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The potential increased risk of developing Alzheimer's disease in menopausal women due to decreased estradiol levels

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Foreword

This thesis focuses on how women after menopause show an increased risk of developing AD and how decreasing estradiol levels might cause this increased risk. The basis for this thesis initially stemmed from my interest in ageing and how this affects the brain. While I am growing up, I watch my grandparents decline physically and mentally. It shows what impact ageing has, not only on the person him- or herself but also on their loved ones. Studying the effects of ageing is very relevant. We all hope to grow old, and research will contribute to healthy and comfortable ageing. During my bachelor, I have learned how hormones influence almost everything in our body, making it incredibly interesting and complex. Therefore studying the estradiol drop in ageing women and the many effects it has was very interesting.

Summary

This thesis aims to answer the research question: Does the decrease in estradiol due to menopause increase the risk of developing Alzheimer's disease (AD)? And specifically, if this is the case, why does a subgroup of women suffer from the drop in estradiol while others seem resilient? AD is the most common cause of dementia, and therefore this thesis focused on AD only [1]. Older women have a higher risk of developing AD than age-related men [2]. Since 1980, researchers have looked at the effects of estradiol on the brain. Estradiol turned out to have a protective effect on brain structures. It plays an important role in maintaining the cholinergic system, an essential system for cognition. Furthermore, high levels of estradiol are thought to decrease the risk of developing neurodegenerative diseases [3].

Menopause is characterised by a sharp decrease in estradiol in the periphery and the brain [4]. Based on estradiol's protective effects, the drop in estradiol is thought to influence the brain negatively [5]. Since researchers suggest that pathological changes in the brain occur 15 years prior to the clinical onset of AD, the onset of AD and menopause is around the same time. This led to a hypothesis that proposed that when estradiol levels in the brain decline, it makes neurons more susceptible to age-related neurodegenerative processes; for example, the forming of amyloid-β plaques [6]. This is one of the best-known characteristics of AD [7].

All women experience this drop in estradiol during menopause; however, not all women will develop AD. Studies show that AD patients have increased cholinergic dysfunction and decreased estradiol levels compared to age-related controls [8]. A fascinating finding is that AD patients show significantly less aromatase mRNA compared to the control. This could be part of the cause of this additional decreased estradiol level seen in AD patients [9]. Based on the positive effects of estradiol, estradiol replacement treatment (ERT) was a popular treatment for postmenopausal women in the 1990s. Studies showed that ERT could reduce or even prevent AD. However, later studies found that ERT had either no benefits or saw an increased risk of developing AD [3]. It is now believed that the timing and duration of ERT are crucial. ERT should be administered during a critical time period [10]. Some research has suggested that ERT increases the risk to develop certain cancers [3]. The optimal duration of ERT is unclear, long term effects are not well understood. Based on the information available, ERT should not be used to treat AD since it is unclear whether the positives outweigh the negatives [11]. However, there is substantial evidence the decrease in estradiol due to menopause can increase the risk of developing AD. It seems that women do need underlying problems to set off the development of AD; such as decreased aromatase activity in the brain or disproportional cholinergic dysfunction compared to age-related controls.

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Introduction

Dementia is a clinical syndrome in which a progressive decline is seen in at least two cognitive domains [13]. These deficits in cognition are severe enough to interfere with daily life [14,15]. The disease has a significant impact on the quality of life. It does not only affect patients but also their caregivers. Studies have shown that caregivers of those with dementia report more stress than other caregivers [16]. Dementia is associated with advanced age; 80% of the cases occur in people older than 75 [16]. Currently, roughly 50 million people worldwide are diagnosed with a form of dementia [1]. As the world population is ageing, it is predicted that by 2050, 132 million people will have developed dementia. Alzheimer's disease (AD) is the most common cause of dementia, and it contributes to 60-70% of all dementia cases. Therefore this thesis will focus on AD only. Although age seems to be an important risk factor for developing AD, AD is not an inevitable consequence of ageing. Around 95% of the world's elderly population will not develop a type of dementia [13]. As the number of people with AD will increase significantly in a relatively short time, one of the consequences might be that health and social services get overwhelmed. There is no treatment to cure or prevent AD at this moment, only treatments that can increase the quality of life for the patient and the caregiver. However, these treatments do not change the rate of decline or the course of the disease [17]. It is essential to find out more about this disease to prevent the system from getting overwhelmed, to allow better care for people with dementia, and develop a more effective treatment [17].

Research has shown that the incidence of AD is higher in women than in men. Nearly two-thirds of all AD patients are women. This cannot entirely be explained by the higher longevity of women compared to men [1,2]. This led researchers to question what could cause women to have a higher risk of developing AD than men. One of the hypothesised causes is the clear difference between women and men in sex hormones. Therefore researchers started to investigate the influence of different sex hormones like testosterone and estradiol on cognition. Even though both hormones are found in either gender, the levels of each hormone in men and women differ massively. While testosterone in males decreases gradually, women experience an abrupt drop of estradiol during menopause [3,18]. Estradiol turned out to be a fascinating hormone in AD research. Before 1980, it was mainly associated with ovulation and reproductive behaviour. The fact that estradiol could also impact higher-order brain functions was not considered [19]. However, research showed that estradiol affects an extensive range of brain regions and has receptors distributed all over the brain. Furthermore, estradiol plays an essential role in the neurobiology of ageing and cognition [20].

New studies suggest that pathological changes start occurring in the brain 15 years prior to the clinical onset of AD [20]. This is around the same time menopause starts, and this drop in estradiol is seen. Therefore, this thesis aims to answer the research question: Does the decrease in estradiol due to menopause increase the risk of developing AD? To answer this question, it is also essential to answer the question: If the estradiol drop is essential, then what causes a subgroup of women to suffer from this while others are resilient to this menopausal drop in estradiol?

What is Alzheimer's disease?

AD is a neurodegenerative disease that mainly affects older adults. It is the most common form of dementia, and it is expected that its prevalence will increase in a short period [20]. Research has uncovered a lot about AD, but unfortunately, it is still unknown which biological changes exactly cause AD. It is thought that the pathophysiological process of AD begins years before the diagnosis is made [21]. Researchers believe that the key to prevent or slow down AD is to detect it early. Therefore studies focus on biomarkers that can indicate this early pathophysiological process. However, it remains unclear how and to which extend biomarkers can predict AD [22]. Because early detection is not possible at this moment, AD is usually diagnosed after symptoms of dementia start occurring. Commonly, the initial symptom of AD is that patients gradually lose the ability to remember new information. Apart from this, a decline in thinking abilities and memory loss is often also visible. The rate at which these symptoms advance differs between individuals [23].

Several diagnostic tests are performed on the patient to diagnose AD, including neuropsychological tests, functional assessments, and a brain scan. Several disorders can cause similar symptoms. Therefore some tests are also used to rule out other disorders being the cause. An MRI scan shows the atrophy caused by AD. The pattern of atrophy in the various neurodegenerative diseases differs. In AD, the atrophy has an insidious onset and inescapable progression. It typically begins in the medial temporal lobe, specifically in the entorhinal cortex. It progresses to the hippocampus, amygdala and the para-hippocampus. Other limbic lobe structures are also affected early in the disease, such as the posterior cingulate. In later stages of the disease, it spreads to the temporal neocortex and later to all the neocortical association areas [24]. At the end-stage of the disease, the entire brain is affected and a significant loss, up to 30%, of brain volume seen (Figure 1) [24].

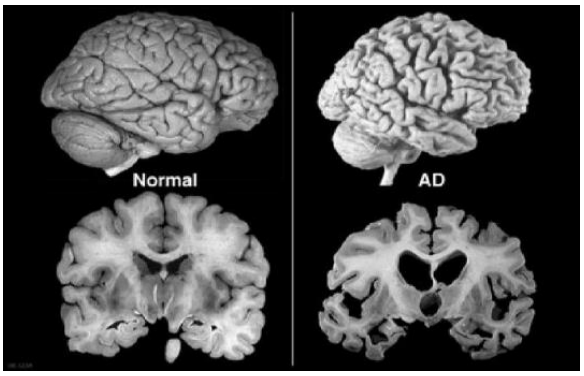

Figure 1. Brain atrophy in advanced Alzheimer's disease compared to a healthy brain [25].

A healthy brain contains billions of neurons that communicate constantly. A neuron receives signals from other neurons. If this generates an action potential, an electrical charge is generated, which travels down the axon. The neuron will release a neurotransmitter into the synapse, and these will bind to a specific receptor on a dendrite of a nearby neuron. This causes that neuron to be either stimulated or inhibited, based on the signal it receives. Unlike other cells, neurons are long-lived [25,26]. Therefore it is crucial for neurons to maintain healthy by repairing themselves. Neurons are able to adjust; synaptic connections can be strengthened, weakened or even entirely broken down. By a process called neurogenesis, new neurons can be generated. All of this is very important for learning and memory. Glial cells are cells surrounding the neurons; they are vital for maintaining a healthy brain function. In AD, the communication between neurons is disrupted. This causes neurons to lose their function and eventually leads to neuronal death due to apoptosis [27].

Alois Alzheimer was the first to describe AD. He described a 51-year-old woman who showed, among other things, a rapid increase of cognitive disturbances, disorientation and unpredictable behaviour. After she died in 1906, examining her brain under a microscope revealed atrophy of the entire brain

and changes in the internal structure [28,29]. The abnormalities Alzheimer's described became known as amyloid-β plaques and neurofibrillary tangles (Figure 2) [30]. To this day, amyloid-β plaques and neurofibrillary tangles are still used to diagnose AD [29].

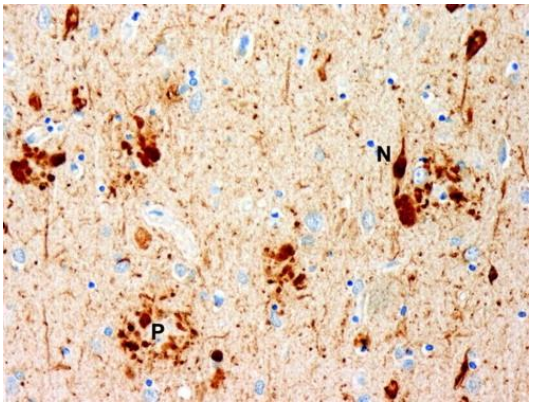

Figure 2. Microscopic picture of the hippocampus, modified Bielschowsky silver impregnation. (P) Amyloid-ß plaques; (N) Neurofibrillary tangles [31].

Amyloid-β plaques are clusters of amyloid-β peptides in the extracellular space. In a healthy brain, the protein amyloid precursor protein (APP) is embedded in the membrane of the neurons. APP is essential for neuronal growth and repair. Like other proteins, it is regularly broken down into peptides and replaced. In AD the amyloid-β peptides misfold and build up between the nerve cells. They aggregate together, forming oligomers and ultimately forming plaques. These amyloid-β plaques can block neurotransmitters in the synapse, and therefore are blocking neuronal communication [32]. Also, with normal ageing, plaque formation can occur. However, AD is associated with large numbers of amyloid-β plaques [33]. It is also thought that these misfolded, aggregated amyloid-β peptides, especially in their oligomeric state, are neurotoxic [34].

Neurofibrillary tangles are aggregates of hyperphosphorylated tau proteins [35]. Microtubules are part of the cytoskeleton of neurons. They are essential for the cell shape of the neurons and transportation within the cell. Tau protein helps to maintain the integrity of these microtubules. In AD, there is evidence that toxic tau enhances amyloid-β through a feedback loop [25,30,31]. On the other hand, amyloid-β peptides activate an intracellular kinase that phosphorylates the tau proteins abnormally. This hyperphosphorylation causes tau proteins to become inactive. As a result, the microtubules start to collapse, disabling intracellular transport. The inactive tau proteins form neurofibrillary tangles in the cell body of the neuron. These intracellular neurofibrillary tangles disrupt the transport in the neuron [36].

Recently, researchers have hypothesised that amyloid-β and tau proteins act as prions [36,37]. Prions are misfolded proteins that spread through tissue in a similar way as an infection. Prions force normal proteins to adopt a similar misfolded shape [36]. Aoyagi et al. (2019) developed sensitive cellular assays to quantify self-propagating conformers of amyloid-β and tau in post-mortem human brain tissue. Their results show that higher levels of these prions are associated with the early-onset form of AD. Based on this evidence, they concluded that amyloid-β and tau are both prions and that AD should be viewed as a double-prion disorder [37]. However, many researchers are hesitant to accept this hypothesis. Instead they rather refer to the spread seen in AD as prion-like [29].

The amyloid-β plaques block the neuronal communication, which causes the neuron to die. The intracellular neurofibrillary tangles disrupt the transport; this causes the neurons to become dysfunctional and die. Together, amyloid-β plaques and neurofibrillary tangles are thought to be the leading cause of AD [38]. The amyloid-β plaques and neurofibrillary tangles spread through the brain during the course of AD (Figure 3) [38].

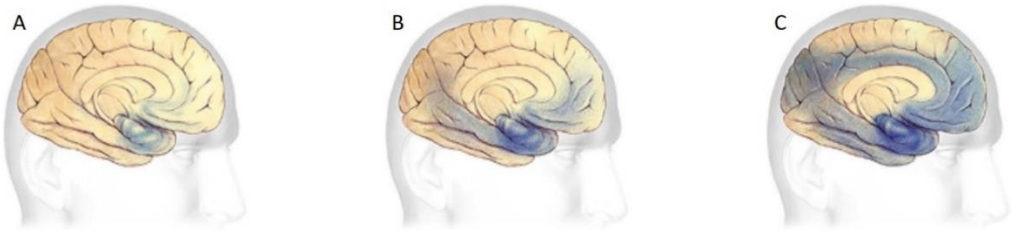

Figure 3. The spread of plaques and tangles (blue areas) through the cortex. (A) Early stages of AD; (B) Mild to moderate stages of AD; (C) Severe stages of AD [38].

What is the function of estradiol, and what does it affect?

It is thought that high levels of estradiol have a protective effect on brain structures during ageing [39]. In women, estradiol is essential for the development of female characteristics and the reproductive system. Nevertheless, it has many other functions as well. In men, the main sex hormone is testosterone. However, a small amount of testosterone is converted into estradiol. Even though the levels of estradiol in women are much higher than in men, estradiol also plays a role in a male's reproductive functions [40].

The hypothalamus secretes gonadotropin-releasing hormone (GnRH). GnRH leads the pituitary to produce the gonadotropins follicle-stimulating hormone (FSH) and luteinising hormone (LH). Together, FSH and LH regulate ovarian function. The concentrations of FSH and LH are controlled by negative feedback from estradiol and progesterone [41]. Estradiol is a sex steroid hormone, and in premenopausal women, it is mainly produced by the granulosa cells of the ovaries [42,43]. Nevertheless, it is also synthesised by non-reproductive tissues like the liver and the brain. Cholesterol molecules can be converted into several androgens, including testosterone. Estradiol can subsequently be synthesised from testosterone by the enzyme aromatase [44]. Estradiol in an adult mainly acts through two types of receptors present in the periphery and the brain; the classical nuclear receptors (ERα and ERβ) and the membrane receptor (GPR30). The nuclear ERs initiate responses slowly, such as hours or days, while membrane ERs respond in a manner of seconds via kinase signalling pathways. GPR30 does not only bind estrogens. Therefore it remains controversial whether or not GPR30 is a genuine novel estradiol receptor [45]. The nuclear ERs regulate genes, and once estradiol binds to its receptor, it activates the production of new proteins. In the periphery, ERα is primarily expressed in the gonadal organs, while ERβ is primarily produced in non-gonadal tissues [46]. However, the nuclear ERα and ERβ are widely expressed in the human brain (Figure 4), and their functions partly overlap. ERα plays an essential role in the neuroendocrine and reproductive systems[47]. The ERα seems to be dominant in the hypothalamus. ERβ are profoundly found in the region AD seems to be initiated; the entorhinal cortex. Also, the nearby located hippocampus contains mainly ERβ [18]. In mice studies, the effect of ERβ was studied, and it showed that blocking the ERβ resulted in increased anxiety and reduced cognitive performance. Also in humans, ERβ is primarily involved in mood and cognitive activities [46].

ER subtypes	Distribution in the brain			
	intense	**moderate**	**weak**	**Absent**
ER α	Amygdala; Bed nucleus of the stria terminals; Periqueductal gray; Preoptic area.	Allocortex; Hypothalamus; Locus coeruleus; Spinal trigeminal nucleus	Hippocampus; Raphe nuclei; Zona incerta.	Anterior tegmental nucleus; Cerebellum; Globus pallidus; Inferior olive nucleus; Isocortex; Ponti nenuclei; Thalamus; Substantial nigra; Superior olive nucleus; Ventral tegmental area.
ER β	Amygdala; Bed nucleus of the stria terminals; Raphe nuclei; Substantial nigra.	Allocortex; Globus pallidus; Hippocampus; Locus coeruleus; Preoptic area; Ventral tegmental area;	Anterior tegmental nucleus; Hypothalamus; Inferior olive nucleus; Isocortex; Periqueductal gray; Pontine nuclei; Spinal trigeminal nucleus; Superior olive nucleus; Thalamus.	Cerebellum; Zona incerta.

Figure 4. Summary of the expression of ERα and ERβ in the different brain regions. ERα is most abundant in the hypothalamus and the amygdala, while ERβ is most abundant in the hippocampus, claustrum and the cerebral cortex [42,43,46].

Estradiol is also synthesised in much smaller amounts by non-gonadal tissues, as the liver and the brain. In the brain, neurons and astrocytes are able to produce brain estradiol from circulating testosterone and cholesterol (**Fout! Verwijzingsbron niet gevonden.**) [48]. Different from ovarian synthesised estradiol, this estradiol acts locally at the site of synthesis [46]. Studies have shown that estradiol has several neuroprotective functions in the brain.

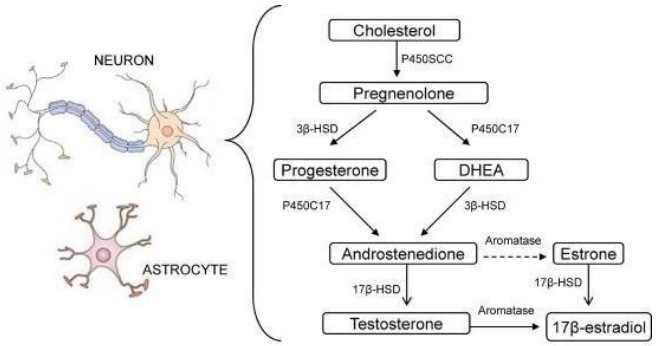

Figure 5. Neurons and astrocytes express all enzymes required for estradiol synthesis to produce brain estradiol [49].

For example, before menopause, women show a lower incidence of strokes compared to age-related men. A reason for this is that estradiol lowers cholesterol levels. This regulation of cholesterol by estradiol also protects women from cardiovascular disease [50]. Mice studies where estradiol is depleted show that estradiol is also involved in neural development, synaptic plasticity and cell survival. The knock-out mice show significant deficits in the spine and synaptic density, especially in the hippocampus [51]. Another mice study showed that estradiol promotes neurogenesis in the hippocampus; this affects spatial memory positively [51].

Acetylcholine (ACh) is a neurotransmitter, and it is crucial for memory and learning processing and attention. Especially in the beginning of AD research, studies focused on the cholinergic system. The cholinergic hypothesis of Alzheimer's disease states that dysfunction of ACh containing neurons in the brain contributes substantially to the cognitive decline observed in AD [52]. The cholinergic system turned out to be an important site of action for estradiol. It is hypothesised that estradiol helps to maintain aspects of attention and verbal and visual memory [53]. How estradiol mediates these effects is not well understood, but research has shown that the majority of cholinergic neurons contain the membrane ER GPR30 [53].

Estradiol also influences blood-glucose utilisation. Glucose is the main energy source for the brain, and it needs to be transported from the peripheral blood. Once glucose is inside the cells, it needs to be transported into the mitochondria. Ageing and declining blood-glucose utilisation lead to decreased mitochondrial function and, as a result, decreased neuronal health [54]. Estradiol promotes the energetic capacity of mitochondria in the brain by maximising aerobic glycolysis [55]. A protective mechanism against the forming of amyloid-β plaques and neurofibrillary tangles in AD is also thought to be estradiol. In vitro studies suggest that estradiol reduces the amyloid-β production and enhances the amyloid-β clearance [56]. Furthermore, estradiol seems to prevent the hyperphosphorylation of tau. However, evidence for this is contradictory [56]. It is hypothesised that when estradiol levels in the brain decline, it makes neurons more susceptible to age-related neurodegenerative processes; for example, the forming of amyloid-β plaques and neurofibrillary tangles [57].

What happens in menopause and what is the link with Alzheimer's disease?

Estradiol serves many important purposes; therefore, it is essential for both men and women. Before menopause, women produce more estradiol than men. The World Health Organisation (WHO) defines menopause as the permanent cessation of menstruation resulting from the loss of ovarian follicular activity of follicle depletion. Postmenopause is the period after menopause, and it begins twelve months after amenorrhea [57]. During menopause, the production of serum estradiol drops significantly

in all women (Figure 6A) [48]. The serum levels of estradiol can decrease with 85-90% [48]. On the other hand, men continue to produce large amounts of testosterone (Figure 6B) [4].

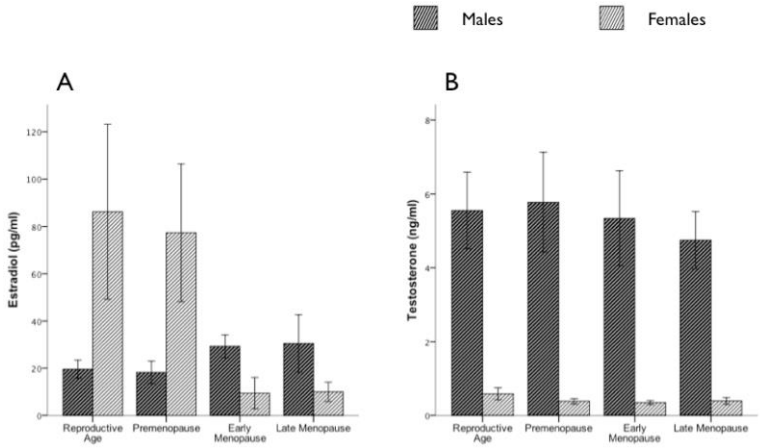

Figure 6. The graphs show the hormone levels in men and women according to women's reproductive stages. (A) Shows the estradiol serum levels. An abrupt decline is seen in women at the start of menopause. (B) Shows the testosterone serum levels. A gradual decline is seen in men around early menopause [58].

After menopause, other sites of estradiol synthesis become the major source. This estradiol often acts locally, therefore serum levels do not represent the estradiol action in postmenopausal women. Furthermore, since AD is primarily a brain disease, it is more relevant to look at estradiol levels in the brain. The levels of estradiol in the female brain mirror the circulating estradiol levels; significant declines of estradiol in the brain are seen compared to premenopausal women [58]. Before menopause, women have relatively more estradiol than men. During menopause, a drop of estradiol in the brain is seen [58]. This drop is only seen during menopause; there is minimal additional decrease after menopause in healthy females [58]. Based on the suggested protective effects estradiol has, this explains why men are more susceptible to certain diseases at a younger age while women that have gone through menopause might be more susceptible. In men, during their lifetime, the estradiol levels increase slightly with age but compared to women the levels remain relatively stable. This is partly because, in men, testosterone levels stay high, and testosterone can be converted into estradiol by aromatase in the brain [9]. Estradiol helps maintain healthy cognitive functions during ageing until menopause. It is hypothesised that due to the drop in estradiol during menopause, women not only lose the advantage that comes with having high estradiol levels, but they also get more susceptible to certain neurodegenerative diseases than men [59].

An important neuroprotective role of estradiol mentioned before is that it is suggested that it can reduce the amyloid-β production and promote clearance. Accumulations of amyloid-β peptides called amyloid-β plaques are one of the two major pathological lesions in the AD brain [60,61]. Multiple mice studies have shown that estradiol effectively lowers amyloid-β levels in an ovariectomised transgenic mouse model [62]. Estradiol also increases the levels of transthyretin in the brain. Transthyretin inhibits the aggregation of amyloid-β into plaques by binding to the amyloid-β. Consequently, it inhibits the amyloid-β plaque induced cellular toxicity [55]. With the drop of estradiol in menopause, a defence mechanism against neurodegenerative diseases is lost. A mice study using a transgenic mouse model of AD showed that an estradiol deficiency accelerates the amyloid-β formation and that it can cause premature plaque formation in brains susceptible to AD [63].

Neurons and astrocytes are both able to produce estradiol. With ageing, alterations in the astrocyte function can occur. These alterations can affect the neuronal viability; aged astrocytes have a reduced ability to maintain neuronal health and, therefore, contribute to neuronal injury in neurodegenerative diseases such as AD [52]. With normal ageing, a gradual loss of cholinergic function is expected due to dendritic, synaptic and axonal degeneration; this leads to age-related functional decline, including

cognitive impairments. In AD, the concentration and the function of ACh are decreased due to severe cholinergic neurodegeneration [64]. There is evidence that amyloid-β may trigger cholinergic dysfunction in AD [52].

Why does a subgroup of women suffer from the drop in estradiol while others seem resilient?

However, this does not explain why a subgroup of women suffers from AD while others seem to be resilient to the drop in estradiol due to menopause. To answer this question, it is important to look at what individual differences are found between AD patients and age-related controls. As mentioned before, ACh plays an important role in memory, and the cholinergic system is an important site of action for estradiol [65]. After menopause, cholinergic abnormalities can occur, such as decreased ACh levels and alterations in choline transport. However, post-mortem studies in AD patients show that cholinergic abnormalities are much more abundant in AD patients than age-related controls. The dysfunction of ACh containing neurons in the brain contribute substantially to the cognitive decline observed in AD [66]. Based on these studies, it can be stated that AD patients show more cholinergic dysfunction compared to age-related controls. There is extensive evidence that amyloid-β can trigger cholinergic dysfunction. This happens in various ways; for example, amyloid-β can interact with nicotinic ACh receptors or interact with acetylcholinesterase. Consequently, it seems that individuals that show an early onset of amyloid-β plaques might be at increased risk to develop AD. Apart from that the amyloid-β plaque formation itself is a risk factor for AD; it also triggers cholinergic dysfunction, which also seems to be a risk factor for AD [63,67].

Astrocytes in the brain are able to produce estradiol. With ageing, alterations in their function can occur. Research has shown that astrocytes already contribute to AD by stimulating synaptic and neuron loss in the earliest stages of AD [68]. During the course of the disease, more astrocytes become activated. A relatively new technique to diagnose AD early on is to study the retinal tissue of the eye; this technique is still being developed. Researchers have reported the accumulation of amyloid-β and phosphorylated tau aggregates in the retinal tissue of human AD patients [69]. However, it should be noted that this solely is not specific enough to diagnose AD since amyloid-β deposits are also found in, for example, macular degeneration. Therefore it is a necessity to investigate other components that influence AD [68]. Due to AD, the number of astrocytes increases abnormally. The protein glial fibrillary acidic protein (GFAP) is up-regulated when astrocytes are activated [68]. Grimaldi et al. (2019) were able to measure the number of activated astrocytes in the retina of AD patients using an immunolabeled anti-GFAP antibody. The amount of activated astrocytes is significantly increased in AD patients compared to the control (Figure 7) [68].

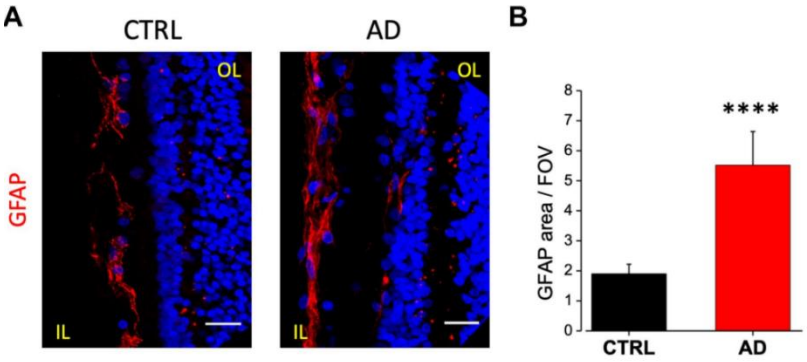

Figure 7. The amount of astrocyte activation is more pronounced in the AD retina compared to the control. (A) images of retinal slices from AD patients and controls immunolabeled with anti-GFAP antibody (red) and Hoechst for nuclei visualisation (blue), bar 20 µm; (B) Quantification of GFAP area using fluorescence intensity [70].

Activated astrocytes typically cause an increase in the synthesis of aromatase. This causes the levels of brain estradiol to increase [70]. Since AD causes astrocytes to become activated, it would be expected

to see increased estradiol levels in an AD brain. Contradictory, a human study found that AD patients had significantly less estradiol in their brains than age-related controls (Figure 8A). These differences were only seen in the brain; there are no differences in serum estradiol levels [9]. A human study by Yue et al. (2005) looked at the mRNA expression of aromatase in the cerebral cortex of female AD patients and female non-AD patients. In post-mortem brains of female AD patients, a considerable reduction of aromatase mRNA levels was seen (Figure 8B). Apart from this, they found a significant negative correlation between the mRNA levels of aromatase and the density of the amyloid-β plaques [71]. Ishunina et al. (2007) looked at the hippocampus specifically, and results showed that hippocampal aromatase is decreased in menopausal AD patients compared to age-related female controls [9,71]. These results lead to the idea that these relatively low brain estradiol levels in female AD patients may be due to impairment of the aromatase expression. It is hypothesised that the decreased estradiol levels in postmenopausal women, combined with suboptimal brain estradiol synthesis may contribute to the inflammatory state seen in AD [9,24]. Lower estradiol brain levels caused an earlier onset of AD and increased the severity of the plaques [72]. The reason for this decrease in aromatase mRNA is not studied yet; however, the alterations of astrocytes that occur due to ageing might change the influence of activated astrocytes on the synthesis of aromatase. Secondly, genetic variations in the gene that encodes aromatase (CYP19A1) might also explain the decrease [73]. Studies have shown that variations in this gene can affect the circulating estradiol levels [73]. The results may support the hypothesis that a combination of insufficient local estradiol synthesis and postmenopausal estradiol decline together increase the risk of developing AD. If aromatase levels are high enough, women might be able to compensate for the decrease in estradiol due to menopause.

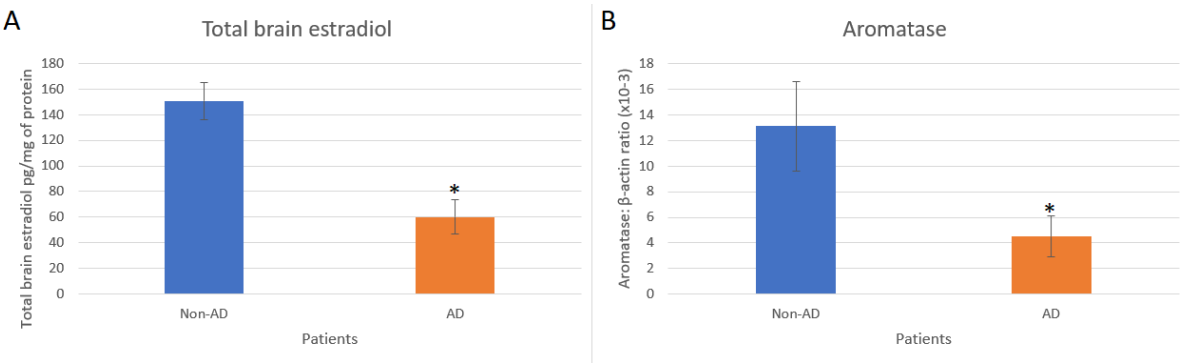

Figure 8. Characteristics of the female study subjects based on Yue et al. (2005), table 1. (A) total brain estradiol pg/mg of protein. AD patients showed significantly lower total brain estradiol levels compared to age-related controls; (B) Aromatase levels in AD patients were also significantly lower compared to age-related controls [40].

What is estradiol replacement treatment and how does it affect Alzheimer's disease?

Estradiol is a significant player in women's health. Many functions are affected by the amount of available estradiol. During menopause, the levels of estradiol decrease while the levels of gonadotropins LH and FSH increase. On average, women begin menopause at 51. Menopause is often characterised by physical and mental symptoms that can start prior to menopause during perimenopause. Usually, menopause starts with the onset of vasomotor symptoms [57,74]. The most significant symptoms that are directly related to estradiol are hot flushes and vaginal dryness. 75% of postmenopausal women have experienced hot flushes, and they can occur at any time. Other symptoms include urogenital symptomatology (50% of postmenopausal women), palpitations and migraines. Vasomotor symptoms can affect a woman's social life, ability to work, sense of well-being and psychological health; therefore, these vasomotor symptoms are the ones women most often seek medical help for [75].

One of the treatments women can receive is estradiol replacement therapy (ERT). Premature menopause can also be a reason to start ERT. Premature menopause can be caused by premature ovarian failure or premature surgical menopause, such as ovariectomy. Prior research has shown that women that experience menopause at younger ages show a reduced cognitive performance in older adults compared to age-related controls [76]. For menopause, this ERT commonly consists of only estradiol (ET) or estradiol and progestin (HT). Most specialists preferably use ET. However, HT is commonly used in women who still have their uterus. The absence of progesterone can result in endometrial pathologies. Studies have shown that treatment with just estradiol (ET) increases the risk of endometrial cancer; a combination of estradiol and progesterone (HT) reduces this risk. However, HT also has disadvantages. For example, HT increases the risk of breast cancer. Therefore it has to be considered carefully which treatment is most suitable for the individual patient. Since estradiol and progesterone can have such extensive effects, specialists preferably give these treatments with a low dose. Trial observational studies that looked at the effect of these treatments saw a lower risk of AD among women who had received ERT. It is hypothesised that ERT might protect against cognitive decline [77]. This idea first came when a study conducted at Rockefeller University showed that women receiving estradiol for six weeks demonstrated cognitive improvements [78]. After this study, many more studies found that ERT was associated with a delay or even preventing AD and other neurodegenerative diseases. Apart from this, ERT showed better maintenance of many aspects of cognition in healthy, ageing women [79]. Thus, observational studies suggest that ET/HT could serve a protective role against some of the risks for developing dementia.

For multiple medical reasons, women can choose to remove their ovaries before menopause, causing a premature estradiol decrease. After ovariectomy in mice, results suggest that ERT can prevent the decline in mitochondrial bioenergetics [80]. A study by Kara et al. (2020) showed the abrupt estradiol decrease due to ovariectomy can increase the risk factors for AD by causing abnormalities in the brain structure, function and metabolism [81]. A mice study by Petanceska et al. (2000) looked at the effect of ovariectomy on amyloid-β peptides specifically. Ovariectomy was associated with an increase in amyloid-β plaques compared to the control. ERT significantly reversed the ovariectomy-induced increase in amyloid-β plaques in the brain. These studies all showed positive results using ERT, and in the 1990s, it was a highly popular treatment [82]. However, other studies with longer trials of ERT found no benefits [83–85]. In 2002, the Women's Health Initiative (WHI) even saw the risk of AD increasing after HT in women aged 65 and above [83–85]. These results were very contradictory to earlier evidence. Questions were raised about this particular study since the WHI used replacement drugs rather than estradiol, and the subjects used were different than in other studies [86].

Nevertheless, these contradictory results caused panic among some users and it led many to question whether ERT should be used as a treatment. This led to a drastic reduction in prescriptions of ERT for postmenopausal symptoms [53,87]. Several recent observational studies have identified that the stage of menopause at which ERT is started modifies the risk of AD. In a mice study, a window of opportunity was suggested [88]. Menopause is characterised by a rapid estradiol depletion. As a result of long-term hormone deprivation, a loss of ERs occurs [61]. Therefore they proposed the so-called critical window hypothesis. The critical window hypothesis proposes that following long-term estradiol deprivation, the brain and cognition become insensitive to exogenously administered estradiol. However, if estradiol is administered during a critical period near the time of cessation of ovarian function, there will be beneficial effects [19]. Results show that when women take ET/HT during the late menopause transition or early postmenopause, they have a lower risk of developing AD [89].

This critical window is related to the duration of estradiol loss. A study by Erickson et al. (2010) examined the hippocampus in postmenopausal women. Results showed that a short interval between the onset of menopause and ERT initiation was associated with larger hippocampal volumes compared to women who received ERT after a longer interval [90]. In a mice study, estradiol was able to increase

the spine density in the CA1 region of the hippocampus in young mice by 30%; in aged mice, estradiol did not increase this number as much [91]. In the mice study by Petanceska et al. (2000), two estradiol doses were used; this study did not show that high-dose estradiol treatment leads to further decrease of the amyloid-β plaques [76]. It is unclear whether a high dosage negatively affects the patient; therefore, specialists preferably prescribe low dosages and only increase when a low dose does not cause symptom relief [92].

Despite the seemingly positive effects of ERT during the critical window period on AD, estradiol also affects many other functions. As mentioned before, ET in postmenopausal women increases the risk of endometrial cancer [85]. Research has also shown that women using HT have an increase in the risk of developing breast cancer. The WHI found that HT influences verbal memory negatively [93]. The results for cardiovascular health are very contradictive; observational studies show a reduced risk for cardiovascular diseases, however, randomised trials have not confirmed this idea [19].

Apart from the timing of treatment, the duration of the treatment also seems to influence the outcome. A study with female monkeys was conducted where they looked at the effects of long-term ET. Results revealed that 2-3 years of ET increased spine density of the CA1 region in both young and aged monkeys [94]. Also human studies have found an inverse relationship between estradiol and AD; the risk of AD decreases when the duration of ET is increased [12]. However, ERT's optimal duration is not clear since other studies found that longer durations caused unwanted effects. The duration of ERT is ideally limited to five years. Research shows that more than five years of HT increases the risk of developing breast cancer. Long-term ET effects are less clear; however, it does not seem to affect breast cancer [11].

Conclusion

This thesis aimed to answer the research question: Does the decrease in estradiol due to menopause increase the risk of developing AD? AD is a terrible neurodegenerative disease that affects millions of people worldwide. AD is the most common cause of dementia, and therefore this thesis focused on AD only. After menopause, women seem to have an increased risk of developing AD compared to age-related men. Research has shown that AD pathology starts 15 years prior to the clinical onset of AD. This means that AD pathology starts around the same time menopause starts. Menopause is characterised by a sharp decrease in estradiol in the periphery and the brain.

A critical function of estradiol is the hypothesised neuroprotective effects it has. This has been tested thoroughly over the last years, and research has discovered that the formation of amyloid-β plaques and neurofibrillary tangles is reduced by estradiol. Menopause is characterised by a sharp decrease in estradiol levels. A hypothesis proposes that when estradiol levels in the brain decline, it makes neurons more susceptible to age-related neurogenerative processes. For example, studies have shown that estradiol reduces amyloid-β production and promotes amyloid-β clearance. Furthermore, estradiol increases transthyretin, which inhibits cellular toxicity. Lastly, it is hypothesised that estradiol helps to maintain aspects of attention and verbal and visual memory by affecting the cholinergic system. This led to the idea that women after menopause indeed have an increased risk of developing AD because they have lower estradiol levels compared to perimenopause.

However, this leaves the important question: what causes a subgroup of women to suffer from this apparent drop in estradiol levels while others are resilient to this menopausal drop in estradiol? AD causes an increase in the number of activated astrocytes. Activated astrocytes cause an increase in the synthesis of aromatase, which results in increased estradiol levels. However, estradiol levels are significantly decreased in AD compared to age-related controls. An interesting finding by Yue et al. (2005) and Ishunina et al. (2007) was that mRNA expression of aromatase is significantly decreased in AD patients compared to age-related controls. Aromatase is responsible for converting testosterone into estradiol. They also found a negative correlation between the mRNA levels of aromatase and the

amyloid-β plaques density. These results suggest that this additional decrease in estradiol seen in AD patients might be because of the reduced aromatase expression. The results may support the hypothesis that a combination of insufficient local estradiol synthesis and postmenopausal estradiol decline together increase the risk of developing AD. The reason for this aromatase mRNA decline is unclear. Possible reasons for this decline are that altered functioning of astrocytes due to ageing result in reduced aromatase expression. A second possible explanation are variations in the gene that encodes for aromatase. However, further research is needed to examine more closely the links between activated astrocytes, decreased estradiol levels and decreased aromatase mRNA.

ERT can be given to women to relieve them from menopausal symptoms. Most studies found positive results using ERT, and in the 1990s, it was a highly popular treatment. However, other studies with longer trials of ERT found no benefits. It was suggested that ERT could increase various cancers, and a study by the WHI showed that women aged 65 and above had an increased risk of developing AD after ERT. Later, the critical window hypothesis was suggested. This hypothesis proposes that following long-term estradiol deprivation, the brain and cognition become insensitive to exogenously administered estradiol, and ERT will lose its beneficial effect.

To conclude, conclusive evidence that ERT decreases the risk of AD is missing. Apart from this, it is not clear if and to what extend ERT increases the risk of other severe diseases as cancer and cardiovascular diseases. In the absence of clear evidence concerning all of this, ERT should be limited to women within the critical period that is at low risk for developing cardiovascular diseases or cancer. Secondly, based on the information available, ERT should not be used to treat AD since it is unclear whether the positives outweigh the negatives. However, there is substantial evidence the decrease in estradiol due to menopause can increase the risk of developing AD. It seems that only women with specific individual characteristics set off the development of AD; such as a decreased aromatase activity in the brain or disproportional cholinergic dysfunction compared to age-related controls.

Even though decreased estradiol levels seem to play a role in developing AD, developing a treatment based on this has not been found. Furthermore, it should be noted that many studies are conducted in mice. However, mice do not go through menopause. This makes research on menopause difficult to conduct. AD is a complicated disease. Due to research, more risk factors are unravelled; however, there are still many unknowns about AD. This makes the development of treatment complicated. As estradiol has neuroprotective effects, other neurodegenerative diseases might also be influenced by the estradiol drop due to menopause. As said before, it is thought that by 2050, 132 million people worldwide will have developed a form of dementia; 30-40% of these cases will not be AD. Based on estradiol's neuroprotective functions, other forms of dementia could also be affected by available estradiol levels. It might be that developing a treatment to prevent those types of dementia will be less complicated. Therefore further studies could focus on other types of dementia as well. Many people will develop another type of dementia than AD, making those studies very valuable as well.

Afterword

The more I studied this topic, the more I enjoyed it. The effect of estradiol or lack of estradiol on neurodegenerative diseases is definitely something worthwhile to explore further.

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