsburgh compound-B (PiB) PET.

METHODS

Participants

KEEPS (NCT00154180) was a multicenter, randomized, double blinded, placebo-controlled clinical trial in recently menopausal women (n = 727) that was conducted between 2006 and

2011. Participants enrolled in KEEPS were between 42 to 59 years of age, within 5 to 36 months past their last menses, and were in good cardiovascular health and did not have a history of hysterectomy or oophorectomy [9]. Estrogens were administered through two different routes: Oral or transdermal. Participants were randomized to either: 1) oral conjugated equine estrogen (CEE; Premarin, 0.45 mg/day); 2) transdermal 17β-estradiol (skin patch, Climara, 50 μg/day); or 3) placebo pills and patch. Progesterone was given orally (Prometrium; micronized progesterone, 200 mg/day) for the first 12 days each month to both active treatment groups. Participants were treated for four years.

Neuroimaging for the current study was conducted from December 2012 through July 2014 and included the subsample of women who were enrolled in KEEPS at the Mayo Clinic, to investigate the effects of the KEEPS hormone treatments on Aβ deposition three years after the end of the trial. This study was approved by the Mayo Clinic Institutional Review Board and all subjects or appropriate surrogates provided informed consent for participation Exclusion criteria for the imaging studies were contraindications for safety and neurologic disorders such as brain tumors, multiple sclerosis, neurodevelopmental abnormalities, or treatments (e.g., systemic chemotherapy) that would affect the brain structure. Apolipoprotein E(*APOE*) genotyping was performed after randomization and clinical examinations were performed at the Mayo Clinic Specialized Center of Research on Sex Differences within three months of the imaging studies. The study was approved by the Institutional Review Board at Mayo Clinic and all participants gave informed consent.

Neuropsychological assessment and cognitive function

A confirmatory factor analysis was used to assess the underlying structure of baseline cognitive data from the KEEPS cognitive and affective study ($n = 662$), and to derive summary scores [12]. Using standard criteria for model fit, the cognitive variables were summarized with a general domain representing global cognitive function at baseline.

A battery of neuropsychological tests three years after the end of the hormone therapy phase were administered within three weeks of the neuroimaging examinations in the Research Psychometrics Resource Laboratory at Mayo Clinic's Center for Translational Science Activities (CTSA) under the direction of a neuropsychologist (JP). Cognitive performance was investigated in four domains: 1) Learning & Memory from the California Verbal Learning Test (CVLT), New York University (NYU) Paragraphs, and Benton Visual Retention Test; 2) Auditory Attention & Working Memory from Wechsler Memory Scale-III Letter-Number Sequencing and Digit Span subtests; 3) Visual Attention & Perceptual Speed from Trail Making Test part A, Color and Word trials of the Stroop test, and Wechsler Adult Intelligence Scale-III Digit Symbol Coding subtest; 4) Speeded Language & Flexibility from phonemic (F, A, S) and category (animals, fruits, vegetables) verbal fluency, Trail Making Test part B, and Color-Word Interference trial of the Stroop.

MRI and PET imaging

MRI studies were performed on a single 1.5T system, with an 8-channel phased-array coil (GE Healthcare). A 3D high resolution MPRAGE acquisition with TR/TE/TI = 7/3/900 ms;

flip angle 8 degrees; in plane resolution of 1.0mm and a slice thickness of 1.2mm was performed for anatomical segmentation and labeling of PiB PET scans.

PET images were acquired using a PET/CT scanner (DRX; GE Healthcare) operating in 3D mode. The participants were injected with 292–729 MBq [^{11}C]PIB. A CT image was obtained for attenuation correction. After a 40-min uptake period, a 20-min PiB scan was obtained. The PiB-PET acquisition consisted of four 5-min dynamic frames, acquired from 40 to 60 min after injection. Standard corrections were applied. The pixel size for PET images was 1.0mm and the slice thickness was 3.3 mm.

Analysis of PiB PET

PiB PET quantitative image analysis was performed using the fully automated image processing pipeline which has been described in detail elsewhere [13]. Briefly, the method includes gray matter (GM) sharpening of PET images using MRI and partial volume correction of cerebrospinal fluid and tissue compartments using Statistical Parametric Mapping unified segmentation algorithm [14]. PiB PET cortical ratio images were calculated by dividing each PiB PET GM voxel value by the median value in the cerebellar GM region in patient's MRI space. PiB retention was calculated by the PiB Standard unit value ratio (SUVR), with the median values of the PiB PET GM ratio from the bilateral parietal, posterior cingulate, precuneus, temporal, prefrontal, orbitofrontal, anterior cingulate GM regions in the in-house modified anatomical labeling atlas.

Statistical analysis

Characteristics of participants were compared across the treatment and the placebo groups using Kruskal-Wallis tests or Fisher exact tests, as appropriate. We also compared the characteristics of the participants and non-participants. Cognitive tests scores were compared across the treatment and the placebo groups using ANOVA and Tukey's honest significant differences test for the *post-hoc* comparisons with adjustments. Age was tested for association with PiB SUVR using Spearman correlations. We performed the comparisons of PiB SUVR values across treatment and placebo groups, adjusting for age, using proportional odds logistic regressions [15]. This semiparametric model mitigates the effect of outliers while allowing for parametric effects of age and treatment, and simultaneously estimates the log (odds) of higher versus lower value of PiB SUVR, at all possible threshold values. Thus, we did not classify the participants into Aβ-positive and Aβ-negative categories based on PiB SUVR.

RESULTS

All women enrolled in KEEPS at the Mayo Clinic in Rochester, Minnesota (n = 118) were considered for participation in the current study. Six participants were excluded due to neurological disorders or MRI contraindications, forty women declined to participate in both MRI and/or PET studies, and four participants were lost to follow-up. Of the 112 eligible KEEPS participants, 68 women (61%) with median age of 60 (range, 52–65) participated in both the MRI and PET studies and were included in the analysis (Fig. 1). Participants included in the analysis did not differ from those who did not participate in the

neuroimaging study on age ($p = 0.09$), education ($p = 0.42$), smoking status ($p = 0.48$), time past from menopause to randomization ($p = 0.55$), or *APOE* status ($p = 0.47$).

The time elapsed between last menses and randomization was on average ten months longer in the oral CEE ($p = 0.05$) and five months longer in the transdermal 17β-estradiol group compared to placebo ($p = 0.04$). The transdermal 17β-estradiol group had a higher proportion of *APOE* ε4 carriers (50%) than the oral CEE (18%; $p = 0.08$) and the placebo groups (18%; $p = 0.03$). All *APOE* ε4 carriers had the ε4/ε3 genotype. Three women declined *APOE* genetic testing (Table 1). All participants were cognitively normal on clinical examination and neuropsychological testing. There were no correlations between neuropsychometric test scores and the PiB SUVR values in the entire group as well as in the oral CEE, transdermal 17β-estradiol and placebo groups separately ($p > 0.09$). However, after adjusting for age, education, *APOE* ε4 status, and time from menopause to randomization, CVLT Total Score was lower in the oral CEE group compared to placebo on ANOVA and *post hoc* Tukey's Honest Significant differences test ($p = 0.03$) (Table 2).

Because of a difference in the proportion of *APOE* ε4 carriers among treatment groups, and the potential impact of this variable on outcome, a stratified analysis in *APOE* ε4 carriers and non-carriers was conducted. There was a significant association of PiB SUVR with age in the whole group ($r = 0.37$; $p = 0.002$), in *APOE* ε4 carriers ($r = 0.48$; $p = 0.046$), and in *APOE* ε4 non-carriers ($r = 0.43$; $p = 0.003$). Therefore, all analyses were adjusted by age (Fig. 2).

The distribution of PiB SUVR varied by treatment group and by *APOE* ε4 carrier status (Fig. 3). Participants who were treated with 17β-estradiol were were more likely to have lower PiB SUVR compared to placebo after adjusting for age [odds ratio (95%CI) = 0.31 (0.11–0.83)]. By use of the proportional odds model, this odds ratio applies to any possible cut-point for PiB SUVR. In the *APOE* ε4 carriers ($n = 18$), transdermal 17β-estradiol treated participants were more likely to have lower PiB SUVR compared to placebo [odds ratio (95%CI) = 0.04 (0.004–0.44)], and compared to the oral CEE treated participants [odds ratio (95%CI) = 0.01 (0.0006–0.23)] after adjusting for age. Treatment with either oral CEE or transdermal 17β-estradiol was not associated with PiB SUVR in *APOE* ε4 non-carriers ($n = 47$) (Fig. 4).

DISCUSSION

This study of recently menopausal women who participated in a randomized controlled hormone therapy trial showed that Aβ deposition measured by PiB retention on PET was lower in women who received transdermal 17β-estradiol for four years compared to placebo. In contrast, oral CEE was not associated with a lower level of PiB retention. Although the oral CEE group performed worse on verbal learning and memory compared to placebo, this finding should be interpreted with caution because of the small sample size and because no correlation was found between PiB retention and cognitive test scores. Stratified analysis by *APOE* ε4 genotype showed that the lower PiB retention in the transdermal 17β-estradiol group was present only in the *APOE*ε4 carriers. Hormone therapy was not associated with PiB retention in *APOE* ε4 non-carriers.

A precipitous decline in endogenous estrogens with menopause is thought to be a major driver of AD risk in women. Hence, hormone therapy with estrogens offers the possibility for preventing or delaying the onset of AD in aging women [6, 8, 16, 17]. Observational studies suggest that estrogen treatment, when administered to recently menopausal women, protects from age-associated cognitive decline and dementia [5, 17–25]. KEEPS was a randomized, placebo-controlled hormone therapy trial designed to test for intervention during the period of rapid estrogen depletion in recently menopausal women. Thus, KEEPS is ideally positioned to investigate the effects of hormone therapy on prevention of AD-related pathology during this “window of opportunity”.

PiB retention on PET imaging is a quantitative measure of Aβ deposition [26]. High Aβ deposition measured on PET imaging or via cerebrospinal fluid is considered to be the earliest biomarker change observed during the preclinical stages of AD [27, 28]. Thus, PiB retention on PET is an appropriate biomarker to investigate whether hormone therapy influences Aβ deposition specifically during the early menopausal years when the effect of Aβ deposition on cognitive function is not yet manifested. We observed no differences in cognitive function among the 17β-estradiol and placebo groups. However, a randomized controlled trial of oral 17β-estradiol in older women (age: 61–87) found less decline in short-delayed verbal recall compared to placebo [29]. Hence, the effects of lower levels of Aβ deposition in the transdermal 17β-estradiol group on cognitive function may be apparent later in life.

Carriers of the *APOE ε*4 allele are at an increased risk of AD dementia; moreover the risk may be higher in women than in men [30, 31]. *APOE ε*4 carriers have increased Aβ deposition at an earlier age than *APOE ε*4 non-carriers, and difference is more pronounced in women than in men [32, 33]. Thus, women who are *APOE ε*4 carriers are at a higher risk for AD-related pathology and may benefit most from preventive interventions at an early age. In the current study, we found that postmenopausal transdermal 17β-estradiol is associated with lower levels of Aβ deposition compared to placebo particularly among women who are *APOE ε*4 carriers. We interpret this finding in two possible ways.

The first possible interpretation is that *APOE* status modifies the effect of transdermal 17β-estradiol on Aβ deposition as a pharmacogenetic effect. This interpretation is consistent with observations where *APOE* also modulates the effect of transdermal 17β-estradiol therapy on Aβ deposition in live mice, [34] and in cultured adult mouse cortical neurons [35]. *APOE ε*4 status appears to modify the effects of hormone therapy on cognitive function and dementia also in humans; however, the findings are conflicting [36–38]. In one observational study, *APOE ε*4 positive women opting to use hormone therapy had lower risk of dementia, however, the forms of hormone therapy were not specified [36]. On the contrary, *APOE ε*4 positive women had more cognitive decline than *APOE ε*4 negative women if they used hormone therapy (primarily with oral CEE) in two other observational studies [37, 38]. Similarly, we did not find an association of oral CEE therapy with Aβ deposition compared to placebo. In fact, *APOE ε*4 carriers treated with oral CEE showed higher levels of Aβ deposition than *APOE ε*4 carriers treated with transdermal 17β-estradiol. However because of the low number of *APOE ε*4 carriers in the CEE group, this finding needs to be interpreted with caution. In WHIMS, oral CEE therapy along with medroxyprogesterone

acetate, initiated in older women (age ≥65) increased the risk of dementia and brain atrophy, which persisted into older ages [1, 2, 39, 40]; however, the *APOE ε*4 status of women in WHIMS was not reported. Because CEE increases serum levels of estrone and of sulfonated conjugates more than transdermal 17β-estradiol, it is not unexpected that the various circulating estrogens would have different efficacy in binding and activation of estrogen receptor mediated events such as the deposition of Aβ [41]. Further work is needed to understand how higher doses of oral CEE (e.g., 0.625 mg/day as used in the Women's Health Initiative), may increase the circulating levels of 17β-estradiol to those comparable to the transdermal 17β-estradiol treatment group.

A second possible interpretation of the finding is that the *APOE ε*4 non-carriers included in our study were too young to show hormone therapy effects on Aβ deposition. Participants recruited to the PET study three years after KEEPS were at a median age of 60 with a range of 52 to 65. In the population-based Mayo Clinic Study of Aging, the estimated age at which 10% of the population had high levels of Aβ deposition was 57 years for *APOE ε*4 carriers and 64 years for *APOE ε*4 non-carriers [42]. Thus, it may be too early to detect transdermal 17β-estradiol effects on Aβ deposition in *APOE ε*4 non-carriers in the current study. Further follow-up of the cohort is planned to determine whether transdermal 17β-estradiol therapy in recently menopausal women is associated with Aβ deposition in older age.

This study was conducted at a single KEEPS site; therefore, the sample size is limited. Our findings need to be confirmed in a larger sample perhaps by including all KEEPS sites. The participation rate (with the exclusions) for this multimodality imaging study is comparable to the imaging participation rate observed in other hormone therapy trials such as the WHIMS-MRI study [40]. A higher proportion of *APOE ε*4 carriers in the transdermal 17β-estradiol group is not surprising given the relatively small number of women included this pilot study. Randomization does not guarantee a balanced allocation across treatment groups when the numbers are small. Lower Aβ deposition in the transdermal 17β-estradiol group compared to placebo cannot be explained by the higher proportion of *APOE ε*4 carriers in the transdermal 17β-estradiol group than the placebo. In fact, the opposite would be expected, because Aβ deposition should be highest in a group with a higher proportion of *APOE ε*4 carriers. Although the study cohort was randomized to hormone therapies and placebo 7 years ago, cardiovascular risk factors and biomarkers remained comparable in the oral CEE, transdermal 17β-estradiol and placebo groups at 84 months (7 years) post-randomization. KEEPS was designed to include women who were in good cardiovascular and neurological health, therefore generalization of our findings to a broader population may be limited. Yet, in a homogenously healthy cohort of women, the potential effects of hormone therapy on Aβ deposition are not confounded by vascular disease and perhaps define a population who might benefit from the use of transdermal 17β-estradiol.

The consequences of Aβ deposition during early menopausal years are not fully understood, and effectiveness of early menopausal hormone therapy in preventing AD-related pathology in the long-term remains unclear. However, reducing Aβ deposition through Aβ-modifying therapies is a widely accepted strategy for preventing AD, and clinical trials are underway in cognitively normal individuals with high PiB retention, [43] and in *APOE ε*4 carriers [44]. The association of transdermal 17β-estradiol therapy in recently menopausal women with

lower Aβ deposition has the potential to change the concepts for preventive interventions that drive the field, and may have a significant impact on women making the decision to use hormone therapy in the early postmenopausal years.

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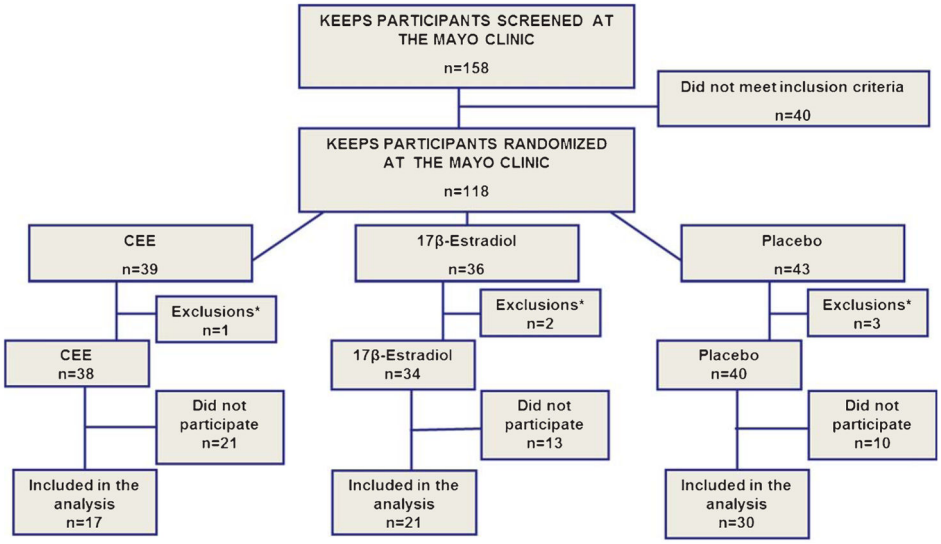

Fig. 1.

Participation flowchart: * There were six exclusions: One woman with an MRI incompatible implant (oral CEE group); one woman with posterior fossa developmental abnormality and hydrocephalus, one woman who developed breast cancer and underwent systemic chemotherapy (transdermal 17β-estradiol group); two women with multiple sclerosis and one woman with a benign brain tumor (placebo group).

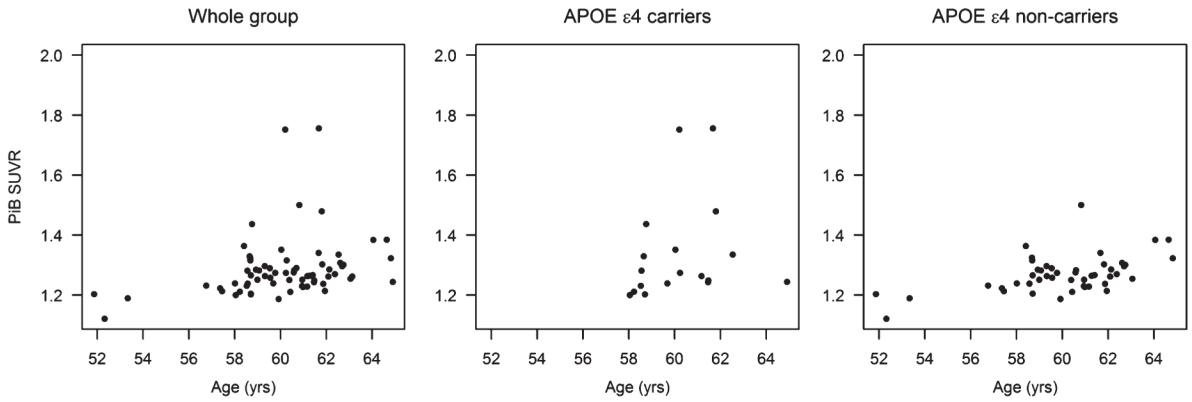

Fig. 2.

Associations of PiB SUVR with age in the whole group of participants, in APOE ε4 carriers, and in APOE ε4 non-carriers.

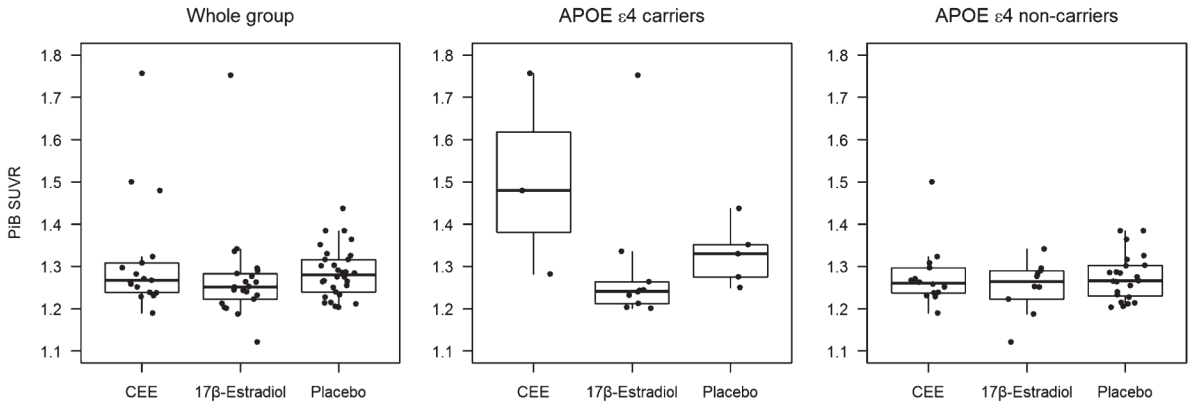

Fig. 3.

PiB SUVR in the oral CEE, transdermal 17β-estradiol, and the placebo groups in the whole group of participants, in APOE ε4 carriers, and in APOE ε4 non-carriers.

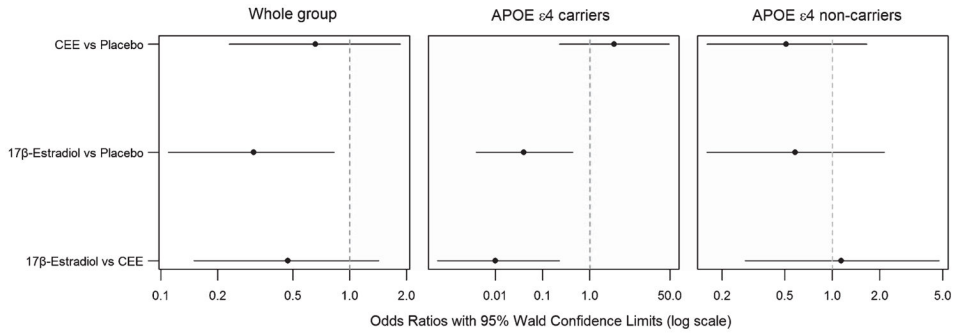

Fig. 4.

Odds ratios for PiB SUVR from proportional odds logistic regression models and 95% Wald confidence limits comparing PiB SUVR in oral CEE, transdermal 17β-estradiol, and the placebo groups in the whole group of participants, in APOE ε4 carriers, and in APOE ε4 non-carriers after adjusting for age. The odds ratio axis is logarithmic to accommodate the entire range of 95% Wald confidence limits.

Table 1

Characteristics of the participants by treatment status

Characteristic[a]	CEE (*N*= 17)	17β-Estradiol (*N*= 21)	Placebo (*N*= 30)	p[b]
Age, year at baseline	54 (46, 58)	53 (45, 58)	53 (45, 58)	0.47
Age, year at the PET scan	61 (53, 65)	60 (52, 65)	60 (52, 65)	0.48
Education, *n* (%)				0.65
High school or less	1 (7)	1 (5)	3 (10)	
Some college/College graduate	12 (80)	12 (63)	17 (57)	
Some graduate/Graduate	2 (13)	6 (32)	10 (33)	
Smoking status, *n* (%)				0.33
Non-smoker	10 (71)	8 (50)	20 (71)	
Smoker	4 (29)	8 (50)	8 (29)	
Time past menopause to randomization (months)	23 (7, 35)*	18 (7, 36)*	13 (5, 36)	0.045
APOE carrier, *n* (%)	3 (18)	10 (50)*	5 (18)	0.04
Migraines, *n* (%)	1 (6)	0 (0)	3 (10)	0.36
Global Cognition at baseline	−0.12 (−1.79, 1.67)	0.38 (−1.84, 1.15)	0.08 (−1.06, 1.83)	0.23
Mean systolic blood pressure, mm Hg at baseline	121 (96, 146)	114 (88, 149)	124 (96, 152)	0.13
Mean systolic blood pressure, mm Hg at the PET scan	88 (77, 116)	84 (68, 104)	93 (68, 116)	0.32
Mean diastolic blood pressure, mm Hg at baseline	78 (66, 91)	72 (60, 87)	76 (60, 88)	0.10
Mean diastolic blood pressure, mm Hg at the PET scan	123 (95, 156)	128 (94, 149)	128 (97, 149)	0.89
Body mass index, kg/m^2 at baseline	26 (20, 36)	25 (18, 34)	26 (19, 33)	0.44
Body mass index, kg/m^2 at the PET scan	77 (62, 96)	76 (60, 88)	79 (60, 93)	0.81
Coronary arterial calcification present, *n* (%) at baseline	0 (0)	2 (10)	4 (13)	0.41
Coronary arterial calcification present, *n* (%) at the PET scan	1 (6)	3 (14)	4 (13)	0.79
Carotid intima-media thickness at baseline	0.69 (0.55, 0.80)	0.64 (0.56, 0.85)	0.66 (0.57, 0.87)	0.91
Carotid intima-media thickness at the PET scan	0.73 (0.61, 0.88)	0.74 (0.56, 0.99)	0.73 (0.58, 1.01)	0.73
Low-density lipoprotein, mg/dL at baseline	121 (79, 163)	117 (64, 172)	114 (53, 178)	0.71
Low-density lipoprotein, mg/dL at the PET scan	124 (91, 191)	117 (66, 181)	120 (66, 166)	0.89
High-density lipoprotein, mg/dL at baseline	70 (45, 84)	70 (54, 89)	68 (50, 122)	0.80
High-density lipoprotein, mg/dL at the PET scan	64 (41, 98)	64 (39, 92)	58 (43, 131)	0.44
Triglycerides, mg/dL at baseline	68 (29, 229)	83 (33, 226)	72 (27, 233)	0.47
Triglycerides, mg/dL at the PET scan	100 (62, 230)	83 (52, 204)	94 (59, 336)	0.60
Fasting Blood Glucose, mg/dL at baseline	76 (65, 100)	78 (67, 94)	78 (68, 94)	0.38
Fasting Blood Glucose, mg/dL at the PET scan	96 (88, 113)	93 (82, 108)	94 (75, 126)	0.59

[a] Unless otherwise indicated, data are given as the median (range).

[b] *p*-values are assessed using Kruskal Wallis and Fisher's Exact Tests.

* Pairwise comparison to placebo $p < 0.05$. Abbreviations: CEE: Conjugated equine estrogen; *APOE*: Apolipoprotein E

Table 2

Cognitive Test Scores at the time of PiB PET imaging

Cognitive scores[a]	Oral CEE (*N*= 17)	Transdermal 17β-Estradiol (*N*= 21)	Placebo (*N*= 30)	*p*-values[b]
NYU Paragraph Immediate Recall Total Score	25 (16, 33)	26 (15, 39)	24 (17, 40)	0.86
NYU Delayed Recall Total Score	16 (5, 24)	14 (7, 23)	14 (9, 32)	0.88
CVLT-II Trials 1–3 Total Score	29 (16, 36)	33 (25, 42)	31 (14, 43)	0.03*
CVLT-II Trial Short Delay Free Recall score	10 (3, 16)	12 (7, 16)	11 (4, 16)	0.06
CVLT-II Trial Long Delay Free Recall score	9 (3, 15)	11 (6, 15)	10 (4, 16)	0.19
WMS-III Digit Span Total Score	15 (10, 22)	18 (10, 26)	17 (8, 26)	0.20
WMS-III Letter Number Sequencing Trial Total Score	10 (6, 14)	11 (6, 15)	10 (7, 17)	0.50
Trail Making Test A (Time to complete in seconds)	24 (15, 44)	23 (15, 43)	24 (15, 39)	0.89
Trail Making Test B (Time to complete in seconds)	56 (33, 83)	59 (35, 249)	57 (33, 135)	0.85
Phonemic Fluency (F,A,S) Total Score	44 (27, 69)	43 (19, 59)	46 (22, 77)	0.40
Semantic Fluency (animals, fruits, vegetables) Total Score	56 (30, 77)	55 (38, 68)	52 (36, 71)	0.35
Stroop Trial Word	99 (69, 136)	105 (70, 120)	100 (69, 140)	0.95
Stroop Trial Color	78 (61, 96)	74 (60, 110)	75 (58, 101)	0.55
Stroop Trial Color-Word	43 (18, 58)	44 (31, 61)	46 (21, 78)	0.78
Digit Symbol Total Score	82 (57, 93)	83 (61, 108)	82 (64, 103)	0.09

[a] Data shown are median (range) of raw scores.

[b] *p*-values were assessed using Analysis of Variance adjusting for age at PiB PET, levels of education, time from menopause to randomization (months) and APOE ε4 carrier status.

* Tukey Honest significant differences test for *post hoc* comparisons: Oral CEE versus placebo ($p = 0.03$); CEE versus transdermal 17β-estradiol ($p = 0.08$); transdermal 17β-estradiol versus placebo ($p = 0.98$).