Published in final edited form as:

Menopause. 2009 ; 16(6): 1167–1177. doi:10.1097/gme.0b013e3181ace484.

Effects of Botanicals and Combined Hormone Therapy on Cognition in Postmenopausal Women

Pauline M. Maki, Ph.D.^{1,2}, Leah H. Rubin, M.A.^{1,2}, Deanne Fornelli¹, Lauren Drogos¹, Suzanne Banuvar, MHA³, Lee P. Shulman, M.D.³, and Stacie E. Geller, Ph.D.⁴

¹ Department of Psychiatry, University of Illinois at Chicago

² Department of Psychology, University of Illinois at Chicago

³ Department of Obstetrics and Gynecology, Feinberg School of Medicine, Northwestern University

⁴ Department of Obstetrics and Gynecology, University of Illinois at Chicago

Abstract

Objective—To characterize the effects of red clover, black cohosh, and combined hormone therapy on cognitive function in comparison to placebo in women with moderate to severe vasomotor symptoms.

Design—In a Phase II randomized, double-blind, placebo-controlled study, 66 midlife women (out of 89 from a parent study; mean age=53 y) with ≥ 35 weekly hot flashes were randomized to receive red clover (120 mg), black cohosh (128 mg), CEE/MPA (0.625 mg conjugated equine estrogens plus 2.5 mg medroxyprogesterone acetate), or placebo. Participants completed measures of verbal memory (primary outcome) and other cognitive measures (secondary outcomes) before and during the 12th treatment month. A subset of 19 women completed objective, physiological measures of hot flashes using ambulatory skin conductance monitors.

Results—There was no impact of either of the botanical treatments on any cognitive measure. Compared to placebo, CEE/MPA led to greater decline in verbal learning (one of five verbal memory measures). This effect just missed statistical significance ($p=0.057$) in unadjusted analyses, but reached significance ($p=.02$) after adjusting for vasomotor symptoms. Neither botanical treatment group showed a change in verbal memory that differed from the placebo group ($ps>0.28$), even after controlling for improvements in hot flashes. In secondary outcomes, CEE/MPA led to a decrease in immediate digit recall and an improvement in letter fluency. Only CEE/MPA significantly reduced objective hot flashes.

Conclusions—Results indicate no effects of a red clover (phytoestrogen) supplement or black cohosh on cognitive function. CEE/MPA reduces objective hot flashes but worsens some aspects of verbal memory.

Keywords

Cognition; Menopause; Hormone Therapy; Red Clover; Black Cohosh; Hot Flash

Reviews of randomized clinical trials have drawn attention to the potential efficacy of black cohosh and phytoestrogens for the treatment of menopausal symptoms^{1, 2}. The need for long-term safety and efficacy studies of these botanical therapies gave rise to a companion article

in this issue of *Menopause* describing a Phase II randomized, double-blind, placebo-controlled trial in which 89 women (mean age = 53) with at least 35 vasomotor symptoms per week were randomized to one of four treatment arms: placebo, CEE/MPA (0.625 mg conjugated equine estrogens plus 2.5 mg medroxyprogesterone acetate), black cohosh (128 mg), or red clover (120 mg)³. Results from the companion article indicated that CEE/MPA, but not red clover or black cohosh, improved hot flashes and that there were no safety concerns associated with any treatment. Here we report findings from an ancillary study aimed at examining the impact of the four treatments on cognitive function.

Although CEE/MPA (.625 mg/2.5 mg) provides substantial hot flash relief⁴, three randomized, double-blind, placebo-controlled trials suggest that this particular preparation and dosage of hormone therapy (HT) may be associated with impairment in verbal memory, with one study showing a trend ($p < .06$) toward a decrease in verbal memory in unadjusted analyses and a significant decrease in adjusted analyses ($p = .04$)⁵, a second showing significant decrease ($p < .01$)⁶, and a third showing a trend toward a decrease ($p = .06$)⁷. In these three CEE/MPA clinical trials, verbal memory refers to the learning and recollection of a word list soon after the initial presentation of the list (immediate recall) and after a delay period of 15 to 20 minutes (delayed recall). Notably, none of the cohorts in the three trials were comprised of women who were experiencing substantial vasomotor symptoms and who therefore were most likely to consider HT for treatment purposes. There are at least two reasons to expect that CEE/MPA might affect memory differently in women with hot flashes. First, in a previous publication based on pre-treatment baseline data from the present Phase II study, we found that decreases in verbal memory were associated with increases in the number of objective hot flashes (i.e., measured with ambulatory monitors)⁸. Therefore, CEE/MPA might improve cognition in relation to improvements in hot flashes. Second, a systematic review of randomized, placebo-controlled clinical trials of estrogen alone found evidence that estrogen enhanced verbal memory in younger postmenopausal women, particularly those with menopausal symptoms⁹. To the extent that estrogen enhances cognition in symptomatic women, estrogen in combination with a progestin might confer the same benefit. On the other hand, preclinical studies suggest that MPA antagonizes beneficial effects of estrogen on hippocampal function¹⁰. Despite substantial relevance for clinical practice, the cognitive effects of CEE/MPA on younger postmenopausal women with moderate to severe hot flashes are unknown.

Clinical trials of phytoestrogens and cognition have involved much smaller samples than trials of CEE/MPA and cognition, but there is evidence of cognitive benefit with certain phytoestrogens. Phytoestrogens are plant-derived compounds that have estrogenic and antiestrogenic properties. The chemical structure of phytoestrogens closely resembles estradiol. The binding affinity of phytoestrogens varies across estrogen receptor (ER) subtypes, tissue types and specific phytoestrogen compounds. The phytoestrogens in soy and red clover (genistein and daidzein) bind more selectively to ER-beta than ER-alpha, and their binding to ER beta can equal that of estradiol while the transcriptional activity equals or exceeds that of estradiol, at least in supraphysiological doses^{11, 12}. Phytoestrogens and estrogen share several common mechanisms of action that could enhance cognitive function, including increased choline acetyltransferase and brain derived neurotrophic factor in the hippocampus and frontal cortex^{13–16}. Consistent with these mechanisms of action, some randomized trials of phytoestrogen supplements have shown benefits to memory¹⁷ and executive function^{17–20} in postmenopausal women aged 66 years and younger. In contrast, no cognitive benefits were evident in a large trial ($n = 191$) involving both younger and older Chinese postmenopausal women²¹ or a large trial ($n = 202$) involving older American postmenopausal women²². Thus, the clinical trial findings are inconsistent with respect to phytoestrogens and cognition.

To date, there have been no studies of the cognitive effects of black cohosh. Preclinical studies indicate that the black cohosh extract used in the present Phase II clinical trial is a mixed

competitive ligand and partial agonist at the human mu opiate receptor²³ and binds to serotonin receptor subtypes 5-HT(1A), 5-HT(1D), and 5-HT(7)²⁴, but does not have estrogenic activity²⁵. In light of these mechanisms of action, it appears unlikely that black cohosh would improve memory.

In the present study we aimed to better understand the impact of botanical menopausal therapies and HT on cognitive function in the context of a Phase II randomized, double-blind, placebo-controlled trial. Our sample represented women who typically initiate HT for clinical purposes - early postmenopausal women with moderate to severe menopausal symptoms. The findings are aimed at complementing results from the companion paper and showing how these treatments not only affect vasomotor symptoms, but also cognition. Our primary outcome was verbal memory. In light of findings from previous clinical trials, and despite the prevalence of vasomotor symptoms in this sample of women, we predicted that CEE/MPA would decrease verbal memory whereas red clover would improve verbal memory. In a subset of women, we also examined the impact of the four treatments on objective, physiological hot flashes measured with ambulatory skin conductance monitors. We predicted that CEE/MPA would have the largest impact on objectively measured hot flashes and that red clover and black cohosh would show a more modest, but significant effect. Information about the potential benefits and risks of CEE/MPA in comparison to botanical therapies is critical in helping women to make informed choices about therapeutic options for menopausal symptoms.

METHODS

Participants

Participants were recruited from a pool of 89 women enrolled in a Phase II randomized clinical trial that compared the effects of black cohosh, red clover, and CEE/MPA (.625 mg/2.5mg) on menopausal symptoms in comparison to placebo (see the companion paper³). In brief, women were recruited into the main clinical trial by advertisements on the internet and bulletin boards and by targeted mailings to women residing in the Chicago area. Inclusion criteria for the parent clinical trial included: 1) last menstrual period 6 months to 10 years before recruitment; 2) reports of a minimum of 35 hot flashes per week as indicated by diaries (i.e., minimum of 2 weeks, most 1 month or more); and 3) intact uterus and ovaries. Exclusion criteria included: 4) use of menopausal therapies, including oral HT within 2 months of study entry, transdermal estradiol within 1 month of study entry, and herbal botanicals within 1 month of study entry; 5) smoking; 6) contraindications to HT (e.g., vascular disease, uncontrolled hypertension, abnormal vaginal bleeding, history of blood clots, diabetes, abnormal mammogram); 7) use of antidepressants, SERMS, and bisphosphonates; and 8) major systemic illness. Additional exclusion criteria for this ancillary cognitive study included: 9) diagnosis of an Axis I psychiatric disorder; 10) any medical condition that affects cognitive function (e.g. stroke, traumatic brain injury); 11) use of prescription or over-the-counter medications that affect cognitive function (e.g., ginkgo biloba); 12) first language other than English (because verbal memory was primary outcome variable); and 13) participation in clinical trials other than the parent trial within 30 days.

From the 89 women (mean age = 53 years) in the parent trial, we completed baseline cognitive assessments on 71 women (mean age = 53 years). Baseline data for one woman was invalid because she fell asleep during cognitive testing, leaving a sample of 70 valid baseline sessions. Eighteen women enrolled in the parent study were not enrolled into this ancillary cognitive study because English was not their first language (n = 9), they had a history of head injury with loss of consciousness for more than 1 hour (n = 2), they had a seizure disorder (n = 1), or their schedule would not accommodate another study (n = 6). Sixty-seven of the 70 women with valid baseline sessions completed their 12-month follow-up assessment. Of the three that did not complete their post-treatment follow-up, one woman was withdrawn because she

stopped taking her study medication, and two women withdrew from the parent study and therefore did not complete follow-up in our substudy. Of the 67 women who completed their post-treatment follow-up assessment, one fell asleep during post-treatment cognitive assessment, so her data were deemed invalid and not included in the final analysis. One woman (red clover) was tested after 92 days of treatment because she then dropped out of the study; her data were included in the final analysis. Thus, the final sample size was 66.

Intervention and Randomization

Using a double-blind approach, eligible participants were randomly assigned to receive CEE/MPA (0.625 mg/2.5 mg, Wyeth Pharmaceuticals, Philadelphia, PA, USA), an ethanolic extract of black cohosh below ground parts (128 mg/day standardized to 7.27 mg triterpene glycosides), an ethanolic extract of the aerial parts of red clover (398 mg/day standardized to 120 mg isoflavone aglycones, specifically 57.5 mg biochanin A, 56.6 mg formononetin, 1.6 mg genistein, and 0.9 mg daidzein), or matching placebo daily over a 12-month period. Further details of these extracts, including preparation and botanical and chemical standardization, are described in the companion paper³ and in other papers^{26, 27} and are based on studies conducted at the University of Illinois at Chicago/National Institutes of Health Center for Botanical Dietary Supplements Research for Women's Health. A computer-generated code randomly assigned women into blocks of eight, with two women in each cluster to each of four treatment arms. Treatment adherence was monitored by pill counts and individual participant diary cards. Institutional review boards at each site approved the study and written, informed consent was obtained from each participant. During the intervention, each participant consumed two capsules each evening containing black cohosh, red clover, CEE/MPA, or placebo. The study capsules were identical in appearance and taste, and there was no detectable odor for any of the preparations.

Measures of Cognitive Function

Each participant met one-on-one with a trained test administrator and completed a cognitive battery twice, once at a pre-treatment baseline and once during the 12th month of treatment. The cognitive test battery was modeled after the cognitive test battery used in the COGNITIVE complaints in Early menopause Trial (COGENT), a study comparing the cognitive effects of HT and placebo in recently menopausal women with subjective cognitive complaints⁷. Each battery lasted approximately 1.5 hours and was administered either at the Center for Cognitive Medicine of the University of Illinois at Chicago (n = 48) or Northwestern University (n = 18). There were no significant differences in the cognitive testing procedures, randomization procedures, management and recruitment of subjects at the two sites, as the core study team was the same at both sites. In the subset of women who completed objective hot flash assessments, hot flash monitors were worn either the day before or the day of cognitive testing at both the baseline and the 12-month assessments. The primary and secondary cognitive outcome variables are described below. Parallel forms were used for the verbal memory outcome measures.

Primary Outcome: Verbal Memory

California Verbal Learning Test (CVLT-modified)²⁸: This modified version of the CVLT was used in recent HT trials, including the Women's Health Initiative Study of Cognitive Aging (WHISCA) and COGENT^{7, 29}. The test provides measures of verbal learning, and short- and long-delay verbal recall. On each of three successive verbal learning trials, the examiner reads aloud a target list of 16 words from four semantic categories (e.g., articles of clothing) and instructs the participant to recall as many words as possible. The examiner then reads aloud a different "interference" list of 16 words from overlapping and different semantic categories and instructs the participant to recall as many words as possible from that list. Next, the

participant is instructed to recall as many words as possible from the original target list (short delay free recall), first without any categorical cues and then with categorical cues. Following a 20-minute delay, the participant is instructed to recall the original target list (long delay free recall), first without any categorical cues and then with categorical cues. Outcome measures included: total verbal learning across trials 1–3, and short- and long-delay free recall.

Logical Memory Subtest of the Wechsler Memory Scale-Revised (WMS-R/LM-R)³⁰: This test yields a measure of immediate and delayed recall of a short story. Participants are read a brief story and are instructed to recall the story both immediately and after a 20-minute delay. Outcome measures included standardized scores of story recall accuracy both immediately after presentation and after a 20-minute delay, where the total scores range from 0 to 25.

Secondary Outcomes

Benton Visual Retention Test (BVRT)³¹: This is a test of short-term figural memory. In each of 10 trials, participants view a geometric line drawing for 10 seconds and then immediately attempt to reproduce the drawing from memory on a blank sheet of paper. Stimulus complexity increases across the 10 trials. The dependent measure was the total number correct.

Modified Card Rotations Test³²: This is a paper-and-pencil test provides a measure of visuospatial ability. On each of trial, participants view a target line drawing of a geometric figure and 8 alternatives representing 2- or 3-dimensional rotations of the target. Participants are instructed to mark the alternatives that show the sample drawing rotated in 2-dimensions (i.e., picture plane) as “same” and to mark those that show the sample drawing item rotated in 3-dimensions (i.e., mirror-images) as “different.” The dependent measure was the number of correct responses minus the number of incorrect responses across two trials of 3 minutes each, with a maximum possible score of 160.

Letter Fluency³¹: This test provides a measure of verbal fluency. Participants are given one minute on each of three successive trials to generate as many words as possible that begin with a particular letter (i.e., F, A, S). They are instructed to refrain from saying proper names, such as the names of people or places, and from saying multiple variations of the same word (e.g., eat, eating). The outcome measure was the total number of words produced across 3 trials.

Digit Span Forward and Backward³⁰: This test provides measures of attention and working memory. In the first set of trials the examiner reads a series of number strings aloud and instructs participants to repeat the string in the order presented (forward). In the next set of trials, the examiner reads a series of numbers aloud and instructs participants to repeat the string in the reverse order (backward). Outcome measures included the number of trials correctly recalled for the forward and backward trials.

Brief Test of Attention - Modified (BTA)^{33, 34}: This test measures auditory attention. On each trial the examiner reads aloud a string of letters and numbers and instructs the participant to state aloud how many numbers were presented in each string. The strings increase in length across 10 trials, and the outcome measure was the total number of correct responses out of 10.

Finding As Test³⁵: This is a test measuring visuoperceptual speed. Subjects are shown five columns of words on a sheet of paper and are instructed to cross out as many words containing the letter ‘A’ as possible in 2 minutes. The outcome measure was the total number of correct responses within 2 minutes.

Estimate of Verbal Intelligence

Primary Mental Abilities Vocabulary test (PMA)³⁶: This is a paper-and-pencil vocabulary test that was included to estimate individual differences in verbal knowledge at baseline. In African Americans, verbal knowledge varies with acculturation, and accounting for acculturation improves the diagnostic accuracy of neuropsychological tests³⁷. For each of 75 items, participants read a target word and circle its synonym out of four alternative responses. The outcome measure was the total number of correctly identified synonyms identified within 5 minutes, with a deduction of .33 for each incorrect answer to correct for guessing.

Memory Functioning Questionnaire (MFQ)³⁸: The MFQ is a 64-item measure of self perception of everyday memory abilities. The outcome measure was total score on the five subscales -Current Memory, Retrospective Memory, Frequency of Forgetting, Seriousness of Forgetting, and Use of Memory Aids & Mnemonics. Lower scores are indicative of more subjective memory problems.

Positive and Negative Affect Scale (PANAS)³⁹: This is a paper-and-pencil self-report measure of positive and negative mood states. Participants rate a list of ten pleasant mood states and ten unpleasant mood states on a 5-point Likert scale based on the extent to which they experienced each state during the previous two weeks. Ratings for each item range from 1 to 5, with 1 indicating “very slightly” and 5 indicating “extremely.” The total score for positive and negative are scored separately and divided by 10 to yield a maximum score of 5 for each scale.

Diary Measures of Vasomotor Symptoms: This was the primary outcome in the parent study. Women were instructed to maintain diaries for a minimum of two weeks prior to randomization and throughout the twelve months of the study. In these daily diaries, they recorded the number of vasomotor symptoms per day as well as the intensity, scored as 1 = slight, 2 = moderate, and 3 = severe. Any night sweat that interrupted sleep received an automatic intensity score of 3.

Objective Measures of Hot Flashes

Participants were fitted with an ambulatory sternal skin conductance monitor (Biolog Model 3991x/2-HFI), with two skin conductance electrodes (UFI) connected to the sternum by adhesive electrode pads (UFI, 1081-HFD) prepared with electrode gel (CustomMed Apothecary). The monitor was placed inside a small pouch for participants to attach to their belt or sling over their shoulder for easy maneuvering throughout the recording session. Participants were instructed to wear the monitor for 24 hours and to refrain from immersing the electrodes in water during the recording period (e.g., by showering). Both objective (i.e., > 2 micromho increase in 30 sec) and subjective (button press) hot flashes were recorded with the monitor according to standard procedures and criteria⁴⁰. Participants were instructed to indicate when they subjectively experienced a hot flash by simultaneously pressing two buttons on the hot flash monitor, resulting in a time-stamp, and by recording the time of occurrence, severity, bother and activity at the time of the perceived hot flash in a diary. In each diary entry, severity and bother were recorded for each subjective hot flash. Participants rated hot flash severity and bother on a Likert-scale ranging from 0 “no bother/not severe” to 10 “very bothersome/very severe.” Activity at time of subjective hot flash was recorded by self report in a diary. For the purposes of this investigation, only frequency data were used, because severity data are not available for objective hot flashes that were not subjectively detected.

Raw hot flash data were transmitted from the Biolog monitor to a PC using the Biolog Interface Box. Skin conductance time series data in μ mho units were read and displayed in a time-locked graph using specialized software (DPS v.1.5, UFI, Morro Bay, CA, USA). Raw objective hot

flash data were analyzed by a combination of automated computer software and data analysts trained by a national expert in this area (Janet Carpenter, PhD). Both the software and analysts defined an objective hot flash as a rapid rise in skin conductance of at least 2 μmho over a 30-second period according to standard criteria⁴⁰. Once an objective hot flash was coded, no other hot flashes were coded for the next 15 minutes according to standard procedures⁴¹. Any discrepancies were discussed and resolved by the two coders before entry of coded data into the database. The primary outcome variables were total numbers of objective and subjective hot flashes during the 24-hour study period.

Statistical Analyses

Differences between the four treatment groups in baseline characteristics were examined using between subjects analysis of variance (ANOVA) for continuous variables and by Chi-square (X^2) tests for categorical variables. The primary efficacy analysis was a multilevel random coefficient model that examined differences in linear rates of change between each of the active treatment groups and the placebo group on verbal memory from the baseline (pre-treatment) assessment to the 12-month assessment¹. Similar analyses were run on secondary outcome measures. Models were analyzed using HLM 6.03. All analyses were two-tail, $p < .05$, but because this is a Phase II study, we also report trends ($p < .10$) and effect sizes (Cohen's d calculated from raw change scores) where warranted⁴².

RESULTS

Characteristics of the Participants

Table 1 shows demographic and clinical information for the 66 participants. Participants ranged in age from 44 to 62 years ($M = 53.13$), and 53% were African-American, 44% Caucasian, 2% Hispanic and 1% Asian. Overall, the four groups were similar across most socio-demographic and clinical variables, although there were significant differences between the groups in race, $X^2 (9, N = 66) = 17.23, p = 0.03$, and body mass index (BMI), $F (1, 62) = 3.85, p = 0.01$. All analyses therefore controlled for race and BMI.

¹The model is as follows:

Level 1: within-individual model

$$\text{Outcome}_{ij} = \beta_{0i} + \beta_{1i}(\text{Time})_{ij} + \varepsilon_{ij},$$

where outcome ij represents the outcome response for individual i at measurement occasion j , for j equal to 0 (baseline) and 1 (12 months); β_{0j} represents the mean outcome at baseline for individual i ; β_{1j} represents the growth rate for person i from baseline to 12 months; and ε_{ij} represents the residual error in the outcome measure for individual i at time j , normally distributed with mean 0 and variance σ^2 .

Level 2: between-individual model:

$$\begin{aligned} \beta_0 &= Y_{00} + Y_{01}(\text{Placebo vs. Red Clover})_j + Y_{02}(\text{Placebo vs. Black Cohosh})_j \\ &\quad + Y_{03}(\text{Placebo vs. CEE/MPA})_j + \mu_{0j}, \\ \beta_{1j} &= Y_{10} + Y_{11}(\text{Placebo vs. Red Clover})_j + Y_{12}(\text{Placebo vs. Black Cohosh})_j \\ &\quad + Y_{13}(\text{Placebo vs. CEE/MPA})_j, \end{aligned}$$

where the coefficient γ_{00} represents the mean outcome for individuals receiving placebo at baseline; γ_{01} represents the mean difference in outcome between red clover and placebo at baseline; γ_{02} represents the mean difference in outcome between black cohosh and placebo at baseline; γ_{03} represents the mean difference in outcome between CEE/MPA and placebo at baseline; γ_{10} represents the mean linear rate of change for individuals randomized to placebo; γ_{11} represents the mean difference in linear rate of change between red clover and placebo; γ_{12} represents the mean difference in linear rate of change between black cohosh and placebo; γ_{13} represents the mean difference in linear rate of change between CEE/MPA and placebo; and μ_{0j} represents the unexplained deviation from group mean random intercept for individual i . Including μ_{0j} in the model allows the level of the individuals response curve to vary from person to person.

Primary Outcome

Verbal Memory—Table 2 shows raw means and standard deviations for the verbal memory measures and shows mean change scores from the baseline to the 12-month, with positive values indicating higher scores at 12 months. At (pre-treatment) baseline, there were no differences between the placebo and treatment groups, except for a trend for the CEE/MPA group to perform worse than the placebo group on CVLT short delay free recall, $t(60) = -1.73$, $p = 0.09$. Irrespective of treatment group, participants showed a significant decline from baseline to 12 months on CVLT short delay free recall, $t(122) = -2.06$, $p = 0.04$, likely due to the introduction of a more difficult parallel form at the second test administration. For the primary analysis of interest, compared to the placebo group, the CEE/MPA group showed greater decline in CVLT total learning from baseline to 12 months, though this effect just missed statistical significance, $t(122) = -1.92$, $p = 0.057$. Neither of the botanical treatment groups showed a change in verbal memory that differed or tended to differ from the placebo group ($ps > 0.28$). The magnitude of the effect size between CEE/MPA and placebo groups in verbal learning on the CVLT, based on Cohen's d , was 0.65 standard deviation units.

As in the parent study, in this ancillary cognitive study, CEE/MPA significantly reduced the number and intensity of hot flashes from baseline to 12-months on the daily diaries compared to the placebo group, $t(122) = -2.57$, $p = 0.01$, and $t(122) = -2.04$, $p = 0.04$, respectively (See Table 3). Therefore a follow-up analysis was conducted to examine whether controlling for the beneficial effect of CEE/MPA on hot flashes would alter the negative effect of CEE/MPA on CVLT total learning. This analysis was accomplished by adding the hot flash outcome from the parent study as a time varying covariate in the standard mixed effect model. After controlling for the vasomotor symptoms, the CEE/MPA group showed a significantly greater decline in total learning compared to the placebo group, $t(120) = -1.84$, $p = 0.02$. Thus, controlling for hot flashes did not mitigate against the negative effects of CEE/MPA on cognition.

Objective Hot Flashes—Figure 1 shows the number of daily subjective hot flashes (i.e., by event markers) and the number of daily objective hot flashes (i.e., by changes in skin conductance) from the subset of 19 women who completed assessments with ambulatory hot flash monitors. At baseline, there were no significant group differences in either subjective or objective hot flashes ($ps > 0.45$). Irrespective of group, subjective hot flashes decreased from baseline to 12 months, $t(122) = -2.64$, $p = 0.01$. For the primary analysis of interest, compared to the placebo group, the CEE/MPA group showed a greater decline from baseline to 12 months in objective hot flashes, $t(29) = -3.19$, $p < 0.001$. The magnitude of change in objective hot flashes from baseline to 12 months was 0% for the placebo group, 33% for the red clover group, and 17% for the black cohosh group. Subjective hot flashes decreased significantly in each group, whereas objective hot flashes decreased significantly only in the CEE/MPA group. The correlation between objective hot flashes at baseline and at 12 months in the placebo group was 0.98.

Secondary Outcomes

Table 3 shows raw means and standard deviations at baseline and 12 months for the secondary outcome measures and shows the mean change scores between baseline and 12 months for the secondary outcome measures, with positive values indicating higher scores at 12 months. Regarding treatment differences, there was a greater decline in performance from baseline to 12 months on the digit span forward task in the CEE/MPA group compared to the placebo group, $t(122) = -2.37$, $p = 0.02$. There was also greater improvement in verbal fluency from baseline to 12 months in the CEE/MPA group compared to the placebo group, $t(122) = 2.02$, $p = 0.045$, and this effect was evident in the model that controlled for the tendency of the CEE/MPA group to perform worse than the placebo group on the verbal fluency task, $t(60) = -1.70$,

$p = 0.09$. Neither of the botanical treatment groups showed a change in secondary outcome measures that differed or tended to differ from the placebo group ($ps > 0.15$).

At baseline, the placebo group reported more retrospective forgetting on the MFQ compared to the CEE/MPA group, $t(60) = -2.12$, $p = 0.04$, and reported a greater seriousness of forgetting compared to the black cohosh and CEE/MPA groups, $t(60) = -1.89$, $p = 0.06$, and $t(60) = -1.70$, $p = 0.095$, respectively. Irrespective of treatment group, women showed a trend towards a significant decline from baseline to 12 months in seriousness of forgetting on the MFQ, $t(122) = -1.97$, $p = 0.05$. Regarding treatment differences, there were trends for the placebo group to report less seriousness in forgetting on the MFQ from baseline to 12 months compared to the black cohosh and CEE/MPA groups, $t(122) = 1.84$, $p = 0.07$, and $t(122) = 1.88$, $p = 0.06$, respectively.

DISCUSSION

The goal of the present Phase II randomized, placebo-controlled trial was to examine the impact of botanical menopausal therapies and standard HT (i.e., CEE/MPA) on verbal memory (primary outcome), other cognitive abilities, and objective hot flashes in midlife women with moderate to severe hot flashes. We predicted that CEE/MPA would decrease verbal memory whereas red clover would improve verbal memory. Results indicated that in comparison to placebo, CEE/MPA decreased verbal learning – one of five measures of verbal memory – though this effect just missed statistical significance ($p = .056$). The negative effect on verbal learning became stronger after controlling for the beneficial effect of CEE/MPA on hot flashes, $p = .02$. By contrast, red clover had no effect on verbal memory. In secondary outcomes, CEE/MPA decreased performance on immediate digit recall relative to placebo and improved performance on a letter fluency task. As predicted, black cohosh had no significant impact on any cognitive measure. Lastly, although each group showed a significant decrease in subjective hot flashes, only the CEE/MPA group showed a significant decrease in objective, physiological hot flashes. The finding that CEE/MPA, at a near-significant level ($p = .056$), decreased verbal learning in younger postmenopausal women who are experiencing substantial vasomotor symptoms parallels findings from three trials involving older and younger women with mild vasomotor symptoms^{5–7}. Although this effect was not statistically significant in this Phase II trial, the effect size of 0.65 standard deviations (Cohen's d) is considered large by common standards⁴², reached significance after controlling for vasomotor symptoms ($p = .02$), and likely would have reached significance in a larger study of this population. In the Heart and Estrogen/progestin Replacement Study (HERS), a trend toward a negative effect on verbal learning ($p < 0.06$) was reported after four years of treatment in 2,763 women (mean age 67 years) with pre-existing coronary disease, and this effect reached statistical significance ($p = .04$) after adjusting for age, education, years postmenopausal, statin use, Geriatric Depression Scale score, alcohol drinks per week, hot flushes, and trouble sleeping⁵. In the Women's Health Initiative Study of Cognitive Aging (WHISCA) a negative effect on verbal memory was evident after long-term treatment (4–5 years) in a generally healthy sample of 1416 women aged 65 and older⁶. Specifically, CEE/MPA led to impairment in verbal learning ($P < 0.009$) and both short- and long-delay free recall ($P < 0.016$ and $P < 0.015$, respectively), whereas memory for figures was improved ($P = .012$). In the Cognitive Complaints in Early Menopause Trial (COGENT), impairments in short- and long-delay free recall ($p < 0.054$ and $p < 0.066$, respectively) were observed after four months of treatment in 180 healthy postmenopausal women aged 45 to 55 years⁷. Like these studies, the present results suggest that CEE/MPA may decrease some aspects of verbal memory, especially word list learning, and this negative effect remains significant even after controlling for improvements in hot flashes. On the other hand, no negative impact of CEE/MPA was seen on other verbal memory measures, including short- and long-delay recall in the present study.

On secondary outcome measures, CEE/MPA decreased performance on Digit Span forward (a test of attention) and improved performance on verbal fluency. Our Digit Span Forward findings differ from COGENT⁷ where no effect was observed, but parallel some findings from WHISCA, where women randomized to CEE/MPA scored slightly lower at the initial assessment on Digit Span Forward ($p = .05$) compared with women randomized to placebo²⁹. Unlike the present study, HERS, COGENT, and WHISCA found no benefit of CEE/MPA on verbal fluency. On the other hand, some findings suggest that loss of estrogen might negatively impact verbal fluency, including the finding that verbal fluency decreases as women transition from premenopausal to perimenopausal stages⁴³.

Similar to previous studies involving larger samples of postmenopausal women, the present findings indicate no cognitive benefits from soy phytoestrogens^{21, 22}. In contrast to findings from a 6-month randomized, placebo-controlled clinical trial involving 30 postmenopausal women aged 60 years and older, we did not find that red clover improved visuospatial abilities or decreased digit recall⁴⁴. Also in contrast to findings from a cross-over study involving 77 young postmenopausal women¹⁹, we did not find an improvement in working memory as measured by Digit Span Backward. Several randomized clinical studies of younger postmenopausal women have reported improvements in executive function measures of planning, set shifting, and category fluency with phytoestrogen supplements^{17–20}. We cannot rule out the possibility that red clover might improve performance on such measures, because they were not included in our test battery.

In a previous report based on baseline measures of cognitive performance from the present sample, we found that objective, physiological hot flashes were associated with worse verbal memory as measured by Paragraph Recall, but not on the CVLT⁸. This finding suggested that interventions that improve hot flashes might benefit memory. Such benefits might be due to fewer night-time awakenings, prevention of any hippocampus dysfunction resulting from the cortisol released following a hot flash⁴⁵, or some other factor. In the present study, we found that CEE/MPA improved objective hot flashes, but nevertheless worsened verbal learning on the CVLT. Thus, the negative impact of CEE/MPA on memory systems appears to override any cognitive benefit from CEE/MPA associated with hot flash improvement. The antagonistic effects of MPA on hippocampal function¹⁰ may be responsible for the opposing effects of CEE/MPA on hot flashes and memory. It remains to be seen whether other treatments that do not antagonize hippocampal function improve both hot flashes and memory function.

To our knowledge, this is the first study to examine the impact of HT and botanicals on objective hot flashes. Due to the small sample size ($n = 19$) the results should be taken with caution. As in a previous report, the present study found no impact of placebo on objective hot flash frequency despite improvements in subjective hot flash frequency⁴⁶. Also, despite a small sample size, CEE/MPA was shown to significantly reduce objective hot flashes from 19 per day to close to 1 per day. Neither red clover nor black cohosh significantly reduced objective hot flashes. For red clover, there was a nonsignificant, 33% reduction in objective hot flashes. For black cohosh, there was a 17% reduction. In light of our small sample size, it is important to point that larger clinical trials of phytoestrogens may reveal significant benefits of phytoestrogens on objective hot flashes.

The objective hot flash findings suggest future directions for clinical research. To our knowledge, this is the first study to look at changes in objective hot flashes within symptomatic naturally menopausal women over a time span of several months. The lack of change in objective hot flashes over a 12-month interval in the placebo group is somewhat surprising in light of expectations that hot flashes should begin to decrease without treatment over time. The high correlation ($r = 0.98$) between baseline and 12-month values for total number of daily objective hot flashes in the placebo group suggests that the measurements were reliable over

time and that they were sensitive to individual differences. However, given the very small sample size there is a need for larger studies exploring the natural history of changes in objective versus subjective hot flashes over time from perimenopausal and early postmenopausal stages to the late postmenopausal stage. Future studies are also needed to explore the extent to which objective hot flash responses in the red clover group may depend on a woman's status as an equol producer versus nonproducer. Intestinal bacteria produce S-equol, a potent phytochemical metabolite, from daidzein, and the amount of equol produced differs across individuals. Although daidzein comprises a small proportion of the red clover extract, formononetin comprises a large proportion of the extract and is a precursor of daidzein⁴⁷. In a recent study of the effects of soy on menopausal symptoms, equol-producing women showed a reduction in hot flashes compared to placebo, but equol-nonproducers showed no reduction⁴⁸.

The present study has several limitations. First, the trial was a Phase II study and therefore has a small sample size (n = 66) for the primary outcomes of interest and an even smaller sample size (n = 19) for the objective hot flash measures. Nevertheless, the results suggest that we are unlikely to find any cognitive benefits with red clover or black cohosh in larger studies because there was no suggestion of a benefit on any cognitive outcome. Second, the cognitive battery lacked executive measures of planning and mental flexibility, which might be particularly sensitive to phytoestrogen treatment. Third, the results are based on a sample in which 53% of the participants were African American. Although we controlled for race, the results may not generalize to other ethnic groups. Fourth, the form of HT under evaluation was CEE/MPA, and the cognitive findings may not reproduce in future studies of other estrogens and progestins, or other drug delivery systems (e.g., transdermal). There is evidence, for example, for improvements in verbal memory with estradiol^{49, 50}, CEE alone⁵¹, cyclic oral piperazine⁵², and estradiol valerate and dinogest⁵³. Larger studies are needed to evaluate the robustness of these Phase II findings and to fully evaluate their clinical significance. Fifth, we did not obtain plasma or urine concentration of primary isoflavones and their metabolites in this study. Although these concentrations are available from published Phase I trial data⁴⁷, it would have been helpful to have such values in this study and to have analyzed data from equol converters versus nonconverters based on those values⁵⁴. Sixth, the loss of 26% of the subject population from the parent trial poses at least a potential threat to the validity of the initial randomized design and introduces a potential source of bias.

Acknowledgments

Source of Funding: This research was supported by NIH/NCCAM grants K01AT002321-01 and R21AT001868-01. The NIH/NCCAM Botanical Dietary Supplements for Women's Health (NIH/NCCAM Botanical Dietary Supplements for Women's Health; Norman Farnsworth, PI) provided pilot funds for this neurocognitive study and funded the parent study.

We would like to thank: Geena Epstein, RN and Denise Romero for their assistance in recruitment and coordination of the parent randomized trial; Pamela Perschler for her assistance as a study coordinator for the cognitive study; Nicole Fender and Erin Eatough for their assistance in data collection. We also wish to thank Northwestern University Feinberg School of Medicine, Northwestern Memorial Hospital, and Northwestern Medical Faculty Foundation for their participation as a site and their contributions toward the completion of the clinical trial.

References

1. Kronenberg F, Fugh-Berman A. Complementary and alternative medicine for menopausal symptoms: a review of randomized, controlled trials. *Annals of Internal Medicine* 2002;137(10):805–13. [PubMed: 12435217]
2. Geller SE, Studee L. Botanical and dietary supplements for menopausal symptoms: what works, what does not. *J Womens Health (Larchmt)* 2005;14(7):634–49. [PubMed: 16181020]

3. Geller SE, Banuvar S, Shulman LP, et al. The Safety and Efficacy of Black Cohosh and Red Clover for the Management of Vasomotor Symptoms: Results of a Phase II Clinical Trial.
4. Weinstein L, Bewtra C, Gallagher JC. Evaluation of a continuous combined low-dose regimen of estrogen-progestin for treatment of the menopausal patient. *Am J Obstet Gynecol* 1990;162(6):1534–9. [PubMed: 2360587]discussion 1539–42
5. Grady D, Yaffe K, Kristof M, Lin F, Richards C, Barrett-Connor E. Effect of postmenopausal hormone therapy on cognitive function: the Heart and Estrogen/progestin Replacement Study. *American Journal of Medicine* 2002;113:543–548. [PubMed: 12459399]
6. Resnick SM, Maki PM, Rapp SR, et al. Effects of combination estrogen plus progestin hormone treatment on cognition and affect. *J Clin Endocrinol Metab* 2006;91(5):1802–10. [PubMed: 16522699]
7. Maki PM, Gast MJ, Vieweg AJ, Burriss SW, Yaffe K. Hormone therapy in menopausal women with cognitive complaints: a randomized, double-blind trial. *Neurology* 2007;69(13):1322–30. [PubMed: 17893293]
8. Maki PM, Drogos LL, Rubin LH, Banuvar S, Shulman LP, Geller SE. Objective hot flashes are negatively related to verbal memory performance in midlife women. *Menopause* 2008;15(5):848–56. [PubMed: 18562950]
9. Maki PM. A systematic review of clinical trials of hormone therapy on cognitive function: effects of age at initiation and progestin use. *Ann N Y Acad Sci* 2005;1052:182–97. [PubMed: 16024761]
10. Nilsen J, Brinton RD. Impact of progestins on estrogen-induced neuroprotection: synergy by progesterone and 19-norprogesterone and antagonism by medroxyprogesterone acetate. *Endocrinology* 2002;143:205–212. [PubMed: 11751611]
11. Belcher SM, Zsarnovszky A. Estrogenic actions in the brain: estrogen, phytoestrogens, and rapid intracellular signaling mechanisms. *J Pharmacol Exp Ther* 2001;299(2):408–14. [PubMed: 11602649]
12. Kuiper GG, Lemmen JG, Carlsson B, et al. Interaction of estrogenic chemicals and phytoestrogens with estrogen receptor beta. *Endocrinology* 1998;139(10):4252–63. [PubMed: 9751507]
13. Pan Y, Anthony M, Clarkson TB. Effect of estradiol and soy phytoestrogens on choline acetyltransferase and nerve growth factor mRNAs in the frontal cortex and hippocampus of female rats. *Proc Soc Exp Biol Med* 1999;221(2):118–25. [PubMed: 10352122]
14. Lephart ED, West TW, Weber KS, et al. Neurobehavioral effects of dietary soy phytoestrogens. *Neurotoxicology and Teratology* 2002;24(1):5–16. [PubMed: 11836067]
15. Lund TD, Lephart ED. Dietary soy phytoestrogens produce anxiolytic effects in the elevated plus-maze. *Brain Res* 2001;913(2):180–4. [PubMed: 11549384]
16. Pan Y, Anthony M, Watson S, Clarkson TB. Soy phytoestrogens improve radial arm maze performance in ovariectomized retired breeder rats and do not attenuate benefits of 17beta-estradiol treatment. *Menopause* 2000;7(4):230–5. [PubMed: 10914615]
17. Duffy R, Wiseman H, File SE. Improved cognitive function in postmenopausal women after 12 weeks of consumption of a soya extract containing isoflavones. *Pharmacology, Biochemistry, and Behavior* 2003;75:721–729.
18. File SE, Hartley DE, Elsabagh S, Duffy R, Wiseman H. Cognitive improvement after 6 weeks of soy supplements in postmenopausal women is limited to frontal lobe function. *Menopause* 2005;12(2):193–201. [PubMed: 15772567]
19. Casini ML, Marelli G, Papaleo E, Ferrari A, D'Ambrosio F, Unfer V. Psychological assessment of the effects of treatment with phytoestrogens on postmenopausal women: a randomized, double-blind, crossover, placebo-controlled study. *Fertil Steril* 2006;85(4):972–8. [PubMed: 16580383]
20. Kritz-Silverstein D, Von Muhlen D, Barrett-Connor E, Bressel MA. Isoflavones and cognitive function in older women: the SOy and Postmenopausal Health In Aging (SOPHIA) Study. *Menopause* 2003;10(3):196–202. [PubMed: 12792289]
21. Ho SC, Chan AS, Ho YP, et al. Effects of soy isoflavone supplementation on cognitive function in Chinese postmenopausal women: a double-blind, randomized, controlled trial. *Menopause* 2007;14(3 Pt 1):489–99. [PubMed: 17308499]
22. Kreijkamp-Kaspers S, Kok L, Grobbee DE, et al. Effect of soy protein containing isoflavones on cognitive function, bone mineral density, and plasma lipids in postmenopausal women: a randomized controlled trial. *Jama* 2004;292(1):65–74. [PubMed: 15238592]

23. Rhyu MR, Lu J, Webster DE, Fabricant DS, Farnsworth NR, Wang ZJ. Black cohosh (*Actaea racemosa*, *Cimicifuga racemosa*) behaves as a mixed competitive ligand and partial agonist at the human mu opiate receptor. *J Agric Food Chem* 2006;54(26):9852–7. [PubMed: 17177511]
24. Burdette JE, Liu J, Chen SN, et al. Black cohosh acts as a mixed competitive ligand and partial agonist of the serotonin receptor. *J Agric Food Chem* 2003;51(19):5661–70. [PubMed: 12952416]
25. Liu J, Burdette JE, Xu H, et al. Evaluation of estrogenic activity of plant extracts for the potential treatment of menopausal symptoms. *J Agric Food Chem* 2001;49(5):2472–9. [PubMed: 11368622]
26. Booth NL, Overk CR, Yao P, et al. The chemical and biologic profile of a red clover (*Trifolium pratense* L.) phase II clinical extract. *J Altern Complement Med* 2006;12(2):133–9. [PubMed: 16566672]
27. Farnsworth NR, Krause EC, Bolton JL, Pauli GF, van Breemen RB, Graham JG. The University of Illinois at Chicago/National Institutes of Health Center for Botanical Dietary Supplements Research for Women's Health: from plant to clinical use. *Am J Clin Nutr* 2008;87(2):504S–8S. [PubMed: 18258647]
28. Delis, DC.; Kramer, JH.; Kaplan, E.; Ober, BA. California Verbal Learning Test - Research Edition. New York: The Psychological Corporation; 1987.
29. Resnick SM, Maki PM, Rapp SR, et al. Effects of combination estrogen plus progestin hormone treatment on cognition and affect. *J Clin Endocrinol Metab*. 2006
30. Wechsler, D. Wechsler Adult Intelligence Scale - Revised. New York: Psychological Corporation; 1981.
31. Benton AL. Differential behavioral effects in frontal lobe disease. *Neuropsychologia* 1968;6:53–60.
32. Wilson J, DeFries J, McLearn G, Vandenberg S, Johnson R, Rashad M. Cognitive abilities: use of family data as a control to assess sex and age differences in two ethnic groups. *International Journal of Aging and Human Development* 1975;6:261–276. [PubMed: 1213852]
33. Schretlen D, Bobholz JH, Brandt J. Development and psychometric properties of the Brief Test of Attention. *Clinical Neuropsychologist* 1996;10:80–89.
34. Schretlen D, Brandt J, Bobholz JH. Validation of the Brief Test of Attention in patients with Huntington's disease. *Clinical Neuropsychologist* 1996;10:90–95.
35. Ekstrom, RB.; French, JW.; Harman, HH. Manual for Kit of Factor-Referenced Cognitive Tests. Princeton NJ: Educational Testing Service; 1976.
36. Thurstone, L.; Thurstone, T. Primary mental abilities. Chicago: Science Research Associates; 1962.
37. Manly JJ, Miller SW, Heaton RK, et al. The effect of African-American acculturation on neuropsychological test performance in normal and HIV-positive individuals. The HIV Neurobehavioral Research Center (HNRC) Group. *J Int Neuropsychol Soc* 1998;4(3):291–302. [PubMed: 9623004]
38. Zelinski EM, Gilewski MJ, Anthony-Bergstone CR. Memory Functioning Questionnaire: Concurrent validity with memory performance and self-reported memory failures. *Psychology & Aging* 1990;5(3):388–399. [PubMed: 2242243]
39. Watson D, Clark L, Tellegen A. Development and validation of brief measures of positive and negative affect: The PANAS scales. *Journal of Personality and Social Psychology* 1988;54:1062–1070.
40. Freedman RR. Laboratory and ambulatory monitoring of menopausal hot flashes. *Psychophysiology* 1989;26(5):573–9. [PubMed: 2616704]
41. Carpenter JS, Andrykowski MA, Freedman RR, Munn R. Feasibility and psychometrics of an ambulatory hot flash monitoring device. *Menopause* 1999;6(3):209–15. [PubMed: 10486790]
42. Cohen, J. Statistical Power Analysis for the Behavioral Sciences. Vol. 2. Hillsdale, NJ: Erlbaum; 1988.
43. Fuh JL, Wang SJ, Lee SJ, Lu SR, Juang KD. A longitudinal study of cognition change during early menopausal transition in a rural community. *Maturitas*. 2005
44. Howes JB, Bray K, Lorenz L, Smerdely P, Howes LG. The effects of dietary supplementation with isoflavones from red clover on cognitive function in postmenopausal women. *Climacteric* 2004;7(1): 70–7. [PubMed: 15259285]
45. Meldrum DR, Defazio JD, Erlik Y, et al. Pituitary hormones during the menopausal hot flash. *Obstet Gynecol* 1984;64(6):752–6. [PubMed: 6095154]

46. Carpenter JS, Storniolo AM, Johns S, et al. Randomized, double-blind, placebo-controlled crossover trials of venlafaxine for hot flashes after breast cancer. *Oncologist* 2007;12(1):124–35. [PubMed: 17227907]
47. Piersen CE, Booth NL, Sun Y, et al. Chemical and biological characterization and clinical evaluation of botanical dietary supplements: a phase I red clover extract as a model. *Curr Med Chem* 2004;11(11):1361–74. [PubMed: 15180571]
48. Jou HJ, Wu SC, Chang FW, Ling PY, Chu KS, Wu WH. Effect of intestinal production of equol on menopausal symptoms in women treated with soy isoflavones. *Int J Gynaecol Obstet* 2008;102(1):44–9. [PubMed: 18395723]
49. Sherwin BB. Estrogen and/or androgen replacement therapy and cognitive functioning in surgically menopausal women. *Psychoneuroendocrinology* 1988;13:345–357. [PubMed: 3067252]
50. Sherwin BB, Tulandi T. “Add-back” estrogen reverses cognitive deficits induced by a gonadotropin-releasing hormone agonist in women with leiomyomata uteri. *Journal of Clinical Endocrinology and Metabolism* 1996;81(7):2545–9. [PubMed: 8675575]
51. Shaywitz SE, Naftolin F, Zelterman D, et al. Better oral reading and short-term memory in midlife, postmenopausal women taking estrogen. *Menopause* 2003;10(5):420–6. [PubMed: 14501603]
52. Hackman BW, Galbraith D. Replacement therapy and piperazine oestrone sulphate (‘Harmogen’) and its effect on memory. *Curr Med Res Opin* 1976;4(4):303–6. [PubMed: 791587]
53. Linzmayer L, Semlitsch HV, Saletu B, et al. Double-blind, placebo-controlled psychometric studies on the effects of a combined estrogen-progestin regimen versus estrogen alone on performance, mood and personality of menopausal syndrome patients. *Arzneimittelforschung* 2001;51(3):238–45. [PubMed: 11304940]
54. Setchell KD, Brown NM, Lydeking-Olsen E. The clinical importance of the metabolite equol—a clue to the effectiveness of soy and its isoflavones. *J Nutr* 2002;132(12):3577–84. [PubMed: 12468591]

(a) Subjective Hot Flashes

(b) Objective Hot Flashes

Note. For subjective hot flashes all women demonstrated a significant decrease from baseline to 12 months. For objective hot flashes only the CEE/MPA group showed a significant linear decline from baseline to 12 months compared to placebo. * $p < 0.001$.

Figure 1.

(a) Number of subjective hot flashes reported per day as a function of treatment and (b) number of objective hot flashes per day as a function of treatment.

Table 1

Patient demographics.

Mean (SD)	Treatment Group			
	Placebo <i>n</i> = 17	Red Clover <i>n</i> = 14	Black Cohosh <i>n</i> = 18	CEE/MPA <i>n</i> = 17
Age, years	52.59 (4.29)	51.64 (5.11)	53.94 (4.04)	53.29 (4.06)
BMI ^a	29.64 (4.93)	30.82 (4.27)	28.89 (3.95)	25.85 (4.11)
Race <i>n</i> (%) ^b				
Caucasian	23	22	56	71
African-American	77	64	44	29
Hispanic	-	7	-	-
Other	-	7	-	-
Months since last period	35.04 (34.72)	49.92 (35.70)	42.62 (31.74)	45.40 (37.49)
Hot flush frequency per wk	52.25 (16.68)	63.22 (22.54)	64.82 (19.57)	73.82 (53.08)
Hot flush intensity (0 = none, 3 = severe)	2.17 (0.66)	2.34 (0.74)	2.49 (0.58)	2.02 (0.66)
Past hormone use, <i>n</i> (%)	47	43	39	41
Months of prior HT use	45.26 (28.81)	35.35 (26.26)	48.84 (30.75)	58.37 (50.20)
Months since last HT use	15.10 (23.06)	26.98 (25.34)	15.84 (16.13)	24.63 (21.58)
Previous tobacco use, <i>n</i> (%)	29	57	56	59
Alcohol use ^a	0.96 (1.74)	0.89 (1.00)	1.51 (1.91)	1.19 (1.66)
Gravida, #	2.65 (1.73)	2.86 (1.79)	2.61 (2.00)	1.88 (1.54)
Number of deliveries	1.53 (1.01)	2.36 (1.50)	1.67 (1.41)	1.24 (1.20)
Total cholesterol	201.35 (31.58)	220.86 (48.81)	213.33 (39.79)	217.88 (28.41)
Estradiol (pg/mL)	24.48 (7.92)	26.84 (5.39)	24.32 (7.81)	24.78 (11.26)
FSH (mIU/mL)	87.69 (35.95)	72.09 (25.86)	88.38 (24.06)	105.21 (38.40)

^aNote. # of drinks per week.^b*p*-value for race from chi-square test for overall homogeneity of race.*
p < 0.05.

Table 3

Mean (SD) and change scores across time for secondary efficacy end points for women randomized to treatment with CEE/MPA, red clover, black cohosh, and placebo.

Test, mean score (SD)	Treatment Group						Black Cohosh (n = 18)						CEE/MPA (n = 17)					
	Red Clover (n = 14)			Black Cohosh (n = 18)			CEE/MPA (n = 17)			Black Cohosh (n = 18)			CEE/MPA (n = 17)			Black Cohosh (n = 18)		
	Baseline	12 months	Change	Baseline	12 months	Change	Baseline	12 months	Change	Baseline	12 months	Change	Baseline	12 months	Change	Baseline	12 months	Change
BVRT	5.71 (1.99)	6.06 (2.11)	0.35 (2.29)	6.00 (2.96)	6.79 (2.42)	0.79 (1.85)	6.78 (1.67)	6.61 (2.17)	-0.17 (1.61)	6.06 (2.28)	5.94 (2.21)	0.06 (1.61)	6.06 (2.28)	5.94 (2.21)	0.06 (1.61)	6.06 (2.28)	5.94 (2.21)	0.06 (1.61)
BTA	8.00 (1.37)	8.29 (1.31)	0.29 (1.31)	7.93 (1.69)	7.71 (2.05)	-0.21 (2.19)	8.22 (1.87)	8.53 (1.66)	0.18 (1.33)	8.35 (1.66)	8.29 (1.21)	-0.06 (1.74)	8.35 (1.66)	8.29 (1.21)	-0.06 (1.74)	8.35 (1.66)	8.29 (1.21)	-0.06 (1.74)
Digit Span Forward	8.12 (2.42)	8.59 (1.84)	0.47 (1.74)	7.43 (1.40)	8.21 (2.16)	0.79 (1.53)	9.17 (2.04)	9.22 (1.56)	0.06 (1.98)	8.71 (1.83)	7.82 (1.21)	-0.88 (1.69)*	8.71 (1.83)	7.82 (1.21)	-0.88 (1.69)*	8.71 (1.83)	7.82 (1.21)	-0.88 (1.69)*
Backward	6.47 (2.85)	6.35 (2.57)	-0.12 (1.62)	5.50 (1.83)	5.77 (1.54)	0.23 (1.92)	7.39 (2.00)	7.61 (2.30)	0.22 (1.40)	6.35 (1.77)	7.06 (2.11)	0.71 (2.23)	6.35 (1.77)	7.06 (2.11)	0.71 (2.23)	6.35 (1.77)	7.06 (2.11)	0.71 (2.23)
Letter Fluency Test	47.35 (13.62)	45.59 (14.96)	-1.76 (6.36)	43.36 (14.21)	44.07 (14.36)	0.71 (9.16)	43.00 (11.30)	43.28 (9.87)	0.28 (6.49)	40.59 (12.45)	43.06 (12.39)	2.47 (6.26)*	40.59 (12.45)	43.06 (12.39)	2.47 (6.26)*	40.59 (12.45)	43.06 (12.39)	2.47 (6.26)*
Card Rotation Test	55.06 (29.14)	58.76 (26.05)	4.75 (15.86)	53.50 (30.33)	67.57 (31.05)	14.07 (20.56)	73.44 (22.95)	83.39 (18.10)	9.94 (16.03)	71.82 (28.13)	81.29 (33.74)	9.47 (12.93)	71.82 (28.13)	81.29 (33.74)	9.47 (12.93)	71.82 (28.13)	81.29 (33.74)	9.47 (12.93)
Finding As	30.53 (6.59)	30.13 (7.94)	-0.56 (5.89)	30.43 (10.48)	31.86 (11.35)	1.43 (4.50)	29.44 (10.04)	30.89 (10.97)	1.44 (7.15)	28.35 (6.75)	30.06 (5.61)	1.71 (5.90)	28.35 (6.75)	30.06 (5.61)	1.71 (5.90)	28.35 (6.75)	30.06 (5.61)	1.71 (5.90)
MFQ	4.60 (0.89)	4.41 (0.85)	-0.19 (0.99)	4.27 (0.92)	4.47 (0.83)	0.20 (0.77)	5.05 (0.85)	5.00 (1.05)	-0.04 (0.61)	4.56 (0.82)	4.64 (0.64)	0.08 (0.83)	4.56 (0.82)	4.64 (0.64)	0.08 (0.83)	4.56 (0.82)	4.64 (0.64)	0.08 (0.83)
General frequency of forgetting	3.34 (1.26)	3.43 (1.10)	0.10 (1.03)	2.96 (1.64)	3.04 (1.34)	0.07 (1.00)	2.99 (1.45)	2.98 (1.36)	-0.01 (0.55)	3.18 (1.34)	2.85 (1.06)	-0.33 (0.92)	3.18 (1.34)	2.85 (1.06)	-0.33 (0.92)	3.18 (1.34)	2.85 (1.06)	-0.33 (0.92)
Mnemonics usage	3.35 (1.03)	3.36 (0.86)	0.01 (1.27)	3.56 (1.00)	3.16 (1.40)	-0.40 (1.49)	3.07 (0.85)	3.13 (1.03)	0.07 (0.91)	2.74 (0.95)	2.76 (0.82)	0.02 (0.73)	2.74 (0.95)	2.76 (0.82)	0.02 (0.73)	2.74 (0.95)	2.76 (0.82)	0.02 (0.73)
Retrospective functioning	4.70 (1.10)	4.22 (0.98)	-0.48 (1.04)	4.30 (1.42)	4.24 (0.99)	-0.06 (1.06)	3.94 (1.42)	4.19 (1.75)	0.25 (1.37)†	3.99 (1.02)	4.18 (0.97)	0.19 (1.12)†	3.99 (1.02)	4.18 (0.97)	0.19 (1.12)†	3.99 (1.02)	4.18 (0.97)	0.19 (1.12)†
Seriousness of forgetting																		
PANAS																		
Positive affect	3.29 (0.89)	3.30 (0.95)	0.01 (0.68)	3.45 (0.78)	3.16 (1.13)	-0.29 (0.73)	3.22 (0.63)	3.27 (0.76)	0.05 (0.65)	3.21 (0.75)	2.99 (0.85)	-0.22 (0.62)	3.21 (0.75)	2.99 (0.85)	-0.22 (0.62)	3.21 (0.75)	2.99 (0.85)	-0.22 (0.62)
Negative affect	1.65 (0.56)	1.61 (0.63)	-0.05 (0.99)	1.72 (0.62)	1.51 (0.69)	-0.21 (0.63)	1.39 (0.49)	1.33 (0.44)	-0.06 (0.60)	1.81 (0.50)	1.42 (0.32)	-0.38 (0.49)	1.81 (0.50)	1.42 (0.32)	-0.38 (0.49)	1.81 (0.50)	1.42 (0.32)	-0.38 (0.49)
Vasomotor Symptoms																		
Frequency per week	54.25 (16.68)	18.53 (15.50)	-33.72 (22.65)	63.22 (22.54)	30.68 (22.67)	-32.54 (22.34)	64.82 (19.57)	42.52 (25.46)	-22.30 (20.03)	73.82 (53.08)	4.54 (7.27)	-89.28 (54.35)*	73.82 (53.08)	4.54 (7.27)	-89.28 (54.35)*	73.82 (53.08)	4.54 (7.27)	-89.28 (54.35)*
Intensity	2.17 (0.66)	1.09 (0.95)	-1.09 (1.02)	2.34 (0.74)	1.66 (0.83)	-0.69 (1.05)	2.49 (0.58)	1.81 (0.99)	-0.67 (0.89)	2.00 (0.66)	0.32 (0.55)	-1.70 (0.78)	2.00 (0.66)	0.32 (0.55)	-1.70 (0.78)	2.00 (0.66)	0.32 (0.55)	-1.70 (0.78)

Note. BVRT = Benton Visual Retention Test; BTA = Brief Test of Attention; MFQ = Memory Functioning Questionnaire; PANAS = Positive and Negative Affect Scale. Vasomotor symptoms are based on daily diaries (see text).

* p < 0.05,

† p > 0.05 and p < 0.10 compared with placebo.