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RESEARCH ARTICLE

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Investigating the synergistic effects of hormone replacement therapy, apolipoprotein E and age on brain health in the UK Biobank

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Abstract

Global prevalence of Alzheimer's Disease has a strong sex bias, with women representing approximately two-thirds of the patients. Yet, the role of sex-specific risk factors during midlife, including hormone replacement therapy (HRT) and their interaction with other major risk factors for Alzheimer's Disease, such as apolipoprotein E (APOE)-e4 genotype and age, on brain health remains unclear. We investigated the relationship between HRT (i.e., use, age of initiation and duration of use) and brain health (i.e., cognition and regional brain volumes). We then consider the multiplicative effects of HRT and APOE status (i.e., e2/e2, e2/e3, e3/e3, e3/e4 and e4/e4) via a two-way interaction and subsequently age of participants via a three-way interaction. Women from the UK Biobank with no self-reported neurological conditions were included ($N = 207{,}595$ women, mean age = 56.25 years, standard deviation = 8.01 years). Generalised linear regression models were computed to quantify the cross-sectional association between HRT and brain health, while controlling for APOE status, age, time since attending centre for completing brain health measure, surgical menopause status, smoking history, body mass index, education, physical activity, alcohol use, ethnicity, socioeconomic status, vascular/heart

problems and diabetes diagnosed by doctor. Analyses of structural brain regions further controlled for scanner site. All brain volumes were normalised for head size. Two-way interactions between HRT and APOE status were modelled, in addition to three-way interactions including age. Results showed that women with the e4/e4 genotype who have used HRT had 1.82% lower hippocampal, 2.4% lower parahippocampal and 1.24% lower thalamus volumes than those with the e3/e3 genotype who had never used HRT. However, this interaction was not detected for measures of cognition. No clinically meaningful three-way interaction between APOE, HRT and age was detected when interpreted relative to the scales of the cognitive measures used and normative models of ageing for brain volumes in this sample. Differences in hippocampal volume between women with the e4/e4 genotype who have used HRT and those with the e3/e3 genotype who had never used HRT are equivalent to approximately 1–2 years of hippocampal atrophy observed in typical health ageing trajectories in midlife (i.e., 0.98%–1.41% per year). Effect sizes were consistent within APOE e4/e4 group post hoc sensitivity analyses, suggesting observed effects were not solely driven by APOE status and may, in part, be attributed to HRT use. Although, the design of this study means we cannot exclude the possibility that women who have used HRT may have a predisposition for poorer brain health.

KEYWORDS
ageing, APOE, cognition, hormone replacement therapy, neuroimaging, UK Biobank

1 | INTRODUCTION

Global prevalence of dementia has a strong sex bias, with women representing approximately two-thirds of the patients with Alzheimer's disease, which is the most common form of dementia (GBD 2019 Collaborators, 2021). In part, this is due to women living on average longer than men and therefore more likely to develop dementia, since age is one of the biggest risk factors (Lin & Beal, 2006; Livingston et al., 2017; Livingston et al., 2020). However, the observed trend remains even after accounting for age-standardised survival rates (GBD 2019 Collaborators, 2021). As such, the aetiology of higher dementia prevalence in women is unclear. Since it is now established that dementia pathophysiology typically starts and remains incipient for years or decades before a clinical diagnosis (Braak & Braak, 1991; Ohm et al., 1995; Zakzanis et al., 2003), the perspective has shifted towards sex-specific risk factors during midlife (de Lange et al., 2021; Ferretti & Santuccione Chadha, 2021). In particular, menopause constitutes a major physiological change in midlife, associated with depleted estrogen levels (Ambikairajah et al., 2022), changes in fat mass and its distribution (Ambikairajah, Walsh, Tabatabaei-Jafari, et al., 2019), and for some individuals, increased risk for dyslipidemia (Ambikairajah, Walsh, & Cherbuin, 2019) and neurodegeneration (Ambikairajah et al., 2020; Ambikairajah et al., 2021). Certain brain regions, including the amygdala and hippocampus, are enriched in estrogen receptors (Barth et al., 2015), with estrogen shown to have neuroprotective properties (Brann et al., 2007). Notably, the hippocampus is one of the first brain regions to be impacted by Alzheimer's disease pathology and experiences the greatest shrinkage over the course of the disease (Braak & Braak, 1991; Planche et al., 2022), with a meta-analysis indicating a 3.33% difference in atrophy rate per year between those with Alzheimer's disease and controls (Barnes et al., 2009). Hence, it is hypothesised that menopausal changes might increase the risk for dementia later on in life (Ambikairajah et al., 2021; Georgakis et al., 2016) and hormone replacement therapy (HRT) may present a viable preventive option for women to reduce their future risk of cognitive decline and dementia (Nelson et al., 2002).

Previous research has indicated that the association between HRT use and brain health may depend on the age of initiation and duration of treatment (Boccardi et al., 2006; Erickson et al., 2005; Erickson et al., 2010; Lord et al., 2008; Nerattini et al., 2023; Resnick et al., 2009; Wnuk et al., 2012). For example, the initiation of HRT at the time of, or immediately after menopause (typically occurring between 46 and 52 years; Schoenaker et al., 2014) has been associated with larger hippocampal volume when compared with women starting HRT 1–18 years after menopause (Erickson et al., 2010). Supporting the hypothesis of a critical window for initiation (Maki, 2013), no association was observed between HRT use and hippocampal volume in women between the ages of 60 and 64 (Low et al., 2006), whereas hippocampal atrophy was associated with HRT use when initiated in women 65 years or older (Resnick et al., 2009). Furthermore, the duration of HRT use may negatively impact hippocampal density, whereby prolonged use can result in decreased posterior hippocampal and parahippocampal grey matter density (Lord et al., 2010). For

dementia, a similar trend for late life HRT initiation (i.e., 65 years and older) has been reported, whereby an ancillary study to the Women's Health Initiative randomised controlled trial (the Women's Health Initiative Memory Study) found a twofold increased risk in developing dementia in those who used HRT (specifically, estrogen and progesterone), relative to placebo (Shumaker et al., 2003). Consistent with this trend, pooled estimates from observational studies indicate HRT use in late life is associated with a 6.8% increase in Alzheimer's disease risk (95% confidence interval [CI]: 2.7%–11.2%) (Nerattini et al., 2023). However, midlife HRT use is associated with a 15.9% risk reduction (95% CI: 24.2%–6.7%) (Nerattini et al., 2023). Taken together, these findings suggest that HRT may have beneficial effects on the brain if treatment is initiated close to the menopause transition, although the exact age thresholds are yet to be established, and that duration of use may have age-dependent implications for brain health.

Another well-known risk factor for dementia is the genetic risk conveyed by apolipoprotein E (APOE)-e4 carriers (Livingston et al., 2017; Livingston et al., 2020). The presence of the e4 allele is associated with increased risk and earlier onset of Alzheimer's disease compared to non-carriers (O'Donoghue et al., 2018). In contrast, the presence of the e2 allele may confer protection against Alzheimer's disease with a lower risk and delayed age of onset compared to e3 homozygotes and e4 carriers (Suri et al., 2013). Meta-analyses indicated that the APOE e2/e3 genotype decreased the risk of Alzheimer's disease, with a 20% greater reduction in odds for women compared to men (women, 49% reduction in odds: 95% CI, 39%–57%; men, 29% reduction in odds; 95% CI, 15%–40%) (Neu et al., 2017). This sex difference was not observed in the e2/e2 genotype, possibly due to insufficient power because of low case numbers. Furthermore, women with the APOE e3/e4 genotype had an increased risk for Alzheimer's disease compared with men between the ages of 65 and 75 years (women, 337% increase in odds: 95% CI, 400%–282%; men, 214% increase in odds; 95% CI, 267%–168%). However, this sex difference was not observed across the lifespan of 55–85 years, nor in the e4/e4 genotype (Neu et al., 2017). The biological mechanisms underpinning this potential sex dimorphism are currently not well understood, but possibly reflect an overlap between unique neuroendocrine processes in women, particularly during the menopausal transition, and the physiological and molecular impact of the APOE-e4 allele, such as effects on neuroinflammation, brain hypometabolism and conduit artery/cerebrovascular function (Kehmeier & Walker, 2021; Mosconi et al., 2021). Moreover, the healthy cell bias of estrogen action hypothesis posits neurons healthy at the time of estrogen exposure exhibit a beneficial response, while in contrast, estrogen exposure to already compromised neurons may exacerbate neurological decline (Brinton, 2008). Therefore, an interaction between APOE, HRT and age may contribute to the observed sex differences (Neu et al., 2017; Riedel et al., 2016).

Evidence relating to a two-way interaction between HRT and APOE for brain health is mixed. In the European Prevention of Alzheimer's Dementia (EPAD) cohort, an interaction between HRT use and APOE status has been detected in some medial temporal-specific regions, including the amygdala and left entorhinal volume, but not the hippocampus (Saleh et al., 2023). Within-group analyses indicated HRT use was associated with better scores on the Repeatable Battery for the Assessment of Neuropsychological Status (RBANS) delayed memory index in APOE-e4 carriers only (Saleh et al., 2023). Although interactive effects remained significant for brain volumes, the interactive effect detected for RBANS delayed memory index did not survive when the model was adjusted for multiple testing (Saleh et al., 2023). In an analysis of UK Biobank data, no interactions between APOE genotype and female-specific factors, including HRT (i.e., use, age of initiation and duration of use), were detected for cognition (Lindseth et al., 2023). A neuroimaging study on the same data set found an interaction between the age of HRT initiation and APOE status on brain age (computed using cortical and subcortical thickness, area and volumes), but this effect did not survive multiple comparisons, although subgroup analyses indicated that earlier age of HRT initiation was protective for APOE-e4 carriers only (de Lange et al., 2020). Notably, APOE-e4 carriers were significantly younger than non-carriers and increasing age was negatively associated with number of carriers (de Lange et al., 2020), which may indicate a potential survival bias in the sample (Heffernan et al., 2016). However, another possibility is that the synergistic (i.e., interactive) effect of HRT and APOE on brain health, depends on age.

In the current study, we used the large UK Biobank data set to investigate the relationship between HRT (i.e., use, age of initiation and duration of use) and brain health (i.e., cognition and regional brain volumes). We then consider the multiplicative effects of HRT and APOE status (i.e., e2/e2, e2/e3, e3/e3, e3/e4 and e4/e4) via a two-way interaction and subsequently age of participants via a three-way interaction. Whilst the hippocampus and amygdala are enriched with estrogen receptors and may therefore be most sensitive for detecting effects related to HRT, in addition to being the most severely affected structures during the entire course of Alzheimer's disease, the selection of brain regions for analyses was also informed by the MRI staging scheme of the structural progression of Alzheimer's disease (Planche et al., 2022). Based on previous studies, we hypothesised that HRT use would be associated with better brain health for women who were carriers of the APOE-e4 allele relative to the most common APOE e3/e3 genotype, and these effects would be more pronounced in women of younger age. Similarly, we hypothesised that the effects of age at initiation and duration of use would depend on APOE status and age.

2 | METHODS

2.1 | Participants

The UK Biobank study is a large population based cohort which consists of 502,359 participants aged 37–73 years at baseline who were recruited from the National Health Service central registers (Sudlow et al., 2015). In the current study, excluded participants were those who did not have at least one completed cognitive measure

($N = 4066$), were males ($N = 229{,}065$), did not have data on e2/e2, e2/e3, e3/e3, e3/e4 or e4/e4 status ($N = 99{,}814$), age ($N = 0$) or responded 'Prefer not to answer', 'Don't know' or NA for ever used HRT ($N = 230{,}640$) or ever had bilateral oophorectomy ($N = 233{,}667$). From the whole sample, we further excluded the following health conditions, including, ever had stroke as diagnosed by doctor ($N = 7666$), or self-reported a neurological condition including brain haemorrhage ($N = 155$), brain abscess ($N = 73$), cerebral aneurysm ($N = 333$), cerebral palsy ($N = 160$), chronic neurological problem ($N = 173$), dementia ($N = 124$), encephalitis ($N = 312$), epilepsy ($N = 4051$), head injury ($N = 1622$), infection of the nervous system ($N = 54$), ischaemic stroke ($N = 17$), meningioma–benign ($N = 117$), meningitis ($N = 1995$), motor neurone disease ($N = 56$), multiple sclerosis ($N = 1777$), neurological injury/trauma ($N = 122$), neuroma (benign) ($N = 148$), other demyelinating condition ($N = 73$), other neurological problem ($N = 2007$), Parkinson's disease ($N = 857$), spina bifida ($N = 72$), subarachnoid haemorrhage ($N = 457$), subdural haematoma ($N = 0$), transient ischaemic attack ($N = 1778$), brain cancer/primary malignant brain tumour ($N = 229$), meningeal cancer/malignant meningioma ($N = 37$), resulting in a final sample of 207,595 participants. Of those, 17,867 had imaging data available. Demographic and health characteristics for included and excluded participants are reported in Table S1. This study follows the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

2.2 | Measures

2.2.1 | Cognition

Visual memory

Participants were asked to memorise the position of as many matching pairs of cards as possible. The cards were presented for 5 seconds, then turned face down on the screen and the participant was asked to touch as many pairs as possible in the fewest tries. Two trials of this task were administered, one with three pairs and one with six pairs of cards. Only the score on the six-pair trial was analysed in the present study, due to the ceiling effect on the three-pair trial. The layout of the cards was purely random and there was no time restriction for the test, that is, the participant was able to match/mismatch the pairs until all were found or the task was abandoned. The number of incorrect matches (UK Biobank data field 399) was used as the measure of visual memory, with a higher score indicating worse performance.

Numeric memory

The participant was shown a two-digit number to remember. Each string was presented on screen for 2000 milliseconds + (the number of digits [i.e., 2] × 500 milliseconds). The number then disappeared and after a wait period of 3000 milliseconds, the participant was asked to enter the remembered number onto the screen. The number became one digit longer each time they remembered correctly (up to a maximum of 12 digits). If the number was 2 digits, the test ends after five successive incorrect answers. If the number was 3 or more digits, the test ends after two successive incorrect answers. The score for analysis was the maximum string length recalled correctly (UK Biobank data field 4282), with higher scores indicating better performance.

Prospective memory

Participants were given the following instruction: 'At the end of the games we will show you four coloured symbols and ask you to touch the blue square. However, to test your memory, we want you to actually touch the orange circle instead'. The participants then completed the pairs matching, fluid intelligence and reaction time tests. A screen then appeared showing a blue square, grey cross, pink star and orange circle, with the instruction to touch the blue square. If the participant touched the orange circle, their response was recorded as 'correct on first attempt'. If they touched the blue square, they were given a prompt on-screen to try to recall what the original instruction was and were asked to respond again. If they correctly selected the orange circle after receiving this prompt, their response was recorded as 'correct on second attempt'. All other responses (including no response) were recorded as incorrect. For the present analyses, data were dichotomised as either 'correct on first attempt' or not (UK Biobank data field 20018).

2.2.2 | Neuroimaging

Image acquisition

A sub-sample of participants were imaged across three imaging centres with identical scanners (3 T Siemens Skyra running VD13A SP4) using a 32-channel head coil (Miller et al., 2016). T1-weighted images were acquired in the sagittal orientation using a 3D magnetization-prepared rapid acquisition gradient echo (MPRAGE) sequence for 5 min; resolution = $1 \times 1 \times 1$ mm^3; field of view = $208 \times 256 \times 256$ matrix (Miller et al., 2016).

Segmentation and image analysis

Images were processed and analysed by the UK Biobank imaging team using the FMRIB Software Library (FSL) v6.0 (http://fsl.fmrib.ox.ac.uk/fsl). More detailed information on the standard MRI analysis protocols have been reported elsewhere (Alfaro-Almagro et al., 2018; Miller et al., 2016). Briefly, the UK Biobank processing pipeline included a linear and non-linear registration to the MNI152 template using FLIRT and FNIRT, respectively. Brain extraction was achieved by using the inverse of the MNI152 alignment warp with a standard-space brain mask transformed into the native space and applied to the image. Automated tissue segmentation was conducted with FAST to segment the brain tissue into grey matter, white matter and cerebrospinal fluid. As part of the segmentation, intensity bias was estimated, which generated a fully bias-field corrected version of the brain-extracted image. The external surface of the skull was then estimated from the T1-weighted image and used to normalise brain tissue volumes for head size, compared with the MNI152 template. Subcortical

structures were derived using FIRST. Brain volumes used in analyses included the hippocampus, amygdala, thalamus and grey matter volumes for the temporal pole, superior temporal gyrus, middle temporal gyrus, inferior temporal gyrus and parahippocampal gyrus. All brain volumes were normalised for head size.

2.2.3 | Genotyping

DNA extraction from stored blood samples and subsequent genotyping was conducted by the UK Biobank genetics team (Bycroft et al., 2018). The genetic data were acquired using two closely related custom arrays: Affymetrix UK Biobank Lung Exome Variant Evaluation (UK BiLEVE) Axiom array or Affymetrix UK Biobank Axiom array, and have undergone quality control procedures as described by the UK Biobank genetics team (Bycroft et al., 2018). APOE genotype was determined based on two single-nucleotide polymorphisms: rs7412 and rs429358.

2.2.4 | Predictors and covariates

Predictors included HRT (i.e., use, age of initiation and duration of use), APOE status (i.e., e3/e3, e2/e2, e2/e3, e3/e4 and e4/e4) (Lyall et al., 2016) and self-reported age. Covariates included time since attending centre for completing brain health measure (i.e. date when brain health measure was completed−date when assessment centre was attended at baseline; Figure 1), self-reported surgical menopause (i.e., ever had bilateral oophorectomy) status, smoking history (i.e., ever or never), body mass index, educational attainment, physical activity (i.e., number of days per week spent doing at least 10 min of continuous vigorous activity), frequency of alcohol intake (i.e., daily or

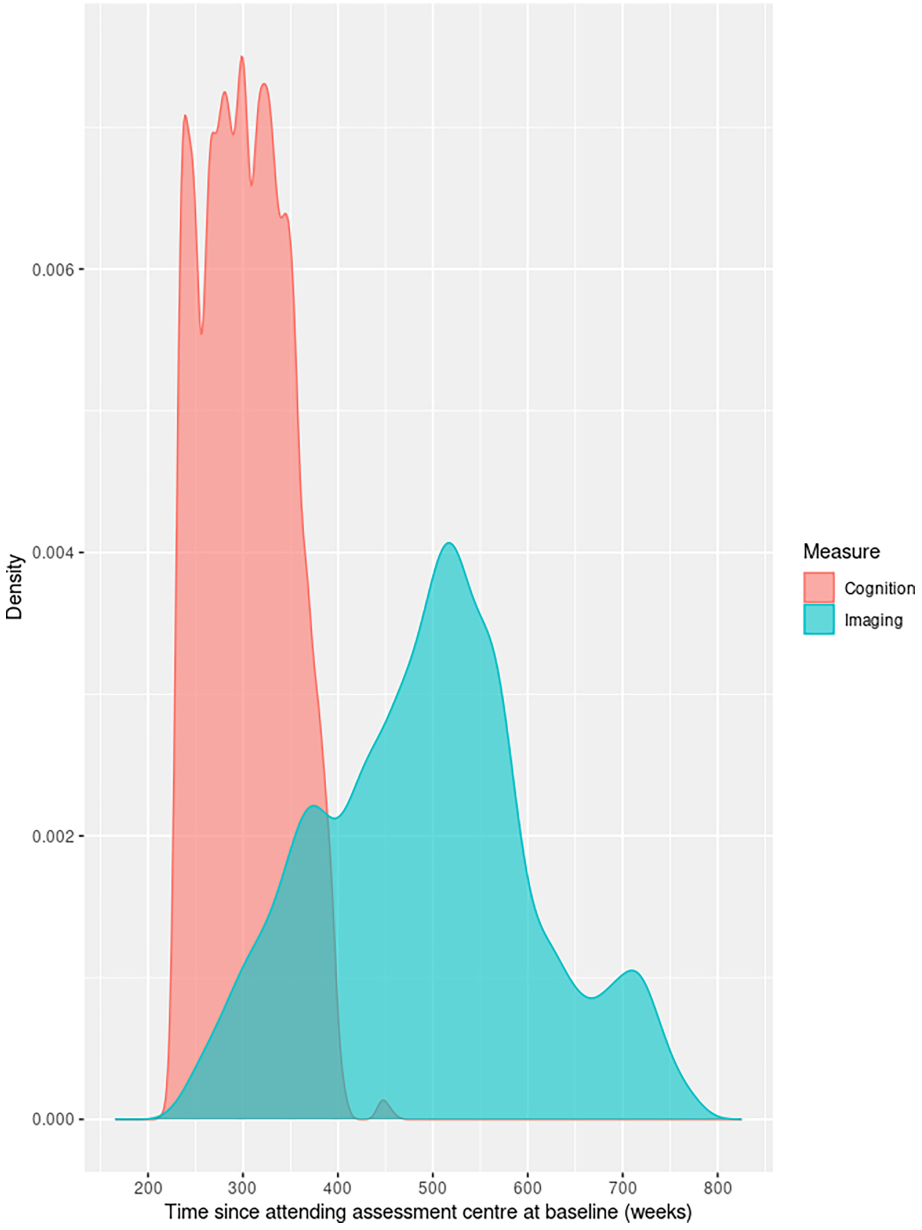

FIGURE 1 Density plot showing the distribution of time since attending centre for completing brain health measure (i.e. date when brain health measure was completed−date when assessment centre was attended at baseline).

almost daily, 3–4 times/week, 1–2 times/week, 1–3 times/month, special occasions only, never or prefer not to answer), ethnicity and socioeconomic status (i.e., Townsend deprivation index). Further covariates included self-reported vascular/heart problems (including heart attack, angina or hypertension) and diabetes, diagnosed by a doctor. Additionally, participants were also classified as hypertensive if they were using blood pressure medication and/or as diabetic if they were using oral anti-diabetic medication or insulin. Analyses of structural brain regions further controlled for scanner site.

2.3 | Statistical methods

All statistical analyses were conducted using R version 4.2.1 (2022-06-23) in Rstudio (version 2023.6.2.561). Log($x + 1$) transformation was applied for visual memory to address skewness.

Generalised linear regression (i.e., multiple linear and logistic regression) models were computed to quantify the association between HRT use and brain health (i.e., cognition and neuroimaging), controlling for APOE status, age and covariates (Model 1). Interactions between HRT use (i.e., ever or never) and APOE status (i.e., e2/e2, e2/e3, e3/e3, e3/e4 and e4/e4) were also tested (Model 2), with HRT (never): APOE (e3/e3) used as the reference category. Three-way interactions for HRT use, APOE status and age were also modelled (Model 3).

For women who had used HRT and not experienced surgical menopause, generalised linear regression models were computed to quantify the association between age-started HRT and duration of HRT use with brain health, respectively. Two-way (HRT*APOE) and three-way (HRT*APOE*Age) interactions were also modelled.

The amount of variance in brain health accounted for by the models (i.e., coefficient of determination: R^2) was reported, in addition to unstandardised beta-estimates, their standard errors, CIs and p values (alpha set to .05). Correction for multiple testing to adjust the p values of analyses was not conducted as it has no impact on the substantive effect size reported nor on the clarity and interpretability of results (Feise, 2002; Hurlbert & Lombardi, 2012). Assumptions of regression models were assessed and met. 'Do not know' and 'Prefer not to answer' responses were treated as missing data. Missing values for covariates (see Table 1), which were not specific to the imaging cohort, were imputed 30 times with 20 iterations by chained equations (Rubin, 1976), which was sufficient for convergence. The 'mice'' package (Version 3.15.0) was utilised with the 'pmm' algorithm for continuous variables, 'logreg' algorithm for dichotomous variables and 'polyreg' algorithm for categorical variables (van Buuren & Groothuis-Oudshoorn, 2011).

3 | RESULTS

The participants' demographic and health characteristics, stratified by HRT use and APOE status, are presented in Table 1 and Table S2, respectively. Differences in brain health measures between APOE genotypes by HRT status are shown in Figure 2, which are further stratified by age in Figure 3.

3.1 | HRT and brain health

Overall, those who have used HRT (i.e., HRT use) performed significantly worse on all cognitive measures than those who have never used HRT (Table 2). Specifically, those who have used HRT have 0.017 (95% CI, 0.01–0.023) more incorrect matches for visual memory, 0.047 fewer digits recalled (95% CI, −0.078 to −0.015) for numeric memory and 6.11% (95% CI, −10.06% to −2.08%) lower odds of correct recall on the first attempt for prospective memory (Table 2). However, HRT use was associated with 98.036 mm^3 larger inferior temporal gyrus volume (95% CI, 12.602–183.469 mm^3) and 57.347 mm^3 larger parahippocampal gyrus volume (95% CI, 17.839–96.855 mm^3) (Table 2).

In women who have used HRT and had not experienced surgical menopause, every 1-year increase in age of HRT initiation was associated with 0.012 more digits recalled (95% CI, 0.006–0.018) for numeric memory and 1.31% (95% CI, 0.6%–2.02%) higher odds of correct recall on the first attempt for prospective memory (Table 2). Similarly, every 1-year increase in age of HRT initiation was associated with 19.023 mm^3 larger temporal pole volume (95% CI, 3.603–34.443 mm^3) and 11.170 mm^3 larger parahippocampal gyrus volume (95% CI, 4.330–18.010 mm^3) (Table 3).

In women who have used HRT and had not experienced surgical menopause, every 1-year increase in the duration of HRT use is associated with 0.010 fewer digits recalled (95% CI, −0.015 to −0.005) and 20.304 mm^3 larger inferior temporal gyrus volume (95% CI, 5.905–34.703 mm^3) (Table 4).

3.2 | APOE and HRT two-way interaction

A two-way interaction between HRT use and APOE status was detected for hippocampal, parahippocampal and thalamus volumes. Specifically, women with the e4/e4 genotype, who have used HRT have 233.912 mm^3 lower hippocampal volumes (95% CI, −454.953 to −12.870 mm^3), 363.162 mm^3 lower parahippocampal volume (95% CI, −595.499 to −130.825 mm^3) and 322.143 lower thalamus volumes (−625.796 to −18.490 mm^3) than those who have never used HRT and have the e3/e3 genotype (Table 2).

In women who have used HRT and had not experienced surgical menopause, a two-way interaction between the age of HRT initiation and APOE status was detected for thalamus volume. Specifically, every 1-year increase in the age of HRT initiation in women with the e3/e4 genotype was associated with 25.895 mm^3 smaller thalamus volume (95% CI, −45.209 to −6.580 mm^3) than those who have the e3/e3 genotype (Table 3).

No two-way interaction was detected between the duration of HRT use and APOE status for all measures of brain health (Table 4).

TABLE 1 Demographic and health characteristics of participants, depending on HRT status.

Characteristics/measures	Never used HRT (*N* = 130,077)	Have used HRT (*N* = 77,518)
Age, years; mean (SD)	53.63 (8.09)	60.65 (5.61)
APOE E2/E2; *N* (%)	753 (0.58%)	459 (0.59%)
APOE E2/E3; *N* (%)	16,149 (12.41%)	9803 (12.65%)
APOE E3/E3; *N* (%)	78,283 (60.18%)	46,714 (60.26%)
APOE E3/E4; *N* (%)	31,678 (24.35%)	18,706 (24.13%)
APOE E4/E4; *N* (%)	3214 (2.47%)	1836 (2.37%)
Duration of HRT use, years; median (Q1, Q3)	-	6 (2, 10)
Age started HRT use, years; mean (SD)	-	47.53 (5.38)
Bilateral oophorectomy, yes; *N* (%)	3149 (2.42%)	13,379 (17.26%)
Education, college/degree; *N* (%)	46,486 (35.74%)	20,396 (26.31%)
Vascular/heart problems, yes; *N* (%)	27,095 (20.83%)	24,827 (32.03%)
Diabetes, yes; *N* (%)	4243 (3.26%)	3085 (3.98%)
Ever smoker, yes; *N* (%)	68,955 (53.01%)	46,542 (60.04%)
Alcohol use, never; *N* (%)	7866 (6.05%)	3802 (4.90%)
Frequency of vigorous physical activity, none; *N* (%)	47,284 (36.35%)	29,939 (38.62%)
Socioeconomic status[a]; median (Q1, Q3)	−2.13 (−3.63, 0.48)	−2.35 (−3.73, 0.05)
Ethnicity, White; *N* (%)	121,028 (93.04%)	75,199 (97.01%)
Body mass index, kg/m^2; median (Q1, Q3)	25.80 (23.15, 29.44)	26.35 (23.77, 29.72)
Visual memory, pairs matching errors; median (Q1, Q3)	3 (2, 5)	4 (2, 6)
Numeric memory, maximum digits correctly recalled; mean (SD)	6.94 (1.45)	6.70 (1.53)
Prospective memory, correct on first attempt; *N* (%)	35,190 (27.05%)	19,069 (24.60%)
Hippocampal volume, mm^3; mean (SD)[b]	10,283.44 (1061.77)	10,015.08 (1055.67)
Amygdala volume, mm^3; mean (SD)[b]	3226.44 (515.92)	3216.77 (500.23)
Thalamus volume, mm^3; mean (SD)[b]	20,342.80 (1567.30)	19,699.60 (1447.04)
Temporal pole grey matter volume, mm^3; mean (SD)[b]	24,702.27 (2649.29)	23,922.53 (2497.66)
Superior temporal gyrus grey matter volume, mm^3; mean (SD)[b]	11,442.45 (1391.68)	10,926.22 (1287.78)
Middle temporal gyrus grey matter volume, mm^3; mean (SD)[b]	29,578.97 (3091.69)	28,520.295 (2798.87)
Inferior temporal gyrus grey matter volume, mm^3; mean (SD)[b]	23,455.03 (2472.31)	22,851.75 (2338.24)
Parahippocampal gyrus grey matter volume, mm^3; mean (SD)[b]	11,803.91 (1151.06)	11,492.19 (1091.27)

Note: Of the overall sample, there were 2145 (1.03%) missing for education, 358 (0.17%) missing for vascular heart status, 403 (0.19%) missing for diabetes, 757 (0.36%) missing for smoking, 227 (0.11%) missing for alcohol use, 11,535 (5.56%) missing for physical activity, 243 (0.12%) missing for socioeconomic status, 614 (0.30%) missing for ethnicity, 599 (0.29%) missing for body mass index, 123 (0.06%) missing for visual memory, 158,764 (76.48%) missing for numeric memory and 136,413 (65.71%) missing for prospective memory. Of those who had used HRT, there were 9895 (12.50%) missing for duration of HRT and 8060 (10.18%) missing for age started HRT.

Abbreviations: APOE, apolipoprotein E; HRT, hormone replacement therapy; N, number; Q, quartile; SD, standard deviation.

[a]Townsend deprivation index.

[b]Brain volumes were normalised by head size.

3.3 | APOE, HRT and age three-way interaction

A three-way interaction between HRT use, APOE status and age was detected for visual memory and numeric memory, such that compared to women with the e3/e3 genotype who have never used HRT, a 1-year increase in age for women with the e3/e4 genotype who have used HRT was associated with 0.002 (95% CI, −0.005 to 0) less incorrect matches for visual memory. Similarly, a 1-year increase in age for women with the e2/e2 genotype who have used HRT was associated with 0.077 more digits recalled (95% CI, 0.015–0.138) for numeric memory, compared to women with the e3/e3 genotype who have never used HRT.

In women who have used HRT and had not experienced surgical menopause, a three-way interaction between the age of HRT initiation, APOE status and age was detected for hippocampal volume and superior temporal gyrus volume, such that compared to women with the e3/e3 genotype, a 1-year increase in age of HRT initiation and age for women with the e2/e2 genotype was associated with 16.638 mm^3 lower hippocampal volume (95% CI, −30.906 to −2.371 mm^3). However, a 1-year increase in the age of HRT initiation

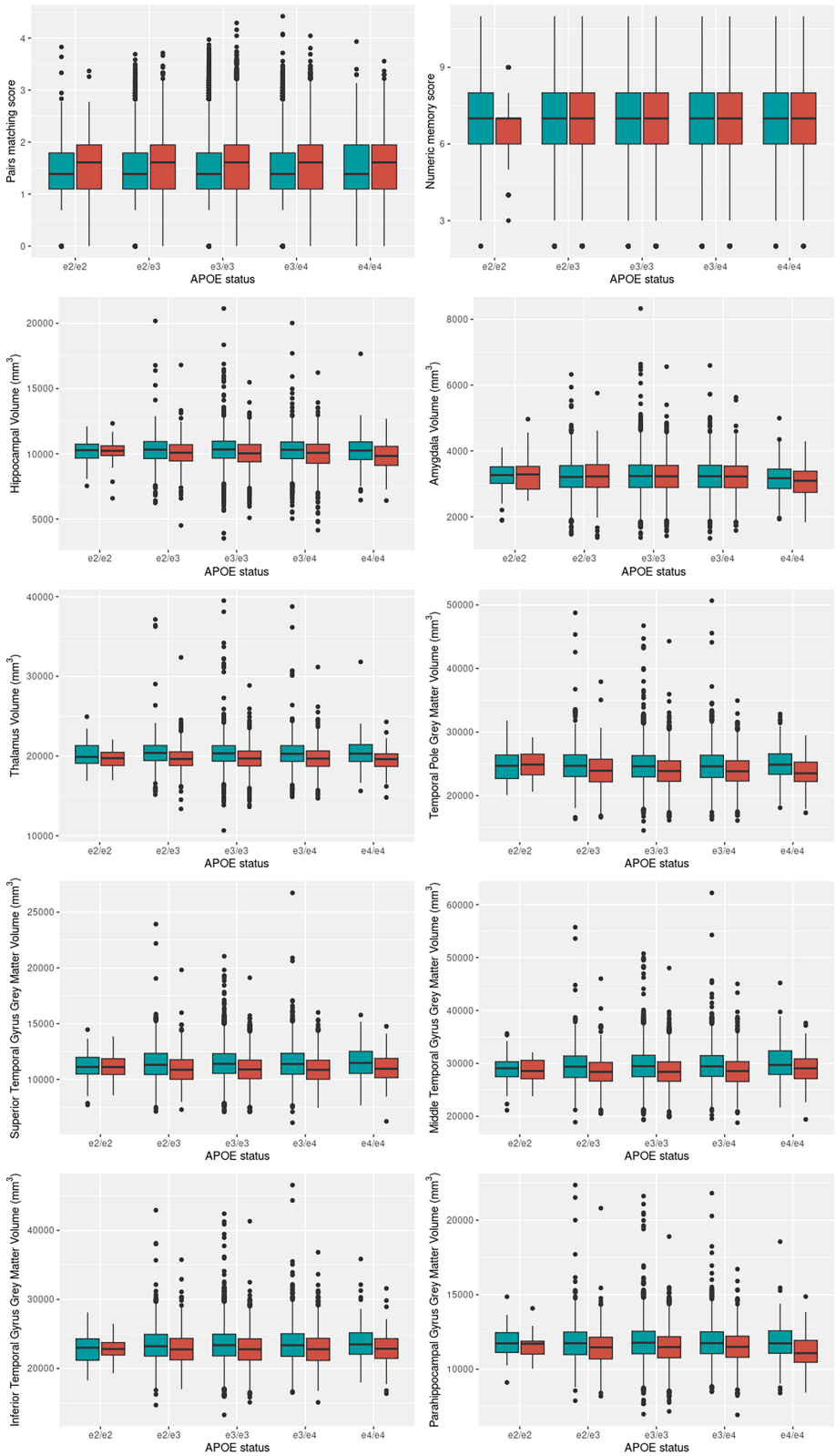

FIGURE 2 Boxplots showing differences in brain health measures between APOE genotypes by hormone replacement therapy status. Teal = women who had never used hormone replacement therapy; Red = women who have used hormone replacement therapy. Log + 1 transformation was applied for visual memory (i.e., pairs matching score). Brain volumes are adjusted for head size.

and age for women with the e3/e4 genotype was associated with 3.155 mm^3 higher superior temporal gyrus volume (95% CI, 0.661–5.650 mm^3).

In women who have used HRT and had not experienced surgical menopause, a three-way interaction between duration of HRT use, APOE status and age was detected for numeric memory and thalamus volume, such that compared to women with the e3/e3 genotype, a 1-year increase in the duration of HRT use and age for women with the e2/e2 genotype was associated with 0.014 fewer digits recalled (95% CI, −0.027 to −0.001) for

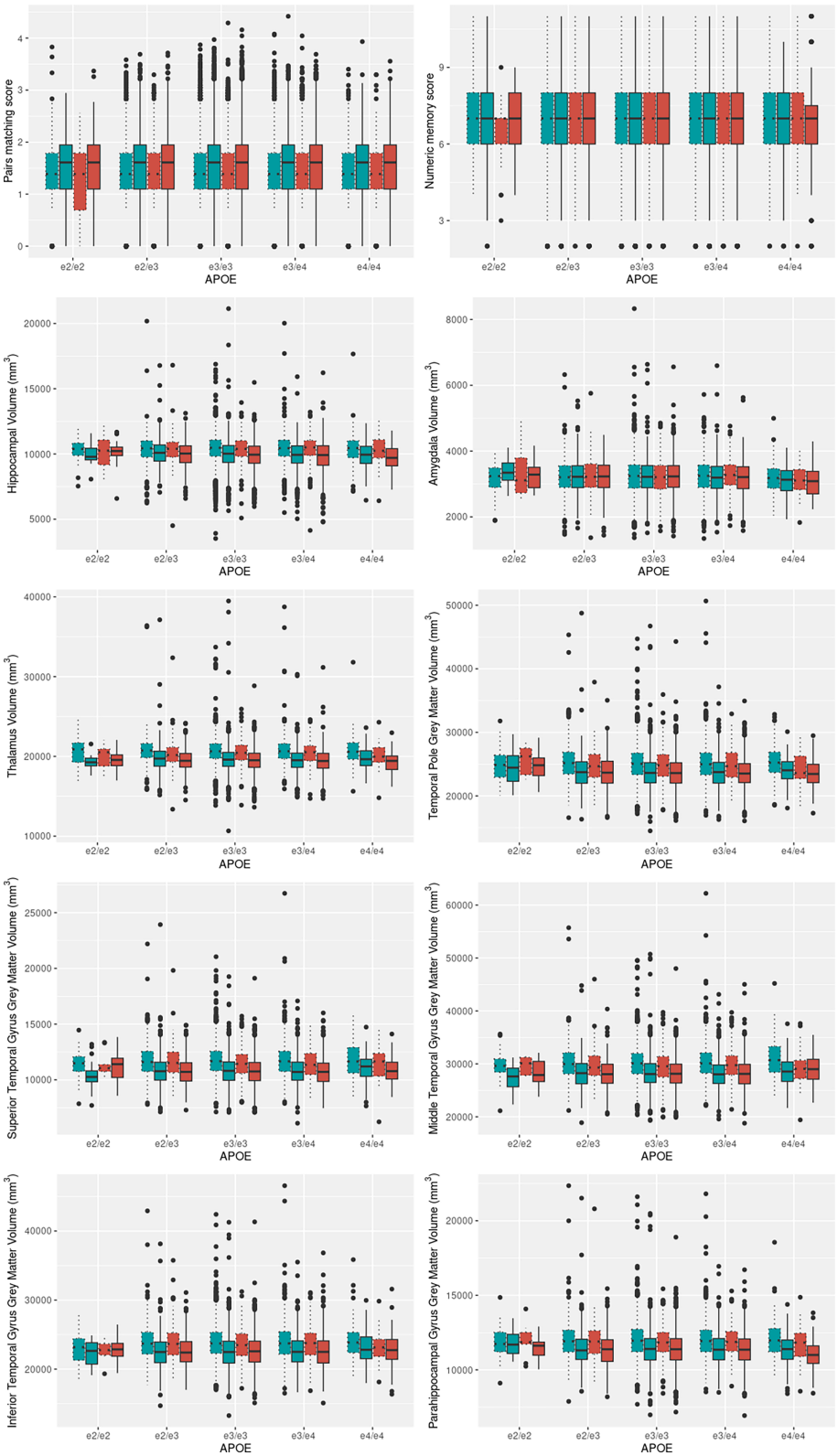

FIGURE 3 Boxplots showing differences in brain health measures between APOE genotypes by hormone replacement therapy status and age. Teal = women who had never used hormone replacement therapy; Red = women who have used hormone replacement therapy; dotted lines = age ≤ 55 years; solid lines = age > 55 years. Log+1 transformation was applied for visual memory (i.e., pairs matching score). Brain volumes are adjusted for head size.

numeric memory. Similarly, a 1-year increase in the duration of HRT use and age for women with the e3/e4 genotype was associated with a 4.446 mm^3 lower thalamus volume (95% CI, −8.214 to −0.679 mm^3), compared to women with the e3/e3 genotype.

4 | DISCUSSION

Women with the e4/e4 genotype who have used HRT showed 1.82% lower hippocampal, 2.4% lower parahippocampal and 1.24% lower thalamus volumes than those with the e3/e3 genotype who had never

TABLE 2 HRT use, APOE status, age and brain health.

Brain health	Predictors	Estimate	SE	95% CI	*p* Value	R^2
Visual memory (Model 1)	Yes—used HRT	0.017	0.003	0.010–0.023	**<.001**	.022
Visual memory (Model 2)	HRT*APOE E2/E2	−0.020	0.038	−0.094 to −0.055	.608	.022
	HRT*APOE E2/E3	−0.001	0.009	−0.019 to −0.017	.910	
	HRT*APOE E3/E4	0.004	0.007	−0.009 to 0.018	.541	
	HRT*APOE E4/E4	−0.007	0.019	−0.044 to 0.030	.715	
Visual memory (Model 3)	HRT*APOE E2/E2*Age	0.010	0.006	−0.002 to 0.021	.101	.022
	HRT*APOE E2/E3*Age	0.000	0.001	−0.003 to 0.003	.972	
	HRT*APOE E3/E4*Age	−0.002	0.001	−0.005 to 0.000	**.036**	
	HRT*APOE E4/E4*Age	0.002	0.003	−0.004 to 0.008	.475	
Numeric memory (Model 1)	Yes—used HRT	−0.047	0.016	−0.078 to −0.015	**.003**	.044
Numeric memory (Model 2)	HRT*APOE E2/E2	−0.014	0.184	−0.375 to 0.347	.939	.044
	HRT*APOE E2/E3	0.067	0.043	−0.016 to 0.151	.114	
	HRT*APOE E3/E4	−0.012	0.033	−0.077 to 0.053	.718	
	HRT*APOE E4/E4	−0.065	0.098	−0.257 to 0.128	.509	
Numeric memory (Model 3)	HRT*APOE E2/E2*Age	0.077	0.031	0.015–0.138	**.015**	.044
	HRT*APOE E2/E3*Age	−0.011	0.007	−0.025 to 0.003	.123	
	HRT*APOE E3/E4*Age	0.002	0.006	−0.009 to 0.013	.730	
	HRT*APOE E4/E4*Age	−0.006	0.017	−0.040 to 0.027	.722	
Prospective memory (Model 1)	Yes—used HRT	−0.063	0.022	−0.106 to −0.021	**.003**	.128[a]
Prospective memory (Model 2)	HRT*APOE E2/E2	−0.196	0.247	−0.679 to 0.288	.428	.128[a]
	HRT*APOE E2/E3	−0.091	0.059	−0.206 to 0.025	0.125	
	HRT*APOE E3/E4	0.032	0.046	−0.058 to 0.122	.480	
	HRT*APOE E4/E4	−0.006	0.125	−0.251 to 0.239	.962	
Prospective memory (Model 3)	HRT*APOE E2/E2*Age	−0.044	0.044	−0.130 to 0.042	.313	.128[a]
	HRT*APOE E2/E3*Age	−0.007	0.010	−0.026 to 0.012	.468	
	HRT*APOE E3/E4*Age	−0.006	0.008	−0.021 to 0.009	.451	
	HRT*APOE E4/E4*Age	−0.005	0.021	−0.045 to 0.035	.801	
Hippocampal volume (Model 1)	Yes—used HRT	11.010	19.176	−26.577 to 48.597	.566	.079
Hippocampal volume (Model 2)	HRT*APOE E2/E2	124.722	228.010	−322.199 to 571.644	.584	.076
	HRT*APOE E2/E3	43.150	50.404	−55.647 to 141.948	.392	
	HRT*APOE E3/E4	−16.179	39.597	−93.792 to 61.435	.683	
	HRT*APOE E4/E4	−233.912	112.771	−454.953 to −12.870	**.038**	
Hippocampal volume (Model 3)	HRT*APOE E2/E2*Age	−8.230	38.487	−83.668 to 67.208	.831	.082
	HRT*APOE E2/E3*Age	−5.890	8.592	−22.731 to 10.591	.493	
	HRT*APOE E3/E4*Age	−7.062	6.732	−20.258 to 6.133	.294	
	HRT*APOE E4/E4*Age	−22.173	20.137	−61.644 to 17.298	.271	
Amygdala volume (Model 1)	Yes—used HRT	9.959	9.510	−8.681 to 28.599	**.295**	.011
Amygdala volume (Model 2)	HRT*APOE E2/E2	80.146	113.082	−141.505 to 301.797	.478	.011
	HRT*APOE E2/E3	21.350	24.998	−27.649 to 70.348	.393	
	HRT*APOE E3/E4	−7.365	19.638	−45.857 to 31.128	.708	
	HRT*APOE E4/E4	−71.839	55.929	−181.465 to 37.788	.199	
Amygdala volume (Model 3)	HRT*APOE E2/E2*Age	−21.489	19.115	−58.957 to 15.978	.261	.012
	HRT*APOE E2/E3*Age	−0.861	4.267	−9.225 to 7.503	.840	
	HRT*APOE E3/E4*Age	−6.533	3.344	−13.087 to 0.020	.051	
	HRT*APOE E4/E4*Age	−6.572	10.001	−26.175 to 13.031	.511	
Thalamus volume (Model 1)	Yes—used HRT	−14.904	26.343	−66.538 to 36.730	**.572**	.184

TABLE 2 (Continued)

Brain health	Predictors	Estimate	SE	95% CI	*p* Value	R^2
Thalamus volume (Model 2)	HRT*APOE E2/E2	−159.500	313.225	−773.452 to 454.453	.611	.185
	HRT*APOE E2/E3	−99.522	69.243	−235.244 to 36.200	.151	
	HRT*APOE E3/E4	−30.524	54.396	−137.146 to 76.097	.575	
	HRT*APOE E4/E4	−322.143	154.917	−625.796 to −18.490	**.038**	
Thalamus volume (Model 3)	HRT*APOE E2/E2*Age	3.017	52.942	−100.753 to 106.788	.955	.185
	HRT*APOE E2/E3*Age	−1.548	11.818	−24.713 to 21.617	.896	
	HRT*APOE E3/E4*Age	−5.612	9.260	−23.763 to 12.539	.544	
	HRT*APOE E4/E4*Age	−37.252	27.699	−91.546 to 17.041	.179	
Temporal pole volume (Model 1)	Yes—used HRT	70.437	45.910	−19.551 to 160.425	**.125**	.127
Temporal pole volume (Model 2)	HRT*APOE E2/E2	751.383	545.934	−318.702 to 1821.467	.169	.128
	HRT*APOE E2/E3	−76.372	120.684	−312.924 to 160.180	.527	
	HRT*APOE E3/E4	−16.227	94.803	−202.050 to 169.596	.864	
	HRT*APOE E4/E4	−507.644	270.016	−1036.901 to 21.613	.060	
Temporal pole volume (Model 3)	HRT*APOE E2/E2*Age	−83.885	92.280	−264.763 to 96.994	.363	.128
	HRT*APOE E2/E3*Age	20.102	20.601	−20.278 to 60.483	.329	
	HRT*APOE E3/E4*Age	1.755	16.142	−29.884 to 33.395	.913	
	HRT*APOE E4/E4*Age	16.274	48.285	−78.369 to 110.917	.736	
Superior temporal gyrus volume (Model 1)	Yes—used HRT	11.160	23.787	−35.465 to 57.785	**.639**	.152
Superior temporal gyrus volume (Model 2)	HRT*APOE E2/E2	424.174	282.887	−130.312 to 978.660	.134	.152
	HRT*APOE E2/E3	26.529	62.533	−96.042 to 149.100	.671	
	HRT*APOE E3/E4	−22.020	49.122	−118.305 to 74.265	.654	
	HRT*APOE E4/E4	−97.729	139.910	−371.966 to 176.508	.485	
Superior temporal gyrus volume (Model 3)	HRT*APOE E2/E2*Age	65.305	47.821	−28.428 to 159.038	.172	.152
	HRT*APOE E2/E3*Age	−13.782	10.675	−34.706 to 7.143	.197	
	HRT*APOE E3/E4*Age	0.987	8.364	−15.408 to 17.381	.906	
	HRT*APOE E4/E4*Age	−8.708	25.020	−57.749 to 40.334	.728	
Middle temporal gyrus volume (Model 1)	Yes—used HRT	39.232	52.574	−63.819 to 142.283	**.456**	.146
Middle temporal gyrus volume (Model 2)	HRT*APOE E2/E2	481.122	625.240	−744.410 to 1706.654	.442	.146
	HRT*APOE E2/E3	7.942	138.215	−262.974 to 278.857	.954	
	HRT*APOE E3/E4	19.867	108.574	−192.948 to 232.682	.855	
	HRT*APOE E4/E4	−368.122	309.239	−974.260 to 238.017	.234	
Middle temporal gyrus volume (Model 3)	HRT*APOE E2/E2*Age	7.195	105.673	−199.934 to 214.325	.946	.147
	HRT*APOE E2/E3*Age	−20.450	23.590	−66.690 to 25.789	.386	
	HRT*APOE E3/E4*Age	−10.211	18.484	−46.441 to 26.019	.581	
	HRT*APOE E4/E4*Age	7.551	55.291	−100.825 to 115.926	.891	
Inferior temporal gyrus volume (Model 1)	Yes—used HRT	98.036	43.587	12.602–183.469	**.025**	.093
Inferior temporal gyrus volume (Model 2)	HRT*APOE E2/E2	347.077	518.354	−668.948 to 1363.101	.503	.093
	HRT*APOE E2/E3	18.009	114.586	−206.591 to 242.610	.875	
	HRT*APOE E3/E4	−51.677	90.012	−228.109 to 124.755	.566	
	HRT*APOE E4/E4	−299.722	256.371	−802.235 to 202.791	.242	
Inferior temporal gyrus volume (Model 3)	HRT*APOE E2/E2*Age	−25.417	87.619	−197.158 to 146.324	.772	.094
	HRT*APOE E2/E3*Age	−29.535	19.560	−67.874 to 8.805	.131	
	HRT*APOE E3/E4*Age	−18.328	15.326	−48.368 to 11.711	.232	
	HRT*APOE E4/E4*Age	13.689	45.844	−76.170 to 103.547	.765	
Parahippocampal gyrus volume (Model 1)	Yes—used HRT	57.347	20.156	17.839–96.855	**.004**	.109

(Continues)

TABLE 2 (Continued)

Brain health	Predictors	Estimate	SE	95% CI	*p* Value	R^2
Parahippocampal gyrus volume (Model 2)	HRT*APOE E2/E2	−66.371	239.661	−536.130 to 403.389	.782	.110
	HRT*APOE E2/E3	−3.748	52.979	−107.592 to 100.096	.944	
	HRT*APOE E3/E4	−6.336	41.618	−87.911 to 75.238	.879	
	HRT*APOE E4/E4	−363.162	118.533	−595.499 to −130.825	**.002**	
Parahippocampal gyrus volume (Model 3)	HRT*APOE E2/E2*Age	−35.472	40.504	−114.863 to 43.919	.381	.110
	HRT*APOE E2/E3*Age	−2.523	9.042	−20.246 to 15.200	.780	
	HRT*APOE E3/E4*Age	−1.693	7.084	−15.579 to 12.193	.811	
	HRT*APOE E4/E4*Age	−26.095	21.191	−67.634 to 15.443	.218	

Note: Model 1 is adjusted for APOE status, age, time since attending centre for completing brain health measure, surgical menopause status, smoking history, body mass index, Townsend deprivation index score, diabetes history, vascular/heart problems, education, physical activity, alcohol use and ethnicity. Model 2 includes an interaction term for HRT use and APOE status. Model 3 includes an additional interaction term for age. Analyses of structural brain regions further controlled for scanner site. All estimates are unstandardised. $p < .05$ considered significant and presented in bold text.
Abbreviations: APOE, apolipoprotein E; CI, confidence interval; HRT, hormone replacement therapy; SE, standard error.
[a]Nagelkerke's R^2_N.

used HRT. However, this interaction was not detected for measures of cognition. Whilst statistically significant three-way interactions between HRT, APOE status and age were detected for some brain health measures, the effect sizes from statistical models were small and not clinically meaningful when interpreted relative to the scales of the cognitive measures used and normative models of ageing for brain volumes in this sample (Nobis et al., 2019). Similarly, results indicated that HRT use and earlier age of initiation were associated with worse cognitive performance, but effect sizes from models were small and not clinically meaningful. For example, in those without surgical menopause, while every 1-year increase in the age of HRT initiation was significantly associated with 0.012 more digits recalled for numeric memory, the scale for this measure ranges from 2 to 12. This would mean it would take approximately an 83-year difference in age of HRT initiation to observe a 1-point difference for numeric memory.

The current findings, which included women aged 39–70 years and rigorously adjusted for relevant covariates, are consistent with previous studies showing no interactions between HRT use and APOE status for cognition (Gleason et al., 2015; Jacobs et al., 1998; Lindseth et al., 2023). Notably, previous studies have extended the age range of participants to the early eighties (Jacobs et al., 1998) and delineated possible effects between never, past and current HRT use (Lindseth et al., 2023) and used other measures of cognition (Gleason et al., 2015; Jacobs et al., 1998; Lindseth et al., 2023). By contrast, other studies have shown HRT*APOE interactions on cognition, such as Yaffe et al. (2000), who reported a significant interaction between estrogen use, APOE-e4 status and cognitive decline. However, this effect was no longer observed in subsequent analyses after the consideration of covariates such as age, education, race and stroke history (Yaffe et al., 2000). In the EPAD cohort, an interaction was detected for the RBANS delayed memory index, although this effect did not survive multiple comparisons (Saleh et al., 2023). Finally, Kang et al. (2004) extended the follow-up period of their initial study by 2 years and suggested women who were carriers of the APOE-e4 allele and currently using HRT had worse rates of cognitive decline compared with those without the APOE-e4 allele; however, this interaction did not meet the statistical threshold for significance (Kang & Grodstein, 2012). It remains to be elucidated whether these different findings are due to differences in cohort characteristics, types of cognitive test used or statistical considerations, including adjustments for covariates. Still, based on our results in a very large cohort, we find no evidence of an interactive effect between APOE and HRT (i.e., use, age of initiation and duration of use) on cognition and this was not found to depend on age.

Available evidence regarding the interaction between HRT and APOE for structural brain measures is limited. In the EPAD cohort, an interaction between HRT use and APOE status was detected in some medial temporal-specific regions, including the amygdala and left entorhinal volume, but not the hippocampus (Saleh et al., 2023). A neuroimaging study on the UK Biobank data set found an interaction between the age of HRT initiation and APOE status on brain age (computed using cortical and subcortical thickness, area and volumes); however, interactive effects sizes were small (de Lange et al., 2020). Specifically, every 1-year increase in the age of HRT initiation was associated with a 0.038-year increase in brain age relative to chronological age. This means it would take a 27-year difference in the age of HRT initiation to observe a 1-year change in the brain age gap (de Lange et al., 2020). Given the age of HRT initiation is normally distributed with a mean of 47.53 years and a standard deviation of 5.38 (see Table 1), 2 standard deviations from the mean, which includes 95% of the sample, is 21.52 years. This is approximately 5.5 years less than the difference in age of HRT initiation required to observe a 1-year change in brain age. While these findings align with our results indicating small effect sizes that are not clinically meaningful, we did observe that women with the e4/e4 genotype who have used HRT have 1.82% lower hippocampal, 2.4% lower parahippocampal and 1.24% lower thalamus volumes than those with the e3/e3 genotype who had never used HRT. Differences in hippocampal volume equate to approximately 1–2 years of hippocampal atrophy observed in

TABLE 3 Age started HRT, APOE status, age and brain health.

Brain health	Predictors	Estimate	SE	95% CI	*p* Value	R^2
Visual memory (Model 1)	Age started HRT	0.000	0.001	−0.001 to 0.001	.737	.011
Visual memory (Model 2)	Age started HRT*APOE E2/E2	0.013	0.007	0.000–0.026	.055	.012
	Age started HRT*APOE E2/E3	0.000	0.002	−0.003 to 0.003	.933	
	Age started HRT*APOE E3/E4	0.000	0.001	−0.003 to 0.002	.915	
	Age started HRT*APOE E4/E4	0.000	0.003	−0.007 to 0.007	.953	
Visual memory (Model 3)	Age started HRT*APOE E2/E2*Age	0.000	0.001	−0.002 to 0.002	.984	.012
	Age started HRT*APOE E2/E3*Age	0.000	0.000	−0.001 to 0.000	.159	
	Age started HRT*APOE E3/E4*Age	0.000	0.000	0.000–0.000	.952	
	Age started HRT*APOE E4/E4*Age	−0.001	0.001	−0.002 to 0.000	.084	
Numeric memory (Model 1)	Age started HRT	0.012	0.003	0.006–0.018	**<.001**	.040
Numeric memory (Model 2)	Age started HRT*APOE E2/E2	−0.020	0.033	−0.085 to 0.046	.558	.040
	Age started HRT*APOE E2/E3	0.008	0.008	−0.008 to 0.024	.341	
	Age started HRT*APOE E3/E4	−0.006	0.007	−0.019 to 0.007	.360	
	Age started HRT*APOE E4/E4	0.000	0.021	−0.041 to 0.041	.997	
Numeric memory (Model 3)	Age started HRT*APOE E2/E2*Age	−0.003	0.005	−0.014 to 0.008	.590	.041
	Age started HRT*APOE E2/E3*Age	0.000	0.001	−0.003 to 0.003	.959	
	Age started HRT*APOE E3/E4*Age	0.001	0.001	−0.001 to 0.003	.469	
	Age started HRT*APOE E4/E4*Age	−0.003	0.003	−0.010 to 0.004	.385	
Prospective memory (Model 1)	Age started HRT	0.013	0.003	0.006–0.020	**<.001**	.102[a]
Prospective memory (Model 2)	Age started HRT*APOE E2/E2	0.041	0.041	−0.040 to 0.121	.322	.102[a]
	Age started HRT*APOE E2/E3	0.004	0.010	−0.016 to 0.023	.707	
	Age started HRT*APOE E3/E4	−0.007	0.008	−0.023 to 0.009	.371	
	Age started HRT*APOE E4/E4	−0.030	0.022	−0.074 to 0.014	.181	
Prospective memory (Model 3)	Age started HRT*APOE E2/E2*Age	0.001	0.007	−0.013 to 0.015	.877	.102[a]
	Age started HRT*APOE E2/E3*Age	0.003	0.002	−0.001 to 0.006	.123	
	Age started HRT*APOE E3/E4*Age	0.000	0.001	−0.003 to 0.002	.831	
	Age started HRT*APOE E4/E4*Age	0.000	0.003	−0.006 to 0.007	.891	
Hippocampal volume (Model 1)	Age started HRT	3.788	3.347	−2.773 to 10.349	.258	.092
Hippocampal volume (Model 2)	Age started HRT*APOE E2/E2	10.746	42.368	−72.317 to 93.808	.800	.092
	Age started HRT*APOE E2/E3	−0.926	9.711	−19.965 to 18.112	.924	
	Age started HRT*APOE E3/E4	−8.459	7.356	−22.880 to 5.962	.250	
	Age started HRT*APOE E4/E4	−31.211	21.178	−72.730 to 10.307	.141	
Hippocampal volume (Model 3)	Age started HRT*APOE E2/E2*Age	−16.638	7.277	−30.906 to −2.371	**.022**	.098
	Age started HRT*APOE E2/E3*Age	−1.825	1.437	−4.643 to 0.993	.204	
	Age started HRT*APOE E3/E4*Age	1.640	1.042	−0.403 to 3.682	.116	
	Age started HRT*APOE E4/E4*Age	3.557	3.342	−2.996 to 10.110	.287	
Amygdala volume (Model 1)	Age started HRT	2.855	1.652	−0.384 to 6.093	.084	.015
Amygdala volume (Model 2)	Age started HRT*APOE E2/E2	−5.356	20.914	−46.357 to 35.645	.798	.016
	Age started HRT*APOE E2/E3	−3.098	4.794	−12.496 to 6.300	.518	
	Age started HRT*APOE E3/E4	−3.808	3.631	−10.926 to 3.311	.294	
	Age started HRT*APOE E4/E4	4.089	10.454	−16.406 to 24.584	.696	

(Continues)

TABLE 3 (Continued)

Brain health	Predictors	Estimate	SE	95% CI	*p* Value	R^2
Amygdala volume (Model 3)	Age started HRT*APOE E2/E2*Age	−4.018	3.599	−11.074 to 3.039	.264	.018
	Age started HRT*APOE E2/E3*Age	0.568	0.711	−0.826 to 1.962	.424	
	Age started HRT*APOE E3/E4*Age	0.352	0.515	−0.658 to 1.363	.494	
	Age started HRT*APOE E4/E4*Age	2.312	1.653	−0.929 to 5.553	.162	
Thalamus volume (Model 1)	Age started HRT	3.937	4.486	−4.857 to 12.731	.380	.139
Thalamus volume (Model 2)	Age started HRT*APOE E2/E2	49.296	56.743	−61.949 to 160.541	.385	.140
	Age started HRT*APOE E2/E3	9.882	13.006	−15.616 to 35.379	.447	
	Age started HRT*APOE E3/E4	−25.895	9.852	−45.209 to −6.580	**.009**	
	Age started HRT*APOE E4/E4	−9.535	28.363	−65.141 to 46.071	.737	
Thalamus volume (Model 3)	Age started HRT*APOE E2/E2*Age	−16.361	9.767	−35.508 to 2.786	.094	.143
	Age started HRT*APOE E2/E3*Age	3.184	1.929	−0.598 to 6.966	.099	
	Age started HRT*APOE E3/E4*Age	0.270	1.398	−2.472 to 3.011	.847	
	Age started HRT*APOE E4/E4*Age	8.043	4.486	−0.752 to 16.837	.073	
Temporal pole volume (Model 1)	Age started HRT	19.023	7.865	3.603–34.443	**.016**	.095
Temporal pole volume (Model 2)	Age started HRT*APOE E2/E2	−12.337	99.583	−207.569 to 182.895	.901	.096
	Age started HRT*APOE E2/E3	15.331	22.826	−29.418 to 60.080	.502	
	Age started HRT*APOE E3/E4	−20.576	17.289	−54.472 to 13.320	.234	
	Age started HRT*APOE E4/E4	−33.117	49.778	−130.707 to 64.473	.506	
Temporal pole volume (Model 3)	Age started HRT*APOE E2/E2*Age	−14.678	17.141	−48.283 to 18.927	.392	.098
	Age started HRT*APOE E2/E3*Age	0.632	3.386	−6.006 to 7.270	.852	
	Age started HRT*APOE E3/E4*Age	−3.985	2.454	−8.796 to 0.826	.104	
	Age started HRT*APOE E4/E4*Age	−3.912	7.873	−19.348 to 11.523	.619	
Superior temporal gyrus volume (Model 1)	Age started HRT	1.563	4.079	−6.434 to 9.561	.702	.094
Superior temporal gyrus volume (Model 2)	Age started HRT*APOE E2/E2	9.699	51.656	−91.573 to 110.971	.851	.094
	Age started HRT*APOE E2/E3	−3.390	11.840	−26.602 to 19.821	.775	
	Age started HRT*APOE E3/E4	3.635	8.968	−13.947 to 21.218	.685	
	Age started HRT*APOE E4/E4	11.918	25.821	−38.703 to 62.539	.644	
Superior temporal gyrus volume (Model 3)	Age started HRT*APOE E2/E2*Age	−12.067	8.887	−29.490 to 5.355	.175	.097
	Age started HRT*APOE E2/E3*Age	2.347	1.755	−1.095 to 5.788	.181	
	Age started HRT*APOE E3/E4*Age	3.155	1.272	0.661–5.650	**.013**	
	Age started HRT*APOE E4/E4*Age	5.421	4.082	−2.581 to 13.423	.184	
Middle temporal gyrus volume (Model 1)	Age started HRT	11.012	8.832	−6.302 to 28.326	.213	.087
Middle temporal gyrus volume (Model 2)	Age started HRT*APOE E2/E2	−10.961	111.807	−230.158 to 208.236	.922	.088
	Age started HRT*APOE E2/E3	30.526	25.628	−19.718 to 80.769	.234	
	Age started HRT*APOE E3/E4	−20.874	19.412	−58.931 to 17.182	.282	
	Age started HRT*APOE E4/E4	1.698	55.889	−107.871 to 111.267	.976	
Middle temporal gyrus volume (Model 3)	Age started HRT*APOE E2/E2*Age	26.778	19.249	−10.960 to 64.517	.164	.089
	Age started HRT*APOE E2/E3*Age	7.048	3.802	−0.406 to 14.502	.064	
	Age started HRT*APOE E3/E4*Age	0.901	2.756	−4.502 to 6.304	.744	
	Age started HRT*APOE E4/E4*Age	3.607	8.842	−13.727 to 20.941	.683	
Inferior temporal gyrus volume (Model 1)	Age started HRT	−0.717	7.568	−15.555 to 14.121	.925	.058

TABLE 3 (Continued)

Brain health	Predictors	Estimate	SE	95% CI	*p* Value	R^2
Inferior temporal gyrus volume (Model 2)	Age started HRT*APOE E2/E2	52.358	95.833	−135.521 to 240.238	.585	.058
	Age started HRT*APOE E2/E3	13.555	21.965	−29.508 to 56.618	.537	
	Age started HRT*APOE E3/E4	−8.377	16.638	−40.997 to 24.243	.615	
	Age started HRT*APOE E4/E4	25.614	47.902	−68.298 to 119.526	.593	
Inferior temporal gyrus volume (Model 3)	Age started HRT*APOE E2/E2*Age	0.080	16.493	−32.255 to 32.414	.996	.061
	Age started HRT*APOE E2/E3*Age	1.091	3.258	−5.296 to 7.478	.738	
	Age started HRT*APOE E3/E4*Age	−3.734	2.361	−8.364 to 0.895	.114	
	Age started HRT*APOE E4/E4*Age	−10.793	7.576	−25.645 to 4.059	.154	
Parahippocampal gyrus volume (Model 1)	Age started HRT	11.170	3.489	4.330–18.010	**.001**	.096
Parahippocampal gyrus volume (Model 2)	Age started HRT*APOE E2/E2	8.984	44.180	−77.631 to 95.600	.839	.096
	Age started HRT*APOE E2/E3	2.404	10.125	−17.447 to 22.254	.812	
	Age started HRT*APOE E3/E4	−7.790	7.670	−22.827 to 7.247	.310	
	Age started HRT*APOE E4/E4	−14.421	22.082	−57.713 to 28.870	.514	
Parahippocampal gyrus volume (Model 3)	Age started HRT*APOE E2/E2*Age	−10.053	7.599	−24.951 to 4.845	.186	.100
	Age started HRT*APOE E2/E3*Age	−0.142	1.501	−3.084 to 2.800	.925	
	Age started HRT*APOE E3/E4*Age	0.333	1.088	−1.799 to 2.466	.759	
	Age started HRT*APOE E4/E4*Age	2.446	3.490	−4.396 to 9.288	.483	

Note: Model 1 is adjusted for APOE status, age, time since attending centre for completing brain health measure, smoking history, body mass index, Townsend deprivation index score, diabetes history, vascular/heart problems, education, physical activity, alcohol use and ethnicity. Model 2 includes an interaction term for age-started HRT and APOE status. Model 3 includes an additional interaction term for age. Analyses of structural brain regions further controlled for scanner site. All estimates are unstandardised. $p < .05$ considered significant and presented in bold text.
Abbreviations: APOE, apolipoprotein E; CI, confidence interval; HRT, hormone replacement therapy; SE, standard error.
[a]Nagelkerke's R^2_N.

typical health ageing trajectories in midlife (i.e., 0.98%–1.41% per year) (Barnes et al., 2009; Fraser et al., 2015). However, this interactive effect was not found to depend on age. These findings may, in part, be explained by the healthy cell bias of estrogen action, which posits neurons healthy at the time of estrogen exposure exhibit a beneficial response, while in contrast, estrogen exposure to already compromised neurons may exacerbate neurological decline (Brinton, 2008). Given the presence of the e4 allele is associated with increased risk and earlier onset of Alzheimer's disease compared to non-carriers (O'Donoghue et al., 2018), it is possible that the neurons in women with the e4 allele were already compromised and the use of HRT contributed to the lower brain volumes observed. Although, since women who have never used HRT with the e3/e3 genotype were used as the reference category, it is possible that the poorer brain health observed in women with the e4/e4 status who have used HRT is driven by the well-documented negative effects of APOE e4 carrier status on brain health (Liu et al., 2015), instead of HRT use. To assess this, post hoc sensitivity analyses were conducted to test within APOE e4/e4 group effects. Analyses in women with APOE e4/e4 status revealed HRT use was associated with lower hippocampal, parahippocampal and thalamus volumes compared with those who have never used HRT (Table 5). Effect sizes were consistent with those reported in our main findings and were statistically significant for parahippocampal and thalamus volumes, but not the hippocampus. These findings suggest that observed effects were not solely driven by APOE status and may, in part, be attributed to HRT use. However, the design of this study means we cannot exclude the possibility that women who have used HRT may have a predisposition for poorer brain health. For example, women who experience symptoms of neurological decline may be more likely to seek treatment and be prescribed HRT. Future studies should investigate this possibility, in addition to whether the findings from this study are consistent longitudinally, particularly when considering other factors relating to HRT use, including composition (i.e., estrogen vs estrogen and progesterone), mode of administration (i.e., oral or transdermal) and dosage.

4.1 | Limitations

The cross-sectional nature of this study limited causal inferences. Despite robust exclusion criteria and comprehensive set of covariates, the presence of confounders is still possible. HRT use was obtained by self-report and therefore, may not be accurate. The UK Biobank is also limited in its representativeness of the general population, given the cohort consists of relatively healthy participants who are predominantly white (94.6%) (Fry et al., 2017). The cognitive tests used in the Biobank might not be as sensitive and/or specific as classical neuropsychological tests, which may help explain why a two-way

TABLE 4 Duration of HRT use, APOE status, age and brain health.

Brain health	Predictors	Estimate	SE	95% CI	*p* Value	R^2
Visual memory (Model 1)	Duration of HRT	0.000	0.001	−0.001 to 0.002	.351	.012
Visual memory (Model 2)	Duration of HRT use*APOE E2/E2	0.004	0.007	−0.010 to 0.018	.588	.012
	Duration of HRT use*APOE E2/E3	−0.001	0.002	−0.004 to 0.002	.430	
	Duration of HRT use*APOE E3/E4	−0.001	0.001	−0.003 to 0.002	.539	
	Duration of HRT use*APOE E4/E4	0.004	0.003	−0.002 to 0.010	.227	
Visual memory (Model 3)	Duration of HRT use*APOE E2/E2*Age	0.001	0.002	−0.003 to 0.004	.741	.012
	Duration of HRT use*APOE E2/E3*Age	0.000	0.000	0.000–0.001	.376	
	Duration of HRT use*APOE E3/E4*Age	0.000	0.000	−0.001 to 0.000	.312	
	Duration of HRT use*APOE E4/E4*Age	0.000	0.001	−0.001 to 0.001	.778	
Numeric memory (Model 1)	Duration of HRT use	−0.010	0.003	−0.015 to −0.005	**<.001**	.039
Numeric memory (Model 2)	Duration of HRT use*APOE E2/E2	0.062	0.039	−0.015 to 0.139	.116	.040
	Duration of HRT use*APOE E2/E3	−0.004	0.008	−0.020 to 0.012	.622	
	Duration of HRT use*APOE E3/E4	−0.008	0.006	−0.021 to 0.005	.217	
	Duration of HRT use*APOE E4/E4	−0.026	0.020	−0.066 to 0.013	.195	
Numeric memory (Model 3)	Duration of HRT use*APOE E2/E2*Age	−0.014	0.007	−0.027 to −0.001	**.039**	.041
	Duration of HRT use*APOE E2/E3*Age	0.000	0.002	−0.003 to 0.003	.929	
	Duration of HRT use*APOE E3/E4*Age	0.000	0.001	−0.002 to 0.003	.832	
	Duration of HRT use*APOE E4/E4*Age	0.003	0.004	−0.004 to 0.010	.399	
Prospective memory (Model 1)	Duration of HRT use	0.005	0.003	−0.001 to 0.012	.105	.100[a]
Prospective memory (Model 2)	Duration of HRT use*APOE E2/E2	−0.019	0.052	−0.121 to 0.083	.713	.100[a]
	Duration of HRT use*APOE E2/E3	−0.019	0.010	−0.038 to 0.000	.053	
	Duration of HRT use*APOE E3/E4	−0.002	0.008	−0.017 to 0.014	.818	
	Duration of HRT use*APOE E4/E4	0.023	0.022	−0.021 to 0.067	.299	
Prospective memory (Model 3)	Duration of HRT use*APOE E2/E2*Age	−0.012	0.014	−0.039 to 0.014	.359	.100[a]
	Duration of HRT use*APOE E2/E3*Age	0.002	0.002	−0.002 to 0.006	.448	
	Duration of HRT use*APOE E3/E4*Age	0.001	0.002	−0.002 to 0.004	.584	
	Duration of HRT use*APOE E4/E4*Age	0.002	0.004	−0.006 to 0.011	.592	
Hippocampal volume (Model 1)	Duration of HRT use	−1.665	3.239	−8.014 to 4.684	.607	.092
Hippocampal volume (Model 2)	Duration of HRT use*APOE E2/E2	−0.092	52.971	−103.941 to 103.758	.999	.092
	Duration of HRT use*APOE E2/E3	−2.338	9.531	−21.024 to 16.348	.806	
	Duration of HRT use*APOE E3/E4	−8.754	7.542	−23.540 to 6.031	.246	
	Duration of HRT use*APOE E4/E4	2.798	25.788	−47.760 to 53.356	.914	
Hippocampal volume (Model 3)	Duration of HRT use*APOE E2/E2*Age	13.189	7.654	−1.817 to 28.194	.085	.095
	Duration of HRT use*APOE E2/E3*Age	0.536	1.742	−2.880 to 3.952	.759	
	Duration of HRT use*APOE E3/E4*Age	−2.552	1.431	−5.358 to 0.254	.075	
	Duration of HRT use*APOE E4/E4*Age	−3.431	5.904	−15.005 to 8.143	.561	
Amygdala volume (Model 1)	Duration of HRT use	−0.359	1.597	−3.490 to 2.772	.822	.016
Amygdala volume (Model 2)	Duration of HRT use*APOE E2/E2	−27.297	26.116	−78.498 to 23.903	.296	.016
	Duration of HRT use*APOE E2/E3	2.234	4.699	−6.979 to 11.447	.635	
	Duration of HRT use*APOE E3/E4	−0.375	3.718	−7.665 to 6.915	.920	
	Duration of HRT use*APOE E4/E4	−12.343	12.715	−37.271 to 12.585	.332	

TABLE 4 (Continued)

Brain health	Predictors	Estimate	SE	95% CI	*p* Value	R^2
Amygdala volume (Model 3)	Duration of HRT use*APOE E2/E2*Age	6.506	3.775	−0.895 to 13.907	.085	.019
	Duration of HRT use*APOE E2/E3*Age	−0.906	0.859	−2.591 to 0.779	.292	
	Duration of HRT use*APOE E3/E4*Age	−0.788	0.706	−2.172 to 0.596	.264	
	Duration of HRT use*APOE E4/E4*Age	1.546	2.912	−4.163 to 7.255	.596	
Thalamus volume (Model 1)	Duration of HRT use	−0.580	4.348	−9.103 to 7.944	.894	.139
Thalamus volume (Model 2)	Duration of HRT use*APOE E2/E2	−59.526	71.097	−198.912 to 79.861	.403	.140
	Duration of HRT use*APOE E2/E3	−2.835	12.792	−27.913 to 22.244	.825	
	Duration of HRT use*APOE E3/E4	12.522	10.123	−7.324 to 32.367	.216	
	Duration of HRT use*APOE E4/E4	−20.031	34.613	−87.889 to 47.827	.563	
Thalamus volume (Model 3)	Duration of HRT use*APOE E2/E2*Age	5.864	10.279	−14.288 to 26.016	.568	.142
	Duration of HRT use*APOE E2/E3*Age	2.044	2.340	−2.543 to 6.631	.382	
	Duration of HRT use*APOE E3/E4*Age	−4.446	1.922	−8.214 to −0.679	**.021**	
	Duration of HRT use*APOE E4/E4*Age	3.096	7.928	−12.446 to 18.639	.696	
Temporal pole volume (Model 1)	Duration of HRT use	10.083	7.621	−4.858 to 25.024	.186	.096
Temporal pole volume (Model 2)	Duration of HRT use*APOE E2/E2	−79.341	124.620	−323.658 to 164.976	.524	.097
	Duration of HRT use*APOE E2/E3	30.349	22.425	−13.616 to 74.314	.176	
	Duration of HRT use*APOE E3/E4	9.380	17.744	−25.408 to 44.168	.597	
	Duration of HRT use*APOE E4/E4	51.374	60.677	−67.584 to 170.332	.397	
Temporal pole volume (Model 3)	Duration of HRT use*APOE E2/E2*Age	6.595	18.030	−28.753 to 41.944	.715	.098
	Duration of HRT use*APOE E2/E3*Age	−0.063	4.105	−8.111 to 7.985	.988	
	Duration of HRT use*APOE E3/E4*Age	0.427	3.371	−6.182 to 7.037	.899	
	Duration of HRT use*APOE E4/E4*Age	−15.730	13.909	−42.999 to 11.538	.258	
Superior temporal gyrus volume (Model 1)	Duration of HRT use	1.170	3.952	−6.578 to 8.917	.767	.093
Superior temporal gyrus volume (Model 2)	Duration of HRT use*APOE E2/E2	−73.075	64.623	−199.769 to 53.619	.258	.093
	Duration of HRT use*APOE E2/E3	0.063	11.629	−22.735 to 22.861	.996	
	Duration of HRT use*APOE E3/E4	9.420	9.201	−8.618 to 27.458	.306	
	Duration of HRT use*APOE E4/E4	10.956	31.463	−50.728 to 72.640	.728	
Superior temporal gyrus volume (Model 3)	Duration of HRT use*APOE E2/E2*Age	−6.315	9.348	−24.642 to 12.012	.499	.095
	Duration of HRT use*APOE E2/E3*Age	1.908	2.128	−2.264 to 6.080	.370	
	Duration of HRT use*APOE E3/E4*Age	−1.403	1.748	−4.829 to 2.024	.422	
	Duration of HRT use*APOE E4/E4*Age	3.710	7.211	−10.427 to 17.846	.607	
Middle temporal gyrus volume (Model 1)	Duration of HRT use	2.530	8.578	−14.286 to 19.347	.768	.085
Middle temporal gyrus volume (Model 2)	Duration of HRT use*APOE E2/E2	−41.367	140.262	−316.350 to 233.616	.768	.086
	Duration of HRT use*APOE E2/E3	−34.468	25.240	−83.951 to 15.015	.172	
	Duration of HRT use*APOE E3/E4	7.782	19.971	−31.371 to 46.935	.697	
	Duration of HRT use*APOE E4/E4	24.630	68.293	−109.259 to 158.518	.718	
Middle temporal gyrus volume (Model 3)	Duration of HRT use*APOE E2/E2*Age	−9.602	20.291	−49.383 to 30.180	.636	.087
	Duration of HRT use*APOE E2/E3*Age	−8.138	4.620	−17.195 to 0.920	.078	
	Duration of HRT use*APOE E3/E4*Age	−4.226	3.794	−11.665 to 3.213	.265	
	Duration of HRT use*APOE E4/E4*Age	−7.285	15.653	−37.973 to 23.403	.642	
Inferior temporal gyrus volume (Model 1)	Duration of HRT use	20.304	7.345	5.905–34.703	**.006**	.060

(Continues)

TABLE 4 (Continued)

Brain health	Predictors	Estimate	SE	95% CI	*p* Value	R^2
Inferior temporal gyrus volume (Model 2)	Duration of HRT use*APOE E2/E2	−4.115	120.098	−239.567 to 231.338	.973	.060
	Duration of HRT use*APOE E2/E3	−30.472	21.611	−72.842 to 11.897	.159	
	Duration of HRT use*APOE E3/E4	7.665	17.100	−25.860 to 41.190	.654	
	Duration of HRT use*APOE E4/E4	13.811	58.474	−100.827 to 128.449	.813	
Inferior temporal gyrus volume (Model 3)	Duration of HRT use*APOE E2/E2*Age	−20.181	17.367	−54.230 to 13.867	.245	.062
	Duration of HRT use*APOE E2/E3*Age	−6.211	3.954	−13.963 to 1.541	.116	
	Duration of HRT use*APOE E3/E4*Age	−1.479	3.247	−7.845 to 4.887	.649	
	Duration of HRT use*APOE E4/E4*Age	−21.648	13.397	−47.913 to 4.617	.106	
Parahippocampal gyrus volume (Model 1)	Duration of HRT use	−1.149	3.378	−7.772 to 5.474	.734	.095
Parahippocampal gyrus volume (Model 2)	Duration of HRT use*APOE E2/E2	−72.083	55.254	−180.408 to 36.242	.192	.096
	Duration of HRT use*APOE E2/E3	2.835	9.941	−16.655 to 22.324	.776	
	Duration of HRT use*APOE E3/E4	4.124	7.867	−11.298 to 19.547	.600	
	Duration of HRT use*APOE E4/E4	−2.021	26.899	−54.756 to 50.713	.940	
Parahippocampal gyrus volume (Model 3)	Duration of HRT use*APOE E2/E2*Age	8.310	7.993	−7.360 to 23.979	.299	.097
	Duration of HRT use*APOE E2/E3*Age	2.350	1.819	−1.217 to 5.917	.197	
	Duration of HRT use*APOE E3/E4*Age	−1.194	1.494	−4.123 to 1.736	.424	
	Duration of HRT use*APOE E4/E4*Age	1.842	6.165	−10.244 to 13.928	.765	

Note: Model 1 is adjusted for APOE status, age, time since attending centre for completing brain health measure, smoking history, body mass index, Townsend deprivation index score, diabetes history, vascular/heart problems, education, physical activity, alcohol use and ethnicity. Model 2 includes an interaction term for duration of HRT use and APOE status. Model 3 includes an additional interaction term for age. Analyses of structural brain regions further controlled for scanner site. All estimates are unstandardised. $p < .05$ considered significant and presented in bold text.
Abbreviations: APOE, apolipoprotein E; CI, confidence interval; HRT, hormone replacement therapy; SE, standard error.
[a]Nagelkerke's R^2_N.

TABLE 5 HRT use and brain health, within APOE E4/E4 group analyses.

APOE status and brain health	Predictors	Estimate	SE	95% CI	*p* Value	R^2
E4/E4						
Hippocampal volume	Yes—used HRT	−238.302	140.370	−514.292 to 37.688	.090	.157
Thalamus volume	Yes—used HRT	−410.113	191.112	−785.871 to −34.356	**.033**	.225
Parahippocampal gyrus volume	Yes—used HRT	−314.154	148.105	−605.353 to −22.956	**.035**	.215

Note: Model is adjusted for age, time since attending centre for completing brain health measure, surgical menopause status, smoking history, body mass index, Townsend deprivation index score, diabetes history, vascular/heart problems, education, physical activity, alcohol use, ethnicity and scanner site. All estimates are unstandardised. $p < .05$ considered significant and presented in bold text.
Abbreviations: APOE, apolipoprotein E; CI, confidence interval; HRT, hormone replacement therapy; SE, standard error.

interaction between APOE and HRT use was observed for some structural but not functional measures of brain health.

5 | CONCLUSION

Women with the APOE e4/e4 genotype who have used HRT showed 1.82% lower hippocampal, 2.4% lower parahippocampal and 1.24% lower thalamus volumes than those who have the e3/e3 genotype and had never used HRT. However, this interaction was not detected for measures of cognition. No clinically meaningful three-way interaction between APOE, HRT and age was detected when interpreted relative to the scales of the cognitive measures used and normative models of ageing for brain volumes in this sample. Differences in hippocampal volume between women with the e4/e4 genotype who have used HRT and those with the e3/e3 genotype who had never used HRT are equivalent to approximately 1–2 years of hippocampal atrophy observed in typical health ageing trajectories in midlife (i.e., 0.98%–1.41% per year). Effect sizes were consistent within APOE e4/e4 group post hoc sensitivity analyses, suggesting observed effects were not solely driven by APOE status and may, in part, be attributed to HRT use. Although, the design of this study means we cannot exclude the possibility that women who have used HRT may have a predisposition for poorer brain health.

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CONFLICT OF INTEREST STATEMENT

The authors declare no conflicts of interest.

DATA AVAILABILITY STATEMENT

This research has been conducted using the UK Biobank resource under application number 52825. Researchers can apply to use the UK Biobank resource and access the data used. No additional data are available.

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