ORIGINAL CONTRIBUTION

Higher Estrogen Levels Are Not Associated With Larger Hippocampi and Better Memory Performance

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Background: Estrogens may prevent cognitive decline and Alzheimer disease. Animal study findings have shown beneficial effects of estrogen on the brain, particularly on the hippocampus, a structure related to memory performance and early Alzheimer disease.

Objective: To investigate whether higher levels of endogenous estradiol in older women and men are associated with larger hippocampal volumes on magnetic resonance imaging and better memory performance.

Design and Setting: Cross-sectional analysis within the Rotterdam Scan Study, a population-based study in the Netherlands of elderly subjects who do not have dementia.

Participants: Two hundred ten women and 202 men, aged 60 to 90 years, with plasma levels of total estradiol and, in part, 162 women and 149 men also with levels of bioavailable and free estradiol.

Main Outcome Measure: Hippocampal volumes on magnetic resonance imaging and memory performance (delayed recall).

Results: Women with higher total estradiol levels had smaller hippocampal volumes and poorer memory performance –0.29 mL (95% confidence interval, –0.57 to –0.00) and –0.4 (95% confidence interval, –1.3 to 0.5) fewer words in delayed recall testing for the highest tertile compared with the lowest tertile. Similar inverse associations were found among bioavailable and free estradiol levels, hippocampal volumes, and memory. In men, no association was observed between estradiol levels and hippocampal volume, but a trend was found for higher levels of total estradiol to be associated with poorer memory performance.

Conclusion: Our data do not support the hypothesis that higher levels of endogenous estradiol in older women and men are associated with larger hippocampal volumes and better memory performance.

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AFTER MENOPAUSE, women have low levels of circulating estrogens. Observational studies reported a reduced risk of cognitive decline and Alzheimer disease (AD) in postmenopausal women using estrogen replacement therapy.[1,2] In animals, estrogens protect hippocampal neurons exposed to amyloid β,[3] improve synapse formation on dendritic spines in the hippocampus,[4] and promote survival of hippocampal neurons, acting in concert with growth factors.[5] Estrogen receptors are located throughout the brain, but especially in the hippocampus.[6] Given the beneficial effects of estrogens on the hippocampus in animals, it has been hypothesized that estrogens may prevent hippocampal atrophy, a key feature of AD.[7] The hippocampus plays a pivotal role in memory function and is one of the first regions affected in AD.[8] The aim of this study was to investigate whether higher endogenous levels of estradiol were associated with larger hippocampal volumes on magnetic resonance imaging (MRI) and better memory performance. We examined this hypothesis in a population-based study of older women and men who did not have dementia.

METHODS

ROTTERDAM SCAN STUDY

This study is based on all participants in the Rotterdam Scan Study that originated from the Rotterdam Study. The Rotterdam Study is a large population-based cohort study in the Netherlands that started in 1990 and investigates the prevalence, incidence, and determinants of various chronic diseases among elderly participants.[9] From 1995 to 1996, we randomly selected 965 living members (aged, 60-90 years) of this cohort in strata of sex and

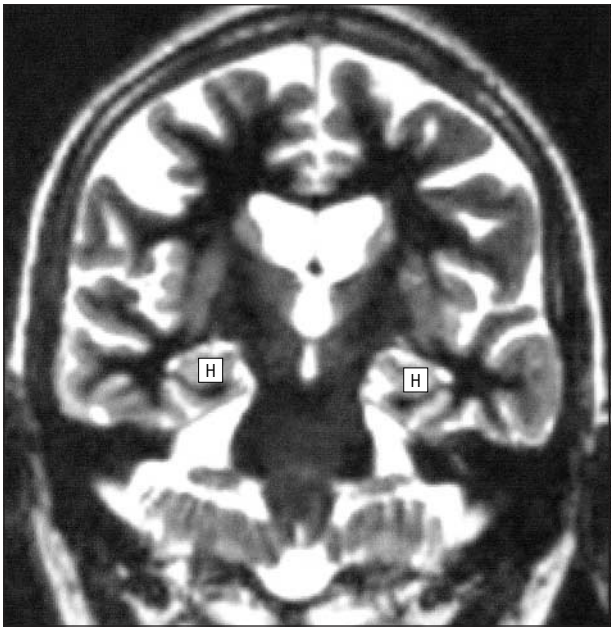

Figure 1. Coronal slice on which the left and right hippocampi (H) are depicted.

age (5 years) for participation in the Rotterdam Scan Study.[10] After exclusion of individuals who had dementia (n=16),[11] or had contraindications to undergo MRI (n=117), 832 persons were eligible for our study. Among these, 563 participants gave their written informed consent to participate in the study (response rate, 68%), which included undergoing an MRI brain scan. The study was approved by the medical ethics committee of Erasmus MC, Rotterdam, the Netherlands.

MRI PROCEDURES

All subjects underwent T1-, T2-, and proton-density–weighted images in a 1.5-T magnetic resonance unit (VISION MR; Siemens, Erlangen, Germany).[12] For volumetric measurements of the hippocampus, a custom-made, inversion recovery–double-contrast, 3-dimensional, half-Fourier acquisition single-shot turbo spin-echo (HASTE) sequence was included (inversion time, 440 milliseconds; repetition time, 2800 milliseconds; 128 contiguous sagittal slices of 1.2 mm; acquisition matrix, 192×256 pixels; and field of view, 256×256 cm). Two HASTE modules were sequentially acquired after the inversion pulse (effective echo times of 29 milliseconds and 440 milliseconds). Each HASTE module combined nonselective radiofrequency excitations to provide a short interecho spacing of 3.9 milliseconds. The first HASTE module was used for the hippocampal volume measurement.

HIPPOCAMPAL VOLUMES

We reconstructed a series of coronal brain slices (contiguous 1.5-mm slices) based on the HASTE sequence, aligned to be perpendicular to the long axis of the hippocampus. All reconstructed slices were transferred to a workstation (Magic View 1000; Siemens) for volumetric assessment of the left and right hippocampi (**Figure 1**). Referencing to an anatomical atlas,[13] we manually traced the boundaries of both hippocampi on each slice using a mouse-driven pointer. We proceeded from posterior to anterior, starting on the slice where the crux of the fornices was in full profile. The alveus could often be used to delineate the boundary of the hippocampal head from the amygdala. Entering the outlined surface areas (expressed in millimeters squared), we multiplied the summed surface areas on each side with slice thickness to yield estimates of the left and right hippocampal volume (expressed in milliliters). In the current analyses the left and right hippocampal volumes were summed.

We also reconstructed a midsagittal slice (thickness 3.0 mm). The midsagittal area, which was used as a proxy for intracranial volume, was measured by tracing the inner table of the skull. Two raters (T.d.H. and a colleague), who were blinded to any clinical information related to the participants, assessed the scans. Studies performed on 14 random scans to evaluate intrarater and interrater correlation showed good overall agreement.

MEMORY PERFORMANCE

All participants underwent neuropsychological testing.[12] The Mini-Mental State Examination was administered to assess global cognitive function.[14] Memory function was evaluated by a 15-word learning test based on the Rey Auditory Verbal Learning Test.[15] This test consists of 3 learning trials in which 15 words have to be remembered. After 15 minutes, the subject is asked to recall as many words as possible (delayed recall). In the present study we used the delayed recall score because this score is most strongly associated with hippocampal volumes[16] and with early AD.[17]

BLOOD MEASUREMENTS

Venipuncture was done in nonfasting subjects between 8:30 AM and 4 PM at baseline examination of the Rotterdam Study (1990-1993). Blood samples were collected in 5-mL tubes containing a 0.5-mL sodium citrate solution. All tubes were stored on ice before and after blood sampling. Platelet-free plasma was obtained by 2-stage centrifugation (10 minutes at 1600*g* at 4°C and 30 minutes at 7000*g* at 4°C). Platelet-free samples were immediately frozen in liquid nitrogen and stored at –80°C. Assays were performed blinded to information on the subject. Plasma levels of estradiol and sex hormone–binding globulin were estimated with double antibody radioimmunoassays (ultrasensitive method for estradiol; Diagnostic Systems Laboratories, Webster, Tex). Because of the small volumes of plasma available, all estradiol levels are single-sample estimations. Intraassay coefficients of variation, determined on the basis of duplicate results of internal quality control pools with 3 different levels of each analyte, were below 4% for sex hormone–binding globulin and 18% for estradiol. Because interassay variations were 14% (sex hormone–binding globulin) and 21% (estradiol), results of all batches were normalized by multiplying all concentrations within a batch with a factor, which equalized results for the internal quality control pools. As measures of the levels of bioavailable and free estradiol, nonsex hormone–binding globulin-bound estradiol, and nonprotein-bound estradiol, respectively, were calculated on the basis of hormone and binding protein levels.[18,19] The median interval from blood sampling (1990-1993) to hippocampal volumes and memory testing (1995-1996) was 2.9 years (range, 1.6-6.6 years).

COVARIATES

Several variables may confound an association between estradiol levels and hippocampal volume or memory performance, such as age at the time of venipuncture, educational level, smoking habits, alcohol intake, body mass index (BMI) (calculated as weight in kilograms divided by the height in meters squared), depressive symptoms, and apolipoprotein E *APOE* genotype; and for women, the variables included type of menopause, age at menopause, and use of hormone replacement therapy (HRT)

(ie, those who ever used HRT or those who never used HRT) for menopausal reports (Anatomical Therapeutical Chemical code g03). The level of education was dichotomized into primary education and lower vocational training and university education. We categorized smoking status into current, former, and never and made dummy variables for the analyses. Alcohol intake was recorded in grams per day.[22] Depressive symptoms were assessed using the Center for Epidemiologic Studies–Depression Scale (CES-D)[23] at the time of assessment of memory performance. Apolipoprotein E genotyping was done according to standard methods[24] and was coded into non-*APOE* ε4 carrier and *APOE* ε4 carrier. Type of menopause was classified into natural or artificial (surgically or chemically induced). Age at natural menopause was defined as the age at which menses had not occurred for at least 1 year. Type and age of menopause were combined in 1 categorical variable with 4 groups: (1) artificial menopause, (2) natural menopause before the age of 48 years, (3) natural menopause between ages 48 and 52 years, or (4) natural menopause after the age of 52 years.

STUDY SAMPLE

To obtain the study samples for the analyses, we excluded 4 women who used HRT at the time of blood drawing, leaving 559 subjects (**Figure 2**). Because estradiol levels were measured as part of several blood measurements and small amounts of plasma were available, we randomly missed total estradiol levels for 147 subjects. Thus, 412 subjects for the analyses on total estradiol levels were included. Because of missing data on binding protein levels (n=101), we had bioavailable and free estradiol levels in 311 subjects. These 311 subjects with bioavailable and free estradiol levels were similar to the 248 subjects who were dropped from these analyses for age, hippocampal volume, and delayed recall score.

DATA ANALYSIS

All analyses were performed in women and men separately. We first investigated whether there was a threshold in the relation between estradiol level and outcome by analysis of covariance (ANCOVA). Tertiles of total, bioavailable, and free estradiol were the group variable and we calculated adjusted mean hippocampal volumes and delayed recall scores in each tertile. Three consecutive models were used. The first adjusted for age and—in the hippocampal analyses—midsagittal area to account for intracranial volume.[25] After separately investigating several covariates, it appeared as if the BMI was the strongest confounder in the associations so the second model was adjusted, in addition to age and midsagittal area, for BMI. In the third model we additionally entered educational level, smoking, alcohol use, CES-D score, *APOE* genotype, and, for women, age at menopause, type of menopause, and ever taking HRT. Because the ANCOVA did not suggest a specific threshold in the relation between estradiol levels and outcome, we performed a multivariate linear regression analysis to calculate the difference in hippocampal volume and delayed recall score per SD increase in estrogen levels. To investigate whether there was an indication of differential associations in age strata (<70 years and ≥70 years) or *APOE* genotype, we included in the multivariate linear model an interaction term and performed stratified analyses. Finally, we repeated the analyses excluding women with a history of HRT use.

RESULTS

Of the 210 women and 202 men with total estradiol measurements, 24 women and 16 men had no hippocampal volume assessment (because they developed claustrophobia during the MRI) and 6 women and 8 men had no reliable delayed recall data (mostly owing to motivational problems). Characteristics of the study sample are given in **Table 1**. There were no differences in the characteristics between subjects with total estradiol levels and subjects without total estradiol levels. Estradiol levels of women were lower than those of men, reflecting the fact that women were postmenopausal and were not taking HRT at the time of venipuncture.

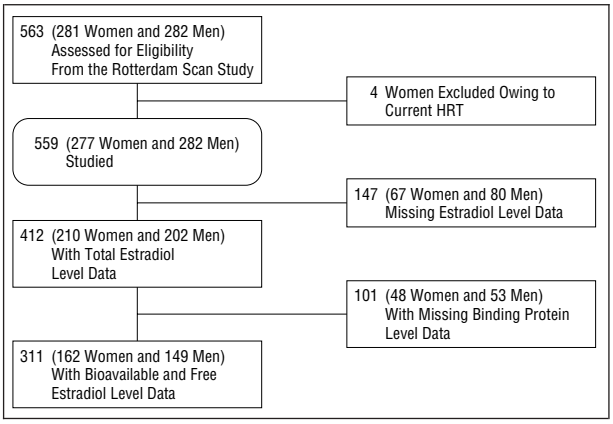

Figure 2. Description of study sample. HRT indicates hormone replacement therapy.

In women, estradiol levels were negatively correlated with age and positively correlated with BMI. In men, estradiol levels were negatively correlated with age and positively correlated with alcohol intake and CES-D score (**Table 2**). Hippocampal volumes and delayed recall scores decreased with increasing age (both in women and men, –0.03 mL/y and –0.1 words per year).

WOMEN

Estradiol Level and Hippocampal Volume

In women, the age- and midsagittal-adjusted mean (SE) hippocampal volumes decreased across higher tertiles of total estradiol levels from 6.24 (0.10) mL to 6.22 (0.10) mL to 6.07 (0.10) mL, but there were no significant differences between tertiles. When the BMI was included as a covariate, the mean (SE) hippocampal volumes were 6.28 (0.10) mL, 6.21 (0.10) mL, and 6.04 (0.10) mL, respectively, but differences were again not statistically significant. **Figure 3**A shows that after additional adjustments for all other covariates the negative association between the total estradiol level and hippocampal volume became statistically significant (difference between the highest and lowest tertile of total estradiol –0.29 mL [95% confidence interval (CI), –0.57 to –0.00]). When we analyzed the total estradiol level as a continuous measure, we found a nonsignificant decrease in hippocampal volume per SD increase in total estradiol level (**Table 3**). There was a nonsignificant inverse relation between the bioavailable estradiol level and hippocampal volumes; the age- and midsagittal-adjusted means of hippocampal volume across higher bioavailable estradiol tertiles was 6.22 (0.12) mL, 6.17 (0.11) mL, and 6.00

Table 1. Characteristics of Study Sample

Characteristic	Women (n = 210)	Men (n = 202)	Subjects Without Total Estradiol Levels (n = 147)
Age, mean (SD), y	70 (8)	69 (8)	71 (8)
BMI, mean (SD), kg/m^2	26.8 (3.9)	26.1 (2.9)	26.3 (3.2)
Subjects with a primary education, %	37	23	34
Subjects currently smoking, %	16	24	23
Alcohol consumption, mean (SD), g/d	7.6 (11.5)	17.4 (10.9)	13.7 (17.0)
CES-D scale score, mean (SD)	7.0 (6.6)	4.6 (5.3)	5.0 (5.7)
APOE genotype, % *APOE* ϵ4 carrier	27	32	27
MMSE score, mean (SD)	27.6 (2.3)	27.8 (2.1)	27.7 (2.0)
Total estradiol level, median (range), pg/mL	3.7 (0.0-17.7)	12.3 (0.0-42.8)	NA
Bioavailable estradiol level, median (range), pg/mL*	2.4 (0.0-13.4)	9.3 (0.0-32.7)	NA
Free estradiol level, median (range), pg/mL*	0.09 (0.00-0.47)	0.33 (0.00-1.14)	NA
Midsagittal area, mean (SD), cm^2†	143.1 (8.8)	155.2 (9.9)	149.3 (11.1)
Hippocampus, mean (SD), mL†	6.17 (0.81)	6.59 (0.89)	6.36 (0.88)
Delayed recall score, mean (SD), No. of words‡	6.2 (2.8)	5.5 (2.4)	5.4 (2.6)

Abbreviations: *APOE*, apolipoprotein E; BMI, body mass index; CES-D, Center for Epidemiologic Studies–Depression Scale; HRT, hormone replacement therapy; MMSE, Mini-Mental State Examination; NA, not available.
To convert estradiol values to pmol/L, multiply by 3.67.
*Values were available for 162 women and 149 men.
†Values were available for 186 women and 186 men.
‡Values were available for 204 women and 194 men. Higher scores reflect better performance.

Table 2. Correlations Between Several Continuous Variables in 210 Women and in 202 Men*

Variable	Total Estradiol Level	Age	Educational Level	BMI	Alcohol Consumption	CES-D Scale
Age, per year	–0.49†	. . .‡	. . .	. . .	. . .	. . .
	–0.62†	. . .	. . .	. . .	. . .	. . .
Education, per grade	–0.08	–1.02†	. . .	. . .	. . .	. . .
	–0.87	*–0.22*	. . .	. . .	. . .	. . .
BMI, kg/m^2	0.97†	0.24	–0.05†	. . .	. . .	. . .
	1.04	*0.04*	*–0.06*	. . .	. . .	. . .
Alcohol consumption, per g/d	0.01	–0.13†	0.01	–0.03	. . .	. . .
	0.33†	*–0.04*	*–0.00*	*0.02*	. . .	. . .
CES-D score, per point	–0.27	0.20†	–0.02	–0.04	0.02	. . .
	0.77†	*–0.05*	*0.02*	*0.03*	*0.20*	. . .
Midsagittal area, per cm^2	0.23	–0.22†	0.02†	0.01	–0.05	–0.10†
	0.14	*–0.07*	*0.02*	*–0.01*	*–0.11*	*0.05*

Abbreviations: BMI, body mass index; CES-D, Center for Epidemiologic Studies–Depression Scale.
*Values are unadjusted regression coefficients in women and men (italics). The variables in the upper row are dependent variables.
†$P<.05$.
‡Ellipses indicate not applicable.

(0.11) mL. When the BMI was included in the model, a significant smaller hippocampal volume was found in women with bioavailable estradiol levels in the highest tertile of bioavailable estradiol compared with the lowest tertile (difference, –0.37 mL [95% CI, –0.71 to –0.03]). Additional adjustments did not change this inverse relation (Figure 3A). When we analyzed bioavailable estradiol levels as a continuous measure, we found a nonsignificant decrease of hippocampal volume of –0.09 mL (95% CI, –0.25 to 0.06) per SD (10-pmol/L) increase. The relation between free estradiol levels and hippocampal volumes was similar to that of bioavailable estradiol levels (Figure 3A). The inverse associations between estradiol levels and hippocampal volumes were primarily seen in the oldest age group (in age<70 years: per SD increase in total estradiol level the fully adjusted difference in hippocampal volumes was 0.07 mL [95% CI, –0.09 to 0.23]; in age≥70 years: –0.24 mL [95% CI, –0.44 to –0.04]; *P* of interaction term .04). The negative associations between estradiol level and hippocampal volumes were more pronounced in *APOE* ϵ4 carriers than in non-*APOE* ϵ4 carriers, although the interaction term was not statistically significant (**Table 4**).

Estradiol Level and Memory

The age-adjusted delayed recall score decreased nonsignificantly across total estradiol tertiles from 6.5 (0.3) to 6.1 (0.3) to 6.1 (0.3) words. Figure 3B shows that additional adjustments for other covariates did not substantially change these results. The fully adjusted difference in delayed recall words between the highest and lowest tertile of total estradiol levels was –0.4 (95% CI, –1.3 to 0.5). When we analyzed the total estradiol level as a con-

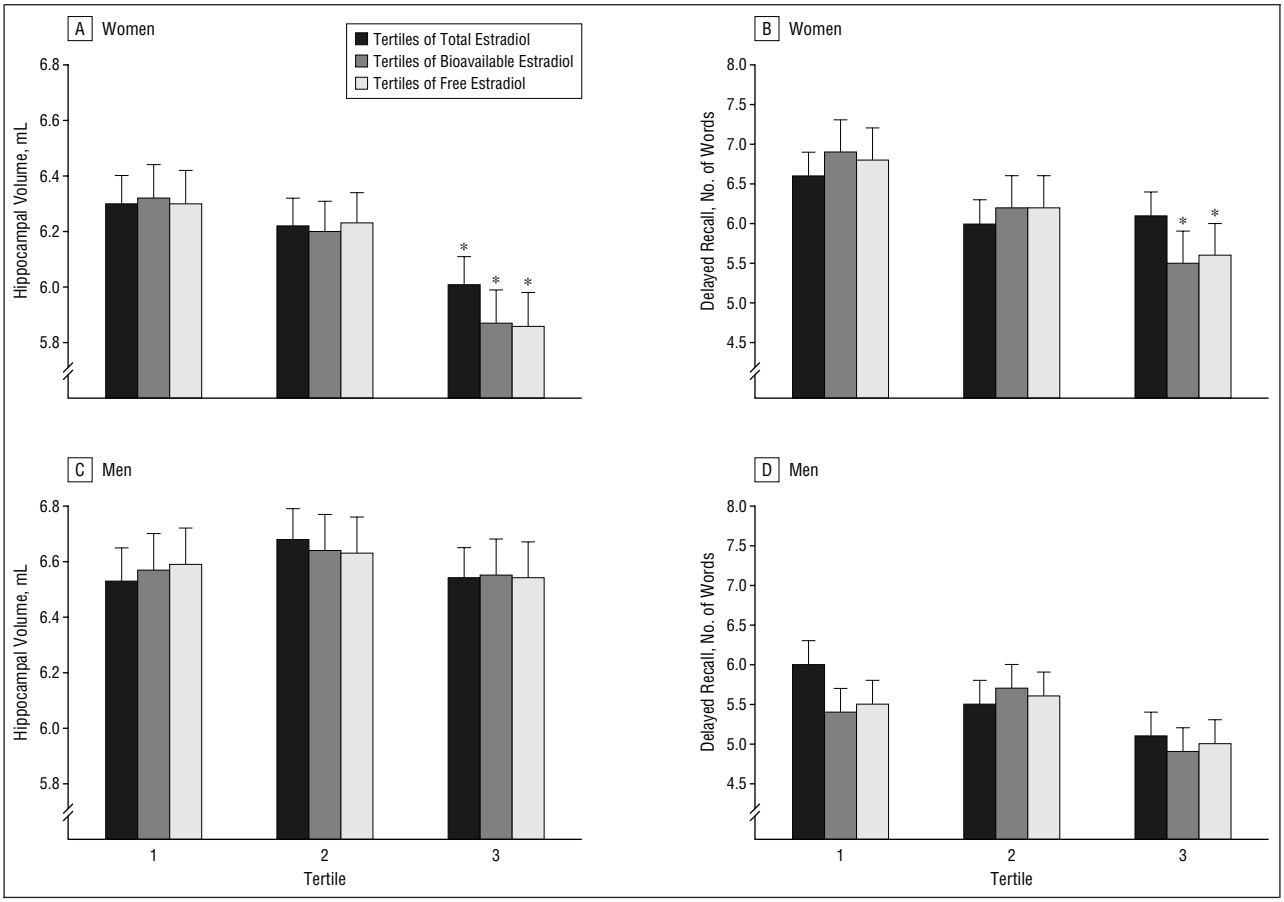

Figure 3. Adjusted hippocampal volumes (SEs) (A and C) and delayed recall (SEs) (B and D) across tertiles of total, bioavailable and free estradiol levels in women and men, respectively. A and B, In women means are adjusted for age, educational level, body mass index (calculated as weight in kilograms divided by height in meters squared), alcohol consumption, smoking, Center for Epidemiologic Studies–Depression Scale score, type of menopause, age at menopause, ever use of hormone replacement therapy, apolipoprotein E genotype, and the hippocampal volumes–midsagittal area. Tertile ranges (in picograms per milliliter) were for total estradiol levels 0.0-1.9, 1.9-5.2, and 5.2-17.7; for bioavailable estradiol levels 0.0-1.5, 1.5-3.8, and 3.8-13.4; and for free estradiol levels 0.00-0.05, 0.05-0.14, and 0.14-0.47. Asterisks indicate statistically significantly ($P<.05$) smaller than lowest tertile. C and D, In men means are adjusted for age, educational level, body mass index, alcohol, smoking, Center for Epidemiologic Studies–Depression Scale score, apolipoprotein E genotype, and the hippocampal volumes–midsagittal area. Tertile ranges (in picograms per milliliter) were for total estradiol level 0.0-9.8, 9.8-14.2, and 14.2-42.8; for bioavailable estradiol level 0.0-7.4, 7.4-10.9, and 10.9-32.7; and for free estradiol level 0.00-0.26, 0.26-0.41, and 0.41-1.14.

tinuous measure, we found a nonsignificant decrease in delayed recall words (Table 3). There was a significant inverse relation between bioavailable estradiol tertiles and delayed recall (Figure 3B), although when we analyzed bioavailable estradiol levels continuously, we found a nonsignificant decrease in delayed recall words of –0.2 (95% CI, –0.7 to 0.2) per SD increase. The results were similar for the relation between the free estradiol level and delayed recall score (Figure 3B). Again, the inverse associations between the estradiol level and memory were stronger in the oldest age groups (data not shown). The associations were not different in *APOE* ϵ4 strata (Table 4). Exclusion of women who had used HRT in the past (n=17) did not change any of the above results.

MEN

Estradiol Level and Hippocampal Volume

In men, there was no association between estradiol levels and hippocampal volumes (age and midsagittal-adjusted hippocampal volumes in total estradiol tertiles: 6.55 [0.11] mL, 6.68 [0.11] mL, and 6.52 [0.11]) mL). Additional adjustments for the other covariates did not change these estimates (Figure 3C and Table 3). There was also no association between bioavailable or free estradiol levels and hippocampal volume (Figure 3C). There were no differential associations in age strata or in *APOE* ϵ4 strata (data not shown).

Estradiol Level and Memory

When we investigated the relation between estradiol levels and memory performance in men, a similar pattern as in women emerged. The age-adjusted delayed recall scores decreased across increasing total estradiol tertiles from 6.1 (0.3) to 5.4 (0.3) to 5.1 (0.3) words. When we analyzed the total estradiol level as a continuous measure, we found a decrease in delayed recall with increasing levels (Table 3). Additional adjustments for covariates did not change this association (Figure 3D and Table 3). The association of delayed recall score with bioavailable and free estradiol levels showed a similar pattern as with the total estradiol level (Figure 3D). There were no

Table 3. Associations Between Total Estradiol Levels and Hippocampal Volumes and Memory Performance*

Women	Per Increase of 1 SD (3.8 pg/mL) in Total Estradiol Level	
	Hippocampal Volume, mL	Delayed Recall, No. of Words
Adjusted for		
Age and midsagittal area†	−0.01 (−0.12 to 0.10)	−0.1 (−0.4 to 0.3)
Age, midsagittal area† and BMI	−0.04 (−0.16 to 0.08)	−0.1 (−0.5 to 0.3)
Age, midsagittal area,† BMI, education, alcohol consumption, smoking, CES-D, *APOE* genotype, age, and type of menopause, ever use of HRT	−0.06 (−0.18 to 0.06)	−0.1 (−0.5 to 0.3)

Men	Per Increase of 1 SD (6.3 pg/mL) in Total Estradiol Level	
	Hippocampal Volume, mL	Delayed Recall, No. of Words
Adjusted for		
Age and midsagittal area†	0.02 (−0.10 to 0.15)	−0.4 (−0.7 to −0.0)
Age, midsagittal area,† and BMI	−0.01 (−0.05 to 0.03)	−0.4 (−0.7 to 0.0)
Age, midsagittal area,† BMI, education, alcohol consumption, smoking, CES-D, and *APOE* genotype	0.04 (−0.09 to 0.18)	−0.3 (−0.7 to 0.1)

Abbreviations: *APOE*, apolipoprotein E; BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CES-D, Center for Epidemiologic Studies–Depression Scale; HRT, hormone replacement therapy.
*Values are adjusted regression coefficients (95% confidence interval) of the relation between total estradiol level and hippocampal volume/delayed recall score.
†Values adjusted for only the hippocampal analyses.

Table 4. Associations Between Total Estradiol Levels and Hippocampal Volumes and Memory Performance in Women According to Apolipoprotein E (*APOE*) ε4 Strata*

Variable	Hippocampal Volumes, mL		
	Non-*APOE* ε4 Carrier	*APOE* ε4 Carrier	*P* Interaction Term†
Adjusted for			
Age, midsagittal area, BMI, educational level, alcohol consumption, smoking, CES-D, age, type of menopause, and ever use of HRT	0.01 (−0.16 to 0.17)	−0.25 (−0.46 to −0.03)	.07

	Delayed Recall, No. of Words		
	Non-*APOE* ε4 Carrier	*APOE* ε4 Carrier	
Adjusted for			
Age, BMI, educational level, alcohol consumption, smoking, CES-D, age, type of menopause, and ever use of HRT	−0.10 (−0.61 to 0.41)	0.44 (−0.21 to 1.09)	.29

Abbreviations: BMI, body mass index (calculated as weight in kilograms divided by height in meters squared); CES-D, Center for Epidemiologic Studies–Depression Scale; HRT, hormone replacement therapy.
*Values are adjusted regression coefficients (95% confidence interval) of the relation between total estradiol level (per 1 SD increase) and hippocampal volume/delayed recall score.
†*P* value of interaction term (*APOE* ε4 strata × total estradiol level) in the multivariate linear regression analysis.

differences in the associations in age strata or in *APOE* ε4 strata (data not shown).

COMMENT

In this population-based study in elderly subjects with no dementia, we could not support the hypothesis that higher endogenous levels of estradiol are associated with larger hippocampal volumes on MRI and better memory performance. Women with higher estradiol levels had smaller hippocampal volumes and poorer memory performance than women with lower levels. In men, there was no relation between estradiol level and hippocampal volumes, but memory performance was worse in those with higher levels of total estradiol compared with those with lower levels.

The strengths of this study are that it is population based and involves a large number of volumetric hippocampal assessments. Our study has several methodological limitations that need to be discussed. First, owing to the small amounts of plasma available, we had a large number of missing data on bioavailable and free estradiol levels. Although these missing data were random, the smaller sample size will have resulted in less precise estimates of the associations. Second, blood was drawn several years before MRI and memory performance. It is difficult to determine if this influenced our results. It may have led to biased associations if subjects

with estradiol levels on one side of the distribution selectively died between blood drawing and MRI measurement. This does not seem likely, however, because the interval between blood drawing and MRI was not long, and the relation between endogenous estradiol levels and mortality is not strong.[26] Third, HRT use in the Netherlands is infrequent compared with, for example, the United States. Our results were based on observations in non-HRT users and they may not be generalizable to HRT users.

The most consistent support for the hypothesis that estrogens prevent dementia comes from animal studies that showed that estrogens protect against cell loss in the hippocampus, increase neurite outgrowth, and have antioxidative properties.[3,27] In humans, observational studies on endogenous levels of estrogen in relation to cognitive function have been inconclusive. Some reported negative effects of higher endogenous estrogen levels on delayed visual reproduction[28] and attention tasks[29] in women and on the results of the Mini-Mental State Examination, memory tasks,[30] and spatial performance[31] in men, while others showed in women positive effects of higher endogenous estrogen levels on verbal memory[32] or cognitive decline.[33] Population-based studies on exogenous estrogens showed that women who used HRT had a reduced risk of AD,[1,2,34] but these studies may have been confounded by healthy-user effect. Also, other studies did not confirm this observation.[35-37] Recent randomized trials with HRT in patients with AD showed no[38-40] beneficial effect of exogenous estrogen on cognitive decline.[41] Together, the biological plausibility of the estrogen hypothesis in dementia is its strongest plea, whereas studies in humans are far from conclusive. Our results are contrary to what we expected in that we observed a small negative effect of higher estradiol levels on both hippocampal volumes in women and memory performance in women and men. As yet, we do not have a biologically plausible explanation for these results. When interpreting these data, one has to keep in mind that the endogenous levels in our study sample of postmenopausal women are much lower than the levels obtained after supplemental use of exogenous estrogens. Therefore, our data do not reject the possibility that exogenous estrogens are related to larger hippocampal volumes and better memory performance.

CONCLUSION

This study does not support the hypothesis that higher endogenous estrogen levels in older women and men without dementia are associated with larger hippocampal volumes and better memory performance.

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