

## Review Article

# Potential role of estrogen in the pathobiology and prevention of Alzheimer's disease

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**Abstract:** Over a decade of converging findings from clinical, observational and basic science research indicate that estrogen administration during the menopausal transition exerts beneficial effects on cognition and decreases a woman's risk of developing Alzheimer's disease (AD) later in life. This review article stresses the research focus of AD prevention, and introduces hormone therapy (HT) as a probable catalyst that may achieve this goal. Furthermore, this article outlines 3 mechanisms proposed to mediate estrogen's beneficial effects, discusses the controversy surrounding HT administration, and presents the most promising estrogen related research in AD prevention and treatment. Although controversial, cumulative evidence suggests that the potential of estrogen initiated during perimenopause to prevent AD needs to be systematically evaluated.

**Key Words:** Estrogen, estrogen therapy, hormone therapy, cognition, Alzheimer's disease, postmenopausal cognition, women's health initiative

## Introduction to Alzheimer's disease and hormone therapy

### Background

With the expected surge of the elderly population in the coming decades, the prevalence of Alzheimer's disease (AD) is projected to increase dramatically [1, 2]. These estimates emphasize the importance of early detection, intervention and ultimately prevention rather than treatment. A delay in the onset of disease by as few as five years is projected to have a substantial beneficial effect, reducing the prevalence of AD by 50% in one generation [1]. Basic science, observational, epidemiological and clinical research suggests that hormone therapy (HT) may prevent cognitive decline associated with AD in postmenopausal women up to 34% ([3] see Brann et al. [4] Miller et al. [5] Hogervorst et al. [6] Fillit [7] and Gleason et al. [8] for reviews). Multiple mechanisms have been posited to explain estrogen's protective effects

on AD. The current review will focus on the importance of AD prevention in terms of three of the most highly promising mechanisms, including neuromodulation, neuroprotection, and cerebrovascular regulation. Additionally, basic science, observational and clinical studies examining the relationship between HT administration and AD prevention will be discussed.

The main symptom of AD is episodic memory loss, with deficits in other cognitive domains, such as working memory and language, which usually appear later. At present, drugs designed to treat AD, are mainly cholinesterase inhibitors, which work by preventing synaptic breakdown of acetylcholine in the brain. However, cholinesterase inhibitors alleviate only some AD symptoms and a positive treatment response is seen in a considerably small subset of the affected population. While an ideal pharmacologic treatment for AD should be directed towards multiple pathophysiologic

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mechanisms, have the potential to favorably alter disease neurobiology and be administered as early in the disease process as possible, the most promising defense against AD onset and progression is AD prevention.

### ***Prevention vs. treatment***

There is increasing evidence that the pathology of AD starts decades before the onset of the clinical symptoms. Once the symptoms of AD manifest, significant neuronal loss has already occurred and, as of now, disease progression cannot be reversed. As such, the ideal time for both early detection and intervention is at the beginning of the prodromal (and functionally "silent") period of neuropathologic change, rather than years later at the appearance of the dementia syndrome. Though research has yet to pinpoint exactly when neuropathologic changes begin, there is compelling evidence that the drastic drop in estrogen levels during the menopausal transition is closely linked to an increased risk of cognitive decline and subsequent AD in women. Thus, beginning a HT regimen during the menopausal transition is likely to delay the onset of AD, and could possibly prevent AD in a significant number of women.

**Table 1** illustrates clinical studies investigating the cognitive actions of estrogen in healthy, postmenopausal women. For the most part, clinical data indicate cognitive benefits in women using HT. Such findings led to questions regarding HT's potential to reduce the risk of developing AD later in life. The results reported in **Table 1** suggest that the beneficial effects of estrogen play a role in AD prevention, because the studies were conducted in women without AD [9-11]. Retrospective and observational studies are also consistent with these positive outcomes, particularly in women who began HT during the menopausal transition [10, 12, 13]. The comparable benefit seen as a result of estrogen/HT suggest that for the most part, the data within the observational studies were derived from women with healthy neurological status [14].

### **Controversies in hormone therapy**

#### ***Background***

While the majority of research studies suggest that beginning a HT regimen during the menopausal transition is likely to delay and decrease the risk of developing AD later in life, the subject of HT administration remains controversial [15]. The source of much debate surrounding HT can be traced back to findings from the seminal Women's Health Initiative [16] (WHI) and its two ancillary studies, the WHI Memory Study [17] (WHIMS) and the WHI Study of Cognitive Aging [18] (WHISCA), which characterized the cognitive efficacy and adverse effect profile of conjugated equine estrogen (CEE) plus medroxyprogesterone acetate (MPA) in older postmenopausal women. The WHIMS found an increased risk for dementia in postmenopausal women aged 65 and older, treated with CEE and MPA, though prior and subsequent research consistently shows that HT improves cognition, likely via multiple neural mechanisms, and may reduce a woman's risk for dementia and [19-21]. The surprising findings from WHIMS indicated an increased risk of dementia and cognitive decline with prolonged administration of CEE [17, 18, 22-24], and prior findings from cohort studies were attributed to inherent flaws in epidemiological research, such as the 'healthy-user' bias. However, support for potential salutary effects of estrogen on cognition and risk for dementia continues to mount from observational (including WHIMS data) [25] as well as prospective cohort studies [3, 10, 26-34]. Some researchers have argued that the WHI was not a true primary prevention study, given the advanced age of participants and the likelihood that they were beyond the opportunity to 'prevent' disease [35]. Moreover, it is speculated that the type of estrogen employed in the WHI, opposed CEE, might underlie the increased risk for dementia and cerebrovascular changes [36].

#### ***HRT preparation***

The WHI employed the most commonly used form of HT, oral Prempro® and Premarin®. Rather than mimicking premenopausal hormone profile or cycles, these hormones elevate steady-state levels of estrone and nine other estrogens [35, 37], and in the case of Prempro®, a synthetic form of progesterone, MPA. Prior to menopause, the predominant circulating estrogen is ovarian-produced estradiol, while after menopause the primary source of estradiol is peripheral conversion of

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**Table 1.** Summary of Clinical Studies Examining the Cognitive Effects of HT

	INVESTIGATOR(S)	OUTCOMES	
Estradiol Preparations	Caldwell & Watson	Improvement in learning and memory	Benefit
	Rauramo et al.	No improvement on tests of memory or reaction time (RT)	No Benefit
	Fedor-Freyburgh	Improvement on RT, visual attention, selective attention, & memory	Benefit
	Sherwin	Treatment improved verbal memory & abstract verbal reasoning	Benefit
	Sherwin & Phillips	Treatment improved verbal memory	Benefit
	Phillips & Sherwin	Treatment improved verbal memory	Benefit
	Rudolph et al.	Benefited subjective well-being & to a lesser extent conc. & atten	Benefit
	Linzmayr et al.	Improved verbal & visual memory & information processing speed	Benefit
	Saletu	Treatment benefited sleep and selected cognitive abilities	Benefit
	Polo-Kantola et al. & Alhola et al.	No differences between treatment & placebo with 3 mo intervention. Follow-up 6 years later, still no difference	No Benefit
	Hogervorst et al.	Trend for improved verbal memory on treatment	Benefit
	Wolf et al.	No group differences but correlation between estrogen level & cognitive performance on verbal memory measures	Benefit
	Duka et al.	Treatment improved memory & nonverbal reasoning	Benefit
	Viscoli et al.	2 <sup>nd</sup> -ary prevention trial in women with recent history of stroke. Less decline over 3 years on MMSE for cognitively normal women on HT. But no benefit for entire group (when baseline MMSE not considered)	Benefit
	Taxel et al.	Men on hormone suppression for prostate CA received E2 or placebo	No Benefit
	Dunkin et al.	Only weak support for overall benefit of E2; but data suggest that women with increased lifetime exposure or recent exposure benefited	Benefit
	Schiff et al.	12 wks of TX (cross-over design) improved RT, but no other ability	No Benefit
	Almeida et al.	20 weeks of 2mg/day tx on cognition, mood and QOL questionnaires	No Benefit
	Yaffe et al.	1 and 2 yrs of an "ultra low dose" ( 0.014 mg/d)	No Benefit
	Dumas et al.	After 3 mos tx administered Scopolamine a cholinergic challenge Women on E2 do better on measures of atten, RT & speeded fx	Benefit
Krug et al.	3-day treatment resulting in E2 levels similar to mid-cycle premenopause resulted in improved performance on tests of prefrontal cortex executive function	Benefit	
Hackman & Galbraith	Treatment improved score on memory test , using piperazine estrone sulfate	Benefit	
Campbell & Whitehead	Improved memory with treatment	Benefit	
Estrone Preparations	Honjo et al.	Treatment group improved on screening tests for dementia	Benefit
	Ditkoff et al.	No benefit on tests of learning, & auditory & visual atten (WAIS)	No Benefit
	Janowsky et al.	No improvement on working memory with treatment	No Benefit
	Binder et al.	No benefit with treatment on comprehensive battery of neuropsychological tests	No Benefit
	Grady et al.	No benefit on cognitive tests for women with coronary heart disease	No Benefit
	Woo et al.	Improved atten, delayed recall & score on global cognitive fx	Benefit
	Shaywitz et al.	CEEs improved reading ability and verbal memory	Benefit
	Pan et al.	Nonsignificant trend for benefit on global cognitive measures	Benefit
	WHIMS	Decline on 3MSE & twice the risk for dementia with CEE	Harmful
	WHISCA	Less decline on visual spatial construction & memory, but greater declines on learning & recall with opposed CEE	Benefit & Harm
Maki et al.	Borderline significance suggesting minimally worse verbal memory with CEE. (notably enrollment and study completion adversely affected by WHIM per study authors).	Harm	
Vanhulle & Demol	<b>Estriol</b> had no effect on intell, atten & conc or speeded processing	No Benefit	

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androstenedione [38]. Estradiol is a very potent naturally occurring estrogen, while estrone and estriol are reported to have lower binding affinities, and thus may fail to induce the full array of neuronal changes induced by estradiol [38]. The dramatic decrease in circulating estradiol, and subsequent increase in gonadotropins may explain the increased risk for cardiac disease and osteoporosis, and possibly for dementia [39-41]. Thus, in order to enhance receptor-dependent cognitive actions of estrogen and to more closely mimic the premenopausal state, estradiol may be a more appropriate replacement strategy. This assertion is supported by **Table 1**, which shows that trials using estradiol preparations more often demonstrated cognitive benefits than did investigations utilizing a CEE.

Preliminary findings from recent studies suggest that, unlike oral conjugated estrogen, transdermal estradiol enhanced cognitive function for postmenopausal women with AD [15, 42]. This may be related to the absence of venous thromboembolic complications [43], and plasma markers of inflammation [44] associated with transdermal estrogen administration. Thus, it may be that not only is the form of HT significant, but the route of administration may be equally critical. Many in the scientific community agree that while the risk of dementia associated with the administration of Prempro® has been clarified for women over the age of 65, research regarding the use of other forms of HT for primary prevention of dementia is highly promising, particularly if estradiol is administered during the menopausal transition, before the period of AD associated neuropathologic change begins [45, 46].

### **Mechanisms mediating the estrogen - Alzheimer's disease relationship**

#### ***Estrogen and neuromodulation***

##### *The Alzheimer's disease brain - estrogen receptors*

Many of the structural and functional aspects of the AD brain are influenced by both endogenous and exogenous estrogen levels. Examination of the AD brain at autopsy reveals marked atrophy in the basal forebrain and mesial temporal structures (i.e. the amygdala, entorhinal cortex and particularly the hippocampus). There is a significant loss of

large pyramidal neurons but the primary sensory cortices are largely spared [47], and the amount of neuronal loss correlates well with functional dementia and cognitive test scores [48]. Moreover, the clinical symptomology corresponds closely to the regional progression of brain change over time (i.e. hippocampus and memory). Also, there is considerable synaptic loss in the association neocortex as compared to control brains. Therefore, AD manifests as selected neuronal loss and decreased synaptic connections in a temporally selective manner.

There is compelling scientific evidence indicating the neuromodulatory efficacy of estrogen and HT, which is directly relevant to AD neuropathology and prevention. Estrogen exerts its biological actions through estrogen receptors (ER) distributed selectively throughout the brain. Several of these brain regions, namely the hippocampus, mediate various cognitive functions including memory, and are selectively affected by AD pathology. These brain regions include the association cortices, midbrain, brainstem, basal forebrain, pituitary gland, hypothalamus, amygdala and particularly the hippocampus [49-53].

##### *Estrogen's effects on brain regions important to memory*

Estrogen has been shown to increase synaptic protein expression [54], synaptic structural plasticity [55] and density of pyramidal cells in the hippocampus [56, 57] via the activation of ER $\alpha$  and ER $\beta$  expressed in the CA1 neuronal field [58-61] and prefrontal cortex [62]. Consistent with these structural changes, investigators report enhanced working memory performance as measured by the Morris water maze task within the time frame of estrogen-induced increases in hippocampal pyramidal cell density [63]. Additionally, estradiol has recently been shown to induce changes in the structural mechanics of cells via mRNA expression levels within the dorsal hippocampus, which could be conducive to promoting memory encoding and consolidation [64]. These results suggest that estrogen-dependent changes in the hippocampus are, at least in part, responsible for the hormonal effects on cognitive functions seen across the menopausal transition [65]. Thus, estrogen's salutary action on these brain regions is a highly promising mechanism in AD prevention.

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### *Estrogen's interaction with neurotransmitter systems*

In addition to estrogen's actions on specific brain regions involved in memory and vulnerable to AD, there is cumulative scientific evidence portraying the facilitative role of estrogen on various neurotransmitter systems (See van Amelsvoort et al. [66] and Yaffe [67] for reviews). Among others, neurotransmitters influenced by estrogen include acetylcholine, serotonin, and the catecholamines (dopamine, epinephrine, and norepinephrine), all of which have been implicated in the modulation of the cognitive processes specifically affected by the pathology of AD [68]. Of particular importance is the cholinergic system, a system integrally involved in several cognitive processes, including attention, learning, memory, and arousal. In patients with AD, notable and early deficits are found in the basal forebrain, the primary seat of cholinergic activity. As noted earlier, the current FDA approved treatments for the disease consist mainly of medications designed to enhance cholinergic function. Importantly, basic research suggests that estrogen facilitates the actions of the cholinergic system [69-75]. Thus, the precipitous drop in estrogen during menopause could contribute to cognitive dysfunction based on the deregulation of acetylcholine, suggesting that HT administration during this important period may combat cognitive decline and later AD. Estrogen appears to also influence neuronal communication through the serotonergic (5-HT) [70, 76-78], dopaminergic [79-82], noradrenergic and adrenergic [83-86] neurotransmitter systems. Thus, estrogen appears to have multiple and complex effects via neuromodulation, and therefore likely serves as a probable contender in regard to AD prevention.

### ***Estrogen and neuroprotection***

Neuroprotection is the process of protecting the brain from neuronal injury or the clinically relevant delaying of disease progression [87]. Here, we discuss some of the neuroprotective effects of estrogen, which have direct implications for AD prevention. Basic science has established that estrogen, particularly 17 $\beta$ -estradiol, protects the brain from various types of insults and injury, including ischemic stroke [88, 89], beta-amyloid (A $\beta$ ) neurotoxicity [90, 91] oxidative damage [92] and apoptosis

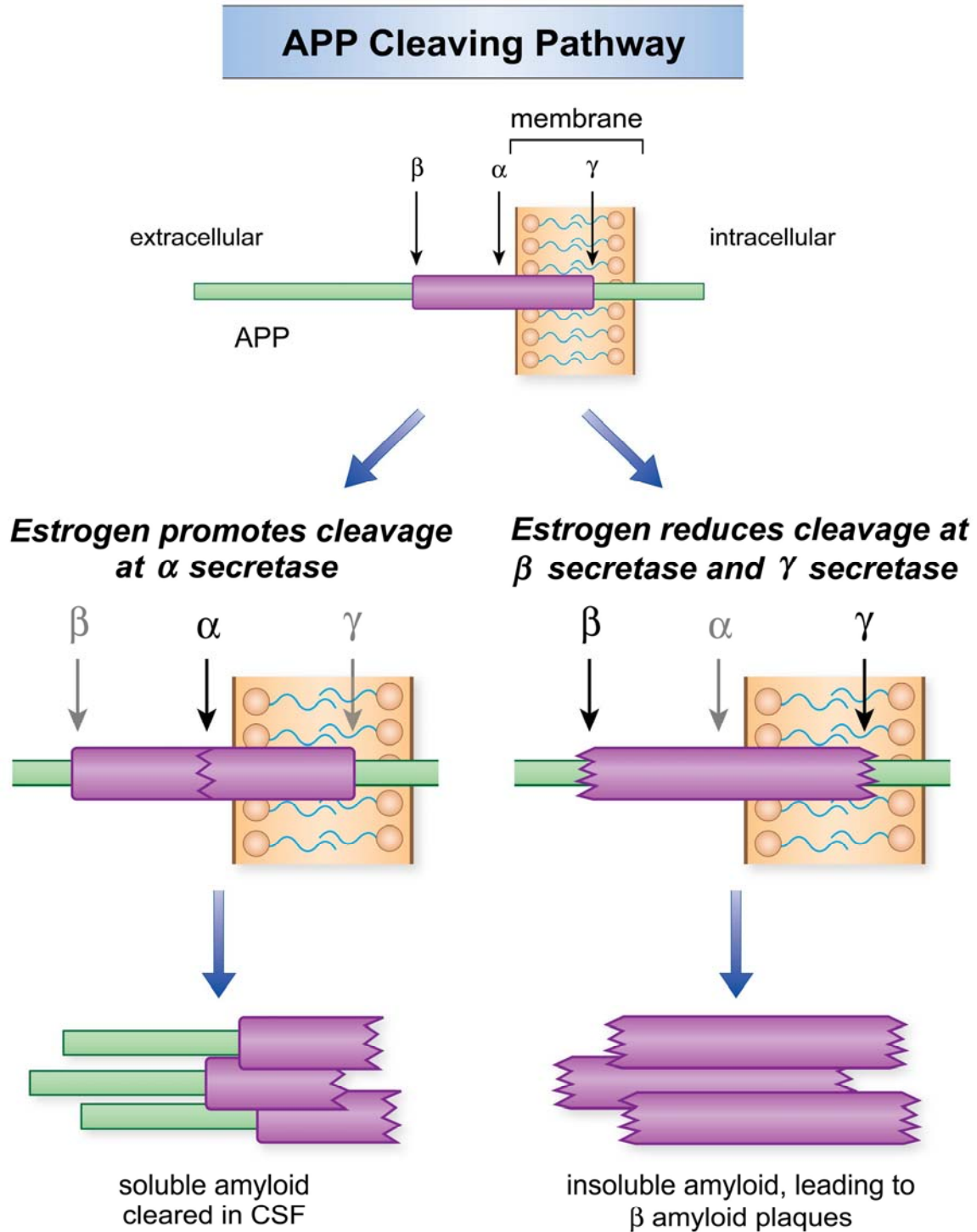
[80]. Estrogen has also been shown to enhance recovery from traumatic brain injury following cerebral ischemia [93] and to reduce brain inflammation, [94] especially inflammation due to A $\beta$  [95]. Additionally, a recent study by Brinton confirmed that estradiol regulates mitochondrial function and enhances aerobic glycolysis [14], which have been shown to be precipitating factors in age-associated neurodegenerative diseases such as AD [96, 97].

### *Estrogen's effect on beta amyloid (A $\beta$ )*

Plaques are one of two primary types of pathology associated with AD. The neuritic plaques are extracellular protein aggregates of A $\beta$ , decorated with complement components, lipoproteins, and a variety of acute phase proteins which are surrounded by degenerating nerve endings and activated microglia and astrocytes [98]. A $\beta$  deposition is a hallmark of AD, and used as a biomarker of AD risk and development. Many studies suggest that the estrogenic component of HT is responsible for reducing A $\beta$  levels [99]. Thus, A $\beta$  reduction is a principal biomarker by which many basic scientists gauge the beneficial effects of HT. As mentioned earlier, estrogen has been shown to reduce inflammatory responses to A $\beta$  in humans. In addition, studies show that estrogen improves CSF clearance of insoluble A $\beta$ , while increasing amounts of non-toxic soluble A $\beta$  [35] (See **Figure 1**). Also, in vitro findings from cultured neurons suggest that estrogen may prevent AD pathogenesis by protecting against A $\beta$ -mediated neurotoxicity, and enhancing A $\beta$  clearance [90]. Based on the widely acknowledged relationship between estrogen, A $\beta$  and AD, there is little doubt that the postmenopausal decline in estrogen levels contribute to detrimental cognitive effects and a higher risk profile for AD development. Hence, prolonging premenopausal estradiol levels could serve to delay, or perhaps prevent AD by preventing A $\beta$  neurotoxicity.

### *Estrogen and apolipoprotein E (ApoE)*

The E4 allele of the apolipoprotein E (ApoE) gene is a well known genetic risk factor for AD. The influence of estrogen on ApoE expression is a relatively new area, and while some studies have found that estrogen exerts beneficial actions on ApoE upregulation and



**Figure 1.** Illustration of amyloid precursor protein (APP) cleaving pathway. Estrogen reduces cleavage at  $\beta$  secretase and  $\gamma$  secretase, shown in the right arm of the figure, which reduces accumulation of toxic  $\beta$  amyloid plaques.

expression, the true relationship remains unclear [100]. First, some research suggests an upregulation of ApoE with estrogen

administration [101-103]. However, a related line of research reports that only the E4 carriers experience the beneficial cognitive

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effects of HT [19, 104], while others have failed to find a statistically significant interaction between HT's neuroprotective effects and ApoE status [10]. Wang et al. has reported that ER $\alpha$  increases and ER $\beta$  decreases ApoE expression in the hippocampus in rats [105]. They have argued that the interaction of E4 allele status and estrogen receptor type may partially explain the complex results in human clinical trials of HT on cognitive function. Other studies have reported that HT increases the risk of AD in those with the E4 allele and to decrease risk in those with the E2 or E3 allele [106, 107]. Wang suggested that an increase of ER $\alpha$  combined with HT will increase ApoE which would selectively increase the risk of cognitive impairment in ApoE 4 positive individuals, in a heterogeneous population in a large clinical trial [105]. Indeed, research investigators examining the effects of estrogen on AD treatment and prevention should consider ApoE4 status and the potential impact this AD risk factor may have on the results of estrogen studies.

### ***Estrogen's Neurovascular Effects***

One of the most promising areas of research linking HT administration to AD prevention involves estrogen's salutary effects on cerebral blood flow (CBF). Estrogen's influence on CBF can in part be attributed to the hormone's multiple and well-documented actions on cerebral vasculature. These effects have been shown to be mediated through ER $\alpha$  and ER $\beta$  [108-110] as well as numerous non-genomic influences on signaling pathways, induced by activation of membrane receptors [111-114] and alterations of membrane ion channels [115-117]. Thus, estrogen imparts both rapid and delayed effects upon cerebral vessels [113]. One means through which estrogen increases CBF is through its effects on dilation of blood vessels via improved vascular reactivity [118, 119] and endothelial function [120-124]. Both smooth muscle and endothelial layer cerebral vessels express estrogen receptors [118]. Cerebral vascular dilation related to improved endothelial function appears to result from estrogen's influences on vasoregulators, such as nitric oxide (NO) and prostanoids. For example, estrogen has extensive effects upon eNOS and nNOS regulation and activity [125-133], influencing eNOS production of NO through

various processes, including sensitizing eNOS to calcium activation through phosphorylation [114] and increased calmodulin production [129], and decreasing inhibition of eNOS by the caveolin-1 protein [113, 129]. Of particular relevance to the current review, estrogen effects on NO production have been found to occur in the hippocampus [134] and forebrain areas [135], primary regions showing neurodegeneration in AD.

Estrogen also increases vasodilation through its effects on prostanoids (e.g., prostacyclin (PGI<sub>2</sub>)). The influence of PGI<sub>2</sub> upon vasculature appears to occur through increased eNOS activity, possibly mediated by the enzyme cyclooxygenase-1 [136-138] (COX-1). Additional mechanisms through which estrogen optimizes endothelium vasodilatory responses include interactions with vasoreactive substances other than NO [139, 140] and changes in smooth muscle responses [116, 141]. Also, estrogen has been shown to favorably influence several other aspects downstream of cerebral perfusion, including improved mitochondrial energy metabolism [142-145] protection from by-products mitochondrial energy metabolism (reactive oxygen species (ROS) and peroxides) [143], and reduced thrombotic [146] and endothelial inflammatory activity [147-150]. Moreover, there appears to be both rapid [111, 113, 151-157] and long-term [158-160] effects upon cerebral vascular response. While it remains to be proven, the above data along with new evidence of HT effects in ApoE E4 carriers [161, 162], it is plausible that HT-related improvements in CBF may offer some protection or delay the progression of AD in women if administered during the menopausal transition.

### ***Estrogen's Effects on Cognition***

In addition to the neuromodulatory, neuroprotective and the cerebrovascular effects of estrogen on AD prevention, further support can be found within the cognitive neuropsychology literature. Of note, estradiol formulations appear to provide a more promising outcome with regards to cognitive benefits, particularly in older populations. For instance, bioavailable estradiol has been found to be more predictive of positive cognitive changes over time than estrone [6]. There is well-documented and compelling evidence to support estrogen's neurobiological

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role in cognitive processes. (See McEwen [163] for review.) Specifically, estradiol has been linked to improved performance on tasks of attention, visuospatial ability, learning and memory, and particularly verbal ability. The recognition of estrogen's numerous neurotrophic and neuroprotective actions suggests a possible deleterious effect of chronic estrogen deprivation, both in healthy aging and disease processes. The cognitive data also supports the theory that the dramatic decrease in estrogen levels occurring at menopause could result in neuronal dysregulation and loss of protective actions, and perhaps account for women's increased risk for AD [39-41]. In sum, a review of the scientific literature indicates that estrogen has cognitively beneficial properties and administration of HT during the menopausal transition will likely reduce the cognitive decline associated with AD symptomology.

### **Research Investigating the Relationship between Estrogen and Alzheimer's Disease**

#### ***Estrogen and AD Prevention***

Research investigating the effects of estrogen on AD has been comprehensive and interdisciplinary. The large amount of research has spanned from the basic mechanistic effects of estrogen on AD risk and prevention to double-blind, randomized, placebo controlled studies investigating the effects of HT on participants already afflicted with AD. With the exception of a few studies, namely the WHI and the WHIMS, most research supports a beneficial effect of estrogen on cognition and subsequent AD prevention. Although the expanse of literature is beyond the scope of this review, we discuss here, some of the most important and most recent scientific research studies investigating the effects of estrogen on AD.

#### ***Estrogen in Basic Science Models of AD***

Basic science analyses examining the neuromodulatory and neuroprotective effects of estrogen were essential to the development of an estrogen-mediated model of AD prevention [14]. Like epidemiological studies, evidence from in vitro and in vivo animal studies supporting estrogen's favorable neuroprotective effects have generally been conducted using estradiol formulations, as opposed to estrone. Overall, mouse models

have shown that estradiol enhances neuroplasticity, induces anti-inflammatory and cytoprotective effects, reduces A $\beta$  plaques from its precursor protein, and modulates glucose metabolism. In one well designed mouse model of AD, investigators found that estrogen-deficient mice exhibited greatly increased A $\beta$  deposition and production [99]. In the same study, investigators measured estradiol levels in the brain tissue of women with and without AD. Results showed that AD patients had significantly reduced estradiol levels in the frontal cortex, a region characteristically affected in AD [99]. In another well conducted, recent line of research, estradiol was administered intraperitoneally in rats immediately following training on the Morris water maze task. Results showed that estradiol enhanced both spatial reference memory [164, 165] and novel object recognition memory [166] in mice and rats. Finally, a handful of researchers have reported that estrogen exerts neuroprotective actions via preventing the phosphorylation of tau, a microtubule-associated protein closely associated with AD. Specifically, 17 $\beta$  estradiol can reportedly prevent neural tau hyperphosphorylation at multiple AD-related sites and also attenuate the forskolin-induced elevation of cAMP and activation of PKA [167]. These findings, along with the results of similar basic models of AD, provide evidence that estrogen is a viable option for prevention of AD in postmenopausal women.

#### ***Observational Studies in Healthy Participants***

While there is an abundance of observational research investigating the effect of HT on AD, evidence from two large observational studies [161, 162] provide the most direct evidence to date that HT can modify biomarkers of early disease related changes. Investigators found a neuroprotective effect of estradiol in a sample of women 'at risk' for developing AD based on the ApoE E4 genotype. Results indicated that estradiol therapy protected the 'at risk' women from AD associated changes, including hippocampal atrophy and diminished hippocampal neuronal metabolism volumes [161, 162]. Importantly, the neuroprotective effect was limited to neurobiology, as no difference in cognitive performance was detected between HT users and non-users. Of note, while participants in this sample were considered 'at risk,' they were not exhibiting



AD symptoms and had not been diagnosed, so deficits in cognition would not be expected. This study not only lends further support to the growing body of research showing a beneficial effect of estradiol in AD, but it also brings AD prevention and neuroprotection to the forefront. These promising findings offer the most direct link between HT usage and protection from early AD associated brain changes.

### *Estrogen Studies in Women with AD*

While the focus of this review is the prevention of AD, it is worth mentioning that some clinical research has reported a favorable effect of HT administration in patients after a diagnosis of AD has been made. We discuss these studies because they lend further credence to estrogen's salutary properties in regard to AD specifically. Thus far, three clinical studies have supported a cognitive benefit in women with AD [15, 168, 169]. An early, double-blind, placebo-controlled study of 14 women with AD (aged  $83.7 \pm 4.5$  y; mean  $\pm$  SD) suggested that CEE improved performance on the MMSE and the Hasegawa Dementia Scale (HDS-R). The seven women receiving 1.25 mg CEE showed significant memory score improvements in the MMSE after 3 weeks, while the placebo group did not display any significant change in the MMSE or HDS-R score.

Asthana et al. conducted a placebo-controlled, double-blind, parallel-group clinical study to evaluate the cognitive and neuroendocrine response to transdermal 17  $\beta$ -estradiol in women with AD [168]. The treatment group exhibited improved attention, and verbal memory. Moreover, scores were directly correlated with plasma concentrations of estradiol. In a follow-up study, Asthana et al. reported that treatment with a high dose (0.10 mg per day) of 17 $\beta$ -estradiol further enhanced cognitive task performance on tests of attention, verbal memory, visual memory and semantic memory in women with AD. Findings were replicated in a meta-analysis [15] and are consistent with those reported in several uncontrolled estradiol studies. These results provide evidence of a beneficial effect of estrogen on cognition in postmenopausal women with AD, and lend further support to HT prevention models of AD.

### **AD Risk Factors and Research**

Although the current review has only skimmed the surface of the estrogen / AD relationship, it is clear that multiple factors warrant consideration when undertaking a project such as those described here. Several potentially mediating variables related to both AD and HT may clarify conflicting findings surrounding estrogen / AD research. In regard to AD prevention, very few studies have accounted for the multiple stages of AD pathology and ApoE status. Additionally, no data exists on women with other types of dementia (e.g. vascular dementia), or age of dementia onset (e.g. early AD). In fact, age in general is another variable that could potentially confound the effects of an investigation examining the effects of HT on AD prevention. In regard to HT, the differential effects of various hormone preparations and doses, as well as the effect of concomitant progestin therapy, hysterectomy status, prior history of HT exposure should be controlled. In addition, HT initiated during the menopausal transition (the 'critical period' hypothesis) has been associated with beneficial effects on AD biomarkers. Thus, it is crucial to initiate HT several years before the onset of AD cognitive symptoms in order to evaluate the full neuroprotective potential of estrogen.

### **Future Directions**

While decades of basic science investigation of estrogen's actions in the brain and subsequent observational and clinical trials continue to confirm the benefit of estrogen based therapies on cognition, future research investigating the potential salutary effect of estrogen for AD prevention is essential. The prevalence of AD will continue to grow with the increase in the average lifespan in industrialized countries. Sixty-eight percent of individuals diagnosed with AD are women. The same 68% of women comprises roughly 5–10% of our population, and this statistic increases dramatically with age [170, 171]. Based on the aforementioned results, it is likely that a large scale, longitudinal, cyclical, HT study with transdermal estradiol would offer much needed information to the field of AD and hormone therapy research. The KEEPS (Kronos Early Estrogen Prevention Study) and the KEEPS Cognitive and Affective Study (KEEPS C/A) are currently underway and are positioned to address such issues. In

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conclusion, we believe that the KEEPS and KEEPS C/A studies will provide much needed answers regarding the use of estrogen during the menopause and postmenopausal periods for the prevention of AD.

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