Hormone Replacement Therapy and Cognitive Performance: The Role of Homocysteine

Rachel A. Whitmer,¹ Mary N. Haan,² Joshua W. Miller,³ and Kristine Yaffe⁴

¹School of Public Health, University of California at Berkeley, and Kaiser Permanente Medical Care Program, Oakland, California. ²Department of Epidemiology, University of Michigan, Ann Arbor. ³Department of Medical Pathology, University of California at Davis. ⁴Department of Psychiatry, Neurology, and Epidemiology, University of California at San Francisco.

Background. Clinical trials have shown that estrogen reduces levels of homocysteine, and recent work has shown that elevated homocysteine increases the risk of incident dementia. However, no studies have investigated whether reduction of homocysteine is a mechanism by which estrogen enhances cognitive functioning. The aim of this study was to examine whether the association between homocysteine and cognitive functioning varies by use of hormone replacement therapy (HRT) in postmenopausal women.

Methods. Serum values of homocysteine, HRT status, and two measures of cognitive functioning, the Modified Mini-Mental State Exam (3MSE) and the Delayed Word Recall Test (DELREC), were measured in 1041 elderly women of Latino background. Multiple linear regression models were done to examine whether HRT could modify the effect of homocysteine on cognitive functioning.

Results. All findings were adjusted for age, education, income, acculturation, and hysterectomy status. Those subjects on HRT (21%) had 3MSE scores 1.88 points higher (p < .001), DELREC scores 0.12 points higher (p = .06), and homocysteine levels 1.19 μmol/L lower (p < .001) than those not on HRT. Among those not on HRT, the 3MSE was 11.8 points lower and DELREC 1.98 points lower for every 10 μmol/L increase in homocysteine. Among those on HRT, homocysteine was not associated with cognitive functioning. This interaction was not attenuated by further adjustment for lipids, blood pressure, creatinine, folate, Vitamin B₁₂, and cardiovascular disease (p < .001, interaction term coefficient for 3MSE and for DELREC).

Conclusions. Women taking HRT may modify the effects of homocysteine levels on cognitive functioning. Further work in randomized clinical trials is needed to examine whether reducing homocysteine levels with HRT can prevent cognitive decline or incident dementia.

Hormone replacement therapy (HRT) has been associated with a lower risk of cardiovascular disease in a large number of observational studies (1,2), although findings from clinical trials have found HRT not to be protective and to increase the risk of stroke or cardiovascular disease (3,4). Observational studies have found that the use of HRT is associated with a reduced rate of cognitive decline and a decreased risk of dementia (5–9). Trials of HRT and cognitive decline among Alzheimer’s patients have shown inconsistent results (10–12). HRT might affect cognition by its effects on intermediate cardiovascular risk factors such as lipids, blood pressure, or clotting factors (13–15).

Reports from observational and clinical trials show that those on HRT have lower plasma levels of the sulfur amino acid homocysteine, a product of methionine metabolism (16–20). Observational studies have found an increased risk of myocardial infarction and stroke associated with modestly elevated blood levels of homocysteine (21–23). Homocysteine may be a risk factor for Alzheimer’s disease (AD), dementia, or poor cognitive functioning. Case control studies have found higher levels of homocysteine in AD patients (24,25), and, among AD patients, those with higher levels of homocysteine have a greater extent of atrophy of the medial temporal lobe (24). High levels of homocysteine are associated with poorer cognitive functioning in population-based studies of community-dwelling elderly people and AD patients seen in clinics (26–29). A recent report based on the Framingham study demonstrated that high levels of homocysteine (14 μmol/L or greater) are positively associated with the risk of developing AD and dementia (30).

In the present study, we examine the cross-sectional association between HRT and cognitive performance, and the extent to which homocysteine modifies this association in postmenopausal women of Latino background.

METHODS

Subjects

The population is the Sacramento Area Latino Study on Aging (SALSA), an observational cohort study of health and sociocultural factors that influence the risk of dementia and diabetes among community-dwelling elderly people of Latino ancestry. All participants were aged 60 and above in 1998, and they were recruited for the SALSA study from Sacramento, San Joaquin, Solano, Stanislaus, Yolo, and Placer counties in the state of California. Data from 1990 census tracts with at least a 10% eligible population (aged 60+ and Latino) were used to identify target areas for recruitment. Potential participants were contacted by a series
of mailings, phone calls, and door-to-door contacts. Participants were eligible for the SALSA study if they met all three of the following criteria: first, they self-identified as being of Hispanic or Latino Ancestry; second, they were age 60 or above; and third, Spanish, English, or both were their primary languages. Participants were recruited from March 1998 through June 1999, with a response rate of 82.2%. The final sample was highly representative of the target population on measures of age and gender, based on 1998 Census data from the Sacramento Dress Rehearsal. Sampling and recruitment methods have been described in fuller detail elsewhere (31).

Data Collection
Bilingual technicians conducted the interviews in the homes of the participants. The survey was given in the language that the participants identified as their primary language (English vs Spanish); those participants who considered themselves bilingual chose which language version of the survey they felt more comfortable with. Participants answered questions about lifestyle factors, acculturation, and history of medical diagnoses, including stroke, myocardial infarction (MI), and diabetes. Participants were queried about any past history of disease as well as the date of diagnosis, and hospitalizations associated with the diagnosis. Acculturation was measured by using the ARSMA-II scale (32). HRT status was determined through the use of a prescription medicine inventory that involved visual examination of medications to determine exact dose and type. HRT was listed and coded according to a standard pharmaceutical database. Systolic, diastolic, and ankle arm blood pressures were measured, and fasting blood was drawn for measurement of serum lipids, plasma homocysteine, red blood cell folate, plasma B12, and serum creatinine.

Biochemistry Methods
Plasma homocysteine was determined by high-pressure liquid chromatography with postcolumn fluorescence detection (33); red blood cell folate was determined by automated chemiluminescence assay (ACS 180, Chiron Diagnostics, now Bayer Diagnostics, Tarrytown, NY); and plasma B12 was determined by radioassay (Quantaphase II, Biorad Diagnostics, Hercules, CA).

Cognitive Tests
Two tests were used to determine cognitive functioning. These were the Modified Mini-Mental State Exam (3MSE) (34) and the Delayed Word Recall Test (DELREC), which is part of the Spanish and English Verbal Learning Test (SEVLT) (35). The 3MSE has been widely used in epidemiologic cohort studies in several languages, including Spanish. It is a measure of global mental functioning and includes eight subdomains of cognitive functioning (remote memory, recent memory, mental control, time and space orientation, abstract thinking, verbal abilities, verbal comprehension, and spatial cognition). It is scored on a 0- to 100-point scale, with higher scores indicating better performance, and has been found to be a sensitive instrument in detecting differences among nondemented people and those with advanced cognitive impairment.

The DELREC is a word list learning task that utilizes 15 words that are presented for 5 sequential learning trials, followed by a distracter list, and then by delayed recall of the original list. This test was constructed to have similar measurement properties and sensitivity to cognitive impairment for Spanish and English language versions. This test specifically measures the ability to learn and recall verbal information, otherwise known as verbal episodic memory. This test is scored on a 0- to 15-point scale, with a higher score indicating better performance.

Statistical Analyses
Statistical analyses were conducted by using the SAS Version 6.12 computer program (36). Descriptive bivariate associations between those on and off HRT were conducted by using frequencies and chi-square tests. Mean differences were evaluated by using an analysis of variance (ANOVA) for categorical independent variables. Potential confounders were identified by examining HRT-specific differences across demographic and health status variables. Multiple regression was used to examine the association between HRT and cognition as well as homocysteine and cognition. Cardiovascular disease, circulatory conditions, lipids, homocysteine, and sociocultural factors were each separately added to the regression model to assess the independent effect of each potential confounder on the model. Effect modification of the association between HRT, homocysteine, and cognition was done by adding a cross-product term to a series of linear regression models. Adjustments for cardiovascular disease, circulatory conditions, lipids, homocysteine, and sociocultural factors were added in a progressive fashion and were evaluated simultaneously in the final model.

RESULTS
Characteristics of the Sample by Hormone Status
Table 1 presents results from chi-square analyses. The mean age of the sample is 70.3 years, 27% of the subjects are diabetic, and 39% have had a hysterectomy. Twenty-one percent of the women were currently taking HRT. Of those on HRT, 80% were on a similar dose and type, which was 0.625 mg of Premarin, an equine-derived unopposed estrogen preparation (Wyeth-Amherst, Philadelphia, PA). Table 1 shows the frequency of hormone use associated with demographic and health care variables. Younger women who were more educated, with higher household incomes, and born in the United States were more likely to use HRT. Access to medical care (such as insurance and having a doctor) and speaking English as a primary language were associated with HRT use. The majority of women using HRT had had a hysterectomy (69%).

HRT and Cardiovascular–Cognitive Measures
Table 2 presents mean differences by HRT for cognitive measures, lipids, creatinine, folate, glucose, homocysteine, and blood pressures from a series of unadjusted ANOVAs. Those taking HRT had significantly higher scores on global mental status (3MSE) and verbal episodic memory (DELREC). Compared to nonusers, HRT users had lower
low-density lipoprotein (LDL) and homocysteine levels, and higher high-density lipoprotein (HDL), folate, and triglycerides. There was no significant association between HRT and any of the blood pressure measures.

**HRT and Cognition**

Table 3 presents a series of coefficients from a linear regression model examining the association between HRT and the two cognitive tests. Model 1 shows the unadjusted association between HRT and the two cognitive tests. Those on HRT have a nearly 7-point higher score on the 3MSE and a 1-point higher score on the DELREC. Adjustments for cardiovascular disease (CVD; Model 2) had almost no effect on the HRT coefficients, whereas the arterial disease model (Model 3) reduced the HRT coefficients by 17% for 3MSE and nearly 27% (a 4.92-point difference) and on the DELREC (an 0.86-point difference). Among those on HRT, the addition of lipids to the unadjusted model (Model 4) produced a 7-point difference on the HRT coefficients, whereas the arterial disease model (Model 2) had almost no effect. The coefficients remained significant in this model. In Model 6, simultaneous adjustment for income, education, acculturation, and age reduced the HRT coefficient for 3MSE by nearly 40% (a 0.67-point difference), although both HRT coefficients remained significant in this model. In Model 6, simultaneous adjustment for income, education, acculturation, and age reduced the HRT coefficient for 3MSE by nearly 40% (a 0.67-point difference), although both HRT coefficients remained significant in this model.

**Homocysteine and Cognition**

In an unadjusted multiple linear regression model examining the association between homocysteine and cognition, a 10 μmol/L difference in homocysteine was inversely associated with an 8.6-point difference on the 3MSE and a 1.5-point difference on the DELREC ($p < .0001$ for both the 3MSE and the DELREC). The association between homocysteine and 3MSE and the
association between homocysteine and DELREC were not attenuated by adjustment for folate, Vitamin B12, and creatinine. Simultaneous adjustment for HDL, LDL, and triglycerides had no effect on the association between homocysteine and either measure of cognition. An adjustment for age, education, income, and acculturation reduced the association between homocysteine and the 3MSE by 66% (a 2.8-point difference for every 10 μmol/L increase in homocysteine; p < .0001) and 78% for the DELREC (a 0.3-point difference for every 10 μmol/L increase in homocysteine; p = .02).

**Interaction of HRT and Homocysteine**

Table 4 shows the results from linear regression models that include HRT, homocysteine, and an interaction term for HRT and homocysteine (homocysteine × HRT) for both 3MSE and DELREC. A series of progressive adjustments to this model are shown for lipids, CVD-related factors, blood pressure indices, and sociocultural factors, with the final model, Model 5, resulting in simultaneous adjustment for all of these variables. The addition of folate, Vitamin B12, and creatinine had no effect on the interaction of HRT and homocysteine on cognition (Model 2), nor did additional adjustment for lipids (Model 3). After additional adjustments of CVD-related factors and blood pressure indices (Model 4), there was a 15% decrease in the interaction term coefficient for 3MSE from the crude model. For the DELREC, however, adjustments for CVD-related factors and blood pressure indices did not reduce the coefficient for the interaction term of HRT × homocysteine. Lastly, additional adjustments for sociocultural factors (Model 5) reduced the interaction term coefficients for both 3MSE and DELREC (55% and 20%, respectively), but both remain statistically significant in this final fully adjusted final model.

Model 5 from Table 4 is plotted in Figures 1 and 2. Figure 1 shows the relationship between 3MSE scores and increasing standard deviations of homocysteine among both HRT users and nonusers. For those not on HRT, there is 12-point difference in 3MSE scores for every 10 μmol/L increase in homocysteine; for those on HRT, there is a 7.2-point difference in 3MSE scores for every 10 μmol/L increase in homocysteine. The inverse association between homocysteine and 3MSE is significant only for those not using HRT. Figure 2 is a similar graph showing the relationship between DELREC scores and increasing standard deviations of homocysteine among both HRT users and nonusers. For those not on HRT, there is a 1.9-point difference in DELREC score for every 10 μmol/L increase in homocysteine; for HRT users, there is only a 0.2-point difference for every 10 μmol/L difference in homocysteine. The inverse association between homocysteine and DELREC was only observed among those not using HRT.

**Discussion**

The present study found that homocysteine is associated with poorer cognitive performance only among postmenopausal women not taking HRT. This is consistent with recent reports with by Seshadri (30) that homocysteine increases the risk of dementia. In our study, those taking HRT had 3MSE and DELREC scores 11% higher than those not on HRT. Adjustment for CVD, arterial disease, lipids, or homocysteine did not confound the association. However, when income, education, acculturation, and age were simultaneously adjusted for, HRT was no longer a significant predictor of verbal memory (DELREC). Yet, it is meaningful that a statistically significant difference in the 3MSE score persisted when adjusting for all the known factors that make these two groups of women different. It is important to note that HRT is modestly associated with global cognitive performance in Latinas, a group of women.

Table 3. The Association Between HRT and Cognition in SALSA Women From Multiple Regression Models

<table>
<thead>
<tr>
<th>Model</th>
<th>3MSE Regression Coeff.</th>
<th>Adjusted R²</th>
<th>DELREC Regression Coeff.</th>
<th>Adjusted R²</th>
</tr>
</thead>
<tbody>
<tr>
<td>1. Crude</td>
<td>6.79*</td>
<td>0.035</td>
<td>1.11*</td>
<td>0.021</td>
</tr>
<tr>
<td>2. CVD</td>
<td>6.79*</td>
<td>0.051</td>
<td>1.07*</td>
<td>0.032</td>
</tr>
<tr>
<td>3. Arterial disease</td>
<td>5.66*</td>
<td>0.047</td>
<td>0.86*</td>
<td>0.040</td>
</tr>
<tr>
<td>4. Lipids</td>
<td>7.10*</td>
<td>0.042</td>
<td>1.20*</td>
<td>0.032</td>
</tr>
<tr>
<td>5. Homocysteine</td>
<td>4.92*</td>
<td>0.084</td>
<td>0.67*</td>
<td>0.047</td>
</tr>
<tr>
<td>6. Sociocultural</td>
<td>1.88**</td>
<td>0.33</td>
<td>0.12</td>
<td>0.23</td>
</tr>
<tr>
<td>7. All</td>
<td>1.47**</td>
<td>0.40</td>
<td>0.10</td>
<td>0.26</td>
</tr>
</tbody>
</table>

Notes: HRT = hormone replacement therapy; SALSA = Sacramento Area Latino Study on Aging; 3MSE = Modified Mini-Mental State Exam; DELREC = Delayed Word Recall Test; CVD = Cardiovascular disease. Model 2 is adjusted for stroke, diabetes, and myocardial infarction. Model 3 is adjusted for diastolic, systolic, and ankle arm index blood pressures. Model 4 is adjusted for triglycerides and high- and low-density lipoproteins. Model 5 is adjusted for homocysteine, folate, Vitamin B12, and creatinine. Model 6 is adjusted for age, income, education, acculturation, and hystectomy status. Model 7 is adjusted for all variables in Models 2–6 simultaneously.

* p < .0001 and ** p < .001.

Table 4. Interaction of HRT and Homocysteine on Cognition From Multiple Regression Models in SALSA Women

<table>
<thead>
<tr>
<th>Model</th>
<th>Regression Coeff.</th>
<th>3MSE</th>
<th>DELREC</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>HRT</td>
<td>4.12</td>
<td>1.11**</td>
</tr>
<tr>
<td></td>
<td>Homocysteine</td>
<td>1.07*</td>
<td>0.19***</td>
</tr>
<tr>
<td></td>
<td>HRT × Homocyst.</td>
<td>1.02*</td>
<td>0.20*</td>
</tr>
<tr>
<td>2</td>
<td>HRT + folate, B12, &amp; creatine</td>
<td>5.57***</td>
<td>1.53**</td>
</tr>
<tr>
<td></td>
<td>Homocysteine</td>
<td>1.18*</td>
<td>0.22**</td>
</tr>
<tr>
<td></td>
<td>HRT × Homocyst.</td>
<td>1.05*</td>
<td>0.22*</td>
</tr>
<tr>
<td>3</td>
<td>HRT + HDL, LDL, &amp; triglyceride</td>
<td>3.05</td>
<td>0.96**</td>
</tr>
<tr>
<td></td>
<td>Homocysteine</td>
<td>0.92*</td>
<td>0.20**</td>
</tr>
<tr>
<td></td>
<td>HRT × Homocyst.</td>
<td>0.89*</td>
<td>0.23*</td>
</tr>
<tr>
<td>4</td>
<td>HRT + MI, stroke, diabetes, systolic, diastolic, &amp; ankle-arm BPs</td>
<td>5.12</td>
<td>1.62**</td>
</tr>
<tr>
<td></td>
<td>Homocysteine</td>
<td>0.92*</td>
<td>0.20**</td>
</tr>
<tr>
<td></td>
<td>HRT × Homocyst.</td>
<td>0.89*</td>
<td>0.23*</td>
</tr>
<tr>
<td>5</td>
<td>HRT + age, educ., income, accent.; &amp; hysterect.</td>
<td>3.29</td>
<td>1.56**</td>
</tr>
<tr>
<td></td>
<td>Homocysteine</td>
<td>0.43**</td>
<td>0.10**</td>
</tr>
<tr>
<td></td>
<td>HRT × Homocyst.</td>
<td>0.46**</td>
<td>0.159**</td>
</tr>
</tbody>
</table>

Notes: HRT = hormone replacement therapy; SALSA = Sacramento Area Latino Study on Aging; HDL and LDL = high- and low-density lipoprotein; MI = myocardial infarction; BP = blood pressure; 3MSE = Modified Mini-Mental State Exam; DELREC = Delayed Word Recall Test. * p < .0001, ** p < .001, and *** p < .05.
previously neglected in the HRT literature. However, when considering the effect of HRT on cognition in this study, one should note that over 80% of the women were on an estrogen-only preparation of HRT because of the high prevalence of hysterectomies. More data are needed to determine if the risks and benefits for this treatment are similar to those of an estrogen and progesterone combination of HRT (4).

The present study found an association between homocysteine and cognition that is consistent with previous research (26–29). Homocysteine was inversely associated with both global cognitive functioning (3MSE) and verbal memory (DELREC). This is not surprising, because homocysteine could be a marker of subclinical vascular disease and cognitive decline. These associations were stronger after adjustment for folate, Vitamin B12, and creatinine, whereas lipids, blood pressure indices, and cardiovascular disease had no effect. This is the first study to suggest an association between homocysteine and verbal memory (as measured by the DELREC), although this effect was attenuated by adjustment for sociocultural factors.

Evidence from these analyses suggests that HRT may exert its effect on cognition, at least in part, by influencing plasma homocysteine levels. Those taking HRT had significantly lower levels of homocysteine, which is consistent with previous research (16–20). Although it is known that homocysteine is a risk factor for cardiovascular disease (21–23), it may be by lowering this risk that HRT is associated with improved cognitive performance.

However, the findings also suggest that HRT is associated with cognition due to changes in homocysteine that are independent of cardiovascular mechanisms. Perhaps homocysteine affects cognition through a direct effect on brain functioning. In the present analyses, homocysteine remained significantly associated with cognition after adjustments for all cardiovascular mechanisms (lipids, CVD history, and blood pressure indices). Moreover, adjustments for all cardiovascular mechanisms did not attenuate the interaction effect of HRT and homocysteine on cognition. This suggests that the synergistic effect of HRT and homocysteine on cognition could also be due to noncardiovascular mechanisms.

There are several lines of evidence that indicate that homocysteine plays a role in the progression of AD, and neurological functioning in those without AD. There are higher levels of homocysteine in AD patients versus healthy controls after age, sex, apolipoprotein E genotype, folate, Vitamin B12, and social class are adjusted for (24). Among AD patients, elevated levels of homocysteine are associated with greater atrophy of the medial temporal lobe, and in vivo studies suggest that homocysteine plays a neurotoxic role in cell injury (37). Homocysteine has been found to elicit DNA damage in rat hippocampal neurons (38) and to potentiate amyloid beta peptide and copper-mediated toxicity in mouse neuronal cultures (39,40). These findings demonstrate that, in animal models, homocysteine increases the vulnerability of hippocampal neurons to excitotoxic and oxidative injury. Thus, one of the neurophysiological mechanisms for HRT and improved cognition may be
a reduction of homocysteine levels, which in turn lessens the extent of hippocampal neuronal damage.

In the present study, there was no inverse association between homocysteine and cognition among those using HRT. This suggests that HRT may mitigate the negative effect of homocysteine on cognition. Because elevated homocysteine may reflect inflammation related to CVD or neurological disease, it is likely that homocysteine would be associated with both global mental functioning as well as verbal memory, and is a robust enough effect to remain statistically significant after numerous adjustments. Whereas the interaction effect of HRT and homocysteine was present for both cognitive outcomes, the 3MSE and the DELREC, the association between only HRT and the DELREC diminished after numerous adjustments. This suggests that HRT and homocysteine interact to affect not only global cognition but verbal memory, a specific domain of cognition. One of the drawbacks of the current analysis is that other domain-specific tests of cognition were not included; future studies will determine if HRT and homocysteine have a similar confluence on other cognitive domains.

A strength of our study is the diverse bilingual sample of postmenopausal Latinas and the extent of medical and cardiovascular risk factors and cognitive information measured. However, longitudinal data from a clinical controlled trial could more accurately assess whether a reduction in homocysteine, resulting from HRT use, is associated with retardation of cognitive decline. The results from the present study merit further investigation into the role of homocysteine in mediating the putative effect of HRT on risk of cognitive impairment. The current findings suggest a possible mechanism between HRT and cognitive performance in a population of women where no previous research has examined this association.

ACKNOWLEDGMENTS

This work was supported by a research grant from the Women’s Center on Health in the Department of Obstetrics and Gynecology, University of California at Davis School of Medicine, and Public Health Service grant T32 HL07365-22. Part of this work was presented at the American Heart Association Epidemiology and Prevention Meeting in March 2001.

Address correspondence to Rachel A. Whitmer, PhD, Division of Research Kaiser Permanente, 2000 Broadway, Oakland, CA 94612. E-mail: raw@or.kaiser.org

REFERENCES


Received July 25, 2002
Accepted September 25, 2002