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COMMENTARY

THE NATURE OF THE EFFECT OF FEMALE GONADAL HORMONE REPLACEMENT THERAPY ON COGNITIVE FUNCTION IN POST-MENOPAUSAL WOMEN: A META-ANALYSIS

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Abstract—We reviewed epidemiological and experimental studies of female gonadal hormone replacement therapy (HRT) on cognitive function in post-menopausal women and carried out meta-analyses. In healthy ageing women, HRT has small and inconsistent effects that include enhancement of verbal memory, abstract reasoning and information processing. Epidemiological studies show larger effects than experimental studies, which is not related to sample size. Important confounds may be that women who start using HRT are healthier than women who do not. Also, controlling for socio-economic status diminishes the effect of HRT. The effects of HRT may depend on the age and type of menopause and the therapeutic intervention used, with the most widely used drug, Premarin, having least effect. However, the effects are independent of mood and climacteric symptom alleviation. There is a paucity of experimental studies that include healthy elderly women. The evidence for an estrogen deficiency in women with dementia and cognitive dysfunction is inconsistent. Nevertheless, epidemiological studies suggest that HRT protects against the development of clinically diagnosed Alzheimer's disease. However, poor recall of HRT use by patients and altered physician behaviour may have confounded the effects. Surprisingly, both healthy and demented women with low education seem to benefit most from HRT. Three recent controlled experimental studies using Premarin showed no effects of HRT in preventing further cognitive decline in women who already have Alzheimer's disease. Duration of treatment seems to play an important role, with beneficial effects declining—and even reversing—with longer treatment in women with Alzheimer's disease.

Future research should further investigate the cognitive effect of different HRT preparations, serum estrogen levels, and the interactions of HRT with age, menopausal status and existing protective (e.g. education) and risk factors (e.g. smoking and apolipoprotein E genotype) for cognitive decline and Alzheimer's disease.

Key words: hormone replacement therapy, estrogens, progestagens, cognition, Alzheimer's disease, meta-analyses.

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Abbreviations: AD, Alzheimer's disease; ApoE, apolipoprotein E; ARIC, Atherosclerotic Risk in Communities; BIMC, Blessed Information Memory and Concentration test; BSRT, Buschke's Selective Reminding Test; BVRT, Benton Visual Retention Test; CEE, conjugated equine estrogen; C.I., confidence interval; CVLT, Californian Verbal Learning Test; DSST, Digit Symbol Substitution Test; E1, estrone; E2, estradiol; HRT, female gonadal hormone replacement therapy; MANOVA, multivariate analysis of variance; mMMSE, modified Mini-Mental Status Examination; MMSE, Mini-Mental Status Examination; O.R., Odds Ratio; RBC, the Rancho Bernardo Cohort; SOF, Study of Osteoporotic Fractures; TMT, Trail Making Test; VaD, vascular dementia; WHIMS, the Women's Health Initiative Study; WISDOM, Women's International Study of long Duration Oestrogen after the Menopause; WMD, Weighted Mean Difference; WMS, Wechsler Memory Scale.

1. INTRODUCTION

The attitude towards hormone replacement therapy (HRT) and its effect on cognitive function in elderly women has seen a turbulent change over the last decade. While the early nineties showed a growing enthusiasm[68,100,181] for the potential of HRT to treat Alzheimer's disease (AD) and cognitive ageing, the tide seems to be turning.[12,202] We aim to review critically the work that led to this initial enthusiasm and the reasons for its current decline.

Nearly all cognitive functions, such as memory and information processing, decline with age. However, large inter-individual variability exists, from little decline to dementia. The determinants of this variability are uncertain,[82,145] but women have a higher risk of developing AD than men[40,96] and their age-specific prevalence of AD is twice as high.[153] After the menopause, women have much lower levels of gonadal hormones than men.[106] This could suggest that maintaining high levels of hormones in women with HRT may protect against AD.[100,181] HRT usually consists of an estrogen alone or combined with a progestagen to reduce the risk of endometrial hyperplasia.[28] Estrogens have a potential role in maintaining brain structures and protecting functional systems affected in AD.[26,56,101,103,104,115,169,183,196] In fact, "there are almost innumerable (biological) reasons why estrogens should be protective against both benign memory loss and senile dementia".[12]

Despite estrogen's multiple biologically plausible protective mechanisms, the initial enthusiasm seems to have outrun the actual data. Recent reviews[124,202] stated that the human studies had substantial methodological problems and had produced conflicting results. This year, two short-[67,187] and one long-term[127] well-designed controlled studies showed that the most widely used HRT, Premarin, did not protect against the progression of AD nor treat its symptoms. However, three questions remained which we will try to answer in the current review.

The primary question is whether HRT protects against the development of AD in healthy post-menopausal women. A definitive answer to this question should be provided by two large population studies, the Women's Health Initiative Study (WHIMS)[168] and the Women's International Study of long Duration Oestrogen after the Menopause (WISDOM)[199] that are under way. However, these studies will not report until 2006 and 2010. Until these data are available, we need to make the best use of existing data. Our review will discuss first whether available evidence indicates that a decline in estrogen levels impairs cognition in elderly women. The second question concerns the nature of the effect of HRT on cognition in healthy women. In particular, we evaluate whether HRT acts selectively on particular cognitive functions (e.g. verbal memory),[164] or non-selectively (e.g. by increasing the speed of information processing). It is possible that enhancing estrogen levels with HRT has effects only during a limited window of time, or in specific groups (e.g. surgically menopausal women, or only in those with depressive or climacteric symptoms). We will also briefly discuss different types and dosages of HRT. The third question is whether HRT could be protective against or effective in treating AD. Almost all the observational studies showed a decrease in the risk to develop AD with HRT use.[68,154,181] Moreover, almost all the earlier studies showed positive treatment effects of HRT in AD.[74,131–133] Surprisingly, given these positive findings, recent controlled trials have not shown any effect of HRT in AD.[67,127,187]

Our goals were: firstly, to examine existing results to determine whether reports of estrogen and HRT's effects on cognition in elderly women are valid; secondly, to try to define the reasons for inconsistencies in the data; and thirdly, to identify crucial questions which could form the basis of future research.

To identify relevant studies, we performed initial searches (Medline, PsychLit, Embase 1966–2000) using the terms "HRT, estrogen, progestagens, cognition, memory, dementia and Alzheimer". We also identified studies from references from the initial searches. Several workers have already reviewed HRT's effects on prevention of cognitive decline and dementia.[18,64,124,135,171,202] We have extended this work via meta-analyses of existing studies and by critically evaluating the selection of subjects, diagnostic techniques, cognitive tests, designs and statistics. As most studies have methodological problems, rather than excluding these, we have tried to systematically investigate the role of confounds by including as many studies as possible. Meta-analyses were done using the statistical program Review Manager (version 3.1, Cochran library system for systematic reviews). Other analyses were done using SPSS 8.0.

2. THE EFFECT OF FEMALE GONADAL HORMONES ON COGNITIVE FUNCTION IN HEALTHY POST-MENOPAUSAL WOMEN

Before the menopause, estradiol (E2) is the predominant estrogen which enters the circulation. E2 in women derives mainly (95%) from the ovaries. About 25% of E2 is converted to estrone (E1). Estrogen receptors are more sensitive to E2 than E1, while estriol, E2's other metabolite, has low receptor affinity.[130] From the age of 40, the production of E2 becomes erratic. There is then a rapid decline in estrogen levels when ovulation ceases at a mean age of 51 years.[85] After the menopause, the metabolism of androstenedione yields small quantities of E2 via E1, which

is then the predominant estrogen. This conversion occurs mainly in adipose tissue.[130]

2.1. Biological plausibility

It is biologically plausible that a decline in estrogen levels has a detrimental effect on brain functions which subserve cognition and mood. There are many physiological mechanisms through which estrogens may exert protective and enhancing effects on cognition. Estrogens can enhance neurotransmitter functions, which decline in ageing and dementia.[81,101,103–105,170] They can facilitate outgrowth of dendritic spines.[51,196,197] They can protect neurons against oxidative stress, both directly[55] and indirectly via actions on the vascular system.[115,156,157] Detailed reviews of this topic are already available.[15,43,77,115,117,124,171] These reviews make it clear that estrogen could potentially modulate or protect brain systems that subserve important psychological functions. However, this potential is not proof of their importance in the clinical domain. We now turn to review studies relating estrogen levels to cognitive measures.

2.2. Subjective cognitive complaints after the menopause

About 80% of women experience negative consequences of the menopause, including hot flushes, night sweats and complaints of impaired cognition.[21,90] A number of studies have evaluated the association between estrogen levels and cognitive function in elderly women. An early study[25] found peaks in cognitive complaints and mood disturbances before the age of 50. It suggested that these peaks related to the decrease in estrogen levels. Because little is known about normal midlife changes in women, a large-scale study has recently investigated these in more detail. Its preliminary findings were surprising.[45] Contrary to earlier views,[85] heavier women had more menopausal complaints than thin women, which may have been because of reduced tolerance to flushes.[95] Also, fewer women complained of symptoms 12 months after the menopause than before it. Menopausal complaints may thus relate not to a decline in estrogen levels, but rather to transient excess estrogen during the transitional period.[146]

2.3. Objective cognitive function after the menopause

The relationship of subjective complaints to objective test performance is unclear. Halbreich *et al.*[62] tested whether age-related deterioration of performance on objective tests accelerated after the menopause. They found an inverse correlation between age and performance on a number of tests in post-menopausal, but not in younger, women. These results supported their hypothesis. However, large population studies do not show an abrupt decline of cognitive function in women during the fourth or fifth decade, when 95% of the women are perimenopausal.[84] Further strong evidence against Halbreich's hypothesis is that, in the Atherosclerotic Risk in Communities (ARIC) study,[31] middle-aged women showed better cognitive performance than men. Elderly men maintain higher E2 and testosterone levels than post-menopausal women.[106] Furthermore, the finding that elderly men (>65 years of age) have a steeper decrement with age than women on several memory tests[11] indicates that the menopausal decline in estrogen levels does not necessarily cause cognitive function to decline. However, these studies have not systematically investigated the association between levels of hormones and cognitive function while controlling for potential confounds.

2.4. Post-menopausal hormone levels and cognitive function

Five studies have tested whether higher endogenous hormone levels relate to better cognitive function in healthy elderly women not using HRT (Table 1). The results were inconsistent, but surprisingly they included two inverse associations. The large Study of Osteoporotic Fractures (SOF)[204] found that higher E1 levels related to slower information processing [Digit Symbol Substitution Test (DSST) at baseline], even after adjustment for numerous potential confounds. Higher E1 levels also related to greater decline in information processing speed (Trail Making Test: TMT-B) over five years. The SOF[204] found no relation between total endogenous E2 and any measure of baseline performance or decline, including the modified Mini-Mental Status Examination (mMMSE). However, in a later report of this group, bioavailable E2 was associated with less cognitive decline as measured with the mMMSE.[201] No effect of testosterone was found. In contrast, the Rancho Bernardo Cohort (RBC) cross-sectional study found no positive effects on a large battery of tests (including the MMSE) of either total or bioavailable E2. They did report an inverse relation between E2 and Visual Reproduction memory.[13] This is consistent with findings that elderly men showed a greater decline in memory functions (including Visual Reproduction and verbal memory) than post-menopausal women,[11,30] despite having higher E2 levels than women. The RBC study[13] found no significant association between E1 levels and cognition (although a positive association between E1 and verbal memory approached significance, $P = 0.07$). Interestingly, they found that in women with suspected dementia, testosterone levels were lower. E2 can be converted from testosterone in the brain.[120,183] The two smaller studies[30,143] showed no relation between endogenous total E2 or testosterone[30] and a range of cognitive variables.

In summary, there is no consistent evidence that low levels of endogenous estrogens related to poor cognitive performance in the elderly, but it has been suggested that bioavailable E2 is a more relevant variable than total E2 to predict cognitive change over time.[201]

3. STUDIES OF THE EFFECT OF HORMONE REPLACEMENT THERAPY ON COGNITION IN HEALTHY POST-MENOPAUSAL WOMEN

In the United States, about 20% (ranging from 5% to 50%) of menopausal women use HRT to treat menopausal symptoms.[21,95] HRT reduces menopausal problems such as hot flushes, night sweats and osteoporosis,[57,184] but is not indicated clinically to maintain cognitive function. While natural hormone levels do not seem to show a clear positive association with cognition (Section 2.4), this does not rule out the possibility that higher estrogen levels due to HRT maintain or enhance cognitive functions. For instance, one observational study that reported positive effects of both total E2 and bioavailable E2 levels[42] did not exclude HRT users from the overall analyses (Table 1).

Analysing HRT effects has several advantages over simply correlating estrogen levels with cognition. It can partly unconfound effects of hormonal loss and ageing, it permits an experimental approach, and it boosts hormone

Table 1. Epidemiological studies of hormone levels (estrone/estradiol/progesterone/testosterone) and gender and cognitive function in healthy elderly subjects

Author	Year	*n*	Subjects	Age	Outcome	Adjusted	Exclusion
Yaffe *et al.*	1998	532	PM, women	72 (5)	Neg association E1 with DSST (at baseline),TMT-B decline at 5 years FU. No association E2.No association with MMSE. 7 AD at FU: no diff E2/E1	Age, education, depression, stroke history, alcohol consumption, weight, weight change since age 50	< 65 years of age, black, bilateral hip replacement, dementia, HRT use
Yaffe *et al.*	2000	425	Healthy women	72 (5)	Bioavailable and non-protein-bound E2 had positive association with mMMSE, T had no effect	Age, education, BMI, HRT use, history surgical menopause, baseline cognitive score	< 65 years of age, black, bilateral hip replacement, dementia
Barret-Connor	1999	393	Healthy elderly women, middle-high SES	74 (55–89)	No association E2/E1: Blessed, MMSE, BSRT, Visual Reprod, Fluency, TMT-B (only adj. effects: MMSE, below cutpoint: T levels 2× lower, low E2 higher Visual Repro)	Age, education, BMI, alcohol, smoking	< 55, institutionalized, HRT use
Polo-Kantola *et al.*	1998	66	PM, healthy women	56 (47–65)	No association of E2: Paired Associates, Object Recall, Naming, Digit Span, Block Design, DSST, BVRT	Equal age, education, SES, marital status, employment, subjective health	Pre-menopausal, head injury, neurological, cardiovascular, endocrinological, mental disease, hyperlipidaemia, malignancies, drug abuse, abnormal sensory, HRT use
Carlson and Sherwin	1998	86	Men (31), women using HRT (14), non-users (41)	72 (6)	No correlation E2, T, DHEAS: with Paragraph Recall, Paired Associates, BSRT, Visual Reproduction and memory HRT and men > non-users: Digit Span; women > men : Word Fluency. No effect of duration of HRT use	HRT, higher SES and education (covariate), equal age	< 65 years of age, major acute medical or psychiatric illness (stroke, recent heart disease, diabetes, vascular disorders, psychiatric disease,drugs)
Barret-Connor	1999	1351	Men (551), women (800)	76 (7)	Men steeper decline on Buschke's, Heaton Visual Reproduction, Fluency. No gender effect on MMSE, serial 7's, world backward, Blessed, TMT-B	Age, education, depression, estrogen use (stratification hypertension, stroke, myocardial history)	Black, lower social class, non-ambulatory
Included HRT users:							
Paganini *et al.*	1996	214	PM, healthy women	80 (6)	Neg association P on Clock Drawing, no association E2 or HRT use	Age, weight, height, P treatment and levels, cholesterol levels, acetaminophen use	Black, lower social class, surgical menopause, myocardial infarction, angina, stroke, diabetes
Drake *et al.*	2000	39	Healthy, non-demented elderly women	79 (7)	High E2: verbal del. recall, TMT-A; low E2: visual memory, line orientation,visual span; low E1: word association, line orientation; High T: Fluency, Word association, TMT-A; low T: visual span backward, P no effect	3 current ERT, 21 past users, 19 never users: age, educ., depression, HRT use, practice effect (testsession) controlled	Dementia (CDR), age <65

BMI, body mass index; CDR, clinical dementia rating; DHEAS, dihydroxyepiandrosterone; FU, follow-up; P, progesterone; PM, post-menopausal; SES, socio-economic status; T, testosterone.

levels and so avoids "floor effects" (see Section 2.4). We identified 32 studies (18 epidemiological and 14 experimental/clinical trials) which investigated the effect of HRT on cognitive function in healthy elderly women (see Tables 1–3). Overall, 24 studies found one or more positive effects of HRT on cognition, eight found no effects and none found negative effects. At first sight, this overall pattern seems to indicate strongly that HRT favourably affects cognitive functions.

3.1. Selectivity of effects of hormone replacement therapy on specific tests of cognitive function

A major consideration which weakens the overall impression that HRT may improve cognition is that of the total number of tests in these studies, less than half (45%) showed a positive effect of HRT. One explanation for this could be that HRT selectively affects specific cognitive functions. To test this possibility, we initially divided the tests into memory and non-memorial categories. Twenty-one of 44 tests of memory (48%) and 24 of 51 non-memorial tests (47%) were positive. Also, two tests of overall cognitive function [the MMSE in three of four studies and the Blessed Information Memory and Concentration (BIMC) in one of two studies] showed effects of HRT. There is no evidence of specificity at this level.

Sherwin[164] has proposed that HRT selectively enhances verbal memory. Meta-analyses could be done only over a limited number of studies, as many did not show the actual data to assess the Weighted Mean Difference (WMD) between HRT users and non-users. The test for the heterogeneity of studies is displayed by χ^2. Meta-analyses showed significant positive overall effects on the following verbal memory tests. Paragraph immediate recall [WMD = 4.15, 95% confidence interval (C.I.) = 2.58–5.71] and delayed recall [WMD = 3.16, 95% C.I. = 0.98–5.34; heterogeneity test: $\chi^2(2) = 0.63$, $P = 0.73$], the Buschke Selective Reminding Test (BSRT) immediate recall (WMD = 2.51, 95% C.I. = 0.88–4.14) and delayed recall (WMD = 0.69, 95% C.I. = 0.18–1.19), Paired Associate memory immediate recall (WMD = 2.06, 95% C.I. = 0.48–3.64, all $z > 2.5$, $P < 0.01$). For almost all of the tests that had a significant z-score, the test for heterogeneity (χ^2 test) was also significant. This indicates that the evidence for HRT's beneficial effect on verbal memory is quite inconsistent. The fact that there was no effect on Paired Associates delayed recall [WMD = 0.23, 95% C.I. = −0.57 to 1.04; heterogeneity test: $\chi^2(2) = 2.77$, $P = 0.25$, $z = 0.57$, $P = 0.6$], a classic test of verbal memory, reinforces the inconsistency of HRT's effect.

Two of the above studies[30,142] also provided data of a test of non-verbal memory (the Visual Reproduction test) and both were negative [WMD = 1.32, 95% C.I. = −0.90 to 3.53; heterogeneity test: $\chi^2(1) = 1.99$, $P = 0.16$, $z = 1.17$, $P = 0.2$]. Therefore, the WMD indicates that HRT has selective effects on verbal memory. However, these meta-analyses may over-estimate HRT's effect on verbal memory, because studies that had non-significant effects were less likely to report their data in a form that we could include in our analyses. Also, several studies used different tests for the same cognitive function or used different parameters of the same tests, which made it impossible to include these in meta-analyses. To avoid this limitation of the meta-analytic method, we further investigated the selectivity of HRT by comparing the proportions of positive tests of verbal and non-verbal memory (Tables 2 and 3). Twelve of 28 tests (43%) of verbal memory (when including Paragraph recall, Paired Associates and word list tests) were positive, compared to only three of 14 tests (21%) of non-verbal memory. Overall, word list recall showed an effect only in four of 11 studies, but there were differences between different word lists. While the BSRT only showed an effect in one of four studies, the Californian Verbal Learning Test (CVLT) was found to be sensitive to HRT's effect in two of three studies. Other word lists used were not well known or described tests.[59,61,93,152] Also, different tests of non-verbal memory give different results. While the Visual Reproduction of the Wechsler Memory Scale (WMS) was not affected in four studies, the Benton Visual Retention Test (BVRT), another test of non-verbal memory, was significantly better in HRT users in two of four studies.[148,149] These two large epidemiological studies employed a different scoring method than the two experimental studies[144,185] that found no effect, which made it impossible to compare them using meta-analyses. Hence, the data do not clearly support Sherwin's hypothesis, but also do not provide firm grounds for its rejection. However, there is evidence that HRT affects cognitive functions other than verbal memory (see below).

Sherwin's hypothesis[164] predicts that HRT enhances verbal memory, but that it should not affect other stages of information processing. Existing data refute this latter prediction. Twenty-four of 51 tests (47%) of non-memorial functions (speed of information processing, visuospatial skills, abstract reasoning, etc.) were reported to show positive effects of HRT. HRT was found to reduce reaction times on 13 of 22 parameters tested. However, some speed of information processing tests seem insensitive to HRT. For instance, the DSST, a test of general speed of information processing, showed an effect only in one of five studies and the TMT-B, a test of attention, showed no effect at all in six studies. HRT enhanced focused attention on some tests (Stroop test in three of five studies), but showed no effect on other specific attention tests.[46,158] Visuospatial abilities improved (Clock Drawing, Block Design, etc.) on three of seven parameters employed. Also, abstract reasoning was enhanced in four of six studies. We thus conclude that HRT can enhance a wide range of functions and its effects are not specific to verbal memory.

The above overview makes it clear that HRT had more positive effects on cognitive tests than could occur by chance alone. However, even for verbal memory, which showed the highest proportion of positive effects, more than 50% of this type of tests used in studies showed no effect of HRT. Moreover, no tests gave unanimously positive results except Paragraph Recall. The possibility that some verbal memory tests, such as Paragraph Recall, are particularly sensitive to HRT is interesting for three reasons. Firstly, these tests have high face validity as ecologically sound measures of useful day-to-day memory. Secondly, Paragraph recall depends more than other verbal memory tests (such as the word list tests) on contextual processing. Craik and Byrd[36] have shown that elderly subjects perform poorly on tasks with little environmental or contextual support. The hippocampus may subserve contextual processing[194] and the hippocampal function deteriorates early in AD.[173] Thirdly, both Paragraph Recall and verbal Fluency decline in dementia.[70,128,178] The Fluency showed an effect of HRT in three of five studies.

Table 2. Epidemiological studies of the effect of hormone replacement therapy use on cognitive function in healthy elderly post-menopausal women

Author	Year	*n*	% users	Age	Design	Tests	Outcome	Adjusted	Exclusion
Barrett-Connor *et al.*	1993	800	34	77 (7)	FU 16 years	Memory (BSRT, Visual Reproduction, Fluency), attention (Blessed, TMT-B), MMSE	HRT no effect 1/6 tests, Fluency only after 20 years use	Age, education, current /past use, duration of use, dose	Hospitalization?
Robinson	1994	144	50	67 (7)	Cross-sect	Memory (word recall, Name–face recall)	HRT positive effect 1/2 tests, Name recall (HRT users more variance)	Matched for age, education, approximately equal: depression (GDS), MMSE, Vocabulary score (no check for duration)	Use insulin, psychotrophic med, < 27 MMSE
Kampen and Sherwin	1994	71	39	65 (5)	Cross-sect	Memory (Paragraph Recall, Paired Associates, word recall BSRT), language, attention, visuospatial memory	HRT positive effect 1 (Paragraph)/14 tests, 2/6 parameters (one-tailed testing)	Equal age, education, SES, martial status, age at menopause, more surgical menop users than non-users (no check for duration)	Age <55, post-menopausal <2 years, history head injury, stroke, heart attack, alcoholism, drug/psychother. depression
Kimura	1995	54	61	58 (2)?	Mean on/off vs controls	Verbal fluency (= making sentences, color naming), perceptual speed, articulatory/motor, visuospatial, interference, verbal memory (word recall)	HRT (CEE) positive effect overall on all 10 test scores, no duration effect	Matched age, education, vocabulary score, no corr. duration of use, equal years since menopause, surgery as covariates, no effect on mood	Age <50, post-menopausal <1 year, major health problems, use other confounding medication
Szklo *et al.*	1996	6110	18 (natural), 49 (surgical)	57 (6)	FU 3 years	Memory (word recall: DWR, Fluency), speed (DSST)	HRT no effect 1/3 tests, Fluency only in younger (<58 years), surgical menopausal women, duration effect (only in current users)	Age, race, education, marital status, subjective health, depression, smoking, drinking, hypertension, diabetes, fibrogen, BMI, sport, years since menopause	History of stroke or TIA, use antipsychotic medication
Schmidt *et al.*	1996	210	50	60 (6)	Cross-sect	Visuospatial and verbal memory (LGT-3), STM (Digit Span), abstract reasoning (Wisconsin), attention (Alterskonzentrat.), speed (TMT, CRT), visuopractical (Purdue)	HRT > abstract reasoning + visuopractical 2/8 tests (attent./RT), WHI lower in users, no relation WHI and cognition	Age, education, blood pressure, self-reported activation (no association WHI and test performance, duration effect for WHI)	Awareness of study reason, <45, age >75, neuropsychiatric/neurological problems, disease, dementia, drug abuse, CNS med, lab abnorm
Paganini-Hill and Henderson	1996	292	41	80 (6)	Cross-sect	Visuoconstruction (Clock Drawing)	HRT no effect 0/1 test, neg effect P; abnormal clocks: age effect	Adjusted for age (no check for duration)	Surgical menopause, myocardial infarction, angina, stroke, diabetes
Resnick *et al.*	1997	288	40	65 (10)	FU 2 years	Memory/ visuoconstruction (BVRT)	HRT less errors 1/1 test; FU: performance maintained. No duration effect	Age (matched, 10-year age bands), education as covariate	Age <40, use vaginal creams, dementia, health < good?
Jacobs *et al.*	1998	727	5 (current) 11 (ever)	74 (7)	FU 2.5 years	Memory (word recall:Buscke; word finding: Naming), abstract reasoning	HRT pos effect all tests 3/3, women using >1 year: better outcomes	Age, education, ethnicity, ApoE genotype, sample	Dementia, Parkinson's disease, stroke
Resnick *et al.*	1998	32	46	66 (6)	Cross-sect	Memory (BVRT, word recall CVLT, Fluency, Naming, prospective, Digit Span), speed (TMT-A, B), mental rotations (Card Rotations), MRI/PET (verbal + figural recognition tests)	HRT pos effect 4/10 (memory tests:CVLT, BVRT, recognition memory (2 tests) PET brain activation	Matched for age, education, vocabulary score (no check for duration)	Age <55, disease (CNS/ severe cardiac/metastatic cancer), left handedness

Table 2 (*continued*)

Author	Year	*n*	% users	Age	Design	Tests	Outcome	Adjusted	Exclusion
Matthews *et al*.	1999	9651	14% (27% past users)	72 (5)	FU 4–6 years	Overall cognition (mMMSE) attention/speed (TMT-B, DSST)	HRT current/past use > MMSE (especially in low educ/older age), DSST (use: >10 yrs better performance < 5 yrs) 2/3 tests	Age, education, functional limitations, depression, (stroke). No effect current HRT use on cognitive decline, effect past use on MMSE and TMT-B decline	Age <65 years, black women, disabled, bilateral hip replacement
Steffens *et al*.	1999	2338	54 (ever) (of which 29% current)	75 (7)	Cross-sect	Overall cognitive performance (3MSE)	HRT pos 1/1 (3MSE) current/ past use >non users. HRT users more limitation of activity, greater depression	Age, education, activity limitation, ApoE genotype, depression, head injury. No evidence for effect of duration (1-, 2-, 5-year cutpoint)	Age <65 years, dementia, stroke, black women
Hogervorst *et al*.	1999	342	7	55 (6)	Cross-sect	Memory (word recall CVLT), simple and complex speed of information processing (Stroop/TMT A-C)	HRT positive effect 1/3 tests, simple speed (sensorimotor)	Adjusted for age, education, subj. and objective health (no check for duration)	Medical conditions that interfere with cognition (CNS/CVA/tumors/ neurological/ psychosis/ psychotropic med)
Yaffe *et al*.	2000	2716	11% current, 12% past users	71 (5)	FU 6 years	Overall cognitive decline (change 3MSE)	HRT pos effect 1/1 (3MSE) decline only in ApoE4 neg women, not in ApoE4 carriers, though carotid atherosclerosis?	ApoEe4, interaction ApoEe4 × HRT, age, education, race, stroke history both as intercept term and interacted with time	< 65 years of age, use of P, move out of area, not able to give informed consent
Rice *et al*.	2000	837	16% current E only, 8% E + MPA	71 (5)	FU 2 years	CASI change (= MMSE + HDS) 9 subscores: orientation, language, drawing, Fluency, short and long term verbal memory, mental tracking, attention, abstract reasoning	HRT E users (mainly CEE) CASI change > HRT never users > HRT E + MPA (worse), better Fluency, Abstract Reasoning (E + P: decline mental tracking, surgical < natural menopause) 2/9 tests	Adjusted for age, education, language spoken, surgical menopause, baseline CASI score (no check for duration). Also check depression, income, health check-ups, history cancer	< 65 years of age, <50% Japanese ancestry, dementia at baseline
Grodstein	2000	2138	33% current, 35% past users	74 (70–78)	FU 21 years (5 years between use assess/testing)	General test (TICS), 10 word list recall, story recall (immediate and delayed) and verbal fluency by telephone	HRT positive effect 1/4 (Animal Fluency), better with use >5 years, no diff E, E +P	Adjusted for age at interview, age at menopause, eduction, high blood pressure history, diabetes, vit E use, NSAIDS use, BMI, smoking, antidepressant use, mental health indez and energy-fatigue index	Dementia, premenopausal status, cardiovascular disease
Verghese *et al*.	2000	35	10 users vs 25 non users	75 (8)	Case-control	BIMC, WAIS-R VIQ, vocabulary subtest, Block Design, Clock Drawing	HRT positive effect 3/14 tests (Block Design, Clock Drawing, BIMC)	Matched age, education, (HRT slightly higher depression)	Dementia, depression, no surgical menopause (BSO and hysterecomy)

attent, attention; BMI, body mass index; BSO, bilateral salpingo oopherectomy; CASI, Cognitive Abilities Screening Instrument; Cross-sect, cross-sectional; CRT, choice reaction time; CVA, cardiovascular accident; DWR, delayed word recall; educ, education; FU, follow-up; GDS, geriatric depression scale; MPA, medroxyprogestrone acetate; MRI, magnetic resonance imaging; NSAIDS, non-steroidal anti-inflammatory drugs; PET, positron emission tomography; RT, reaction times; STM, short-term memory; TIA, transient ischaemic attack; TICS, telephone interview for cognitive status; WAIS-R VIQ, Wechsler Adult Intelligence Scale-revised version, verbal intelligence quotient; WHI, white matter hyperintensity.

Table 3. Experimental studies and clinical trials with hormone replacement therapy in healthy elderly post-menopausal women

Author	Year	*n*	Age	Menopause	Exclusion	Design	Bli	Pla	Ra	Dur	HRT	Dose	Test	Outcome	Subjective
Rauramo	1975	56 ooph/ 88 (incl con)	49/46 (30–55)	Surgical menopausal (BSO/TAH)	Not available for testing	Parallel	n	n	n	6 months	E2	2 mg	Memory (Integrative memory test); Speed (SRT; CRT; Letter Cancellation); Logical reasoning (Raven Progressive matrices)	No effect HRT 0/5. Depression HRT > con (con = only hysterectomy, were younger)	Ooph > con: tearful, more neurovegative (flushes/ perspiration/tachycard/dyspnoe/ headache/sleep disturbances) + nervous sympt (irritable/tired/ tense/distressed), HRT = con
Vanhulle and Demol	1976	26	57.6	Healthy post-menop nuns	Post-menopausal <2 years, somatic problems, tranquillizer use	Parallel	y	y	y	3 months	E3	4 mg	Memory (BVRT; Digit Span; Visuospatial learning: Rey); Speed/Concentration (DSST; calculations; Vigilance test; Bourdon-Wiersma)	HRT > placebo alertness/attention; no effect HRT on memory, concentration 2/7	No difference symptoms (VOEG) except less tiredness in HRT group (trends flush/sweats). Subjects had high education
Fedor-Freyberg	1977a	25	55 (47–67)	Post-menop outpatients gynaecol clinic	Hospitalization needed, history mental illness	Single	y	y	n	1,6 months	E2 + P	2 mg 23 days, then 1 mg 24–28, P 13–22	Speed/ concentration (CRT, Letter cancellation (vigilance: simple or with memory load); KVT; Stroop (simple: color naming, interference); attention test: STM/reasoning: RT/error	HRT not 1 month, 6 months > CRT, Stroop interference,error attention, memory load (not simple versions) 4/9	All symptoms improved (sleep, fatigue, anxiety, depression, irritability, restlessness conc/memory problems), less neurotic, more extrovert (EPI), less depressed (HDS), or distressed
Fedor-Freyberg	1977b	21	57 (47–70)	Post-menop patients (many symptoms)	History mental disease	Parallel	y	y	y	3 months	E2	2 mg	Speed/ concentration (Letter cancellation; KVT; Stroop; CRT; attention test: STM/reasoning)	HRT > placebo on all tests (9 parameters) 9/9	All symptoms improved (sleep, fatigue, anxiety, depression, irritability, restlessness conc/ memory problems), less neurotic, more extrovert (EPI), less depressed (HDS), or distressed
Hackman *et al.*	1977	18	29–68	Mix: 10 BSO/8 post-menop. patients with symptoms	Liver/renal/hypertension/ cardiac failure/malignancy/ thrombosis/HRT use	Parallel	y	y	y	6 months	Piperazine E1	1.5 mg	Memory (Guild memory test)	No effect HRT 0/1	HRT improved flushes and sweats (4/9 plac), vaginal/urinary sympt., and insomnia, no difference in overall symptom improvement or anxiety, depression, lassitude, memory problems. Bleeding in 3/9 women
Sherwin	1988	50	45.4	Surgical menopausal (BSO/TAH) healthy	Not healthy (mental/physiol) problems, <9 years education	Crossover	y	y	y	3 months	E2 (i.m.) T (i.m.)	10 mg/month, 200 mg/month	Memory (Digit Span; Paragraph Recall); Speed (Clerical speed); Abstract reasoning	HRT (T/E2) > placebo on all tests (ANCOVA, age as covar) 4/4	No symptom change measured
Sherwin *et al.*	1990	12	47	Surgical menopausal (BSO/TAH) healthy	Not healthy (mental/physiological problems)	Parallel	y	y	y	2 months	E2 (I.M.)	10 mg/month	Verbal memory (Paragraph Recall, Paired Associates); Visual Reproduction Memory (WMS)	HRT > placebo on verbal memory, no effect on visual memory 2/3	No symptom change measured
Goebel *et al.*	1995	89	> 69	Healthy post-menop.	< 69 years of age, disease/smoking	Parallel	y	y	y	2, 4, 8 months	CEE (+MPA)	0.625	Speed (Trial Making Test:TMT-B)	No effect HRT 0/1	No effect HRT on postural stability (to reduce falls in elderly women)
Philips and Sherwin	1992	19	48 (5)	Surgical menopausal (BSO/TAH-benign)	Not healthy (mental/physiological problems), malignancies, HRT use	Parallel	y	y	y	2 months	E2 (I.M.)	10 mg/month	Verbal memory (Paragraph Recall; Paired Associate learning; Digit Span); Visual Reproduction memory (WMS)	HRT > placebo: verbal memory (not Digit Span), no effect HRT visual memory 2/4	Menopausal index and mood (MAACL): no difference, except flushes (also negative correlation with verbal memory); corr E1/E2 with Paired associates
Ditkoff *et al.*	1991	36	53 (45–60)	Hysterectomized, asymptom.	Medical illness, HRT use, medication	Parallel	y	y	y	3 months	CEE	0.6/1.2 mg	Memory (STM = Digit Span); Speed (DSST)	No effect HRT 0/2	no change MMPI, profile adaptation to life (emotions/ well being/ drug abuse/ physical symptoms/ relationships/ activity), BDI (depression) improved
Polo-Kantola *et al.*	1998	62	56 (47–65)	Hysterectomized, healthy	Medical and mental disease, premenopausal status (8 excl)	Crossover	y	y	y	3 months, 1 wash-out	E2 (transdermal)	2.5 mg/day or 0.05 mg/day	Memory (Digit Span, BVRT) Speed/ Alertness (Stroop, Vigilance (letter cancelation), RT, PASAT, DSST, subtraction)	No effect HRT/ E2 levels 0/8	Depression (BDI) no difference
Shaywitz *et al.*	1999	46	51 (33–61)	Post-menop. healthy	Not healthy, lefthandedness, IQ < 85, pre-menopausal status	Crossover	y	y	y	2 1days, 14 wash	CEE	1.25 mg	Memory (Word and token (non verbal) recall); fMRI	No effect HRT on tests 0/2; HRT effect on hemisphere encoding/ retreival assymmetry (fMRI)	No symptoms measured
Hogervorst *et al.*	1999	22	54 (3)	Healthy	< 45 years, health problems, post-menop <1 year, drug abuse, use psychoactiv.med	Parallel	n	n	n	12 months	E2 + P	2 mg	Memory (Word recall)	HRT positive effect (WS) 1/1, blindness was not maintained (spotting)	HRT > con activation, emotional vulnerability, cognitive complaints, flushes, quality of sleep after 12 months. HRT = con: psychosomatic complaints

Table 3 (*continued*)

Author	Year	*n*	Age	Menopause	Exclusion	Design	Bli	Pla	Ra	Dur	HRT	Dose	Test	Outcome	Subjective
Wolf *et al.*	1999	38	68	Healthy	Medical illness, HRT use, psychoactive, glucocorticosteroids	Parallel	y	y		2 weeks	E2 (transdermal)	0.1 mg/4 days	Verbal memory (Paired word recall, Fluency), Visuospatial memory, mental rotation, Speed (Stroop)	No effect HRT 0/3	No group effect with ANOVA (mood as covariate), baseline diff HRT group, practice effects, median split E2: within HRT group: no diff age, educ, BMI, verbal recall better, low E2 lower mood, no corr mood/ memory. Blind check ok

BDI, Beck depression inventory; Bli, blind; BMI, body mass index; BSO, bilateral salpingo oopherectomy; CRT, choice reaction time; Dur, duration; E3, estriol; EPI, Eysenck personality inventory; fMRI, functional Magnetic Resonance Imaging; HDS, Hamilton Depression Scale; i.m., intramuscular injection; KVT, Konzentrations Verlags Test; MAACL, multiple affect adjective check list; MPA, medroxyprogestrone acetate; MMPI, Minnesota multiple personality inventory; ooph, oopherectomized; P, progestrone/progestagen; PASAT, Paced Auditory Serial Additions Test; Pla, placebo-controlled; post-menop, post-menopausal; Ra, randomized; RT, reaction times; SRT, simple reaction time; STM, short-term memory; T, testosterone; TAH, total abdominal hysterectomy; VOEG, Subjective health questionnaire; WS, within subjects.

These considerations are consistent with the possibility that HRT could prevent dementia (Section 4).

It may be that there are specific factors that explain the inconsistencies between studies, such as the study design, subject selection, lack of power, dosage, type or duration. We will now examine these issues.

3.2. Epidemiological vs experimental studies

A major design factor, which *a priori* we might expect to account for differences between studies, is that some were epidemiological and others were experimental. In epidemiological studies (Table 2), women almost always used HRT for non-cognitive reasons, so this type of study is more ethical and less sensitive to expectation bias. However, these studies are only correlational and cannot control rigorously for confounding factors, such as prior health, medication compliance, dose and type of HRT, and mood of subjects. An important prospective study by Matthews *et al.*[112] found that women who started using HRT after the menopause were already more healthy before it (in terms of blood pressure, cholesterol levels, plasma insulin, weight, alcohol use and exercise) than those who were non-users of HRT after menopause. Another study reported that women who enter HRT trials may have fewer baseline cardiovascular risk factors than the general population.[69] Also, women who use HRT are generally younger and have higher levels of education and socio-economic status than non-users.[12] These factors could introduce a systematic bias into the results of epidemiological studies. Indeed, existing data contain clear evidence of this. The proportion of epidemiological studies which yielded a positive result overall (17 of 18) was higher than that of the experimental studies (seven of 14) [$\chi^2(1) = 8.30$, $P = 0.004$]. This indicates that some systematic bias differentiates the epidemiological and experimental studies. Experimental studies or clinical trials have often controlled for the confounds mentioned above (see Table 3). However, ethical and economic considerations have led to small, short-term trials in highly motivated subjects who may have been biased by expectancy effects. The strengths and weaknesses of these two kinds of studies (epidemiological and experimental/clinical) are complementary. So where there are discrepancies or inconsistencies between the results of one type of study, work from the other type may help to determine the reason.

3.3. Study design and power

The study design and statistical methods can influence whether effects of HRT appear. Epidemiological studies often use parallel or between-subjects designs that require large groups to show treatment effects. Sample size is important because it determines statistical power. However, the possibility that the experimental studies did not detect effects of HRT because they were smaller than the epidemiological studies (Mann–Whitney $z = -3.95$, $P < 0.0001$) seems unlikely because overall study outcome did not relate to sample size (Mann–Whitney $z = -0.91$, $P = 0.38$). Experimental designs that tested HRT effects within subjects can detect very slight changes. For example, the Digit Span (a measure of attention and short-term memory retention[7]) was reported to be significantly higher using a within-subject crossover design,[163] even though the HRT effect was very small (6.7 digits after

HRT, compared to 6.4 digits after placebo). Five other studies reported no effects (of which one other was a crossover study).[144] One other parallel groups study reported significant differences, but HRT users had very high mean values (9.64) while non-users were more within the normal range (7.61).[30] Meta-analyses of five studies also showed that there was no significant overall effect of HRT on the Digit Span [WMD = −0.22, 95% C.I. = −0.48 to 0.03; heterogeneity test: $\chi^2(4) = 28.98$, $P = 0.00001$, $z = 1.73$, $P = 0.08$]. So, although HRT may increase Digit Span, the effect may be too small to be useful.

The statistical analyses in most HRT studies have been sub-optimal. Although most measured performance in several cognitive tests, they used univariate analyses and, in some studies, studied cases were not compared to controls. Such analyses may lack power to detect effects that really exist and they cannot test whether HRT has specific or general effects. For example, disregarding significance levels, the study of Resnick *et al.*[148] found that HRT users had higher mean scores than non-users on 10 of 14 measures. This overall pattern of results approaches significance (binomial $P = 0.09$), but only four tests were actually significant in univariate analyses. Multivariate analysis of variance (MANOVA)[180] would have been able to determine if HRT had specific effects on the few tasks that were significant in univariate tests, or if it had overall effects which only passed the threshold for significance in a few univariate tests. The only study to date which has tested the specificity of HRT's effects statistically is that of Kimura.[93] She transformed all the data from her tests to *z*-scores and showed that there was an overall treatment effect in a repeated-measures univariate ANOVA. This method does not take into account the likelihood that subjects' scores on the different tests were inter-related, and so lacks power compared to MANOVA. We recommend that future research in this field makes more use of MANOVA to test the specificity of HRT's effects on particular cognitive functions and to increase power.

3.4. Subject selection

The results of epidemiological studies are extremely sensitive to the method of subject sampling. We have already seen that HRT users are atypical in being more educated and healthier even before their menopause.[112] This makes it important for studies to control for socio-demographic variables which differ between users and non-users, either directly by matching or indirectly by analysis of covariance.

Education is a major determinant both of HRT use[92] and of performance on cognitive tests.[111] Thus, HRT's apparent effects could be merely secondary to a higher educational level in users. The great majority (17/18) of epidemiological studies controlled for education. Perhaps surprisingly, the only one which did not find an effect of HRT[136] was also the only one which did not control for education. This weakens the notion that HRT's apparent effects mainly reflect pre-existing differences in education. In fact, a recent study found the opposite.[111] It showed that HRT's effects were stronger in women with less education (education × HRT interaction). However, despite their careful analysis of education effects, Matthews *et al.* concluded that it would still be premature to discount the view that education confounds HRT's effect on cognition.

Socio-economic status has independent associations with both educational level and cognitive performance.[12] HRT users in general have higher levels of education and socio-economic status, which could give them more access to medical services and healthier lifestyles.[181] The only study which controlled rigorously for socio-economic status via subject selection[86] found an HRT effect in only one of 14 tests, which is the lowest proportion of positive outcomes in any report. The RBC study[10] controlled socio-economic status by restricting its sampling to a single upper middle class community. It too found only a single small effect of HRT. These findings suggest that, with adequate controls for socio-economic status, HRT's apparent effects on cognition are minimal. Following this line of argument, the use of a random population sample in the Manhattan study[79] may paradoxically have over-estimated HRT's effects. Its cohort represented a broad population, including subjects of lower socio-economic status. It found the highest proportion of positive effects on cognition of any epidemiological study (all of three). Hence, socio-economic status may have been a major confound of HRT's effect and may have lead to the higher rate of positive outcomes in epidemiological compared to experimental studies.

3.5. Age as a potential confound

Age is an important potential confounding factor, because cognitive functions, duration of low estrogen levels and HRT use all decline with age. Overall, age did not relate to the overall outcome of studies (Mann–Whitney $z = -1.49$, $P = 0.14$). However, age may interact with HRT's apparent effects on cognition in two opposite ways, via two contrasting mechanisms. The first mechanism involves tests that do not require fast reactions. Here, younger subjects may be insufficiently impaired to show effects of HRT, due to ceiling effects, so we might expect HRT to show stronger effects in older women. Consistent with this, a recent report by Matthews *et al.*[111] confirmed that HRT had stronger effects in older women on the MMSE (a brief battery to assess cognitive function, including verbal memory). Ceiling effects can thus mask HRT's effects on some tests in younger women. They could also help to explain the low proportion of positive experimental studies: subjects were, on average, 16 years younger (overall mean age 52 ± 7) than those (mean age 69 ± 7) in the epidemiological studies (Mann–Whitney $z = -4.21$, $P < 0.0001$).

The second mechanism involves tests that require fast, accurate reactions. These are more sensitive to detrimental effects of age[144] and performance shows increasing variance with age.[84] Therefore, HRT's effects on information processing and attention may be clearer in younger post-menopausal women than in older women. However, there was no overall age difference in the seven studies that showed an HRT effect on the Stroop, DSST, TMT or general reaction time tests when compared to the four studies that showed no effects (Mann–Whitney $z = -0.83$, $P = 0.41$). Unfortunately, insufficient data were present to perform proper meta-analyses on information processing slowing with age as a confound.

Existing data are insufficient to enable us to draw clear conclusions on when and how age may mask HRT's effects. However, it is important to be clear on this for two reasons. Firstly, most women use HRT for only a few years after their menopause. Secondly, we wish to assess the potential role of HRT in dementia, which, in the majority of cases, begins after

65 years. At present, there are only two experimental studies, one short term[195] and one long term, that used only one cognitive test,[54] which both investigated healthy women over 60 using HRT. Neither found an effect in these groups, both of which had an average age of around 69. In summary, at present there is a paucity of studies that investigated the effect of HRT in older women over a longer period of time.

3.6. Surgical menopause and age

Surgically menopausal women have their menopause younger than naturally menopausal women. Nappi *et al.*[129] have hypothesized that a surgical menopause could induce a premature start of brain ageing. In support of this view, naturally menopausal women recalled more words on a short-term verbal memory task than surgically menopausal women (also see Ref. 35), in whom better memory related to later surgery. Also in line with Nappi's hypothesis, the ARIC study[179] found effects of current HRT use on one of two verbal memory tests (Word Fluency, but not word recall) only in the younger group of surgically menopausal women. Nappi *et al.*'s hypothesis has important implications for the three experimental studies[142,162,163] in which immediate Paragraph Recall was apparently sensitive to HRT, because all those reports used young surgically menopausal women. Also, in the epidemiological study[86] which found that HRT improved Paragraph Recall, a higher percentage of users than non-users had undergone surgical menopause. Paragraph Recall could thus be sensitive to HRT only in young surgically menopausal women (the mean ages in these four studies were less than in the others: Mann–Whitney $z = -2.08$, $P = 0.04$). This conclusion could weaken Sherwin's hypothesis that HRT has specific effects on verbal memory. However, three studies[93,150,201] reported that HRT's cognitive effects (including effects on Word Fluency and memory) remained significant after adjusting for surgical menopause. It also remains to be seen how and if surgical menopause interacts with the age of testing. The Kame project[150] also found, in older (71 ± 5 years) Japanese American women, that those who had undergone surgical menopause had less improvement at follow-up on a battery of global cognitive performance compared to naturally menopausal women. In addition, a recent case control study only investigating older surgical menopausal women (mean age 75 ± 8 years) found protective effects of HRT on the BIMC and two visuopractical tasks, the Block Design and Clock Drawing. These tests have also been reported to be affected in early dementia.[186] Further studies should define whether the effects of HRT on verbal memory and other functions are dependent on the type of menopause and whether this interacts with the age at which cognitive tests take place.

3.7. Treatment duration, type and dose differences

Finally, it is plausible that differences in dose, type or duration of HRT could explain the inconsistencies between studies. The ability to control these factors is a major advantage of experimental trials over epidemiological studies. Five of seven experimental studies which used oral or intramuscular E2 found one or more positive effects on cognition.[46,72,142,162,163] (but see Refs 144 and 195). In contrast, none of the three studies that used conjugated equine estrogens (CEEs, which mainly consist of E1 sulphate)[39,54,161] or other E1 preparations[61] found any effects. Brain estrogen receptors are more sensitive to E2 than E1.[130] Also, several observational studies reported that raised E1 levels were associated with detrimental effects on cognition (Section 2.4). These findings seem to suggest that CEEs are not effective in enhancing cognitive function in elderly women. However, this may have been related to the type of tests used to investigate CEEs' effect (Digit Span, the DSST and TMT) which, in studies using E2, have also not been found to be very sensitive to pick up effects of HRT. Also, equilin, CEEs' most active ingredient, has been found to positively affect neuronal systems.[22] Furthermore, as it is the most widely prescribed drug, it is probable that the great majority of women in the epidemiological studies used CEEs. It is therefore surprising that these studies almost all yielded positive results.

There are three possible explanations for this apparent contradiction. The first is that epidemiological studies were larger and thus sensitive enough to detect smaller effects of CEEs. However, we showed earlier that the size of the studies was not overall related to outcome (Section 3.3). The second is that the women in epidemiological studies used HRT for longer periods and short duration of use may be insufficient to cause effects on cognitive tests.[144,161] Consistent with the latter possibility, both the RBC[10] and Manhattan[79] studies found effects of duration of use on the Fluency test. However, the details of their results were inconsistent. The relationship was present in women who had used HRT for one year or more in the Manhattan study,[79] but for over 20 years in the RBC study.[10] Moreover, the Baltimore Longitudinal Study of Aging[149] and Kimura's[93] studies found no duration effect, while some experimental studies found effects of HRT after as little as two months.[142,163] Hence, duration of HRT use cannot explain the contrasting outcomes of epidemiological and experimental studies. The third explanation is that women in epidemiological studies already have a better health status before they start using HRT (see Section 3.2) and that using HRT (mainly CEEs) in itself does not relate to a better outcome on cognitive tests.[112]

One of the most important considerations in prescribing medication is the optimal dose. HRT doses have been optimized to treat post-menopausal conditions other than cognition (e.g. osteoporosis, hot flushes reduction). Experimental studies could, in principle, determine the effective dose (ED_{50}) of HRT for enhancing cognition. Existing experimental studies have produced a wide range of serum estrogen levels. Among observational studies that showed normal E2 levels in HRT users and non-users, only one found that, in a group of users and non-users combined, E2 levels related to better verbal memory (delayed recall word list) and speed of information processing (TMT-A).[42] Another study combining users and non-users found no effects on Clock Drawing, but their E2 levels were much lower (16 ± 28 pmol/l[136] compared to 50 ± 48 pmol/l[42]). In other studies, HRT produced higher E2 levels in users, but there were no associations between hormone levels and cognitive functions (in pmol/l: users 291, non-users 23;[144] users 116, non-users 28;[30] users 124, non-users 38;[195] users 136, non-users 38[86]). However, one[195] of these, while not finding overall effects of estrogen levels or HRT, found that, in a subgroup of HRT users, higher levels of E2 (>109 pmol/l) related to better verbal memory (delayed recall Paired Associates). Only one study[142] produced very high E2 levels (a factor of 10 higher compared to the other studies;

users E2: 1839 ± 10 pmol/l, non-users 128 ± 25 pmol/l). This study administered E2 injections and also found positive associations of E2 with a verbal memory test (Paired Associates). However, they also reported positive associations of E1 with verbal memory, and there seemed to be baseline differences on the test between users and non-users, so it is not clear that the HRT or high levels of E2 were responsible for the differences in verbal memory.

The possibility that higher doses of HRT producing higher levels of E2 would enhance cognitive function requires further study. However, quite apart from the risks of physical side effects, there is also a risk of detrimental cognitive effects. Two observational studies found that E2[13,42] and E1[42] related negatively to Visual memory, line orientation and Visual span backward. Such detrimental effects may be weak because two other studies found no effects of E2 levels on Visual memory.[30,144]

High levels of E2 seem to be related to route of administration. The two studies that reported strong effects of HRT[142,163] administered intra-muscular depot injections of E2 (10 mg for one to three months) and showed very high serum levels of estrogens, with large differences between treated cases and controls. The only two clinical trials that delivered low-dose E2 transdermally found no effects on cognitive function,[144,195] even though, in one,[195] higher E2 levels related to better verbal memory. Thus, the ED_{50} for E2 may be more than that supplied by transdermal or oral dosing. In summary, at present, there is no consistent evidence that higher levels of E2 cause better cognitive performance. However, there is a striking heterogeneity between the E2 levels in different studies, even for non-HRT using controls. The reasons for this are unclear and cloud interpretation of the few studies that exist.

Most of the experimental studies only used estrogens. However, in the general population, HRT usually includes progestagens to reduce endometrial proliferation, which can result in endometrial cancer.[28,85] It is biologically plausible that progestagens could reverse some positive effects of estrogens on the brain.[6,94,114,115,155,157] Consistent with this possibility, one study found that women with a progestagen added to their HRT had worse performance than those using unopposed estrogens on mental tracking.[150] The study of the Leisure Cohort[136] reported that women who performed poorly on a clock drawing task had higher levels of serum progesterone, but their E2 and E1 levels did not differ. The memory component of clock drawing is probably small, compared to the contribution of planning and motor skills. However, this test is of interest for two reasons. Firstly, clock drawing shows impairment in patients with dementia.[60,174] Secondly, it is biologically plausible that high levels of progesterone opposed effects of E2. In contrast, several studies[72,86] did not find any detrimental effect of adding a progestagen on cognitive function in healthy post-menopausal women. Other large epidemiological studies reported no differences between estrogen users and combination therapy users on verbal memory (Fluency)[59] or the mMMSE.[201] Drake *et al.*[42] also reported no association of progesterone levels with cognitive function. Finally, one of the studies[163] added testosterone (150 mg) to E2 injections. As mentioned, testosterone can be converted to E2 in the brain (Section 2.4), but in this study testosterone combined with E2 did not give better cognitive performance than E2 alone.

In summary, our review indicates that the cognitive effects of HRT in healthy women are small and not selective for verbal memory. It remains to be investigated whether supranormal E2 levels (>1000 pmol/l) produced by HRT could induce stronger cognitive effects in elderly women. The possibility that progesterone can significantly impair cognition remains uncertain, but does not seem likely. However, cognition is not the only psychological domain which HRT may alter. We turn next to discuss its other putative psychological actions.

3.8. Indirect effects: expectancy, subjective well being, mood and climacteric symptoms

Several workers have proposed that HRT may indirectly affect cognitive function via expectancy effects, improvement in mood or the alleviation of climacteric symptoms. Expectancy is more important in experimental trials, since women must know the purpose of the study in order to give informed consent. If women in a trial know that its purpose is to investigate HRT effects on cognition or mood, they may expect changes in those areas. To counter such expectancy effects, it is important to maintain double-blind conditions. However, this is difficult due to withdrawal bleeding after one month of HRT, especially in relatively young post-menopausal women. While this is not the case for surgically menopausal women,[142,163,166] the reduction in flushes and night sweats can unmask participants' blindness to their treatment. Expectancy could have a large impact on the subjective measures of well being and mood. For example, two randomized "double-blind" placebo-controlled trials[28,192] showed that scores on some quality of life variables improved equally with both HRT and placebo. Thus, expectancy effects may enhance performance in placebo controls in experimental studies and so tend to mask any effects of HRT. This mechanism could help to explain the lower proportion of experimental studies that yielded a positive outcome.

HRT could enhance cognition indirectly by improving mood.[204] Arguing against this, several reviews[135,139] concluded that evidence for HRT as a treatment for depressed mood or dysphoria is weak in naturally post-menopausal women. Table 3 shows that HRT has inconsistent effects on mood in healthy women. It had no effect in five studies,[61,140,142,144,185] enhanced mood in four,[39,46,72,195] but impaired it in one.[147] Controlling for depression in the analyses of large studies did not change the outcome of HRT's effect.[59,201] Also, in one study,[195] high E2 related to better memory independent of mood. Overall, there is no consistent evidence that HRT's apparent effects on cognition are merely secondary to changes in mood. Some authors suggested that adding progestagens has detrimental effects on mood and hence indirectly on cognition.[38,107,165] Others found no evidence for this view.[72,86,99] A large controlled study[57] investigating unopposed estrogens and combination therapy also found no compelling evidence for differential effects of various hormone replacement combinations on self-reported cognitive function, anxiety or affect. Hence, we conclude that it is unlikely that HRT (unopposed or combination) affects cognitive function through the enhancement of mood.

The "domino theory"[28] proposes that cognitive dysfunction in menopausal women may result from hot flushes and night sweats impairing sleep.[12] In line with this theory, one double-blind study[39] found that, in asymptomatic women, CEEs had

minimal effects on cognition (only one scale—"income management"—improved). Further evidence supporting the "domino theory" was that hot flushes related inversely to memory scores.[142] However, other evidence weighs against the "domino theory". Firstly, in Phillips and Sherwin's study[142] there were no differences between HRT users and non-users with respect to sleep difficulties. Secondly, in another study,[99] HRT improved all assessments of the quality of life (health, psychological and social well being) superior to symptomatic treatment of hot flushes (veralipride, 100 mg/day). Finally, direct testing of whether climacteric symptoms or mood relate to cognitive function were negative in two studies.[143,195] Thus, the "domino theory" cannot account for impaired cognitive function in menopausal women.

3.9. Summary of the effects of hormone replacement therapy in healthy elderly women

In summary, we conclude that the cognition-enhancing effects of HRT in healthy post-menopausal women are small and inconsistent. They are not specific to verbal memory, nor are they due to improvement of mood, or the reduction of climacteric symptoms. There are significant differences in outcomes between epidemiological and experimental studies that may be due to confounding HRT effects, including a variety of factors, such as age, socio-economic status, prior differences in health status, surgical menopause, the type of HRT and expectancy effects.

There is, at present, a paucity of studies investigating long-term effects of HRT in older (60 +) healthy women. Two large controlled trials are currently under way, the WISDOM[199] and WHIMS.[168] These should answer whether HRT has an effect in older women. Unfortunately, these studies both use CEEs, which contains mainly E1 sulphate. CEEs have not been found to improve cognitive function in other studies (see Section 3.7). In fact, increasing E1 levels may even impair cognitive function (see Section 2.4 and below). These studies will also investigate whether HRT could protect against dementia. In the next section, we will appraise the available evidence for this idea.

4. STUDIES OF THE EFFECTS OF HORMONE REPLACEMENT THERAPY ON COGNITION IN WOMEN WITH DEMENTIA

We turn now to discuss the potential roles of HRT as a prophylaxis against and treatment for dementia. Dementia is characterized clinically by progressive memory impairment and disturbances of other cognitive functions.[83,118] AD is the most frequent cause of dementia. A major difficulty in all studies of AD is that of making the diagnosis. A definite diagnosis requires *post mortem* analysis of brain tissue.[118] Many other disorders can masquerade as AD, especially vascular dementia (VaD), the second most common dementia. The distinction between AD and VaD is often very difficult *in vivo*.[34]

Women have a higher risk for AD (but not VaD) than men.[96] As mentioned earlier, elderly men have much higher levels of E2 and of testosterone, which can be converted to E2 in the brain.[106] In post-menopausal women, estrogen levels drop to almost non-significant levels. If estrogen levels affect the progression in AD, then men with AD should retain better cognitive performance than women not using HRT. Several studies have compared men and women with AD on cognitive tests. One[41] analysed 444 AD cases. The 270 women had worse performance on the AD assessment scale (ADAS-Cog) than the men, controlled for age and education. The AD women using HRT did not differ from the men. In another study,[65] women with AD were worse on several verbal tasks (naming and fluency) than men with dementia, whereas control females were better at these tasks than control men. A later study found that AD women using HRT were even better on the Digit Span and the Token test than AD men matched for age, education and duration of dementia symptoms.[66] While in healthy elderly women an estrogen deficiency is not related to a decline in cognitive performance (see Section 2.4), it may be that demented men and women on HRT retain more verbal skills than women not using HRT during the initial stages of the process.[65]

4.1. Estrogen levels in women with Alzheimer's disease

The evidence for estrogen deficiency in women with dementia compared to controls is inconsistent (Table 4). Meta-analyses showed that women with AD had lower levels of total E2 than controls (WMD = −8.73, 95% C.I. = −9.58 to −7.87, $z = 20$, $P < 0.0001$). However, the heterogeneity was also very high [heterogeneity test: $\chi^2(6) = 544.39$, $P < 0.0001$], suggesting that comparability between studies was low. Two studies found higher levels in patients,[13,73] two found higher levels in controls[49,109] and four found no significant difference.[29,37,75,204] The reasons for the discrepancies between studies are unclear. Some studies may not have adequately diagnosed their cases.[13] However, studies that controlled for many factors and diagnosed their subjects carefully still came up with different results.[73,109] Table 4 shows that one of the major differences between studies is the overall E2 level, which sometimes differs by more than a factor of 3. This may be because different assays were used, with variable sensitivity and specificity. Remarkably, in these studies the difference in E2 levels in controls was larger than that in cases. Clearly, further studies are needed to resolve the question whether E2 is lower in women with AD. Also, as suggested,[201] bioavailable E2, rather than total E2, may be a better measure to assess estrogen deficiency.

Interestingly, one study[37] found that total E1 levels were higher in AD, which is consistent with findings that E1 correlated inversely with speed of information processing[204] and Word Association[42] in healthy elderly women (see Section 2.4). This finding may be important because the most widely used HRT preparation, CEEs, consist mainly of E1 sulphate.[130] This fact, in turn, raises the possibility that HRT could further impair cognition in AD. This is in line with a randomized controlled clinical trial[127] that found negative effects of CEEs on some cognitive variables in AD patients. The levels of E1, the predominant estrogen after the menopause, could hypothetically increase the relative risk of developing AD. Against this hypothesis, most studies[9,75,109] have found no association between AD and E1 levels. In fact, Manly *et al.*[109] found a trend for total E1 to be lower in AD patients. However, so far this hypothesis has not been investigated longitudinally. To further investigate this hypothesis, we did meta-analyses on the epidemiological studies investigating the risk of AD subjects taking HRT. As mentioned earlier (Section 3.7), most women in these studies used CEEs. Secondly, we evaluated studies which tested whether HRT ameliorates symptoms in established dementia and performed meta-analyses over the studies that

Table 4. Cross-sectional studies investigating the differences in estrone/estradiol levels in dementia of Alzheimer's type and controls

Author, year	DAT *n*	Con *n*	Age	DAT E2/E1	Con E2/E1	Sign level	Matched	Remarks
Fillit, 1986A	12	20	AD:78 (5); Con:77 (3)	E2: 45 (26)	E2: 91 (73)	$P < 0.01$	Equal age, weight, tot protein, albumin, Hb, Ca, P, urea nitrogen	E2 and total E: AD < Con 11/12 AD and 7/10 Con: undetectable levels
Honjo, 1989	7	7	AD:80 (1); Con:80 (2)	E2: 15 (7); E1:118 (18)	E2: 11 (4); E1:103 (18)	$P > 0.20$, $P > 0.10$*	Not matched (but equal age), no data given on other variables	E1-S: AD < Con, E2/E1 no diff
Cunningham, 1994A	12	16	AD: 76; Con: 74	E2: 62; E1:230	E2: 70; E1: 155	$P = 0.558$, $P = 0.006$	Equated on age, BMI, alcohol intake, GDS, excl HRT, illness	E2: AD = Con, E1: AD > Con
Manly *et al*., 2000	50	93	AD: 78 (6); Con: 74 (8)	E2: 52 (3); E1: 74 (6)	E2: 70 (4); E1: 98 (8)	$P = 0.005$, $P = 0.06$	Adj age, educ, ApoE allel, race, BMI, exclusion HRT users, premenopausal, history stroke/PD, <65 years	No diff in proportion past HRT users (AD 12% Con 20%, $P = 0.23$), BMI Con > AD, logistic regression: no effect E1, E2 low: risk AD 4–6× than high E2 (adjusted)
Carlson *et al*., 2000	21 + 3 HRT users	20 + 3 HRT users	AD: 77 (6); Con:75 (5)	E2: 27 (11) HRT users; E2: 91 (30)	E2: 27 (14) HRT users; E2: 112 (56)	$P =$ ns, HRT > non users	Matched for age, HRT use, exclusion illness, medication use	Higher E2 less GDS (depression), depression > AD non users
Barret-Connor, 1999	19 MMSE < 23	373	74 (55–89)	E2: 22 (3); E1: 70 (7); T: 0.36 (0.1)	E2: 20 (1); E1: 69 (2); T: 0.61 (0.0)	$P =$ ns, $P =$ ns, $P = 0.01$	Adj age educ, date and duration of serum storage, depression, no corr smoking, alcohol, BMI, exclusion HRT	Lower E2 (<18 pmol/l) better visual reproduction
Yaffe *et al*., 1998	7	406	77 (5)	Overall at baseline 72 years	E2: 24 (17); E1: 84 (43)	$P = 0.43$, $P = 0.65$	Adj age, educ, depression, strokes, alcohol use, weight, excl HRT users	At 5-year FU no diff AD/Con E2/E1, 40% undetectable levels
Hogervorst, 1999A	41	54	75 (9)	E2: 29 (17)	E2: 21 (11)	$P < 0.005$	Adj age, educ, smoking, alcohol abuse, hysterectomy, BMI, thyroid medication, glucocosteroids, storage sample, exclusion HRT users	E2 AD > Con

adj = adjusted for; BMI, body mass index; Ca, calcium; Con, controls; corr, correlation; DAT: dementia of the Alzheimer type; educ, education; FU, follow-up; GDS, geriatric depression scale; Hb, haemoglobulin; P, phosphorus; PD, Parkinson's disease. *As calculated by the author (pmol/l = 3.67 × pg/ml).

reported MMSE values, which was the most widely used cognitive test.

4.2. Potential protective effects of hormone replacement therapy on the development of Alzheimer's disease

We identified 15 studies that tested whether HRT protects against the development of AD (Table 5). However, for the last study[160] not enough data were available, so we could only do meta-analyses of 14 studies. For several studies (see Table 5) we had to infer the actual number of cases and controls receiving HRT or not, as only percentages were given. Also, the earlier studies (before 1994) included men and we had to recalculate the Odds Ratio (O.R.). The meta-analysis supported the hypothesis: the O.R. as an estimator of the Relative Risk of AD was 0.56 overall (95% C.I. = 0.46–0.68), indicating a protective effect of HRT. Strikingly, however, the results of the different studies are significantly heterogeneous [heterogeneity test: $\chi^2(13) = 24.61$, $P = 0.03$, $z = -6.05$, $P < 0.0001$].

There is no evidence that this heterogeneity is due to publication bias.[44] When the sample size was greater than 250, the O.R. was 0.46 (95% C.I. = 0.36–0.59) and the heterogeneity test was no longer significant [heterogeneity test: $\chi^2(7) = 7.82$, $P = 0.36$]. When n was smaller than 250, the O.R. (0.81) was not significant (95% C.I. = 0.59–1.11) and there was considerable heterogeneity in studies [heterogeneity test: $\chi^2(5) = 16.80$, $P < 0.0001$]. Also, the 10 studies after 1994 adjusted for more potential confounds than the four earlier studies. However, despite their greater rigour, the later studies (after 1994) show larger protective effects of HRT against AD [O.R. = 0.49, 95% C.I. = 0.46–0.68; heterogeneity test: $\chi^2(9) = 14.20$, $P = 0.12$] than the earlier studies [O.R. = 1.08, 95% C.I. = 0.68–1.70; heterogeneity test: $\chi^2(3) = 10.42$, $P = 0.02$]. Publication bias clearly cannot explain this finding. A potential explanation for this heterogeneity is that the dose and/or type of HRT may have changed progressively over time,[191] so that early and late studies are correctly estimating the effects of different HRT regimens. However, the evidence for this is weak. For example, two recent longitudinal studies followed women for one to five years[181] and 13–17 years,[188] but they found similar estimates of relative risk. So, the statistical considerations above are consistent with the view that HRT is protective against AD. However, statistics are only one component of validity. Overriding their importance is that of data quality. We shall see below that different aspects of data quality confound and could explain the statistical trends outlined above.

4.3. Diagnosis, surrogate responders, hormone replacement therapy treatment duration effects and prescription bias

The difficulties surrounding the diagnoses of dementia are not too important for the purposes of this review. This is because estrogens have numerous vascular effects and may enhance cerebral circulation.[26,50,156,157] Also, recent evidence has shown that the same factors could contribute to the pathogenesis of both AD and VaD.[33] On these lines, Mortel and Meyer[126] found a similar O.R. for AD (O.R. = 0.6, 95% C.I. = 0.3–1.2) and VaD (O.R. = 0.5, 95% C.I. = 0.2–1.2). Also, the one study[137] that used death certificates to assess dementia found similar confidence intervals to those which used batteries of *in vivo* diagnostic tests. Finally, a recent study[110] found that HRT even decreased the risk of Parkinson's disease with dementia but not Parkinson's disease without dementia. Hence, on the basis of these findings, specificity of diagnosis does not explain the differences in O.R.s.

A potential bias which could confound HRT's apparent protective effect against AD is difficulty in ascertaining HRT use. Six of the 14 studies[1,19,23,71,97,172] ascertained HRT use in their AD patients by questioning their family or friends (surrogate responders; Table 5). This approach may be inaccurate, since the surrogate responders may not have full information about the participants' previous HRT use. Six studies ascertained HRT use directly, by questioning the subjects themselves.[8,68,91,126,137,181] This approach, however, can also be inaccurate, since AD patients may not recall their HRT use. Two others used medical records or pharmacy files.[20,188] We tested whether the protective effect of HRT in the 14 studies related to the method of ascertaining HRT use. The O.R. was less in the six studies that questioned subjects directly [O.R. = 0.43, 95% C.I. = 0.32–0.57; heterogeneity test: $\chi^2(5) = 7.55$, $P = 0.18$] than in the six that used surrogate responders [O.R. = 0.66, 95% C.I. = 0.48–0.91; heterogeneity test: $\chi^2(5) = 11.94$, $P = 0.04$] or computerized files [O.R. = 0.78, 95% C.I. = 0.51–1.19; heterogeneity test: $\chi^2(1) = 5.12$, $P = 0.02$]. This finding indicates that either surrogate responders systematically over-estimate HRT use in AD patients, or the patients themselves systematically under-estimate their HRT use. This review cannot choose between these alternative explanations, but *a priori*, the latter seems more likely. Thus, the studies not using surrogate responders may possibly have over-estimated HRT's protective effect. However, another explanation for this pattern of results is that the use of surrogate responders itself relates to publication before 1994. However, this was not the case (see Table 5). Hence, analytical rigour over time, unreliable estimates of use by asking patients and sample size all confound this methodological issue (Section 4.1). Clearly, further work is needed to resolve if and how the method of ascertaining HRT use may bias its apparent protective effects against AD.

If HRT consistently protects against AD, then this should depend reliably on the duration and dose of treatment. However, duration effects are very variable. Five studies tested the relationship of HRT's protective effect to its duration of use: one[91] reported that duration had no effect, one found a nearly significant effect for current users[20] and the other three found protective effects only after six months,[188] one year[181] or seven years,[137] respectively. Hence, it is unclear if there is a critical duration of HRT use for it to be protective. Only two studies tested whether higher doses of HRT were more protective. One[188] was negative and the other[137] reported that a minimum daily dose of 1.25 mg CEE was necessary. The difficulties of ascertaining dose and duration are even greater than those of ascertaining HRT use itself (see above). However, subjects are more likely to recall the route of administration. Of studies which analysed the relative risk of AD in relation to the route of HRT administration, two studies found that oral use was more effective than "topical" use,[20,188] while one found protective effects of both oral and transdermal use.[91] Clearly, existing data concerning the risk of AD in relation to the dose and duration of HRT are insufficient to inform firm recommendations for its clinical prescription.

The apparent reduction of the risk of AD in women who take HRT may be partly an artefact of physician behaviour.

Table 5. Epidemiological studies describing the Odds Ratio as an estimator of the Relative Risk of developing Alzheimer's disease with use of hormone replacement therapy

First author	Year publ.	*n*	HRT info	Age (years)	AD:Con HRT (%)	Risk	O.R.	95% C.I.	Covariates	Study sample	Diagnostic evaluation	Duration/type/ dose effects
Heyman	1984	84	Surrogate	61 (51–71)	15: 7.5	×0.5	2.38 (adj)	(0.7–7.8)*	Age, sex, ethnicity (AD had higher levels of educ, SES and were healthier)	Durham; AD: Medical centre, con: phone nr. sampling	$n = 28$, "rigorous criteria" for clinical diagnosis	Not checked
Amaducci	1986	329	Surrogate	40–80	10:13	×1.3	1.7 (adj) population	(0.4–5.9)*	Age, sex, region	Italy; AD: 7 neurol centres, con: clinic/acquaintance	$n = 116$ AD, Blessed dementia scale, HDS, clinical history, neurological, np/lab tests	Not checked
Borenstein	1990	120	Surrogate	65	18:16	×1.1	1.15 (adj)	(0.5–2.6)	Age, sex	Washington; AD: clinic, con: friends (MMSE > 26)	$n = 60$ AD, NINCDS/DSM-III (no: PD, mood disorder, hypothyroidism, stroke)	Not checked
Broe	1990	212	Surrogate	79 (7)	8:11	×1.4	0.78 (adj)	(0.4–1.6)*	Age, sex, clinic	Sydney; AD: 2 dementia clinics, 4:1 con:family doctor	$n = 106$ AD, clinical history, neurological, MMSE (<26), np/lab tests	Not checked
Brenner	1994	227	Pharmacy files	77 (7)	50:50	×1.0	1.1 (adj) for oral 0.7 ns, vagin 1.3 ns	(0.6–1.8)	Age (matched on), sex, education, married, ethnicity,smoking, P use, hysterectomy	Seattle, Puget Sound Health coop group; con: MMSE >28	$n = 107$ AD, medical history, physical, neurological examination, np/lab tests, imaging	Oral use: O.R. 0.7 (C.I. 0.4–1.5), vaginal 1.3 ns, current 0.6 (C.I. 0.3–1.2), former 1.7 (C.I. 9–3.2), current oral 0.4 (C.I. 0.2–1.1)
Henderson	1994	235	Self/care giver	76 (9)	7:19	×2.7	0.35 (adj)	(0.1–0.8)	Age,education, symptom duration, type menopause, thyroid medication	UCLA; AD/con: MMSE = 29 (1) FU study	$n = 143$ AD, neurological, np/lab examination, MMSE = 7 (8)	Not checked
Mortel	1995	306	Self	73	11:20	×1.8	0.48 (adj, VaD; O.R.: 0.6 ns)	(0.2–1.2)	Age	Houston; Veterans Medical Center FU 12 years	$n = 94$ AD (O.R. 0.6, C.I. 0.3–1.2), 65 VaD (O.R. 0.5, C.I. 0.2–1.2)	Not checked
Paganini-Hill	1994	534	Self	87 (6)	38:46	×1.2	0.69 (adj 0.7, 95% C.I. 0.4–1.2)	(0.5–1.0)	Age, HRT dose and duration, weight, age menarche/menopause, medication use, blood pressure, stroke	Leisure Cohort; AD: death certificate FU 11 years	$n = 71$ AD, 65 other dementias (e.g. "senile dem", "dementia"), death certificate	Duration >7 years and dose (>1.25 mg) effect
Tang	1996	1124	Self	74 (7)	5:15	×3.0	0.40 (adj 0.5)	(0.2–0.9)	Age, education, ethnicity, ApoE genotype	Manhattan; community based FU 1–5 years	$n = 34$ AD, medical records, np tests, imaging	Duration >1 year lowest risk, delays onset
Kawas	1997	472	Self	62 (28–94)	27:46	×1.9	0.46 (adj)	(0.2–1.0)	Age, education, age menarche/menopause (type/duration)	Baltimore Longitudinal Study Aging; FU 16 years	$n = 167$ AD, neurological, lab, imaging	No duration effect (only oral + transdermal HRT effective)
Lerner	1997	246	Surrogate	74 AD, 78 Con	25:45	×1.8	0.41 (adj 0.58 age, 0.9 ns educ)	(0.3–0.9)	Age, education (combined with smoking, O.R. = 0.37, C.I. 0.0–0.7)	Cleveland case-control study	$n = 88$ AD, AD center/neuro geriatrics	Duration effect not checked, possible synergy between smoking and HRT in protection against AD
Balderischi	1998	1568	Self	65–84	3:12	×4.0	0.28 (adj 0.28)	(0.1–0.8)	Age, age menarche/ menopause, education, smoking, alcohol, weight, kids	Italian Longitudinal Study on Aging, cross-sectional	$n = 92$ AD, MMSE, DSM-III, NINCDS, 59 other dementias, 1417 controls (the latter 2 combined O.R.)	Duration not checked, no effect age menopause, BMI, kids, AD later menarche
Slooter	1999	238	Surrogate	58 (7)	10:20	×2.0	0.29 (adj 0.34)	(0.1–0.8)	Age, education (and ApoE genotypes) O.R. same without CVD cases	Rotterdam and Utrecht Aging Study	$n = 109$ AD, early-onset NINCDS/ADRDA	Duration not checked, no interaction ApoE genotype and HRT, although stronger OR with ApoE4 and ApoE2

Table 5 (*continued*)

First author	Year publ.	*n*	HRT info	Age (years)	AD:Con HRT (%)	Risk	O.R.	95% C.I.	Covariates	Study sample	Diagnostic evaluation	Duration/type/dose effects
Waring	1999	444	Medical record	82 (57–96)	5:10	×2.0	0.42 (adj 0.47)	(0.2–1.0)	Age, education, age at menopause, parity	Rochester; medical records of Mayo Clinic, FU 13–17 years	$n = 222$ AD, neurologist (NINCDS), clinical and lab	Duration effect: Con more likely use HRT >6 months, to have oral HRT use + more likely to have breast cancer. No dose effect
Seshadri	2000A	110,931 HRT 110,931 Con	GP data base	66 (10)	Matched 59 cases to 4 controls	×1.0	1.1 (adj)	(0.5–2.9)	Age, gender, physician practice, time enrollment	United Kingdom population based cohort	$n = 59$ probable AD (NINCDS?)	Duration not checked, no other variables (educ, weight, illness etc)
O.R. for HRT and Parkinson's disease												
Marder	1998	167	Surrogate	71/79	4%: 16% (comp PDND: 17%)	×4	0.22 (adj)	(0.1–1.0)	Age, education, ethnicity, ApoE genotype		$n = 87$ with PD + dementia (DSM), $n = 80$ with PD only, $n = 989$ controls	No effect PD, but lower risk dementia in PD. No effect ApoE status

BMI, body mass index; Con, controls; CVD, cerebrovascular disease; DSM, Diagnostic and Statistical Manual of Mental Disorders; E, estrogens; FU, follow-up; HDS, Hamilton Depression Scale; lab, laboratory; np, neuropsychological; NINCDS/ADRDA, National Institute of Neurological and Communicative Disorders and Stroke/Alzheimer's Disease and Related Disorders Association; ns, non-significant; P, progestagens; PD, Parkinson's disease; PDND, Parkinson's disease, no dementia; RT, reaction times; SES, socio-economic status; UCLA, University of California Los Angeles; VaD, Vascular dementia *O.R. and confidence intervals had to be recalculated as the orginal figures included men.

Physicians may be more likely to prescribe HRT to healthy women than to those with dementia, for two reasons. Firstly, women with dementia may be less likely to ask for HRT. Secondly, doctors may be less likely to prescribe HRT due to concerns over consent and compliance. So, doctors may be less likely to start HRT in women with established dementia. This could contribute to HRT's apparent protective effect against dementia. Moreover, physicians may be more likely to stop HRT in women who develop dementia. This could explain findings in which longer HRT use related to less risk of dementia.[137,181,188]

4.4. Educational level and risk factors for Alzheimer's disease

We have already seen that HRT users often have higher levels of education and socio-economic status.[12] Higher levels of education and socio-economic status can affect health-related lifestyles and both these factors protect against AD.[181] It is presently unclear whether HRT's protective effect against AD is direct or merely a correlate of health-related lifestyles. This is because poorly educated women are more likely to develop AD and they are less likely to take HRT. On this reasoning, studies that control for education should yield smaller (but more accurate) estimates of the potential protective effect of HRT. Paradoxically, however, studies that adjusted for education found greater protective effects of HRT against the development of AD [O.R. = 0.46, 95% C.I. = 0.36–0.58; heterogeneity test: $\chi^2(7) = 13.47$, $P = 0.06$] than the ones that did not [O.R. = 0.79, 95% C.I. = 0.58–1.07; heterogeneity test: $\chi^2(5) = 11.15$, $P = 0.05$]. The reason for this paradox is unclear, but the relationship seems strong. In the one study[71] that found a non-significant doubling of the risk of AD in HRT users, the AD subjects were unusual in having more education, higher socio-economic status and healthier lifestyles than healthy controls. Moreover, these findings in studies on the risk of AD are consistent with the recent report by Matthews *et al.*[111] that HRT had larger protective effects on MMSE scores in women with less education. Further studies are needed to clarify the reasons for these surprising, but consistent, findings.

In summary, there are several sources of potential bias in existing case control studies which could explain, partly or wholly, the apparently protective effect of HRT against AD. This list is not exhaustive. We will consider two further possibilities, smoking and apolipoprotein E4 (ApoE4) status. Smoking could bias the relationship between HRT use and dementia. Smokers have an earlier menopause,[89] so they have lower levels of estrogens at an earlier age than non-smokers. Smokers are also less likely to be well educated and are poorer,[63,193] so they may be less likely to use HRT. Recent work has shown that smokers are more likely than non-smokers to develop AD.[121] Finally, HRT may be less effective in smokers, since smoking induces hepatic enzymes, which metabolize estrogen.[119] One study[20] which controlled for smoking found no protective effect of HRT against AD. In contrast, another study[97] reported a possible synergistic effect of smoking and HRT in risk reduction. Further work should test if the apparent protective effect of HRT against AD in other studies is due partly or wholly to the independent relationships of both AD and HRT with smoking. ApoE4 is a strong risk factor for vascular disease and AD. In the Manhattan cohort, women with an ApoE4 allele had a higher risk to develop AD, but this was substantially decreased when

these women were taking HRT. The risk was 0.13 for women who were ApoE4 heterozygote and 0.4 for other ApoE genotypes. None of the ApoE4 homozygotes had taken HRT.[181] Also, in another study, the inverse association between HRT and early-onset AD seemed stronger in ApoE4 carriers (O.R. = 0.37) than women with the ApoE3 genotype (O.R. = 0.60). However, interactions between ApoE4 genotype and HRT use were not significant.[172] Interestingly, Yaffe *et al*.[203] found, in healthy women, that HRT use was associated with a risk reduction for cognitive decline in ApoE4-negative women, but there was no risk reduction of HRT in women with an ApoE4 allele. Steffens *et al*.,[176] in contrast, found that controlling for ApoE genotype did not change the higher MMSE scores in HRT users over time. To further complicate matters, smoking was found to be only a risk factor for AD in subjects without an ApoE4 allele.[134] Clearly, the interactions between HRT and risk factors and protective factors (such as education) need more investigation.

4.5. Effects of hormone replacement therapy on cognitive function in established dementia in epidemiological studies

We turn now to evaluate the effect of HRT on cognition in established dementia. We identified five epidemiological studies (counting Refs 40 and 41 as one study) that compared cognition in demented patients who were taking HRT or not (Table 6). All five found better cognition in users than non-users (binomial $P = 0.03$). The importance of these studies is that confining analysis of HRT's effects to women with dementia automatically excludes some confounds which complicate comparing healthy women with demented women. The remaining potential confounds are those that relate to HRT use in healthy controls (Section 2.3) and to physicians' willingness to prescribe HRT in dementia (Section 4.3). However, it seems unlikely that the former confounds accounted for the cognition-enhancing effects of HRT, since better cognitive function with HRT use survived adjustment for education in four of five studies.[40,66,68,159] It remains possible that physician factors could contribute partly (or wholly) to the apparent cognition-enhancing effects of HRT (see Section 4.3). To exclude this problem requires randomization of demented patients to receive HRT or not. However, within this limitation, the results of the epidemiological studies indicate that HRT may ameliorate the cognitive symptoms of established dementia (Table 6).

4.6. Experimental studies of the effect of hormone replacement therapy on dementia

The results of epidemiological studies, outlined in Tables 5 and 6, indicate that HRT may have therapeutic effects in established AD. However, the interpretation of epidemiological studies is beset with difficulties (such as the confounding effects of educational level and problems ascertaining HRT use). Experimental studies of AD patients can avoid these difficulties (Table 7). We identified 16 such studies. Of these, one study did not use cognitive tests,[87] but only the Hospital Adjustment Scale, which was filled in by nurses. This study is therefore not included in the analyses (Table 7).

It is important first to test whether the overall positive effect (nine of 15) of the studies is due to publication bias.[44] The data show a trend to such bias (see Fig. 1). Initial tests of any treatment usually contain fewer subjects and less rigorous controls than later tests.[44] This is the case for studies on the effect of HRT in AD: the sample size showed a trend to increase over time (Spearman's rho = 0.49, $P = 0.06$). In addition, most of the earlier studies have methodological difficulties. Three tested only AD women using HRT,[48,132,190] three others tested AD patients using HRT vs those without HRT,[75,131,133] one was a crossover study,[47] and eight studies randomized women to receive HRT or placebo and were blinded (Table 7). Of these, two were preliminary results and were not published in full format.[5,17] Only five of eight studies that were controlled showed positive effects and, excluding the abstract reports, this leaves six controlled published studies, of which three found a positive effect of HRT. Most of the earlier studies lacked adequate statistics (e.g. they compared treated subjects and untreated subjects separately against their own baselines, rather than testing treatment × time interaction analyses with MANOVA). This criticism also applies to two of the three controlled studies[17,76] (but see Ref. 4, which reported effects of HRT in treating AD). In contrast, most of the recent controlled studies that did adequate statistics did not find any positive effect of HRT.[67,127,187] Furthermore, the only study that used a sensitive crossover design[47] did not find effects of HRT. Therefore, the apparent positive effects of HRT against AD in earlier reports could well have been due to publication bias.

Five studies[4,76,127,132,133] reported MMSE values, which allowed us to perform meta-analyses. The WMD was significant (2.52, 95% C.I. = 1.67–3.37), but there was significant heterogeneity [$\chi^2(4) = 27.28.12$, $P = 0.0001$], which may reflect publication bias. The two studies[132,133] in which HRT had positive effects on MMSE [WMD = 3.95, 95% C.I. = 2.90–5.00, $z = 7.37$, $P = 0.00001$; heterogeneity test: $\chi^2(1) = 5.40$, $P = 0.02$] had much lower MMSEs (average 14 ± 2 in untreated subjects) than the three negative reports [average 20 ± 5 in controls; WMD = 0.17, 95% C.I. = −1.61 to 1.28, $z = 0.23$, $P = 0.8$; heterogeneity test: $\chi^2(2) = 1.96$, $P = 0.38$]. Hence, it appears that significant differences between studies related more to their untreated subjects or controls than their cases. It is difficult to draw firmer conclusions, since the two positive reports[132,133] were not placebo controlled, randomized or blinded and only compared users to untreated subjects.

Within the limitations of the data, we attempted to define if there is an optimal duration, type or dose of HRT treatment. Three studies indicated[27,87,127] that longer duration (>12 months) HRT does not maintain positive effects. In fact, in the one recent well-designed controlled study,[127] some tests, such as the sensitive Fluency test, showed that after one year subjects on HRT were actually worse than those on placebo. Moreover, this trial also showed that the MMSE was better with HRT after two months, but detected no difference after 12 months. Hence, if there are effects of HRT in established AD, these seem to be short-lived and duration is probably the most important limiting factor on HRT's effects. It is uncertain whether the type of HRT is important. In the controlled trials, CEEs were effective in two of five studies,[17,76] but these used inadequate statistics and measured only up to nine months. Similarly, E2 was only effective short term (eight weeks),[4,5] but not in the long term.[27] There was some indication from two earlier studies that adding a progestagen was detrimental,[132,159] but the only controlled experimental study showed no negative effect of adding a progestagen.[17] The dose of HRT also seemed unimportant, as in the studies

Table 6. Cross-sectional studies assessing use of hormone replacement therapy and cognitive function in demented women

Author	Year	*n*	Source	Age	Design	% users	Neuropsychological test	Covariates
Funk	1991	158	Study of ageing, stroke and dementia: CVD ($n = 51$) vs controls	40–90	Retrospect, longitudinal case-control study	29	HRT users + CVD no cognitive decline (Cognitive Capacity Screening Examination = like MMSE) and trends for increased cerebral perfusion after 18 months	Time onset menopause (controlled: stroke, hypertension, smoking, alcohol, heart disease, diabetes, hyperlipidaemia)
Henderson	1994	235	Community volunteers UCLA clinic	74 (7)	Cross-sectional study	8	AD + HRT (n = 10) MMSE: 15 > AD non-users (n = 128) MMSE 7	Age, education, dementia symptoms, medication
Henderson	1996	35	Community volunteers/ UCLA clinic	74.5 (6)	Cross-sectional study	26	AD + HRT (MMSE: 16 S.D. 7) > AD non-users (MMSE: 12 S.D. 8): better verbal memory (Naming, Digit Span, Fluency) language (Token test). No effect: verbal word list, non-verbal STM, depression (GDS)	Matched age, education, age onset dementia, symptom duration dementia
Schneider	1996	118	Multicenter trials mild to moderate AD	Users: 67 (8) non-users 74 (8)	Clinical trial tacrine	13	HRT users + tacrine ($n = 8$) better response ADAS-Cog (trends in MMSE) after 30 weeks than non-users on placebo ($n = 50$) or tacrine alone ($n = 50$). No effect duration HRT + P use ($n = 8$) worse	Age, education
Doraiswamy	1997	270	Multicenter clinical AD trials (very healthy selection: many excl. criteria)	73 (0.5)	A subset not using psychoactive medication	17	HRT current users better ADAS-Cog, Comprehension, Construction Praxis, Ideational Praxis	Age, education
Doraiswamy	1997	970	Multicenter clinical AD trials (very healthy selection: many excl. criteria)	72 (8)	Clinical drug trials	9	HRT users better ADAS-Cog (and CIBIC = AD scales)	Age, education, age at onset dementia

ADAS-Cog, Alzheimer's Dementia Assessment Scale—cognitive section; CIBIC, Clinician Interview-Based Impression of Change; CVD, cerebrovascular disease; GDS, geriatric depression scale; P, progestagen.; STM, short-term memory; UCLA, University of California Los Angeles.

Table 7. Experimental studies and clinical trials of the effect of hormone replacement therapy in women with Alzheimer's disease

First author	Year publ.	Design	Treated	Controls	Severity	Age	Treatment	Pla	Duration	Outcome
Caldwell	1952	Randomized single blind	13?	15 (match age, educ)	Nursing home	Mean 75	2 mg E2 i.m./week (+20 mg T after 6 months)	y	18 months	WS: improved verbal IQ, comprehension, memory (Wechsler) Stroop, Naming after 12 mnth, then plateau or decline (2/10 tests). Placebo: all decline
Kantor	1973	Randomized double blind	25?	25?	Nursing home	?	0.625 mg CEE	y	3 years	WS: Hospital Adjustment Scale (nurse scores) at 6 and 19 months treatment, peak 12 months then decline to placebo
Fillit	1986	Uncontrolled trial	7 SDAT	None	MMSE 3 (severe) to 14 (mod)	66.5 (5.0)	2 mg E2	n	6 weeks	WS: 3 (older, higher MMSE, lower GDS)/7 pts improved MMSE (14 → 20), memory (Randt test), mood (HDS), no improvement Global Deterioration Scale, Blessed, Mattis Dementia Scale (2/5 tests)
Weiss	1987	Uncontrolled not blinded	5 SDAT	None	MMSE = 12–23, moderate	?	1 mg E2 + nalmefene (>14 days)	n	28 days	Cases: 3 no improvement (MMSE, GDS, Buschke, TMT, DSST, Fluency, HDS = mood), 1 detrioration, 1 drop-out (0/5 tests)
Fillit	1994	Crossover	8 SDAT	None	?	?	0.05 mg E2 transdermal	n	3 months	WS: no improvement MMSE, Paired Associates, mood (Bradburn Affect Balance Scale) (0/3 tests)
Honjo	1989	Untreated controls	7 SDAT	7 SDAT/ 7 Con (nursing)	?	80.1 (2.9)	1.25 mg CEE (= E1-S)	n	6 weeks	WS: 6/7 pts improved memory, orientation, calculation (NSD), 5/7 pts HDS after 3 and 6 weeks (2/2 tests). Untreated + controls no improvements
Honjo	1995	Randomized double blind	7 SDAT	7 SDAT	MMSE 17/18 (mild)	83.7 (4.5)	1.25 mg CEE + P (>4 weeks)	y	7 weeks	WS: 7/7 improved MMSE (18 → 22), NSD, WS + BS: HDS (immediate memory) after 3 weeks (3/3 tests). Slight decrease scores 6th week (P). Controls no change (MMSE 17 → 18, HDS, NSD no improvement)
Ohkura	1994a	Not random not blinded	15 SDAT	15 SDAT	MMSE = 12, mild to severe	71.9 (2.4)	0.625 mg CEE	n	6 weeks	WS: 10 (mild to moderate):15 improved MMSE (12 → 14), 11:15 HDS, CBF increase, EEG pathology decrease. No change in controls, reversal to baseline after stop
Ohkura	1994b	Not random not blinded	10 SDAT	10 SDAT	MMSE = 18, mild to moderate	70.7 (2.1)	0.625 mg CEE	n	5 months	BS: only after 5 mnths HRT less decline MMSE (19 → 21), Con (18 → 15) (for recall and visuoconstruction: 2/4), HRT > Con HDS: for verbal memory and recall events (2/3): 4/7 parameters, 2/2 tests
Ohkura	1995	Uncontrolled not blinded	7 SDAT		Mild to moderate	56–77	0.625 mg CEE + P (in 4 pts)	n	5 months	Cases: 4/7 pts improved MMSE, HDS, 2/7 moderate response, 1/7 no response. Reversal after stop. +P: more subdued/ physical condition worse
Birge	1997	Randomized double blind	10 SDAT	10 SDAT	Mild, CDRS < 2	> 70	0.625 mg CEE + MPA (P)	y	9 months	WS: HRT improved CIBIC (8/10 pts), also improvement Blessed (orientation, concentration, memory), TMT, Paired Associates 3/3 tests. 5/10 controls declined, none improved

Table 7 (*continued*)

First author	Year publ.	Design	Treated	Controls	Severity	Age	Treatment	Pla	Duration	Outcome
Asthana	1999	Randomized double blind	5 SDAT	5 SDAT	MMSE = 20, mild	77–84	0.1 mg E2 transdermal	y	8 weeks	WS: improved verbal memory (Buschke, trends for Naming, Visual Paired Associates) (1/3 tests). No improvement in controls
Asthana	1999	Randomized double blind	6 SDAT	6 SDAT	MMSE = 21, mild	79 (8)	0.05 mg E2 transdermal	y	8 weeks	(M)ANCOVA repeated measures: HRT improved (verbal) memory (Buscke delayed recall, trends for Token test, Visual Reproduction) and attention (Stroop self corrections). No effect on Paragraph Recall, Fluency (S), TMT-A/B, MMSE, Blessed (2/10 tests). E2/NE corr verbal memory. In placebo group no difference
Henderson	2000	Randomized double blind	21 SDAT	19 SDAT	MMSE = 20, mild	78 (1)	1.25 mg CEE	y	16 weeks	BS: no difference ADAS-Cog, CGIC, ADL/IADL, mood, of 13 × 2 test: HRT > TMT (week 4), Pla > Digit Span, Visual memory, verbal memory (delayed Paragraph Recall) (1/13 tests). Correlation E2 level and NE with memory
Wang	2000	Randomized double blind	23 SDAT	24 SDAT	MMSE = 16, mild/moderate	72 (9)	1.25 mg CEE	y	12 weeks	BS: no difference CASI (cognitive battery), MMSE-CE, CIBIC-plus, CDR, HDRS, HARS, BEHAVE-AD, SPECT (0/8 tests)
Mulnard	2000	Randomized double blind	81 SDAT	39 SDAT	MMSE 12–28, mild/moderate	56–91	0.625 and 1.25 mg CEE	y	15 months	BS: only after 2 months HRT better MMSE. After 12 months no diff ADCS-CGIC, MMSE, ADAS-Cog, face recogition, new dot test (memory) Letter cancellation, TMT, SDMT (attention), ADL, mood, Pla > HRT: CDR, Category Fluency, Finger Tapping (0/11 neg: 3/11)

ADAS-Cog, Alzheimer's Dementia Assessment Scale—cognitive section; ADCS, Alzheimer's disease co-operative study; ADL, activities of daily life; BEHAVE-AD, behavioural abnormalities in AD rating scale; BS, between subjects; CASI, Cognitive Abilities Screening Instrument; CBF, cerebral blood flow; CDRS, Clinical dementia rating scale; CGIC, clinical global impression of change 7-point scale; CIBIC, Clinician Interview-Based Impression of Change; Con, controls; educ, education; EEG, electroencephalogram; E1-S, estrone sulphate; GDS, geriatric depression scale; HARS, Hamilton anxiety rating scale; HDS, Hamilton depression scale (mood) or Hasegawa Dementia Scale; IADL, instrumental activities of daily living; i.m., intramuscular injection; MPA, medroxyprogestrone acetate; NE, norepinephrine; NSD, New Screening test for Dementia; P, progestrone; Pla, placebo-controlled; pts, patients; SDAT, senile dementia of the Alzheimer type; SDMT, symbol digital modalities test; SPECT, single photon emission computed tomography; T, testosterone; WS, within subjects (versus baseline).

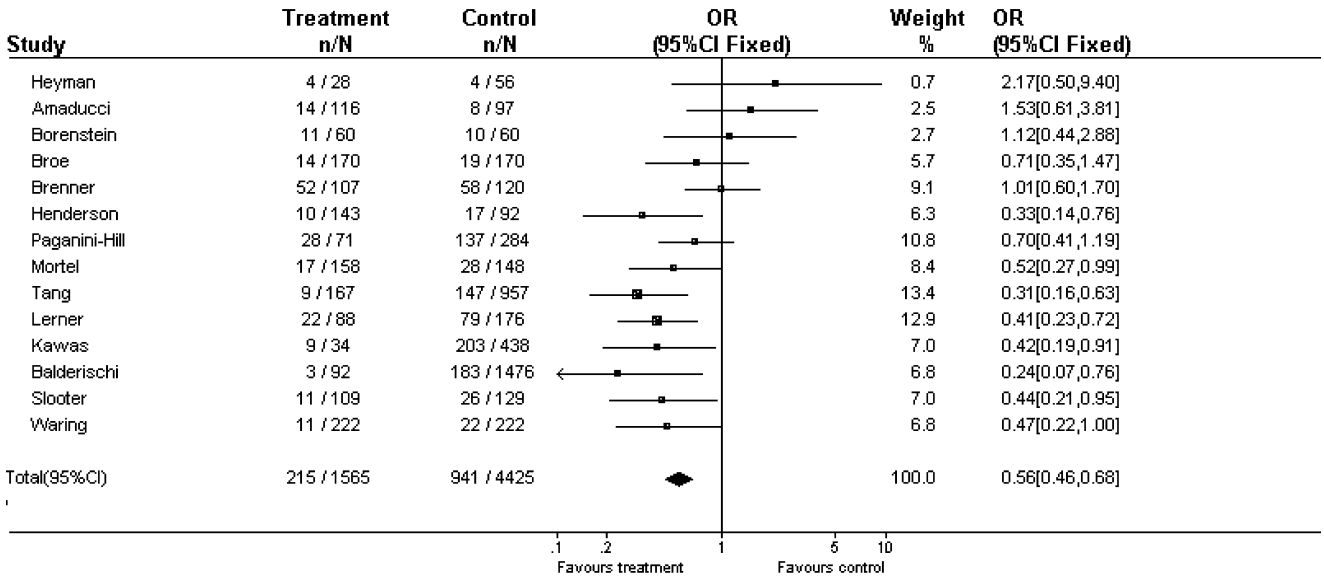

Fig. 1. The meta-analyses of Odds Ratios (O.R.) and 95% confidence intervals (C.I.) of studies investigating the risk of developing AD with the use of HRT. Studies are ordered by year of publication.

involving healthy women (see Section 3.7), the minimum effective dose of transdermal E2 for AD seemed to be about 50 μg per day. Two studies used this low dose: one[4] found cognitive improvements, the other did not.[47]

Finally, two studies evaluated the effects of HRT in combination with putative cognition enhancers. One[190] was negative. The other[159] found that HRT users who received the anticholinesterase tacrine had better cognition than non-users who received placebo or tacrine alone. This interactive effect of tacrine and HRT was maximal at 24 weeks and had lessened by 30 weeks. This is in line with HRT's time-limited effects mentioned earlier.

4.7. Biological plausibility

The cognitive decline in AD is associated with the loss of synaptic connections, the death of neurons and the deposition of amyloid and of neurofibrillary tangles. One or more of these processes could be a potential target for a protective effect of estrogens. Potential effects of estrogens relevant to AD include promoting the survival of cholinergic neurons via estrogen–neurotrophin interactions,[77,169,183] reducing the toxicity of amyloid β-peptide on hippocampal neurons,[113] diminishing excitotoxicity-related glutamate release and glucose deprivation,[55] whilst modulating glutamate transporter-1 expression at the blood–brain barrier,[167,205] reducing accumulation of the neurotoxic β-amyloid fragment of amyloid precursor protein,[80,200] protecting neurons via ApoE-dependent mechanisms,[177] *N*-methyl-D-aspartate 2-D receptor gene/estrogen receptor interactions[22,189] and glutathione.[58]

In addition to AD-specific effects, HRT could protect the brain indirectly through many other mechanisms. It could blunt stress-induced and AD-related elevations of glucocorticoid levels,[116] have anti-oxidant actions,[16,113] lower levels of plasma homocysteine,[2,3,52,98,123,125,198] lower blood pressure,[78,108,122] enhance cerebral blood flow[26,138,148] and vascular reactivity,[88] and potentially protect against atherosclerosis and vascular endothelial dysfunction,[32,53,151,175] all of which may contribute to the risk of cognitive decline and dementia. These latter effects may explain the potential action of estrogen in VaD when it presents either alone or in combination with AD. Lastly, estrogens could theoretically maintain cognition in ageing and dementia through enhancing neurotransmitter synthesis and function.[101–105,169]

Progestagens oppose some effects of estrogens, such as those on noradrenergic function,[14,94,141] dendritic outgrowth[115,196] and vascular function.[156] It is, at present, unclear whether progestagen in combination with HRT actually opposes other potential protective effects of estrogens, but we have seen that this combination is unlikely to significantly influence cognition negatively on a short-term basis.[72,86,99]

The list of estrogen's potential beneficial actions against AD is large and impressive. However, it is based largely on short-term studies in animals. Several longer term studies[24,51] have found a down-regulation of estrogen receptors, which could explain the time-limited effect in the experimental studies found above. Toran-Allerand[182] argued that, because long-term continuous exposure to estrogens results in lowering of estrogen binding to its receptors, women should discontinue treatment for three to four days each month to maintain efficacy. Also, Gibbs[51] suggested adding progestagens to avoid down-regulation of receptors.

5. DISCUSSION

Appraising the results of HRT studies on cognition is a minefield of confounding factors and different HRT regimes and, possibly, publication bias. We have chosen a different path through this minefield than other reviewers. The main difference is that we have provided meta-analyses, both of the WMDs of treatment groups and of the proportions of positive outcomes between studies. Previous reviews relied mainly on qualitative analyses. The variety and inconsistency of the reports that we have documented mean that qualitative methods can support a range of conclusions. Our statistical approach, while complementing other reviews, provides a framework to highlight studies which do not fit general trends.

Our conclusions differ from those of some other reviews. For instance, Sherwin[164] concluded that estrogen maintains verbal memory specifically, but our review could not support

this. Barrett-Connor[12] stated that the enthusiasm for the neuroprotective effect of estrogen has outrun the actual data, since all observational studies have limitations and available clinical trial results are few and small. However, we found a sufficient number of trials to test a variety of hypotheses (e.g. concerning differences between epidemiological and experimental studies). Haskell *et al.*[64] stated that "the available clinical trials provide insufficient evidence to support the conclusion that HRT improves cognitive function", but we found evidence of small effects. Yaffe *et al.*[202] also concluded that the effects of HRT in healthy women are probably biased by a reduction of menopausal symptoms. Our review did not find consistent evidence of this. Some of the differences between our conclusions and those of other studies may reflect the different methods which we used (see above).

The main extrinsic limitation of our review, in common with all previously published reviews, was that the quality of many of the target studies is uneven or poor. One reason for this is that research on cognition in the elderly is inherently difficult. Some of this difficulty stems from the heterogeneity of subjects and the severity of age-related cognitive decline. This relates to factors such as education, socio-economic status and health status,[83] which are also important confounding factors in the relation between HRT and cognitive performance. We have seen that such confounds can explain some of the discrepancies between studies. It is almost impossible to eliminate them from epidemiological work because women who used HRT after the menopause were better educated[92] and healthier before it[112] compared to non-users. Previous authors have viewed these confounds as seriously undermining the apparent protective effects of HRT on cognition. However, to our surprise, evidence from studies of both healthy women and those with AD indicated that beneficial effects of HRT on cognition were larger in studies which controlled for education. This paradoxical pattern of results requires further investigation. Large prospective randomized placebo-controlled studies which could address this are currently in progress in the USA (WHIMS)[168] and in the UK (WISDOM). If the finding that HRT is efficacious in less educated women is confirmed, this would add more support to the recommendation of HRT for prophylaxis against AD.

Our review has several intrinsic limitations. These relate to the statistics we used. First, our meta-analyses were biased because negative studies were less likely to report data in a form that we could use. To get around this, we performed further tests that analysed overall study outcomes, regardless of the size of each study or of the size of its positive effects. These analyses could not, therefore, summarize HRT's effects over several studies if each found small non-significant trends in the same direction. This insensitivity parallels the deficiency we have already identified in individual studies, since none used MANOVA to assess HRT's effects over a range of cognitive tests. A second statistical limitation is that our analyses did not take into account the fact that some of the data for individual tests were repeated measures on the same subjects. This approach is statistically invalid, because tests which assume independence are less sensitive to contrasts within subjects. Our justification for this is that our positive findings may therefore under-estimate differences between HRT's effects on different tests.

A further potential limitation of the review is that it gathered only data from published reports, so it may be open to publication bias. This is difficult to assess, since most studies did not report effects in a manner allowing incorporation of their results into meta-analyses appropriate to detect publication bias. The studies that analysed the effects of HRT in AD patients showed some evidence of publication bias, but those that compared the risk of developing AD in HRT users and non-users did not.

Most surprising were the findings that higher levels of estrone related to worse cognition in healthy women and that CEEs, containing mainly E1 sulphate, may even have worsened dementia.[127] This result is paradoxical, given the physiological basis of this research (i.e. that a lack of estrogen may cause cognitive decline and AD; see Introduction). The paradox is even stronger when we recall that, among the epidemiological studies in healthy women, HRT that contains estrone (CEEs) generally enhanced cognition, but among the experimental studies CEEs were ineffective. Further research is needed to decide whether there is a type of HRT that would be effective for enhancing cognition in healthy women or for protecting against dementia. It has been suggested that continuous treatment with HRT down-regulates receptors.[24] Possibly, adding progestagens may reduce this risk,[51] as well as reducing the risk of endometrial cancer.[28] While some studies suggest that progestagens could oppose biochemically many of the protective effects of estrogens, this does not seem likely to affect cognitive performance (see Section 4.6).

6. CONCLUDING REMARKS

In summary, we found small, but inconsistent, effects of HRT on several aspects of cognitive function, such as memory, attention, reaction time speed and abstract reasoning, in healthy women. These effects are not explained by the alleviation of depression or climacteric symptoms, or the use of combination therapy. In general, epidemiological studies showed more positive effects of HRT than experimental studies. This is not related to their sample size. Rather, women are healthier before they start using HRT than those who do not use HRT and controlling for socio-economic status decreases HRT's effects. For AD, diminished recall of HRT use by patients may be an important confound, as well as physician behaviour. Surprisingly, women with low education seem to benefit more from HRT. It is, at present, unclear whether long-term HRT can protect cognition and diminish the risk of AD, as there is a paucity of long-term studies in elderly healthy women. In the AD trials, the HRT effect seemed to be time limited. The large controlled studies currently underway will hopefully address these issues. However, both these studies use Premarin, the most widely prescribed drug, which has not been shown to have effects on cognitive function in experimental studies. Interactions of HRT use with age, menopausal status, and risk and protective factors (such as education, smoking and ApoE genotype), and the actual level of bioavailable estrogen obtained, may be important to assess whether HRT actually affects cognitive function in elderly women over a longer period of time.

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