Cognitive effects of hormone replacement therapy in menopausal women

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ABBREVIATIONS:

- HRT: Hormone replacement therapy
- MPA: Medroxyprogesterone Acetate
- CEE: Conjugated Equine Estrogen
- ERα: Estrogen receptor alpha
- Erβ: Estrogen receptor beta
- PRA: Progesterone receptor A
- PRB: Progesterone receptor B
- Aβ: Amyloid beta
- P: Progesterone
- E: Estrogen
- E2: Estradiol
- E1: Estrone
- m-P: Micronized progesterone
- TD: transdermal
- WHI: Women's Health Initiative
- WHIMS: Women's Health Initiative memory study
- WHIMSY: Women's Health Initiative memory study-young
- HERS: Heart and Estrogen/progestin Replacement Study
- KEEPS: Kronos Early Estrogen Prevention Study

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1. Summary

INTRODUCTION: Hormone replacement therapy (HRT) is prescribed to women for the treatment of postmenopausal symptoms; however, there remains doubt about what kind of influence does it have on cognition. Previous studies, in animal models and observational clinical data, showed that estrogen could have a positive effect on the aging brain; but large clinical trials, such as the Women's Health Initiative Memory Studies (WHIMS), showed a negative effect on cognition in older women. We aimed to explore possible reasons for such inconsistency, based on the results of the clinical studies classified according to patient age, duration and type of hormone replacement therapy.

METHODS: We carried out a literature search for the period between January 1995 to May 2018 in three databases (PubMed, Embase, Cochrane) using the following keywords: progesterone, estrogen, cognition. Inclusion criteria were the following: randomized controlled trial, cognition test(s), no additional pharmacological manipulations, no phytoestrogens/herbal preparations or selective estrogen receptor modulators (SERMs).

RESULTS: After application of the inclusion criteria, a total of 34 (35) articles out of 7,086 were included. Regarding the duration of therapy, 3 studies used acute (≤1 month), 18 subacute (1 month - 1 year) and 14 used chronic therapy (≥1 year). Most of the studies, (20/36) showed that HRT had no effect on cognition; whereas, an additional 6 showed positive influence on verbal cognition in younger women (medroxyprogesterone acetate was not the part of HRT). The remaining 9 studies showed a negative effect on cognition (6/9 done in older patients).

CONCLUSION: There is evidence of a positive effect with the use of unopposed estrogen or estrogen/micronized progesterone in younger women, with acute and subacute therapy. There is a negative effect on cognition with the use of conjugated equine estrogen/medroxyprogesterone acetate in older postmenopausal women. The great majority of studies included in our research showed that HRT had no effect on cognition. Future studies should focus on the research effect of natural progesterone and estrogen in younger postmenopausal women.

2. Sažetak

UVOD: Hormonska nadomjesna terapija (HNT) se prepisuje ženama za liječenje postmenopauzalnih simptoma, međutim utjecaj na kognitivne funkcije i dalje ostaje nejasan. Istraživanja na životinjskim modelima kao i epidemiološke studije ukazuju na pozitivan učinak HNTna kogniciju, ali su velika randomizirana klinička istraživanja poput Women's Health Initiative Memory Studies (WHIMS) pokazala negativan učinak HNT-a na memoriju kod žena. Cilj ovog rada je da se nađe objašnjenje za navedene razlike , te smo rezultate studija klasificirali prema ishodu studije, starosti uključenih pacijentica i duljini i tipu HNT.

METODE: Pretražili smo tri baze podataka (PubMed, Embase, Cochrane), u razdoblju od siječnja 1995. do svibnja 2018., koristeći slijedeće ključne riječi: progesterone, estrogen, kognicija. Uključni kriteriji su bili: randomizirani klinički pokus, postojanje kognitivnih testova. Dok su isključni kriteriji bili: korištenje drugih lijekova, korištenje fitoestrogena/biljnih preparata ili selektivnih modulatorov estrogenskog receptora (SERMs)

REZULTATI: Nakon primjene navedenih kriterija ukupno 34 (35) od 7,086 studija su uključene u naš rad. U svezi sa duljinom liječenja, 3 studije su koristile akutnu (≤1 mjesec), 18 subakutnu (1 mjesec – 1 godina) i 14 kroničnu (≥1 godina) hormonsku terapiju. Većina studija (20/36) je pokazala da HNT nema učinak na kogniciju, 6 je pokazalo pozitivan učinak na verbalnu kogniciju u mlađih žena (medroksiprogesteron acetat nije bio dio HNT). Preostalih 9 studija je pokazalo negativan efekat na kogniciju (6/9 studija je bilo u starijih pacijentica).

ZAKLJUČAK: Rezultati ovog rada upućuju na pozitivan učinak akutne i subakutne terapije estrogenom ili estrogenom/mikroniziranim progesteronom na kogniciju u mlađih žena. Negativan učinak je zabilježen pri terapiji s konjugiranim estrogenom/ medroksiprogesteronom acetatom u starijih postmenopauzalnih žena. Velika većina studija uključenih u istraživanje ipak pokazuju da HNT nema učinka na kogniciju. Buduće studije bi se trebale usredotočiti na istraživanje učinka prirodnog progesterona i estrogena u mlađih postmenopauzalnih žena.

3. Introduction

3.1 Menopausal transition and cognition

The female body goes through great hormonal changes during the period of perimenopause and menopause. The symptoms usually become recognized in 5th decade of their life and the median age of menopause in developed countries is between 50 and 52 years of age (1). That means that women on average spend one third or their lifetime in menopause. Several biological changes occur during the menopausal transition. Even before birth, the number of oocytes start declining. At birth there are about 1 to 2 million and by menarche this number falls to 400,000. By the time of menopause, they reach a critically low level where there is only a few hundred left (2). With that, endocrinological changes are observed. There are rising gonadotropin levels, especially of follicle stimulating hormone (FSH) and declining levels of estrogen; which characterize the menopausal transition. Up to 85% of women experience negative symptoms related to it; such as vasomotor symptoms, vaginal dryness, insomnia, mood and cognition changes (3,4). There is a decrease in bone density and a risk for developing osteoporosis, as well as an increased risk of coronary heart disease and depression. Hormone replacement therapy was shown to be effective for bone density, vasomotor and genitourinary symptoms, but its impact on cardiovascular disease, depression and cognitive dysfunction continues to be a subject of research (5,6).

The nervous system is closely regulated by steroid hormones and during the menopausal transition biochemical, structural and functional changes can be observed in the brain (7). There is, therefore, sufficient evidence to support the fact that estrogen influences cognitive functions in women. Estrogen probably causes a specific effect on cognition; more exactly, enhances aspects of verbal memory where, at the same time, it could have a negative effect on spatial memory (8). Some studies also support that estogen exerts a positive effect on sexually specific cognitive skills, in which women usually stand-out from men (like verbal articulation and fine motor skills) (8).

We can also observe a higher incidence of dementia and, especially, Alzheimer's disease (AD) in elderly women when compared to men; and these differences cannot be explained solely by increased longevity (9). Furthermore, experimental animal studies suggest that estrogen and progesterone exert numerous protective actions which are relevant in prevention of dementia and AD, such as increasing neuron viability and reducing the accumulation of beta-amyloid which is a known factor in the initiation and progression of AD (10). Evidence suggests that these neuroprotective effects could decrease with increasing age due to decrease of neural receptiveness to estrogen. This could decrease the possible therapeutic use of HRT for cognitive problems in elderly women. Estrogen's neuroprotective action is also modulated by progesterone. Whereas continuous progesterone exposure was associated with inhibition of estrogen actions, cyclic delivery could enhance neural benefits of estrogen (11). Regarding the role of HRT in postmenopausal women with Alzheimer's disease there are studies which show, as mentioned before, that estrogen and progesterone depletion in postmenopausal women represents a significant risk for developing AD, which could be reduced by estrogen-based HT. However, the data from recent clinical trials oppose this beneficial effect (12).

In this review article we focused on the effects of HRT on cognition in postmenopausal women without AD. It is still a matter of debate whether estrogen treatment results in improved cognitive function in postmenopausal women. Observational studies showed a halved risk of dementia in women who were treated with estrogens around the age of menopause. This is also in accordance with many animal and cell culture data; however some larger clinical trials, like Women's Health Initiative Memory Studies (WHIMS), found a negative effect on cognition (13).

3.2 Estrogen receptors & cognition

We have 2 types of estrogen classical nuclear receptors namely ERα and ERβ; and a G protein coupled receptor (GPR30 or GPER1) (14). They are located in multiple brain regions. With some slight differences, both ERα and ERβ are mostly located in the medial amygdala, stria terminalis preoptic area, and in different hypothalamic nuclei. Membrane associated ER are especially observed in the prefrontal cortex, dorsal striatum, nucleus accumbens and hippocampus, all which are involved in learning and memory (15). Mechanisms of rapid signalling are employed through GPR30 and through the interaction of membranous ERα and ERβ with metabotropic receptors. Changes in G-protein and Ca2+, increases the kinase activity; including tyrosine kinase (TK), protein kinase A (PKA) and mitogen activated protein kinase (MPKA). This change in the kinase/phosphatase equilibrium promotes phosphorylation of transcription factors including CREB (14) which induces gene transcription. Research suggests that membrane ERα and GPER1 cell survival signalling through AKT and all 3 receptors can activate Ca2+ to extracellular signal regulated kinase (ERK); signalling which is thought to be involved in promoting cell survival and memory (16,17,18). The cascade of rapid signalling of all receptors were also shown to enhance ERα mediated transcription through its phosphorylation (19), which means that in rapid signalling we do not see much of a specific ERα and ERβ action, since all signalling downstream causes ERα dependent transcription.

In the classical nuclear pathway, estrogen binds to an inactive ERα or ERβ monomer receptor in the cytoplasm or nucleus. By binding, the monomer becomes activated and forms a dimer with another activated monomer. Depending on whether it binds to the same receptor type or not, it forms a homodimer or heterodimer. In the last step, the activated dimer, together with co-regulator proteins, binds to an estrogen response element (ERE) in the DNA to initiate the transcription of target genes (18). Structural differences of ERα and β influence estrogen affinity and the interaction with transcription activators and repressors (20). ERα homodimers exhibit increased transcription in comparison to ERβ homodimers or heterodimers with studies suggesting that ERβ can act as a negative regulator of ERα mediated transcription (21,22). Furthermore, studies suggest that ERα is the dominant receptor for synaptogenesis (23) where relative expression of ERα to β may also be important.

Szymczak et al. actually observed a decrease in spine formation with an increase of ERβ in the hippocampus of rat brains (24).

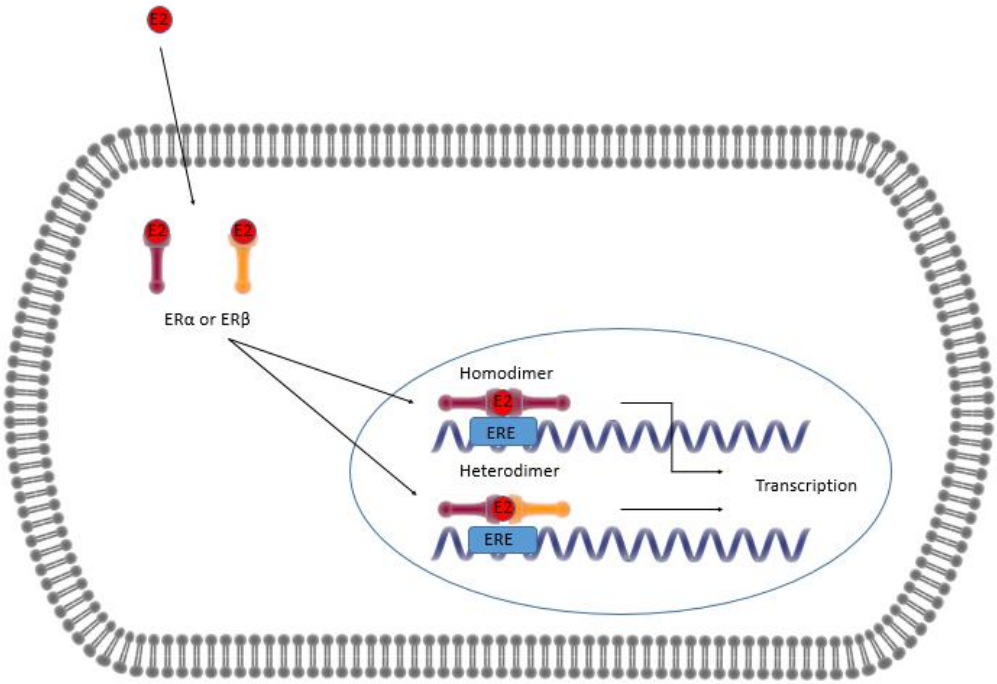

Figure 1. Estrogen nuclear pathway. Estrogen binds to an inactive – estrogen receptor α (Erα) or – estrogen receptor β (Erβ) in the cytoplasm or the nucleus. The activated estrogen receptor monomer forms a dimer with another activated estrogen receptor monomer. Depending on the type of monomers they form either a homodimer or a heterodimer. Dimer then binds to the estrogen response element (ERE) on the DNA and modifies the transcription of target genes. ERβ is acting as a negative regulator of ERα-mediated transcription, therefore the ERα homodimers cause an increased transcription in comparison to hetero- or ERβ homodimers. Summarized from (12, 15, 18).

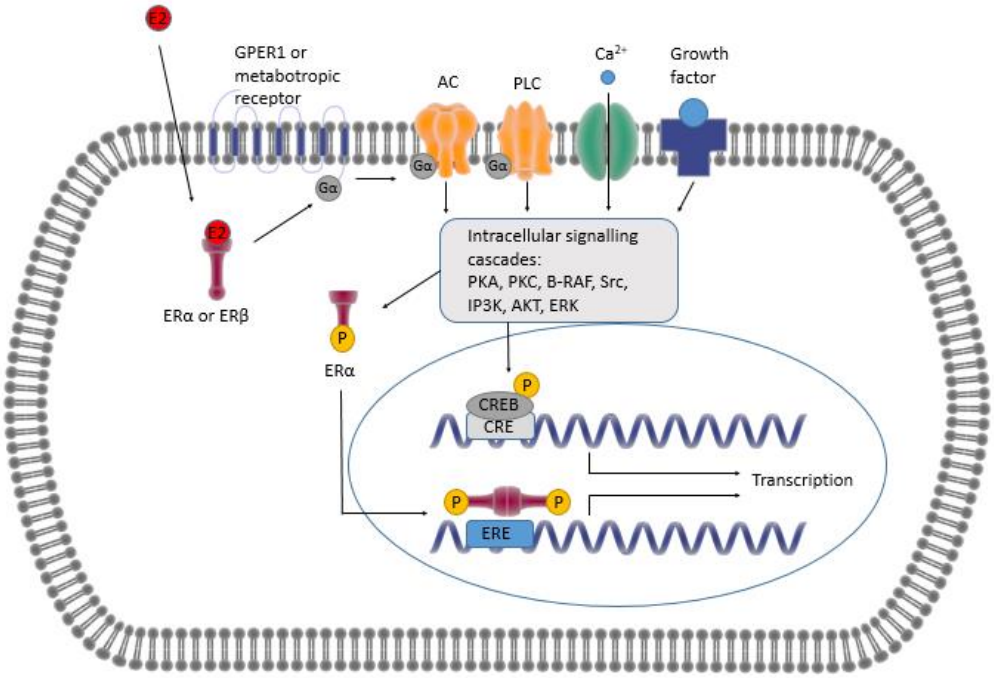

Figure 2. Membranous estrogen receptor pathway. Membrane associated estrogen receptors and associated G-protein coupled receptors are activated by estrogen, which induces intracellular signaling cascades. They then rapidly influence the physiology of neural cells or phosphorylate estrogen receptor-alpha (ERα) or CREB proteins. AC-adenylyl cyclase, PCL–phospholipase C, GPER1-G-protein coupled estrogen receptor, PKA-protein kinase A, PKC-protein kinase C, P-phosphate, CREB- cAMP response element binding protein, CRE-CREB response element. *Summarized from (12, 15, 18).*

Also, the addition of specific ligands for ERα enhances hippocampal learning and memory; conversely, the effect is not observed with the activation of ERβ (23). Fugger H. N. et al. showed impaired memory performance in ERα-knockdown mice; whereas the performance of ERβ-knockdown mice was not compromised (25). Estrogen also induces increased expression of nerve growth factors, brain-derived neurotrophic factor, and choline acetyltransferase in cholinergic neurons (with an increase in NMDA binding sites); all of which are closely connected to cognition (26,27,28). Aditionally, there is evidence that ERα protects against amyloid beta glutamate induced neurotoxicity (29). Amylod beta (Aβ) induces glutamate release from astrocytes and with that extrasynaptic NMDA receptor activation and synaptic loss (30). The exessive activation of glutamate receptors increases the intracellular Ca2+ concentration. This calcium overload causes production of oxygen free

radicals, depolarization of the mitochondrial membrane in neurons and activation of proteases; consequently leading to cell apoptosis and necrosis (31). Because this glutamate induced neurotoxicity is mostly modulated by glutamate NMDA receptors at extrasynaptic sites (32), we can conclude that ERα causes some kind of modulation of extrasynaptic NMDA to attenuate the effect of glutamate's increases in Ca2+ levels. Another mechanism of estrogen protection against Aβ could be through increased expression of Aβ clearance factors mostly insulin degrading enzyme and others, which was shown by experiments performed in cell cultures and samples of female rat brains (33).

3.3 Progesterone receptors & cognition

The classical nuclear progesterone receptor has been localized to multiple regions of the CNS, such as the hippocampus, cortex and cerebellum (34,35). Two isoforms are known, namely progesterone receptor A and B (PRA, PRB). PRA is derived with alternate initiation of translation from the same mRNA transcript, rather than by proteolysis of the larger receptor B protein (36). The classical nuclear PR, like most steroids receptors, exerts its action through interaction with specific response elements (PRE) in the promoters of target genes and then with basal transcription machinery by binding to steroid receptor co-activators and with that initiates transcription of specific genes.

In addition to PRA and PRB, some other splice variants have been identified which are formed with the insertion of intronic exons or exon skipping (37). Some of these uncommon variants have defective DNA-binding domain and nuclear localization signals; and, as such, could be serving as a membrane associated PR (38), which exerts its effect through non-genomic mechanisms such as activation of second messenger signaling cascades (39,40). Another progesterone binding protein with characteristics of G-protein coupled receptor has been identified as a membrane receptor. It is known as 7TMPR due to its 7 transmembrane domains (41,42). Even though the role of progesterone receptors in reproductive function has been extensively studied, the specific receptors that exert neuroprotective effects of progesterone are yet to be identified (38). Probably, the gene transcription through classical PRA and PRB exerts some of the effects; but progesterone binding sites have also been identified on the membrane of hypothalamic and spinal neurons (43). These sites, known as progesterone receptor membrane component 1 (PGRMC1) are the same ones that exert progesterone antiapoptotic actions in granulose and luteal cells in the ovary. There, PGRMC1 exerts its action through activation of SRC family kinases or forms complexes with serpine 1 mRNA binding protein (PAIRBP1) (44); but these signaling actions are not yet determined in neural cells. Similarly to PGRMC1, the before mentioned transmembrane receptor 7TMPR can mediate progesterone signaling. It was shown to activate inhibitory G protein and through its inhibition of cAMP production activates MAPK pathway (45). Through the activation of MAPK, extracellular signal regulated kinase (ERK - which is activated by both progesterone and estrogen) is required for induction of neuroprotection (46,47) and

activation of its substrate cAMP response element binding protein (CREB) is associated with resistance to ischemic injury and, in turn, upregulates antiapoptotic protein bcl-2 (48,49). In addition to the MAPK/ERK pathway, estrogen and progesterone activate the Akt pathway, which also increases neuronal survival (50).

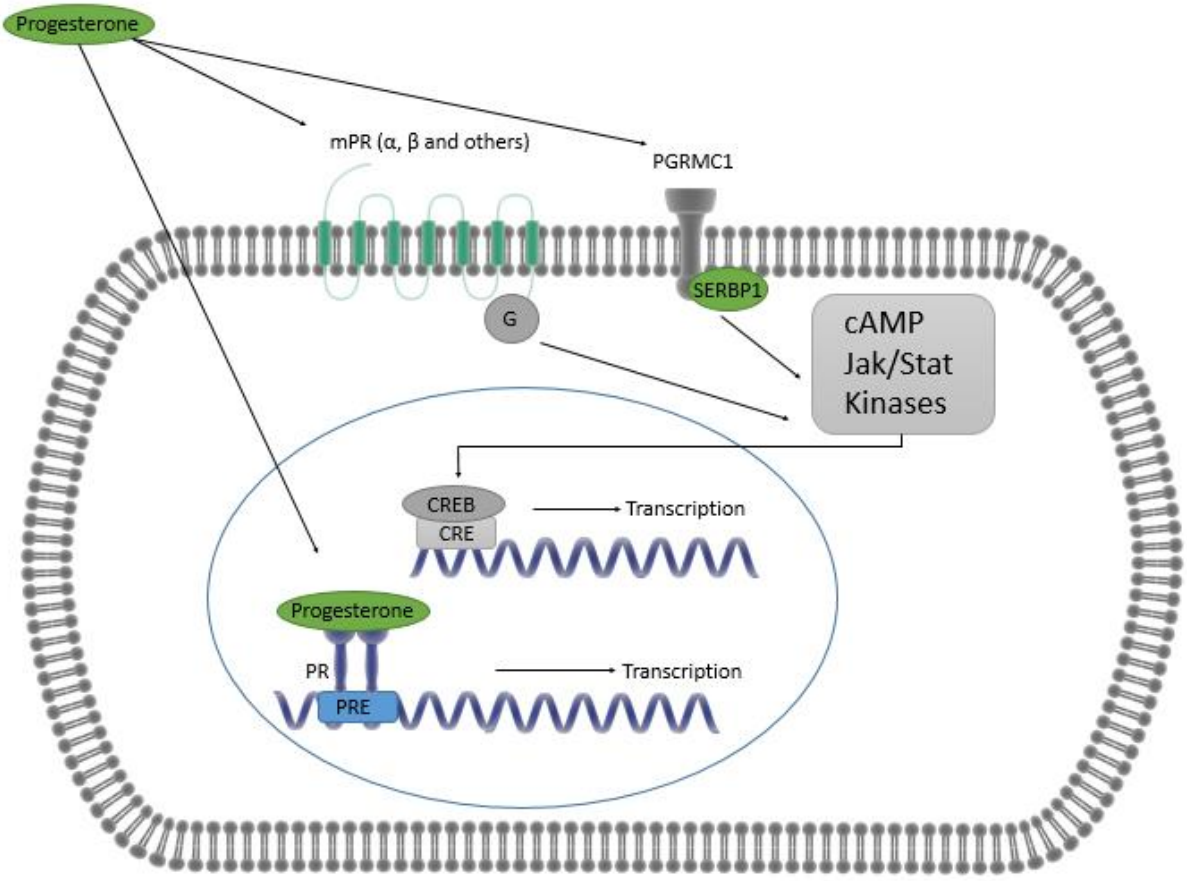

Figure 3: Nuclear and membranous progesterone receptor pathway. Nuclear pathway is similar to estrogen signalization with formation of homo- and heterodimers which activate the transcription of specific genes. Membranous pathway consists of progesterone activating the membranous progesterone receptors and progesterone receptor membrane component 1 (PGRMC1). They activate second messengers and kinases, which in turn activate CREB and initiate transcription. PRE-progesterone response element, PR-progesterone receptor, mPR-membranous progesterone receptor, SERBP1- serpine 1 mRNA binding protein. Summarized from (38).

The effect is not the same with the use of synthetic progesterone. Medroxyprogesterone Acetate does not affect Bcl-2 expression but rather inhibits the one caused by estrogen. Similarly, MPA does not alter Akt phosphorylation, but blocks E2-induced Akt phosphorylation (40). Furthermore, the addition of progesterone after traumatic brain injury has been shown to reduce the cytotoxic surge of inflammatory factors which increase immune cell invasion and cerebral edema (51). In models of spinal cord injury, progesterone neuroprotection was associated with an increase in brain derived neurotrophic factor (BDNF), choline acetyltransferase and a reduction in mitochondrial dysfunction (52,53). Regarding indirect neuroprotective effects, progesterone was also found to decrease leakage of the blood-brain-barrier, glial activation and to increase myelinization (54,55,56). In neural cells, progesterone is transformed into neurosteroid allopregnanolone with sequential action of 5a-reductase and 3a-hydroxysteroid dehydrogenase (38), which then binds to GABA-A receptor, causing an increase in GABA induced chloride conductance (57,12). Because GABA receptor activation inhibits the induction phase of long-term potentiation and can through that impair memory process, the same can be true for allopregnanolone (57). On the other hand, several studies have suggested that allopregnanolone acting on GABA-A attenuates seizure activity and decreases hippocampal neural loss during excitotoxicity (58,59,60). Furthermore, glutamate, the main excitatory neurotransmitter in the CNS, has been shown to be suppressed by progesterone in dose dependent manner, meaning that it can protect the neurons from glutamate excitotoxicity, while estrogen has the opposite effect (61,62). There are some other findings of progesterone and estrogen's antagonistic actions. Progesterone was found to block estrogen induced increases in hippocampal spine density (63) and upregulation of BDNF, neurotrophin 3 and neural growth factor in the entorhinal cortex (64). Bimonte-Nelson H. A. et al. also found that the addition of progesterone reversed the beneficial spatial memory cognitive effects of estradiol in ovariectomized rats (65). In summary, the literature shows a complex interaction of estrogen and progesterone to classic neurotransmitters, where both of the female sex hormones can have either neuroprotective or some neurotoxic effects.

3.4 Hormone replacement therapy regimens: continous versus cyclic treatment

Hormone Replacement Therapy regimens can be cyclical (sequential) or continuous. Continuous involves taking both estrogen and progesterone daily and is recommended for postmenopausal women. Cyclical regimens are recommended for women who have postmenopausal symptoms, but still have their periods. Depending on their menstrual cycle, these regimens could be monthly (where estrogen is taken every day with progesterone only the last 14 days of the cycle) or three-monthly (where estrogen is taken daily with progesterone only 14 days every three months). The first is generally recommended for women with regular periods and the second for women experiencing irregular periods who then have menstrual bleeding once every three months (66).

Literature shows that continuous versus cyclic progesterone exposure has significant differences on gene expression profiles in the brain. A regimen of estrogen with cyclic progesterone exposure mimics the physiologic female hormone pattern better and induces gene expression profiles consistent with the ovary-intact brain. By contrast, the regimen with continuous progesterone exposure induces gene expression profiles of the ovarian-hormone-deficient brain (67). In a transgenic mouse model of Alzheimer's disease, treatment with estrogen prevented the increase of tau hyper phosphorylation, beta-amyloid accumulation and impaired hippocampal-dependent behaviour, where continuous progesterone blocked its beta-amyloid lowering action. In contrast, cyclic progesterone alone was able to decrease beta-amyloid levels and improved rather than inhibited estrogen effects (68,69). Barron A. M. et al. studied the effect of HT regimens on neuron viability and sprouting, after entorhinal cortex lesion in rats and found that a combination of estrogen and cyclic progesterone had the greatest neuroprotective efficiency (70).

3.5 The »timing hypothesis« (window of opportunity)

Due to before mentioned discrepancies of observational data, which showed decreased mortality in women using HRT (71,72) and larger clinical trials, such as WHI and the Heart and Estrogen/Progestin Replacement Study (HERS), which showed an increase of cardiovascular adverse events and mortality, the idea of a window of opportunity was developed (73). This “timing hypothesis” advocates that the cause of contradictory results is the time of initiation of HRT; where different clinical effects occur if treatment is started close to the onset of menopause, rather than years later. In support of this hypothesis, women in observational data started with HRT at younger age of onset to treat their postmenopausal symptoms, where women in randomized clinical trials were older (74). Clarkson TB tested this hypothesis on a monkey model which showed a 70% reduction in coronary atherosclerosis in monkeys immediately treated with CEE, whereas the delay of 2 years showed no significant changes (73,75). The ELITE trial studied the vascular effects of early and late postmenopausal HRT and found that oral estradiol, when initiated within 6 years after menopause, was associated with slower progression of subclinical atherosclerosis; whereas a beneficiary effect was not observed when HRT was initiated 10 or more years after menopause (76). Carrasquilla D. G. et al. also studied the effect of timing of HRT on the incidence of coronary heart disease and found that only early initiation (<5 years) had a protective effect (77). A study by Pereira R. I. shows that timing could also have an effect on insulin action; where transdermal estradiol was able to increase glucose disposal rate in women who were treated less than 6 years, but not in women more than 10 years after menopause (78). The effect of timing of HRT is not so clear for neuroprotection and cognition; however, studies done on animal models show that some neuroprotective effects such as CHIP-mediated degradation of brain ERα and increased miRNAs expression that regulate BDNF, glucocorticoid receptor, and SIRT-1 genes which are important for memory and stress regulation are also in accordance with the timing hypothesis (79,80). An observational longitudinal study, by Whitmer R. A. et al., found that women who use HRT in midlife had a 26% reduced risk of dementia; where, on the contrary, women using HRT only in older age had a 48% elevated dementia risk compared to women who never used HRT (81).

In summary, there is evidence from both animal and clinical studies to support the timing hypothesis, but new clinical studies should be developed to study the time period until when after menopause is the initiation of therapy purposeful. The belief of the North American Menopause Society, stated in their 2017 guidelines, is that for women who initiate hormone therapy more than 10 years after menopause, the benefit-risk ratio appears less favorable due to greater absolute risks for CHD, stroke, thromboembolism and dementia (82).

3.6 Pharmacokinetics of hormone replacement therapy

The pharmacokinetics of HRT is dependent both on the route of administration (oral or non-oral) and nature of hormone (natural or synthetic). If we want, for example, to achieve early follicular phase levels of estradiol with oral preparation, we have to use an approximately 15 times larger amount as produced daily by the ovary (83). Since micronized estradiol is almost completely absorbed, that is not the reason for poor bioavailability but rather metabolic inactivation which happens in the bowel mucosa and liver. Therefore, the result of oral estradiol ingestion is the exposure of the total oral dose to the liver, which causes an increase in hepatic substances that are sensitive to estrogen, such as renin substrate, lipoproteins, sex hormone binding globulin and other carrier proteins (83). Another difference is the proportion of estradiol (parent compound) and its metabolites (estrone), which differs from physiologic findings in menstrual cycle. Namely, with endogenous estradiol produced by the ovary, the proportion of E2/E1 is always larger than 1, whereas estradiol ingested orally is almost completely metabolized into estrone in the bowel mucosa (83). In the liver, estradiol is in large part further metabolized into estradiol sulfate. Through enzyme 17β hydroxylase in hepatic and extrahepatic sites, E1 is converted back to E2 but preferential direction of this enzymatic reaction results in almost 10 times larger levels of E1 (84). On the other hand, studies have shown physiological E2/E1 ratios when the hormone was delivered transcutaneously (85). The transdermal estradiol is delivered into systemic circulation through stratum corneum at a constant rate for multiple days with avoidance of first-pass hepatic metabolism and can therefore maintain the physiologic levels with low daily doses and less side effects (86). However, the hepatic effects seen with oral estradiol ingestion is not only linked to the oral route of administration, but is also seen when the amount of E2 reaching the liver are increased to amounts seen with oral intake. That happens during the first trimester in pregnancy when E2 levels are largely increased. The same outcome is seen with the use of synthetic estrogens which resist hepatic inactivation, such as ethinyl estradiol or CEE. This was demonstrated by the studies using non-oral administration of mentioned substances (87,88).

Similar principles are true for progesterone where, even with high plasma levels of progesterone after micronized progesterone (m-P) oral ingestion, the effect on the endometrium is not the same as with increase of endogenous P during the luteal phase (89). The reason for that is that recorded high plasma levels with m-P were largely due to high levels of P metabolites, mainly 5b pregnanedione and pregnanolone (83). Due to this poor bioavailability of oral P, synthetic progesterones (such as MPA) were synthetized to resist the enzymatic degradation with oral administration. In addition, P also exerts non-genomic effects, such as enhancing the brain´s GABA channels (57). These neuro-psychological effects are not shared by synthetic MPA, therefore the described principles of pharmacokinetics are more clinically relevant for P than for estrogens.

4. Methods:

We carried out a literature search for a period between January 1995 to May 2018, in three databases (PubMed, Embase, Cochrane) using the following keywords: progesterone OR estrogen AND cognition. We set inclusion criteria, which were the following: the studies had to be randomized controlled trial, used cognition test(s) and had no additional pharmacological manipulations or used selective estrogen receptor modulators (SERMs). Due to the difficult assessment of the amount and concentration of phytoestrogens and herbal preparations, these were also excluded.

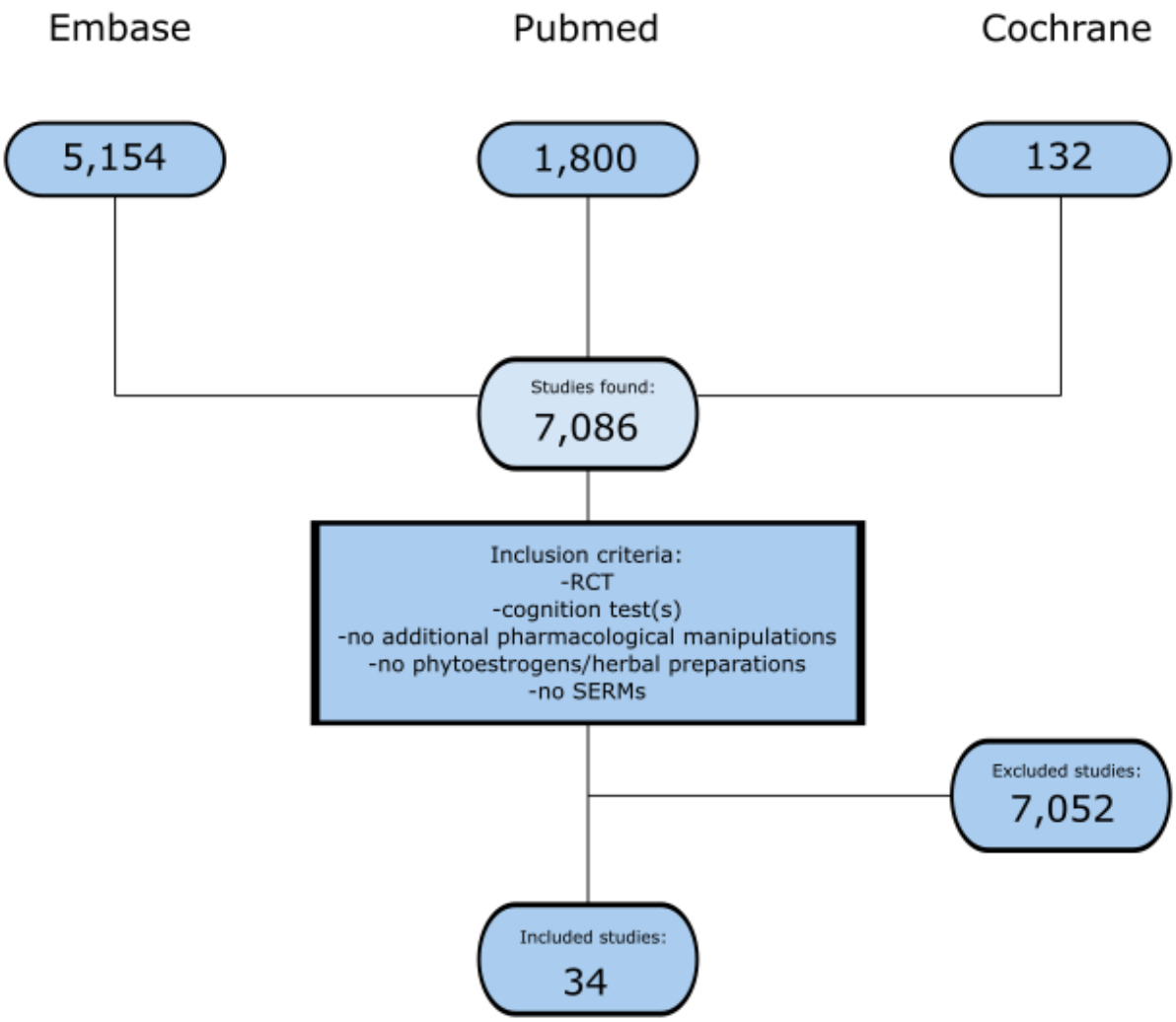

Figure 4: Flow diagram of found studies. RTC- randomized controlled trial; SERMs- selective estrogen receptor modulators.

In Embase and Pubmed databases we found 5,154 and 1,800 studies, respectively. An additional 132 new studies were found while searching the Cochrane database, which uses both the Embase and Pubmed databases (Fig. 1). For additional information, we also consulted previous review papers. After finding a substantial amount of studies, we started reviewing abstracts in order to exclude the ones which are not in accordance with our inclusion criteria. A majority of studies which we excluded were review articles or studies which did not deal with our particular subject of interest.

After application of our inclusion criteria, a total of 34 articles out of 7,086 were included (Fig. 1). The articles that met our inclusion criteria were later divided and categorized by the age of the patients, duration of the therapy, type of HRT, and the effect it had on cognition.

The age of the patient was categorized into older and younger women, where the cut-off age was set to 65 years. The age of 65 has been used in literature as the beginning of elderhood, 75 as old and above 90 as very old age. Likewise, a great majority of our included articles used the same cut-off age value when categorizing patients into older and younger. Since the study “Long-term Effects on Cognitive Trajectories of Postmenopausal Hormone Therapy in Two Age Groups” by Espeland MA (90) presented the effect in 2 different age groups, we regarded both groups as a separate article and with that got an artificial number of 35 articles included in our results.

The duration of therapy was categorized into acute, subacute and chronic; where the cut-off values were: 4 weeks, 12 months and over 1 year, respectively.

The type of therapy was divided later by patients who received natural estrogen or medroxyprogesterone acetate; because we found discrepancies in the effect they had on patient’s cognition.

5. Results:

Studies used different types of hormone therapy; where the most common was combined estrogen and progesterone therapy, which decreases the risk of endometrial carcinoma in women with an intact uterus; and, estrogen only HRT for women who have had a previous hysterectomy and who used a transdermal preparation. Therapies included a variety of natural (estradiol, m-P) and synthetic (MPA, estradiol valerate, ethinylestradiol, norethisterone hormone preparations in generic doses; where some studies also used unconventional doses (Table 1).

Table 1: Types of therapy used in studies with standard doses.

Estrogen		**Progesterone**	
type	*dosage*	*type*	*dosage*
Estradiol (17beta-estradiol)	Oral: **0.25mg***, 0.5mg, **1mg**, **2mg**,	Micronized progesterone (m-P)	Oral: 100mg, **200mg**, 300mg IM: 50mg
Conjugated equine estradiol (CEE)	Oral: 0.3mg, **0.45mg* 0.625mg**, 0.9mg, 1.25mg, 2,5mg IM/IV: (premarin) 25mg/5ml	Medroxyprogesterone acetate (MPA)	Oral: **2.5mg**, 5mg, 10mg, 100mg IM: 150mg/ml, 400mg/ml
Estradiol valerate	Oral: 1mg, **2mg**, 3mg IV: 10mg/1ml, 20mg/1ml, 40mg/1ml	Norethisterone (Norethindrone)	Oral: 0.1mg, 0.35mg, 0.5mg, **1mg*** 5mg
Ethinylestradiol	Oral: 0.02mg, 0.05mg, 0.5mg	Dienogest	Oral: 2mg, **3mg**
Transdermal estradiol	Patch: 0.025mg, **0.05mg**, 0.075mg, **0.1mg**, **0.014 mg/d***, **50 µg /d***, **100µg /d*** Cream: 0.1mg	Drospirenone	Oral: 0.25mg, 0.5mg, 1mg, **2mg*** 3mg

Note: doses in bold were used in included studies. Summarized from https://www.medscape.com/

**not a typical dose of HRT*

After the application of our inclusion criteria, a total of 35 articles from 7,086 were included. Regarding the duration of therapy, 3 studies used acute (≤1 month), 18 subacute (1 month-1 year) and 14 used chronic therapy (≥1 year) (Table 2), (Table 3), (Table 4). Most of the studies, (20/36) showed that HRT had no effect on cognition; whereas, an additional 6 showed positive influence on verbal cognition in younger women. The remaining 9 studies showed a negative effect on cognition.

Table 2: Studies with neutral effect, dividend based on duration of therapy

STUDIES WITH NO EFFECT ON COGNITION (20)		
Duration	**Author**	**Treatment/dose**
ACUTE THERAPY		
4 weeks	Kocoska-Maras Lj et al (91)	Estradiol 2 mg/d
SUBACUTE THERAPY		
6 cycles of 28 days	Gorenstein C et al (92)	CEE 0.625 mg/d
24 weeks	Wolfa OT et al	Estradiol valerat 2mg/d
10.9±2.4 months	Guvenal T et al (93)	CEE 0.625 mg/d + MPA 2.5 mg
9 months	Binder EF et al (94)	CEE 0.625 mg/d +MPA 5 mg
6 months	Alhola P et al (95)	Estradiol valerat 2mg/d+ 1 mg nore-thisterone
4 and 8 weeks	LeBlanc ES et al (96)	Estradiol 2 mg/d
3 months	Polo-Kantola P et al (97)	Estradiol 2,5 mg per os Estradiol 3.2 mg TD
6 months	Alhola P et al (98)	Patient choice
20 weeks	Almeida OP et al (99)	Estradiol 2 mg/d
6 months	Davison SL et al (100)	estradiol 1 mg/drospirenone 2 mg
3 months	Smith YR et al (101)	ethinyl estradiol 5 ug + 1 mg nore-thindrone acetate
10 weeks	Dunkin J et al (102)	Estradiol 0.1 mg /d TD
6 weeks	Schiff R et al (103)	Estradiol 50 µg /d TD
CHRONIC THERAPY		
7 years	Espeland MA et al (WHIMS) (90)	CEE 0.625 mg/d + MPA 2.5 mg
4 years	Gleason CE et al (KEEPS) (104)	CEE 0.45-mg/d or estradiol 50 µg /d TD + m-P 200-mg/d
4 years	Kantarci K et al (105)	0.45 mg CEE or-estradiol 50 µg /d TD
2 years	Yaffe K et al (106)	0.014 mg estradiol/d TD
3 years	Pefanco MA et al (107)	Estradiol 0.25 mg/d
2.5 and 5 years	Henderson VW et al (108)	estradiol 1 mg/d

Data from included studies, with neutral effect on cognition (91, 92, 93, 94, 95, 97, 98, 99, 100, 101, 102, 103, 90, 104, 105, 106, 107, 108).

Table 3: Studies with neutral effect, dividend based on duration of therapy

STUDIES WITH POSITIVE EFFECT ON COGNITION (6)		
Duration	**Author**	**Treatment/dose**
ACUTE THERAPY		
3 days	Krug R et al 20 (109)	TD Estogen 100 mg/d
SUBACUTE THERAPY		
90 days	Berent-Spillson A et al (110)	Estradiol 1 mg/d or P 200 mg/d
12 weeks	Sherwin BB et al (8)	CEE 0.626 mg/d or CEE 0.626 mg/d + m-P 2,5 mg/d or 0.626 mg/d + MPA 2,5 mg/d
2 months	Linzmayer L et al (111)	Estradiol valerate 2mg/d or Estradiol valerate 2mg/d + progesterin dienogest 3mg/d
12 weeks	Joffe H et al (112)	TD Estradiol 0.05 mg/d
CHRONIC THERAPY		
2 and 4 year	Ryan J et al (113)	different types of HT /patient choice

Data from included studies, with positive effect on cognition (109, 110, 8, 111, 112, 113).

Table 4: Studies with neutral effect, dividend based on duration of therapy

STUDIES WITH NEGATIVE EFFECT ON COGNITION (9)		
Duration	**Author**	**Treatment/dose**
ACUTE THERAPY		
4 weeks	Kocoska-Maras Lj et al (114)	Estradiol valerate 2 mg/d
SUBACUTE THERAPY		
4 months	Maki PM et al (4)	CEE 0.625 mg/d + MPA 2.5 mg
3 months	Newhouse PA et al (115)	Estradiol (1 mg/d for 1 month, then 2mg/d for 2 months)
CHRONIC THERAPY		
5.5 years	Espeland MA et al (WHIMS) (90)	CEE 0.625mg/d +- MPA 2.5 mg
3 years	Espeland MA et al (WHIMS) (116)	CEE 0.625 mg/d
4.2 +- 0.4 years	Grady D et al (HERS) (117)	CEE 0.625 mg/d + MPA 2.5 mg
4.2 years	Stephen RR et al (WHIMS) (118)	CEE 0.625 mg/d + MPA 2.5 mg
3 years	Resnick SM et al (WHIMS) (119)	CEE 0.625 mg/d + MPA 2.5 mg
7 years	Espeland MA et al (WHIMSY) (120)	CEE 0.625mg/d +- MPA 2.5 mg

Data from included studies, with negative effect on cognition (114, 4, 115, 90, 116, 117, 118, 119, 120).

Pie charts from figure 2 and figure 3 represent the different content of younger and older patients in studies, with positive and negative effect on cognition. Figure 3 shows that 6 out of 9 negative studies were done on older patients (>65 years); whereas, figure 2 represents an even more homogenous group of positive studies, where all except one study were done on younger patients.

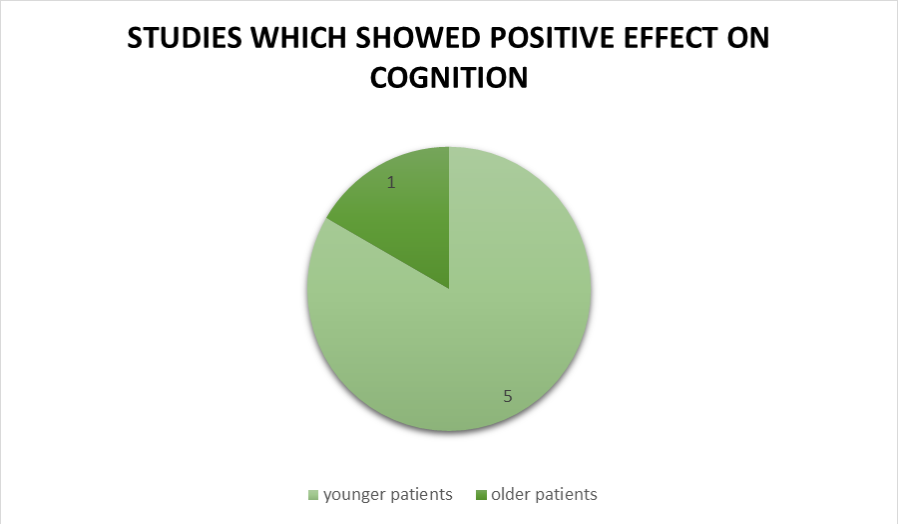

Figure 5: Pie chart of positive studies, divided on patients' age. 5 studies with younger patient (<65 years), only one with older patient (>65 years)

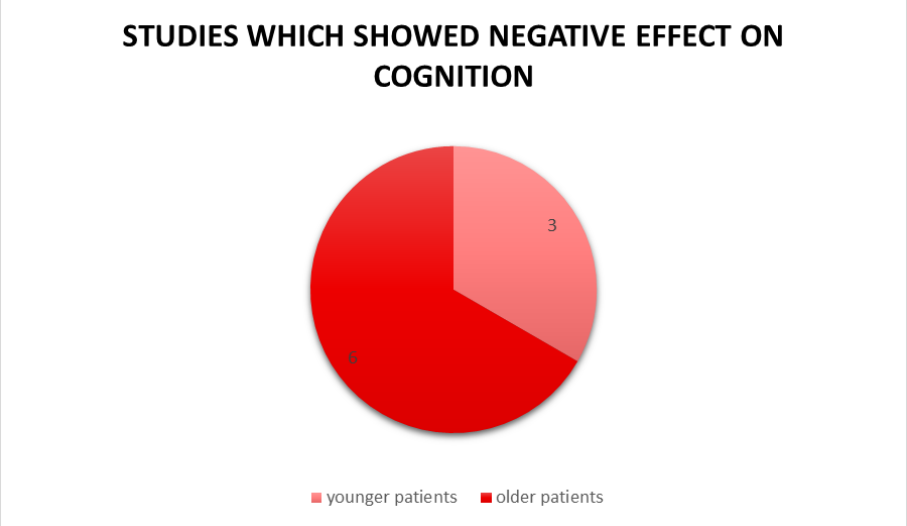

Figure 6: Pie chart of negative studies, divided on patients' age. 6 studies with younger patient (<65 years), 3 studies with older patient (>65 years)

Table 5: Manner of progesterone use in found studies and its connection to cognition. Cyclic progesterone hade neutral or positive effect on cognition while most of the studies that used continuousprogesterone had negative effect on cognition

Effect on Cognition	Continuous Progesterone Regimen	Cyclic (sequential) Progesterone Regimen
Neutral	Guvenal T et al (93) Alhola P et al (95) Davison SL et al (100) Espeland MA et al (WHIMS) (90)	Binder EF et al (94) Alhola P et al (95) Smith, Y. R. (101) Gleason C. E. (104)
positive	Berent-Spillson A et al (110) Linzmayer L et al (111)	Sherwin BB et al (8)
negative	Espeland MA et al (WHIMS) (90) Espeland MA et al (WHIMS) (120) Grady D et al (HERS) (117) Stephen RR et al (WHIMS) (118) Resnick SM et al (WHIMS) (119) Espeland MA et al (WHIMSY) (116) Maki PM et al (4)	ø

Data from included studies, which used progesterone therapy (93, 95, 100, 90, 110, 111, 120, 118, 119, 116, 4, 94, 95, 101, 104, 8)

In table 5, we can see which studies, divided by their effect on cognition, used continuous or cyclic progesterone treatment. As mentioned before, the literature suggests that the manner of progesterone use impacts gene expression in the brain (67). Cyclic progesterone treatment is more physiologic and beneficial for cognition and, contrary to the continuous treatment, increases the neuroprotective effects of estrogens (68,69,70). Only 3 positive studies used progesterone treatment; 2 continuous and 1 cyclic. Out of 7 neural studies with progesterone treatment, a study by Alhola P (95) used 2 groups with both continuous and cyclic methods, so we can say that half used continuous and the other half the cyclic manner of treatment. The most interesting finding here is that all 7 negative studies which used progesterone treatment did so using the continuous manner.

6. Discussion

Most of the studies we included (20/36) did not show any effect on cognition. Out of these 20 neutral studies, 6 were done on older patients and 3 used therapy with MPA. Seven used chronic, 1 acute and the other 12 subacute durations of therapy (table 2). Even though emerging evidence suggest that older women should not be receiving therapy with MPA, a study by Ellen F. Binder (94) on a smaller sample size (n=34) showed no negative decline in women whose mean age was 81 years. In the study, women received daily CEE (0.625mg/day), with a higher dose of MPA (5mg/day) prescribed to women without a prior hysterectomy in a cyclic manner for 13 days every third month. Tevfik Guvenal was studying the effects of different postmenopausal HRTs on cerebral blood flow and cognitive functions, and found no significant differences in pre- and posttreatment values in groups using placebo, unopposed CEE or a combination of CEE and MPA; however, the sample size was small (n=11-13 per group) and was done on younger patients with a mean age of 46 years (93). Studies which did not show any effect on cognition had generally a smaller sample size – with some studies using less than 10 patients and only 6 studies had group sample sizes larger than 40 participants. The largest study in the group (n=701) is a divided part of the study done by MA Espeland et al. who studied the effect of postmenopausal HRT in 2 age groups. Where the treatment of older women was associated with long term decrements in global cognitive function, working memory and executive function, younger women (50-54 years) had no significant long term effect on cognition (90). Another larger study by Gleason C. E., an ancillary cognitive and affective study of the Kronos early estrogen prevention study (KEEPS), examined the effect of menopausal HRT of longer duration (4 years) on mood and cognition in recently postmenopausal women. Patients were randomized into 3 groups to receive a combination of oral CEE (0.45mg/day) and cyclic therapy of micronized progesterone (m-P) (200mg/day), transdermal estradiol (50 µg/day) and cyclic therapy of oral m-P, or a placebo. The study found that none of the HRTs had any effect on cognition, only a small beneficial effect on mood was noted with the CEE therapy (104). Kantarci K. also performed the KEEPS ancillary study (105), which investigated the effects of hormone therapy on brain structure.

As mentioned above, there was no difference in global cognitive function across the groups; but, a statistically significant (p=0.01) higher rate of ventricular expansion was observed in the CEE group, which correlated with the rates of decreasing brain volume and with rates of increase in white matter hyperintensity which was observed with the fluid-attenuated inversion recovery (FLAIR) sequence (105). Yaffe K., Schiff R. and Dunkin J. studied the effect of transdermal estrogen preparations; and, none of the 3 studies found any benefit of HRT on cognitive functions. Schiff R. however found that depressive symptoms could be reduced with transdermal estradiol preparation, which was not only due to its known positive effect on female climacteric changes (106,103,102).

Out of the 6 studies which showed evidence for positive influence on cognition, 5 of them were done on younger women (<65 years) and only one on older (>65 years) (Fig. 2). Additionally, all of them except one used acute and subacute time of therapy (Table 3). Studies that positively influenced cognition used therapy with unopposed estrogen or a combination of estrogen and micronized progesterone. In a study by Ryan J et al., “Characteristics of hormone therapy, cognitive function, and dementia: the prospective 3C Study”, the type of therapy was not specifically detailed; rather, the women used different types of HRTs of their choice. This is also the only study out of the group to show a positive influence on cognition; which was done on older postmenopausal women and with chronic therapy (2 and 4 year follow-up) (113). Based on these characteristics, it might be reasonable to exclude it and we would be left with an even more homogenous group of studies with a positive effect where all of them are done on younger women, of acute and subacute duration of therapy and used unopposed estrogen or estrogen with micronized progesterone (Table 3). Two studies from the positive group used transdermal estrogen preparations as a mode of therapy, which could also be the reason for decreasing side effects of HRT while still exerting positive effect on the brain. Estradiol is the usual estrogen compound used in transdermal patches. Substantially lower doses can be used since the metabolism in the skin is low and estradiol bypasses hepatic metabolism and extensive gut degradation (121).

Transdermal preparations are, therefore, more benign metabolically with less adverse effects on clotting factors, blood pressure, triglycerides and thyroid-binding globulin (122). Randomized studies, which compared oral and transdermal estrogen preparation, showed a larger increase of activated protein C with oral therapy (123). Increased levels of protein C are at least partly responsible for the increased risk of VTE and PE; and, in 2016, the IMS released recommendations to use transdermal preparations in women who are at risk for VTE (124). In addition to observed positive cognitive effects in these 5 studies, a study by Joffe H. used fMRI with blood oxygenation level dependent (BOLD) contrast imaging and found an increase in prefrontal cortex activation during verbal recall tasks, in women who were using estradiol transdermal patches for 12 weeks (112). It is important to note that in addition to the small number of studies which showed positive effect, this is also the group of studies which had overall the smallest sample sizes. Only the study by Ryan J. had therapy group sizes of over 30 participants (113). Apart from health bias, where women who enroll in clinical trials are on average in better health, we can also mention bias between age groups. Older women have decreased numbers of neuron and expression of estrogen receptors (18) and that could be the reason for decreased therapeutic effectiveness of HRT for cognition.

On the other side of the spectrum, we have 9 studies which showed negative influence on cognition. Interestingly, 6 of those were done on older patients and used chronic duration of therapy (>1 year). It is important to point out that 5 of the 9 studies used the database from the same large clinical trial: Women's Health Initiative Memory Studies (WHIMS) (Table 4, Fig. 3).

The WHIMS are an ancillary study to the Women's Health Initiative (WHI) trial. The WHI was designed to evaluate the benefits and risks of menopausal HRT in the prevention of different chronic diseases, including cardiovascular diseases and breast cancer in postmenopausal women. The trial enrolled 27,347 postmenopausal women at 40 US clinical centers. Women with an intact uterus were randomized to receive continuous CEE (0.625 mg/d) + MPA (2.5 mg/d) or placebo; whereas, women who had a hysterectomy were randomized to receive either continuous CEE alone or a placebo. The trial was stopped earlier than initially planned, because of increased risk of stroke for the CEE + MPA arm after a median of 5.6 years and in the CEE-only arm after a median of 7.2 years. The average age of women who participated in WHI trial was 63 years, which is significantly higher than the average age of menopause in the west. That is in contrast with most observational studies, which showed a positive effect of HRT used in younger patients closer to the onset of menopause. Therefore, the WHI was not representative of younger symptomatic women from observational studies.

With stratification of WHI by age there was a reduction in all-cause mortality and MI in women aged 50 to 59 years, treated with CEE alone. For the CEE + MPA group, a risk of MI depended on the time since menopause. This supports the “timing hypothesis” which was first described in animal primate studies in 1990s (125). Following the before mentioned WHI sub analysis, KEEPS and ELITE clinical trials were conducted to evaluate the safety of menopausal HRT in early and healthy postmenopausal women; and found mainly a positive effect on health with HRT (126).

The Women’s Health Initiative Memory Study (WHIMS) a WHI ancillary study was the first double-masked, randomized, placebo-controlled, long-term clinical trial, which was designed to found out if HRT reduces the incidence of all-cause dementia in women aged 65 and older. Women underwent annual cognitive assessments, with the Modified Mini-Mental State (3MS) Examination, and found an increased risk of dementia – especially in the group with combined CEE + MPA therapy. Another article, by Espeland MA et al., studied the effects on Cognitive Trajectories of Postmenopausal HRT in two age groups. Just as in the before mentioned studies, they found a decline in global cognitive function, working memory and exectutive functions when HRT was precribed to older women (65-79 years).

When, on the other hand, HRT was prescribed to women aged 50-54 years, HRT had no significant effects on cognitive function and on changes in cognitive function (90). Interestingly, 6 of the 9 studies which showed a negative cognitive effect, used medroxyprogesterone acetate in the therapy (Table 4). Medroxyprogesterone acetate is a synthetic progesterone, related in chemical structure to progesterone. It differs mainly in that it has a methyl group and acetate group at its 6^{th} and 17^{th} carbons, respectively. This chemical difference increases its oral bioavailability and results in higher progestational activity. Progesterone is an important steroid for the brain, due to its neuroprotective effect when used to treat traumatic brain injury. Studies on animal models showed that, in contrast to natural progesterone, MPA does not share the same neuroprotective effect. Studies in rat hippocampal cells found that MPA actually antagonizes the neuroprotective effect of estradiol (40,47). Jodhka et al. found that, in contrast to progesterone, which induces neuroprotective brain-derived neurotrophic factor (BDNF) in the explants of the cerebral cortex, MPA did not have the same effect and does not protect against glutamate toxicity (127).

Irwin et al. studied the effect of post-ovariectomy restoration of mitochondrial function by estradiol and progesterone. Again, MPA was found to have an antagonizing effect on estradiol (128). In addition to direct its negative influence on the brain, the negative effect could also be secondary; with studies on primates showing that MPA negates the positive effect estradiol has on vascular function (129). The clinical significance of the before mentioned animal studies need to be investigated; but they are supporting the same negative effect pattern on cognition as in the groups treated with MPA in our review.

Regarding the manner of progesterone treatment, all of our included studies, which used progesterone treatment and showed negative influence on cognition, used continuous daily administration of progesterone. Only 3 studies, which showed a positive effect, used progesterone treatment. Out of these, 2 used continuous and 1 used cyclic progesterone therapy. Eight neutral studies also used combined therapy with progesterone. Four used continuous and 3 used cyclic progesterone therapy (Table 5). The 8th study, by Alhola P et al., used both types (130). In premenopausal women, the HRT was estradiol and cyclic norethisterone for 12 days and in postmenopausal the therapy was continuous for both estradiol and norethisterone (95). From the before mentioned literature which suggests that cyclic progesterone treatment mimics the physiologic female hormone pattern better is neuroprotective, induces expression of different genes in the brain and is able to decrease beta-amyloid levels, we can say that our results, where all negative studies used continuous of progesterone treatment, could be also, at least partially, explained with the manner of therapy (Table 5).

7. Conclusion

The great majority of studies included in our research showed that HRT had no effect on cognition (Table 2). We found some evidence of positive effect with the use of unopposed estrogen and combination of estrogen with natural micronized progesterone in younger women (<65 years); where the duration of therapy was of the acute and subacute type (mostly less than 3 months) (Table 3, Fig. 2). There is evidence of negative effect with the use of conjugated equine estrogen/medroxyprogesterone acetate in older postmenopausal women and a continuous manner of progesterone therapy (Table 4, Table 5, Fig. 3). Our study shows that physicians should not be afraid to prescribe HRT to younger symptomatic patients; however, further studies focusing research on the effect of natural progesterone and estrogen in younger postmenopausal women should be done.

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9. Biography

I was born in Kranj, Slovenia on 18th of June 1993. My father Brane Kovačič is an economist and my mother Klara Kovačič a primary school teacher. I grew up in Škofja Loka, city in upper Carniola region, where I finished primary school and secondary school (secondary school of mechanical engineering).

In secondary school I realized that I am very much interested in natural sciences and human body and have in parallel with the last year of secondary school prepared for additional “matura” examination in biology at the Kranj grammar school. After that I started my study at the Faculty of Pharmacy, University of Ljubljana where I successfully completed the first year but then decided to go into medicine.

During my medical studies in Zagreb (2013-2019) besides from studying and enjoying life in Croatia I was presenter of review article and case reports at different summits in Zagreb and was also attending a foreign language school where I became fluent in German language. I was improving my practical medical skills on clinical rotations in Zagreb, Jesenice, Ljubljana, Munich and Hamburg.