

Protective effects of peri-menopausal oestrogen replacement: A test of the critical period hypothesis

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**A thesis submitted to the University of Plymouth in fulfilment of the
requirements for the degree of**

Doctor of Philosophy

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This study was self-financed with the aid of a £3,000 research award from the University of Plymouth to help finance the supplements needed for the completion of study two. This study was completed part time from October 2008 until the submission of March 2013. Relevant scientific seminars and conferences were regularly attended at which work was often presented and one paper is being prepared for publication.

Papers

Jacobs, P., & Pettit, S. (2009). What determines declines in working memory and attention during pregnancy: Mood or oestrogen? *Psychology and Health*, 24(1), 216.

Papers in preparation

Pettit, S., Jacobs, P., & Andrade, J. (2011). Hormone replacement therapy benefits working memory when taken during the peri-menopause: Evidence to support the critical period hypothesis. (in preparation)

Oral presentations

Pettit, S. (2012). Time is critical for beneficial effects of hormone replacement therapy on working memory above other cognitive functions. *9th European congress on menopause and andropause, Athens, Greece 28-31st March*

Pettit, S. (2010). Hormone replacement therapy, soya, and their time of initiation, what we have found so far. *PsyPAG 25th Annual Conference, the University of Sheffield, UK*

Pettit, S. (2009). How realistic is soya for replacement of hormone replacement therapy to relieve menopausal symptoms. *The Vice Chancellors Research and Enterprise Conference, The University of Plymouth, 22nd April*

Pettit, S. (2009). Does time of initiation of hormone replacement therapy (relative to menopausal symptoms) have an effect on cognition? *Staff-Postgraduate Psychology Conference, The University of Plymouth, 26th May*

Pettit, S. (2009). Does hormone replacement therapy have a protective role in cognition? *University of Plymouth Postgraduate Short Conference Series, The University of Plymouth, 17th November*

Poster presentations

Pettit, S., Jacobs, P. (2010). The effect of hormone replacement therapy on working memory and sustained attention, evidence for a right and wrong time of initiation. *Staff-Postgraduate Psychology Conference, The University of Plymouth, 19th May*

Pettit, S. (2009). Effects of oestrogen on the human female brain. *7th Annual London Evolutionary Research Network Conference, The Natural History Museum, London, 14th September*

Pettit, S. & Jacobs, P. (2009). Working memory decline in the third trimester of pregnancy evidence for the role of oestrogen or simply baby on the brain? *Psychology Postgraduate Affairs Group Annual Conference, 29th August 2009, University of Cardiff, Cardiff, UK*

Word count for the main body of this thesis: 64,599

Signed:

Date:.....

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List of Acronyms

AD	Alzheimer's Disease
CEE	Conjugated Equine Estrogen
CFQ	Cognitive Failures Questionnaire
CSpan	Counting Span
CVLT	California Verbal Learning Task
DC	Digit Cancellation
DMTS	Delayed Matching To Sample test
DSS	Digit-Symbol Substitution
FHSA	Family Health Service Association
fMRI	functional Magnetic Resonance Imaging
FSH	Follicle-Stimulating Hormone
GHQ-12	General Health Questionnaire (shortened version)
GnRH	Gonadotropin Releasing Hormone
GQoL	Global Quality of Life scale
HADS	Hospital Anxiety and Depression Scale
HERA	Hemispheric Encoding Retrieval Asymmetry
HRT	Hormone Replacement Therapy
IDED	Attentional set shifting task
KEEPS	Kronos Early Estrogen Prevention Study
LAD	Leuprolide Acetate Depot
LH	Luteinising Hormone

MMSE	Mini-Mental State Examination
MAO	Monoamine Oxidase
MQoL	Menopausal Quality of Life Scale
MRI	Magnetic Resonance Imaging
MWS	Million Women Study
NART	National Adult Reading Test
NMDA	N-methyl-D-aspartate
ONS	Office for National Statistics
OSpan	Operation Span
PASAT	Paced Auditory Serial Addition Test
PET	Positron Emission Tomography
QoL	Quality of Life
RCT	Randomised Controlled Trial
RSpan	Reading Span
SA	Sustained Attention
SART	Sustained Attention to Response Task
SoC	Stockings of Cambridge test
TBI	Traumatic Brain Injury
WHI	Women's Health Initiative study
WHIMS	Women's Health Initiative Memory Study
WM	Working Memory
WMC	Working Memory Capacity
WMS	Wechsler Memory Scale

Acknowledgments

I would like to acknowledge my supervisory team for their contribution to the present thesis. I especially would like to thank my director of studies, Associate Professor Pam Jacobs. I am indebted to her for inviting me to complete a PhD under her supervision. She has taught me to work hard and I miss her very much. I am extremely grateful for Professor Jackie Andrade's help during the writing up period. Her attention to detail is marvellous. And finally Dr Gail Rees who joined the team in the final stages, her encouragement has been of great comfort.

I would like to thank my parents for their continuous love and support and their financial contributions which made a self-funded PhD possible. And thanks to Brad for sharing the final years of completion with me.

**Protective effects of peri-menopausal oestrogen replacement:
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Oestrogen decline during the menopause leads to decline in cognitive performance because oestrogen receptor sites are found in the prefrontal cortex and hippocampus of the female brain, areas associated with memory and attention functions. Extensive research over the past two decades has tested the effects of administering Hormone Replacement Therapy (HRT) to maintain oestrogen levels. MRI studies have shown improvements in hippocampal volume and frontal functions with HRT, but evidence for associated improvements in verbal memory performance has been mixed. Some studies have even found detrimental effects of HRT, leading to the suggestion of a critical period for HRT administration relative to menopause.

Oestrogen receptor sites are found in frontal brain regions associated with working memory (WM) functions including attention. These functions have been researched less than verbal memory, but with similarly mixed findings. The research reported in this thesis tested the critical period hypothesis in relation to WM. Study one tested the prediction that HRT will benefit WM if the therapy is initiated during the peri-menopause, and will harm it if initiated post-menopause. A naturalistic sample of 121 women were recruited, comprising women who varied in the time they had begun taking HRT, and menopausal status-matched controls who had never taken HRT. Participants completed three tests of WM span and the Sustained Attention to Response Task (SART) on two occasions 12 months apart. WM performance supported the critical period hypothesis, with women who had begun the therapy after the menopause displayed worsened WM capacity when compared to peri-menopausal initiators and post-menopausal women with no history of HRT use. At one year follow up, postmenopausal HRT users were still underperforming compared to peri-HRT initiators and those in the post-menopausal stage with no history of HRT use. No significant differences were identified between groups on the SART.

The effects of natural supplements on physical symptoms of the menopause have been researched, but there is little research on their effects on cognitive symptoms and none specifically testing the critical period hypothesis. Study two tested the effects of soya isoflavones on WM during peri- and post-menopausal stages. One hundred and twelve peri- and post-menopausal women were randomly allocated to receive either placebo or 100mg soya supplement in capsules daily for three months. Participants and researcher were blind to this allocation. Participants completed two tests of WM span and two Sustained Attention (SA) tasks at baseline, after three months of soya/placebo, and after a further three months without supplement. There was no effect of isoflavones on cognition, regardless of time of initiation of the supplement.

This thesis offers a unique contribution to the literature, by establishing empirically that HRT may have long-lasting benefits for WM if administered in the peri-menopause period, and detriments if taken post-menopause. There was no evidence that administration of soya-based phytoestrogens for three months peri- or post-menopause replicated these effects of HRT on cognition.

Summary

This thesis assesses the hormone replacement therapy (HRT) literature and ascertains the role of time of initiation of oestrogen therapies in the protection of cognitive function in menopausal women. At present the critical period hypothesis suggests that when HRT is taken during the peri-menopausal stage, frontal lobe function will be protected from the slowing down of neurogenesis that takes place during the menopause. The window of opportunity is poorly defined, therefore the present study will compare HRT users who have initiated the therapy specifically within 12 months of their last menstrual bleed (peri-menopausal stage) with those who initiated the therapy in excess of 12 months since their last menstrual bleed (post-menopausal stage). This tight window of comparison is yet to be explored, as previous studies have used wider age gaps as a defining feature of stage in the climacteric. The present study will also consider the role of HRT on cognition in post-menopausal women, comparing post-menopausal initiators to those in the post-menopausal stage with no history of HRT use. Also of importance to the literature, the first study will be extremely specific in the types of cognitive tasks chosen for exploration, where the literature as a whole struggles to pin point the exact type of frontal lobe function HRT may target and protect, often using large batteries of cognitive tasks without specific predictions about which

will be most sensitive to oestrogen. The thesis will then go on to explore the use of isoflavones (natural phytoestrogens that can mimic oestrogen in the body and brain) as a substitute for HRT in the protection of cognition. A randomised controlled trial (RCT) will explore ideas established in the HRT literature to see if natural phytoestrogens, which alleviate hot flushes, also work to benefit cognition. Wider implications include phytoestrogens as a potential replacement for HRT.

The thesis aims to address the following questions:

- Does early initiation of HRT protect executive function performance as compared to matched controls?
- Does late initiation of HRT damage executive function performance as compared to matched controls?
- Does Sustained Attention (SA), as well as WM, benefit from early initiation of HRT?
- Are isoflavones a realistic substitute for HRT to maintain WM and SA?
- Is there a critical period of initiation for isoflavones?

Oestrogen action in the body and brain

1.1 Oestrogen action

Oestrogen is a female sex hormone produced mainly in the ovaries. Other sex hormones include androgens (the male sex hormones) and progesterone (the 'pregnancy' hormone). Hormones are chemicals that are secreted by the body as a part of the endocrine system. They provide similar messaging systems as neurotransmitters however where neurotransmitters act on specific neurons, hormones can affect the body to induce long-term state changes such as global states of behaviour and this can take much longer. At any time there are more than 50 hormones in the bloodstream which have been secreted by various organs under the control of the hypothalamus. When hormones are secreted into the bloodstream they reach all parts of the body, but only certain target cells have receptors that allow them to respond to particular hormones. In these, the specific hormone can bind to the receptor cell and trigger a change in the cell's chemical behaviour (Carlson, 2012; Toates, 2007; Wickens, 2009). The synthesis and secretion of oestrogens (and progesterone) is stimulated by Follicle-Stimulating Hormone (FSH) and Luteinising Hormone (LH), and controlled by the Hypothalamic Gonadotropin Releasing Hormone (GnRH). When levels of oestrogen (and progesterone) exceed a threshold, they inhibit FSH and LH synthesis (Stevenson, Cox, & Britt, 1981).

Generally the hypothalamus works to maintain homeostasis and keep a balance of hormones in the body. In the case of oestrogens, this balance varies through natural rhythms, such as the menstrual cycle and events such as pregnancy and menopause. These are points during a female lifespan where oestrogen levels will naturally change. During the menstrual cycle levels of oestrogen fluctuate from their highest points mid cycle (during the ovulation phase) to their lowest points at the end of the cycle and through the menstrual bleed (during the menstrual phase). In a healthy menstruating female the fluctuations are relatively steady throughout the female life span until she reaches the menopause, with the exception of pregnancy. During pregnancy oestrogen levels increase gradually, reaching their highest levels in the third trimester of pregnancy. At this point in the female's life span she will naturally experience her highest levels of oestrogen. During the menopause oestrogen levels decline in both the body and the brain reaching the lowest natural point in the female's life span (Melton, 2000). This decline can be responsible for a range of reported problems or menopausal symptoms experienced during this transitional stage, one of the largest reported problems overall being disturbed memory (Philips & Sherwin, 1992).

Figure 1.1 shows oestrogen change as experienced during the different stages of the menopause.

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Figure 1.1: Oestrogen levels during different stages of the menopause, cited from: <http://www.34-menopause-symptoms.com/osteoporosis-causes.htm>

Oestrone (E1), oestradiol (E2) and oestriol (E3) are the major occurring oestrogens in women, and these diffuse across cell membranes to bind to and activate oestrogen receptors (Shao, Cheng & Jin, 2012). The actions of these major oestrogens are therefore dependent on and mediated by the oestrogen receptor in that cell (Okada et al., 2008). These three forms of oestrogen have differing potency levels that dominate depending on stage in a female's life. For example, during normal reproductive function oestradiol is most active and most potent of the major oestrogens but it changes to oestriol during pregnancy and oestrone during the menopause (Jansson & Holmdahl, 1998).

Oestrogen receptors act by entering the cell cytoplasm, binding to specific oestrogen receptor sites via the regulation of transcriptional processes, which leads to the up- or down-regulation of gene transcription (Simoncini et al., 2004). This *genomic* ability is unique to nuclear receptors, and allows direct interaction with, and control of genomic DNA. Oestrogen receptors have also been shown to have *non-genomic* actions that are independent of these interactions with DNA (Losel & Wehling, 2004), binding to cytoplasmic oestrogen receptors causing changes in cell function that are independent of changes in

gene transcription (Aquila et al., 2004; Heinlein & Chang, 2002). Thus, genomic effects are those that involve triggering or changing transcription processes during protein synthesis and non-genomic effects are those acting on cell membranes.

Because of the genomic and non-genomic effects of oestrogens, cells and organs are regulated by an interaction of these two mechanisms, including the cardiovascular and central nervous system (Simoncini & Genazzani, 2003) and oestrogen influences brain mechanisms via both genomic and non-genomic routes.

1.2 Oestrogen creates differences in female brain structure, mechanisms and activity

Exposures to sex-sensitive hormones create differences between males and females, and this can include differences in the macroscopic structure of the brain, which may account for differences in functioning of the brain (Craig & Murphy, 2006; Gorski, 1998). Common differences between male and female behaviour are often attributed to their dominating sex hormones and this can include small differences in brain structure, brain mechanisms and cognitive performance (Epting & Overman, 1988; Kimura & Hampson, 1994). The study of the action of oestrogen in the brain has advanced from known influences in sexual behaviour, reproductive effects and neuroendocrine function to known effects on motor coordination, pain mechanisms, dementia, depressive illness and cognitive function (Brinton, 2001; Brinton, 2009; McEwen, 1999).

A healthy, normally functioning brain contains ten billion neurons, and every neuron has axon connections to thousands of other neurons allowing numerous biochemical receptors that respond to and trigger various actions. The

brain and these receptors are widely responsive to gonadal hormones (McEwen, 1999) where a proportion of the receptors in the female brain are responsive to oestrogens via oestrogen binding sites. In fact, 20 per-cent of the total number of neurons in the cortex, hippocampus and amygdala have oestrogen binding sites (Gustafsson, 2000).

Initial cellular evidence for oestrogen actions in the brain was based upon the technique of steroid autoradiology. Here Pfaff and Keiner (1973) mapped out [3H] oestradiol uptake and retention to locate oestrogen-concentrating neurons in the brain, which were found in the hypothalamus, amygdala, hippocampus and cortical and limbic areas which are involved in memory and learning processes (Gibbs, 2000a; Markou, Duka, & Prelevic, 2007; Wang, Hara, Janssem, Rapp, & Morrison, 2010). The amygdala and hippocampus are both found in the temporal lobe and responsible for specific memory and learning (see figure 1.2). The existence of oestrogen receptor neurons in the hippocampus was further clarified by Loy, Gerlach and McEwen (1988) and Orikasa (2000). Specifically, oestrogens can indirectly stimulate nerve cell growth in hippocampus neurons and this is associated with improved learning and memory (Gazzaley, Weiland, & McEwen, 1996; Wooley, Weiland, McEwne & Schwartzkroin, 1997).

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Figure 1.2: Areas of the brain with oestrogen receptor sites. Cited from:
<http://www.memorylossonline.com/glossary/basalforebrain.html>

Wang and colleagues (2010) have located an abundance of oestrogen receptor sites within the prefrontal cortex (PFC) suggesting that these specific receptor sites are oestrogen responsive. Miller, Conney, Rasgon, Fairbanks and Small (2002) used Positron Emission Tomography (PET) and identified an increase in glucose metabolic rates in the PFC and hippocampus for hormone replacement therapy (HRT) users during the completion of verbal and WM tasks. They suggested that the cortex and hippocampus may be the most important areas of consideration for effect during menopausal oestrogen decline, perhaps due to being highly saturated in oestrogen binding sites.

Tang and colleagues (2004) further clarified the location of oestrogen in the PFC, suggesting oestrogen to be primarily located in regions of the PFC involved in executive function. Monkeys who had undergone ovariectomy immediately received either oestradiol or a placebo. For those receiving oestrogen treatment, spine numbers of dendrites in the PFC significantly increased compared with the placebo group, whose spine numbers did not

increase. The researchers specified this change to be in layer one of area 46 of the dorsolateral PFC, an area involved with working memory (WM) and sustained attention (SA). In contrast, the researchers considered oestrogen change in spine numbers of layer one of area six, in which there were no difference between oestrogen and placebo users, further reinforcing the idea that oestrogen can influence certain neocortical regions in the PFC.

Neuro-imaging studies that compare HRT users to non-users further demonstrate the effect of oestrogen on the brain. For example, Erickson, and colleagues (2005) used high-resolution Magnetic Resonance Imaging (MRI) to examine the effects of HRT on brain volume in postmenopausal women and found HRT to be associated with spared grey matter in prefrontal, parietal, and temporal brain regions and white matter in medial temporal lobe regions. In addition to this, an association was made between longer durations of therapy use and greater sparing of grey matter tissue. The researchers concluded that age-related neural decline in both grey and white matter tissues can be decelerated by HRT use. In agreement, Ghidoni and colleagues (2006) found that women with a history of HRT use had greater white matter than those with no history of HRT use, and this was consistent in women who were current users and past users of HRT. They suggested oestrogen to have a 'trophic effect' on the cerebellum.

Oestrogen's role in the hippocampus further supports the impact of oestrogen change on distinct regions of the female brain. Generally it has been shown that exposure to oestrogen during proestrus (either naturally or with intervention) greatly enhances the density of dendrite spines in the hippocampus (Gould, Woolley, Frankfurt, & McEwen, 1990; Mukai et al., 2010;

Woolley & McEwen, 1993). These changes in spine density have encouraged synapse density and hippocampal transition of electrical signals (Montoya & Carrer, 1997; Warren, Humphreys, Juraska, & Greenough, 1995; Wong & Moss, 1992).

Erickson, Voss, Prakash, Chaddock and Kramer (2010) used MRI in a cross-sectional sample of 102 women to detect hippocampal, amygdala and caudate nucleus volume between those who had never received HRT, those who had initiated the therapy close to the menopause and those who initiated the therapy post-menopause. For both left and right hemispheres, those who began HRT at initiation of menopause had bigger hippocampal volume than either other groups. And in their study Lord, Buss, Lupien and Pruessner (2008) included postmenopausal women currently using HRT, past HRT users and never users to investigate the neuroprotective effects of oestrogens on the hippocampus and amygdala. HRT users exhibited larger hippocampal volumes than both past users and never users, which is consistent with the study by Erickson and colleagues (2010). The information demonstrates oestrogen's role in brain function via increased blood flow and increase in spine density in specific brain regions (Maki & Resnick, 2000; Rasgon et al., 2004).

1.2.1 Exposure to oestrogen creates differences in female brain activity and function

Oestrogen receptors are located in specific parts of the female brain and oestrogen change alters performance of behaviours mediated by that brain area. For example, it has been demonstrated in ovariectomised rats treated with oestrogen therapy that this increase in oestrogen is correlated with superior

performance in memory tasks compared with a placebo control (Simpkins, Singh, & Bishop, 1994).

In a human study, Cutter, Craig, Norbury, Robertson and Whitehead (2003) found that those taking HRT had significantly greater volumes of white matter associated with the cortex. During completion of a verbal memory task, those administered HRT demonstrated increased activation in the right superior frontal gyrus during item retrieval, a higher level of hippocampal and parietal lobe activation during item storage and greater left hemisphere activation during encoding, however no differences in task performance itself were found. As a means of comparison, participants were asked to remember non-verbal material. Interestingly there was decreased activity in these brain regions during storage of non-verbal material. The study highlights the effect of HRT on PFC activity during the completion of a verbal task.

Using a similar study design, Shaywitz and colleagues (1999) looked at brain activation during the completion of verbal and non-verbal WM tasks using fMRI to determine whether oestrogen treatment modified expected brain activation patterns during the completion of these tasks. Peri-menopausal women were treated with either HRT or a placebo for two periods of 21 days. During the storage of material from the WM tasks, those receiving the oestrogen treatment had increased activation in the inferior parietal lobe and the superior frontal gyrus, similar to the study by Cutter and colleagues (2003). As with the study by Cutter and colleagues, despite frontal lobe activation improving in the group receiving oestrogen therapy, this improvement was not reflected in task performance itself. The participants had been receiving treatment over a short period only, which perhaps was not long enough to demonstrate effects in

cognitive performance transpiring in the tasks themselves. However both studies demonstrated metabolic and neurological differences in response to levels of oestrogen in the body. Shaywitz and colleagues (1999) concluded that oestrogen received by menopausal women can cause alterations in brain activation patterns during the performance of WM tasks, suggesting that oestrogen affects the PFC, an area of the brain that underlies WM processes. It should be noted that the difference between brain activation of oestrogen users and the placebo controls was greater during the verbal WM task.

In a more recent study, Joffe and colleagues (2006) looked at the effect of oestrogen treatment on WM, learning and executive function against a placebo control in those entering the menopause. The fMRI study revealed increased frontal lobe activation during tests of verbal WM and spatial WM in those receiving oestrogen treatments. The studies further suggest oestrogen's role in the PFC, and how completion of WM tasks can illustrate this effect. In fact several other recent human clinical and animal behavioural studies suggest that oestrogen replacement enhances performance on WM tasks that are reliant on the PFC (Duff and Hampson, 2001; Keenan, Ezzat, Ginsburg, & Moore, 2001; Rapp et al., 2003) and these will be discussed in more detail in section 1.4.

The described studies have used fMRI to show that on completion of a memory task, blood flow and oxygen concentration levels are altered in brain areas gated by oestrogen binding sites such as the PFC, and that blood flow and oxygen levels are dependent on oestrogen levels in the brain (Dumas, Kutz, Naylor, Sites, & Newhouse, 2010; Joffe et al., 2006; Shaywitz et al., 1999). This may be dependent on type of oestrogen receptor.

Keenan and colleagues (2001) recognised poor executive function in women with lower levels of oestrogen, and proposed that oestrogen's involvement with the hippocampus is secondary to these prefrontal-mediated changes. The PFC is associated with higher order cognitive processes such as attention selection, resistance to interference, behavioural inhibition, task switching and decision-making (Dalley, Cardinal, & Robbins, 2004; Ragozzino, 2007). The ability to conduct these processes with normal function and capacity relies on the healthy functioning of the receptor sites and synaptic activity within the PFC.

To date there are at least two types of intracellular oestrogen receptors identified, ER-alpha and ER-beta (McEwen, 2002). These two forms of oestrogen receptors are encoded by separate genes but are similar in binding characteristics (Brinton, 2001). Although the distribution of ER-alpha has been fairly well established by steroid autoradiography and immunocytochemistry (McEwen, 2002; Pfaff, 1980; Stumpf & Sar, 1976) there is still some degree of uncertainty concerning the localization of ER-beta. It has been suggested that ER-alpha is the predominant receptor subtype of cholinergic neurons in the basal forebrain and also the PFC (Wang et al., 2010) whereas it is thought that ER-beta are more abundantly distributed in the hippocampus and cerebellum (Shughrue, Lane, & Merchenthaler, 2008).

ER-alpha and ER-beta are present in dendrite spines and presynaptic terminals to modulate both transmitter release and spine dynamics (Dominguez, Lui, & Baudry, 2007; Hojo et al., 2008). The difference in brain localisation suggests that these two receptors mediate different functions. Oestrogen binding sites are found in the hippocampus and amygdala, and these brain areas

are associated with memory, attention and emotion. Because of the widespread presence of oestrogen receptor sites in various forms throughout the brain, oestrogen actions are also widespread and affect many neurotransmitter systems including the serotonic, noradrenergic, cholinergic and γ -aminobutyric acidergic systems (Sherwin, 2003). It can therefore be concluded that major projecting neural systems (such as noradrenergic, serotonergic, cholinergic and dopaminergic) are subject to the influence of ovarian hormones. This is further discussed in the next section.

As well as oestrogen being needed at oestrogen receptor sites to satisfy activation in these areas and maintain dendrite health, more intricate details of the neuroprotective role of oestrogen to their receptor sites and dendrite spines have been identified. Oestrogens can work with neurotrophins to indirectly stimulate nerve cell growth whereby oestrogens induce an increase in a membrane protein (N-methyl-D-aspartate receptors; NMDA) in hippocampus neurons which results in increased signalling to the excitatory neurotransmitter glutamate. This is associated with long-term potentiation, a proposed model of learning and memory (Gazzaley, et al., 1996; Wooley, et al., 1997). Binding a neurotrophin to their receptors results in a stimulation of the function related to the neurotransmitter production and release. In addition to this, oestrogens also have a neuroprotective action against several toxins that can result in hydrogen peroxide build up in the brain which can cause irreversible damage (Norbury et al., 2003), thus having a protective effect.

1.3 Oestrogen affects neurotransmitter production, duration and destruction

Oestrogen receptor sites help to regulate the production, duration and destruction of neurotransmitters; chemicals that make it possible for neurons to communicate across their dendrites. The neurotransmitters modified by oestrogen action include dopamine, acetylcholine and serotonin (Craig & Murphy, 2006). Changes in concentration of these neurotransmitters can affect their interlinking systems, mainly mood and cognition. For example, oestrogen action alters serotonin transition, which can lead to modifications in mood and cognition (Schloss & Williams, 1998). Sherwin (1994) found that mood varies during the different stages of the menstrual cycle, with improvements in mood during stages of the cycle when oestrogen levels reach their highest. During the menopause, women often report feelings of lowered mood, and mood is often included in menopausal rating scales. Subjective ratings of depression have been shown to increase as oestrogen levels decrease (Sagsoz, Oguzturk, Bayram, & Kamaci, 2001). The relationship between these mood-regulating neurotransmitters and oestrogen change can be explained when considering the location of these receptor sites and neurotransmitters in the brain.

1.3.1 Dopaminergic terminals innervate pyramid cell spines in the PFC

Dopamine production and action is oestrogen responsive and low levels of oestrogen result in a decrease in this neurotransmitter, which is associated with pre-menstrual syndrome, post-natal depression and post-menopausal depression (Fink, Sumner, Rosie, Grace, & Quinn, 1995). In fact Leranth and colleagues (2006) demonstrated a 30% reduction of dopaminergic cells in the brains of non-human primates after being deprived from oestrogen for 30 days.

Tang and colleagues (2004) demonstrated an increase in spine numbers of dendrites in area 46 of the PFC in ovariectomised monkeys when given oestradiol. Kritzer and Kohama (1998) also considered the role of oestrogen deprivation in area 46 of the PFC through ovariectomy of non-human primates. In their study, ovariectomy resulted in the dopaminergic system being compromised in this area, but this was restored with oestrogen treatment. This is because dopamine is facilitated by oestrogen, and dopaminergic terminals innervate pyramid cell spines in the PFC, an area saturated with oestrogen receptors (Lambe et al., 2000). Similar to the study by Tang; changes in dopaminergic action was most profound in layer one, area 46 of the PFC which is dense with both dopaminergic innervation and oestrogen action (Williams and Goldman-Rakic, 1993).

Dopaminergic change plays an important role in mood, but also in information processing during WM tasks (Goldman-Rakic, 1996; Tang et al., 2004) and dopamine reduction in the dorsolateral PFC causes WM deficits in non-human primates (Sawaguchi and Goldman-Rakic, 1995).

1.3.2 Oestrogen change affects the cholinergic system and memory

Studies have demonstrated that oestrogen affects mechanisms that facilitate acetylcholine production, and greater production of this neurotransmitter will lead to better function of specific memory systems (Gibbs, 2003). After the loss of ovarian function, the effect of oestradiol on the cholinergic system diminishes and this results in poorer cognitive function (Craig & Murphy, 2006; Gibbs & Gabor, 2003).

In a study by Voytko (2002), performance on visuospatial attention and reaction time was measured before ovariectomy, two months following the procedure and 14 months after treatment with either HRT or a placebo in non-human primates. Oestrogen treatment resulted in modulation of attentional performance in a delayed response task. It was concluded that visuospatial attention could be altered by oestrogen treatment, perhaps through the cholinergic system.

1.3.3 Serotonin in the PFC is oestrogen responsive, influencing verbal memory and executive function

Like dopamine, the neurotransmitter serotonin is also found in pyramid cells of the PFC and oestrogen receptors in this area facilitate the production of this neurotransmitter (Kugaya et al., 2003). Lower levels of oestrogen during the menstrual cycle are associated with lower levels of serotonin (Hindberg & Naesh, 1992) and this has been related to lower mood during this time (O'Keane & Dinan, 1991).

Kugaya and colleagues (2003) demonstrated an increase in serotonin activity in the right frontal cortex of oestrogen users above any other area, and this correlated with increased plasma levels of oestrogen in the same brain region. Participants receiving oestrogen treatment also performed better on tasks of verbal memory (paragraph recall and verbal paired associates tests from the revised Wechsler Memory Scale (WMS-R; Wechsler, 1987) and executive function (Trail Making Test, Reitan & Davison, 1974).

1.4 Oestrogen affects brain mechanisms which impact on memory systems

Studies have highlighted the importance of oestrogen role in cognition, and in particular, memory (Duffy, Wiseman and File, 2003; Sherwin, 1988; Sherwin, 2012). Oestrogen level also seems to have a large effect on verbal episodic memory (Maki, Zonderman & Resnick 2001; deGroot, Hornstra, Roozendaal & Jolles, 2003; Sherwin, 1988).

Oestrogen receptors can be found in the PFC and the hippocampus (Sherwin, 2003). Structures in the medial temporal lobe, and especially the hippocampus, are involved during and after learning, and this learnt information can be retrieved dependent on the PFC (Squire & Zola, 1996; Ungerlieder, 1995). Changes in brain structure and dendrite activity as a result of oestrogen change account for the relationship between oestrogen levels in the brain and memory performance (Sherwin, 2003). Brain imaging studies have helped to map out the neuronal changes (described in the previous section) onto behavioural changes, observed in the performance of cognitive tasks. For example, concentrations of oestrogen binding sites are found in the PFC (Tang et al., 2004), the amygdala and the hippocampus (Loy et al., 1988; Orikasa, 2000). These brain areas are associated with memory and several studies have demonstrated a change in memory at times when oestrogen levels differ (Jenkins et al., 2004; Resnick, Metter, & Zonderman, 1997; Sherwin, 1988).

Oestrogen levels change during pregnancy and the menopause, and during this time many women complain of memory problems (Craig & Murphy, 2006; Mitchell & Woods, 2001). This is non-surprising when considering the location of oestrogen in the PFC and hippocampus, brain areas associated with

short and long term memory including explicit, implicit and episodic memory, as well as WM and SA. Information regarding oestrogens involvement with implicit, explicit, episodic and in particular verbal episodic memory is long withstanding, and less is understood regarding the role of oestrogen in WM and SA. Current research regarding oestrogen's involvement with these memory systems will be described in the following section.

1.4.1 Explicit, implicit and episodic memory

Learning and memory are intricately linked, whether this is declarative or non-declarative. The temporal lobe, a region of the cerebral cortex located on the left and right hemispheres of the brain, consists of structures that are vital for long term, declarative (sometimes referred to as explicit) memory. The temporal lobe is involved in auditory processing and important for the processing of semantics in both speech and vision (Poeppel, 2003). The main subcortical temporal lobe structures include the limbic cortex, the amygdala (responsible for associating emotion with sensory input) and the hippocampus (responsible for formatting and organising long term memories; Swenson, 2006). Removal of the medial temporal lobes (including the hippocampus and amygdala) results in anterograde amnesia. Lesions of the left temporal lobe result in impaired recall of verbal memory and lesions in the right temporal lobe result in impaired recall of non-verbal material.

Healthy human aging is accompanied by changes in brain structure that varies by sex. For example, a hasty increase in ventricular volume begins in the fifth decade in men but not until the sixth decade in women (Kaye, DeCarli, Luxenberg, & Rapoport, 1992) suggesting that perhaps brain atrophy begins earlier in men than women. However the velocity of the atrophy process

increases with age more rapidly in women than men (Norbury et al., 2003). Generally, age-related loss of brain tissue is greater for females in the hippocampus and parietal lobes (Murphy et al., 1996). Eberling and colleagues (2003) suggest that, due to oestrogen receptor sites in the hippocampus, oestrogen protects against this age-related hippocampal atrophy. Memory for acquisition of new declarative or explicit memories is dependent upon the hippocampus and becomes compromised with increasing age (Small, Stern, Tang, & Mayeux, 1999).

Explicit memory is conscious memory divided into semantic and episodic memory. It refers to memories that we are aware of and can recall. Implicit memory refers to unconscious memory (Andrade & May, 2004). For example, explicit memory refers to the conscious recollection of information, whereas implicit memory involves non-conscious habits and repetition priming (Sherwin, 2002); it can be revealed as changes in behaviour, for example response bias or preference in the absence of conscious recall or recognition of the stimulus. Explicit memory is typically tested by paragraph recall (verbal memory), list learning (verbal memory) or recognition paradigms and is dependent on the hippocampus. It has been speculated that the right extrastriate cortex may play a role in implicit memory, rather than the hippocampus (Keenan, Yaldoo, Stress, Fuerst, & Ginsburg, 1998). Due to the relationship of explicit and implicit memory with the hippocampus, and the location of oestrogen receptors in this brain area, implicit and explicit memory performances during different points of the female lifespan have been explored.

Maki, Rich and Rosenbaum (2002) considered the role of oestrogen on implicit and explicit memory during different stages of the menstrual cycle.

Women aged between 18 and 28 completed tests of memory during their follicular (low levels of oestrogen) and midluteal (high levels of oestrogen) phases. Participants performed better on the category exemplar generation (measuring implicit memory), during their midluteal phase but no differences were found between stage in cycle for the explicit, category-cued recall task. In addition, oestradiol levels correlated positively with verbal fluency.

Using paragraph recall (from the WMS-R) to assess explicit memory, and the word stem completion priming recall task to assess implicit memory; Keenan and colleagues (1998) assessed explicit and implicit memory change in pregnant women during their three trimesters of pregnancy. Pregnant women demonstrated worse implicit and explicit memory as their pregnancy progressed, but this did not reach significance between any trimester. Pregnant women did however perform significantly worse during the third trimester of pregnancy as compared to non-pregnant women matched for age, education and mood. Sharp, Brindle, Brown and Turner (1993) also demonstrated impairments in both implicit and explicit memory during the third trimester of pregnancy.

Similar to the study by Keenan and colleagues (1998), Buckwalter and colleagues (1997) reported paragraph recall to be improved in women after giving birth as compared to performances during their third trimester of pregnancy. The two studies demonstrate that oestrogen change during pregnancy can have adverse effects on explicit memory but this can be corrected when oestrogen levels become normal again.

As well as a relationship between oestrogen levels in the hippocampus and explicit and episodic memory performance, oestradiol helps protect against

the loss of acetylcholine, a neurotransmitter important for the formation of episodic memories (Singh, Meyer, Millard & Simpkins, 1994).

Episodic memory is a declarative memory involving the recollection of discrete episodes and episodic performance is influenced by semantic memory and executive function such as attention and processing speed. Like explicit memory, episodic memory encoding is mediated by the hippocampus and medial temporal lobes (Henderson, 2009). Damage to this area results in a loss of episodic memory (Andrade & May, 2004). However, episodic memory has also been shown to have frontal lobe components (Rasgon et al., 2004; Tulving, Kapur, Craik, Moscovitch, & Houle, 1994) especially verbal episodic memory (Fletcher et al., 1995; Schmidt et al., 2002).

When oestrogen levels are elevated during the follicular stage of the menstrual cycle, there is an increase in right anterior hippocampal volume, and this was associated with better performance during an episodic verbal memory task (Protopopescu et al., 2008).

1.4.2 Verbal memory

Oestrogen levels fluctuate throughout the menstrual cycle where the highest level of oestrogen occurs in the luteal phase. At this stage, verbal articulation is improved relative to when the participant is menstruating (Cutter et al., 2003; Wickham, 1958). Women tend to perform better than men on verbal memory tasks, and women tend to have greater verbal task performance at mid cycle when oestrogen levels are at their highest suggesting that oestrogen facilitates verbal memory (Hurrell & Slade, 2001; Mordecai, Rubin, & Maki, 2008).

Oestrogen receptors are found in the frontal lobes and higher levels of activation are associated with higher concentrations of oestrogen in this brain area. Craig and colleagues (2008) found a positive correlation between serum oestradiol levels and left inferior gyrus activation (in the frontal lobe) in menstruating women during the encoding of verbal information. Dietrich and colleagues (2001) also demonstrated increased activation in the superior parietal cortex of those in the mid-luteal phase during completion of a word-stem task.

Although higher levels of oestrogen are associated with improved verbal memory, too great a level of oestrogen can also hinder verbal memory, further demonstrating an association between oestrogen levels and verbal memory. For example, deGroot, Adam and Hornstra (2003) compared the performance of 13 memory and attention tasks of pregnant women (with high levels of oestrogen) to non-pregnant women (with normal levels of oestrogen). Pregnant women performed worse on language processing and attentional switching, suggesting that, as well as oestrogen deficiency, too great a level of oestrogen can have detrimental effects on verbal memory amongst other cognitive functions. The authors found correlations between test score and education and concluded education to be an important factor to be considered in data analysis.

1.4.3 Executive function

In familiar cognitive tasks we can respond using behaviours developed through learned schemas. However in some cases we may face a situation to which we need to 'override' an automatic process. In such cases we rely on our executive functioning. The PFC is linked to executive function, memory and attention; executive function being an operation of the PFC (Miller & Cohen,

2001). Our understanding of the process of executive functioning has developed from observational studies of patients with frontal lobe damage. Although patients often demonstrate normal learning, memory and reasoning performance they frequently exhibit disorganised strategies and actions for everyday tasks, finding it hard to override prepotent responses and being easily distracted (Robertson, Manly, Andrade, Baddeley, & Yiend, 1997). The cortex facilitates executive functioning by maintaining patterns of activity allowing us to achieve tasks' goals whereby the gain of neurones responsive to the task are increased. For example, focusing on one stimulus over another, the brain would increase the gain of neurones responsive to that stimulus. Types of executive function that depend on this process are WM and SA.

WM is a temporary storage system involving an interaction between attention and memory described as 'the capacity for controlled, SA in the face of interference' (Engle, Tuholski, Laughlin, & Conway, 1999). Rather than simply using rehearsal to embed information into our minds, WM is the ability to store information temporarily, and access this in order to perform complex cognitive tasks, requiring a certain amount of attentional control. Neuroimaging studies show that executive functions such as WM function is dependent on cells found in the PFC of the brain (Kane & Engle, 2003) and since oestrogen receptors are found in the PFC, oestrogen may modulate executive functioning and also WM (Sutcliffe, Marshall, & Neil, 2007).

Attention is a basic function that is often used in a variety of cognitive and neurological functions. In the process of attention, we choose to concentrate on one aspect whilst ignoring or filtering out other things that may or may not be a distraction. According to Miller and Cohen's model (2001) the

PFC can exert attentional control over sensory and response neurons, overriding both memory and emotion. Interaction between the PFC and sensory, limbic and motor cortices exercise a level of cognitive control needed in selective attention and different forms of inhibition. If a focus or behavioural response needs to be maintained (usually during a continuous activity), the attention becomes sustained. SA is the ability to continue this focus over a greater period of time in the face of distraction. SA can become challenged if the task is boring, or has a workload that is too high. WM involves an interaction between attention and memory by storing information temporarily, and accessing this when needed, which requires control and practise of SA. SA becomes problematic in patients with frontal lobe and white matter damage and as such it has been suggested that the cortical basis of executive functioning and attention are situated in the frontal lobes and PFC (Whyte, Grieb-Neff, Gantz, & Polansky, 2006), home to oestrogen receptor sites.

Duff and Hampson (2001) have suggested that some prefrontal functions are sexually differentiated. In their study, male and female students completed a novel multi-trial spatial WM task and a verbal WM task, prefrontal mediated tasks. During completion of the SPWM, females made fewer WM mistakes and reached conclusions significantly faster than the males. Females also outperformed males in the verbal WM task.

In an fMRI study Berman and colleagues (1997) demonstrated that when young women were suppressed of oestrogen, oestrogen withdrawal resulted in reduced blood flow to the dorsolateral prefrontal and parietal cortex during completion of the Wisconsin card sorting task (Grant & Berg, 1948). The

Wisconsin card sorting task is a measure of executive function that can be impaired with frontal lobe damage.

Animal studies have also demonstrated an effect on oestrogen change on executive function. Korol's study (2004) demonstrated, using female rats, that differing oestrogen levels throughout the menstrual cycle alter the learning strategy used to solve a task even when learning rate is unaffected. The authors suggest that it is the quality of the information processed by the brain that becomes regulated, and this is dependent on hippocampal sensitive mechanisms. Here it was demonstrated that, in addition to how much is learnt, oestrogen levels can change what and how this information is learnt, exercising SA and WM. In their study, young female rats were tested on completion of a T-maze task at different stages of their menstrual cycle. The task allowed for free choice of the cognitive strategy used to solve the task. Rats could either solve the task using place strategy (go there) or response strategy (go right). Rats with highest circulating oestrogen were significantly more likely to select place strategies. Rats with lowest circulating oestrogen were more likely to select response strategy and those in the middle of their cycle had no bias. The strategy learning process is a hippocampal mediated process, and in this case oestrogen levels did not affect processing speed.

In human studies, SA is impaired during pregnancy when oestrogen exceeds threshold levels in the brain and this may lead to impairment in other cognitive functions (deGroot et al., 2003). Crawley, Grant and Hinshaw (2005) administered thirteen cognitive tasks and compared pregnant and non-pregnant women. Two tasks were significantly different between groups. Firstly, pregnant women were significantly slower on a verification task (for example, 'all salmon

are fish?'). Secondly, the pregnant women were significantly worse at working out what floor a moving elevator was on when they were given instructions to its movement. The tasks used were not valid tasks but do demonstrate that changes in verbal memory, WM and attention are dependent on oestrogen levels in the body. Specifically, too much oestrogen as experienced during pregnancy can have detrimental effects on these executive functions.

The combination of results from neuropsychological studies, fMRI information, and behavioural changes suggest that oestrogen can enhance performance of executive function and is dependent on the PFC in humans. The effect of oestrogen change on verbal, episodic and explicit memory is well tested, however the relationship with WM and SA is less well explored, and it is assumed that this relationship with executive function may explain memory problems reported by women during the menopause. Changes in oestrogen level during the menopause and the physical and mental effects of this hormonal depletion are explored in more detail in the next section.

1.5 Hormone therapy is used to reduce menopausal symptoms in menopausal women

During the lead up to the menopause, the patterns of neural coding in the temporal lobes are altered steering to the cessation of reproductive cycles. The interaction of ovarian and hypothalamic/pituitary 'pacemakers' becomes progressively dysfunctional and this results in the menopause (Sherwin & Henry, 2008). In addition to this, the pool of ovarian follicles becomes limited until there is an eventual cease in production. These mechanisms combined result in a drastic decline in oestrogen levels which affect many body tissues and can produce menopausal symptoms, such as hot flushes, changes in mood and a

decrease in bone density. Similar changes may be induced by surgical menopause or ovarian failure (Al-Azzawia & Palaciosb, 2009). Although female life expectancy during the past century has increased by 20 years, the average age of the menopause in industrialised countries has remained the same (51.8 years). This means that women live approximately a third of their lives after the loss of ovarian functioning (Sherwin, 2007) in a state of oestrogen deficiency that can challenge Quality of Life (QoL; McEwen, 1999). Current UK estimates for female life expectancy are 82.3 years (Office for National Statistics, 2012) and life expectancy for women in the UK is projected to rise to 87.0 years in 2033 (Rutherford, 2012). This increase in life expectancy will inevitably result in poorer QoL in women overall (Sherwin 2007), and may be helped by HRT.

HRT can reduce menopausal symptoms including both cognitive and physical symptoms (Anderer, Semlitsch, Saletu, & Gruber, 2005). By 1999 an estimated 20 million post-menopausal women worldwide were using HRT (Beral, Banks, & Reeves, 2002) usually in the form of Conjugated Equine Estrogen (CEE) in 0.625 mg daily doses, or as very low doses (0.3 mg daily) of oestrogen combined with progesterone. In a Department of Health Survey conducted in 1998, 38% of women in England aged 45-54 years had used HRT (Department of health, 1998). Since the publishing of the Women's Health Initiative study (WHI, Writing Group for the WHI investigators, 2002) which indicated an adverse effect of HRT use on heart health and cognition; prescribing of HRT has declined substantially (Prescription Statistics Publications, 2005). Other noteworthy studies include the Women's Health Initiative Memory Study (WHIMS; Rapp, Espeland et al., 2003; Espeland et al., 2004; Shumaker et al., 2004) which recruited from the WHI, and the Million Women Study (MWS) which reported

one million women to have stopped taking HRT after a twofold risk of cancer in HRT users was revealed (Beral, 2003). After the results from the WHI became known, a decision was made to stop another large study, the Women's International Study of Long Duration Oestrogen after Menopause (Anon, 2005). The percentage of women using HRT has declined over the past decade; as is shown in figure 1.3 (percentages from Sennik, 2009).

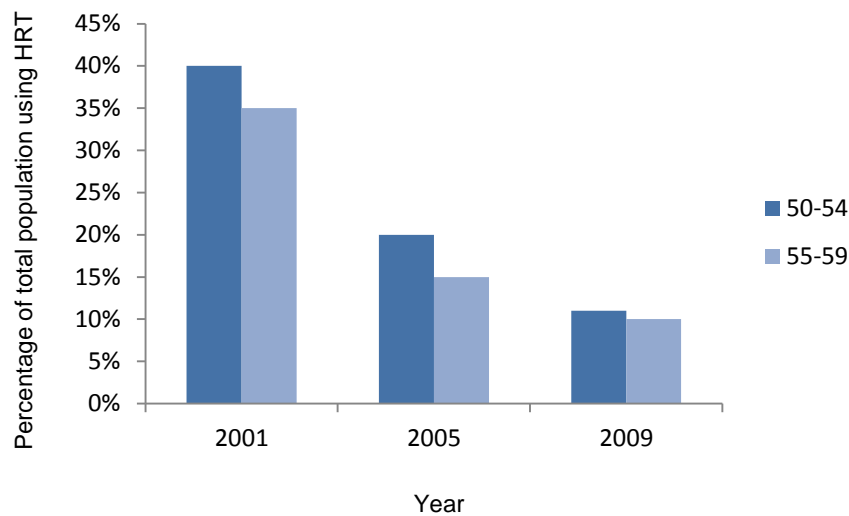


Figure 1.3: Percentages of total female population receiving HRT split for age (50-54 years or 55-59 years only)

Prescription records have indicated that in 2001, six million women in the UK were receiving HRT. This decreased to three million in 2005 and two and a half million in 2009 (Lambert, 2009). Despite this decline due to fears of adverse effects, there are still a large proportion of women using HRT. Due to the continual large number of women initiating HRT at different points in their climacteric, it is important to establish HRT's role in overall health and in particular, characteristics of HRT use which may result in optimal improvement in QoL.

1.5.1 HRT can reduce osteoporosis when initiated within 3 years of completion of the menopause

It has long been recognised that oestrogen deficiency may cause osteoporosis by contributing towards loss of bone mineral content and bone density, as well as encouraging fractures (Lindsay et al., 1976). The use of HRT has been shown to prevent such bone loss and as a result, decrease risk of fracture. For example, oestrogen use for a period of between five and ten years is associated with a 50% reduction in likelihood of fracture (Fogelman, 1991). The protection of bone density by oestrogen use appears to be long lasting. In their study, Lindsay, Hart, Forrest and Baird (1980) demonstrated a significant reduction of height in 100 women who had received a placebo nine years prior during a trial of oestrogen therapy for the prevention of post-ovariectomised osteoporosis. The oestrogen treated group had less central and peripheral bone loss, and a reduction in incidence of vertebral compression. Bone atrophy is likely to occur at one per-cent per annum, and this has been shown to be prevented in HRT users for the first three to five years of treatment (Lindsay et al., 1976). More recent studies with larger cohorts have also demonstrated the beneficial effects of oestrogen use on prevention of osteoporosis. The WHI was the first Randomised Controlled Trial (RCT) to demonstrate reduction of hip fracture risk with oestrogen use (Writing group for the WHI investigators, 2002). But the prevention of osteoporosis through oestrogen use may be limited to time of initiation relative to completion of the menopause. Demonstrated in an early study; oestrogen use in 114 middle-aged women two months post ovariectomy resulted in significant prevention of bone mineral loss compared with a placebo. Treatment delayed to three years resulted in significant

increases in bone mineral density, but this effect was lost when treatment was delayed to six years post operation (Aitkin, Hart & Lindsay, 1973).

1.5.2 HRT can reduce cardiovascular disease in younger menopausal women

Both oestrogen receptors are found in blood vessel cells and facilitate oestrogen inhibition of vascular injury such as atherosclerosis (Pare et al., 2002; Punnonen, Jokela, Dastidar, Nevala, & Laippala, 2000) and this decreases the risk of heart disease (Nadkarni, Cooper, Brancaloeone, Bena, & Penetti, 2011; Rosano & Panina, 1999). Administration of oestrogen in menopausal women has been shown to affect blood flow and the vascular system (Volterrani, Rosano, Coats, Beal, & Collins, 1995) whereby oestrogen use can improve blood flow and protect the cardio vascular system. The increase in blood flow could, in part, account for the change in blood flow recognised in fMRI and PET studies and this is something that needs to be considered in analysis. However it should also be noted that this increase in blood flow is most prominent during the completion of specific, frontal mediated tasks.

Although observational studies have recognised a benefit of HRT use on prevention of cardiovascular disease in menopausal women (Henderson, Paganini-Hill, & Ross, 1991; O'Keefe et al., 1997), two RCTs found no difference in coronary heart disease risk between HRT users and a placebo group (Hsia et al., 2006; Hulley et al., 1998). Both studies considered the role of oestrogen use in post-menopausal women, and risk was lower for younger initiators (aged 50-59, Hsia et al., 2006).

1.6 HRT can protect natural cognitive declines experienced during the menopause

Due to the effects of oestrogen on brain chemistry, it has been proposed that oestrogen use during the menopause may enhance cerebral and cognitive function (Rasgon, Magnuson, Johansson, Pedersen, & Gatz, 2005). Norbury and colleagues (2003) go on to suggest that oestrogens affect brain development in regions crucial to higher cognitive function by interacting with neuronal networks at many different levels.

Animal studies have helped to investigate neuronal changes at a level of detail that is difficult to explore in human studies. As a result of animal studies it is now recognised that oestrogen influences aspects of brain chemistry known to be important for memory functions, such as influence on neurotransmitters (Craig & Murphy, 2006), and it is these functions that are impeded by oestrogen decline during the menopause (Brinton, Chen, Montoya, Hseih, & Minaya, 2000).

During the menopause, oestrogen decline can lead to changes in the structure of the female brain (Cutter, Norbury, & Murphy, 2003) such as decreased blood flow to the hippocampus (Maki & Resnick, 2001) a reduction in the white brain matter (associated with decreased memory functions; Whyte et al., 2006) and a decrease in spine density of dendrites in the PFC and hippocampus (associated with different types of memory and attention decline; Hao et al., 2004; Robertson & Van Amelsvoort, 2001). At oestrogen receptor sites (found in the specific areas of the brain detailed above), oestrogen regulates synaptogenesis (increase in dendrite density and synapse). Oestrogen production during the menopause decreases and this results in decreased

dendrite density and decreased synaptic activity and can result in the cognitive functions mediated by this activity becoming compromised.

After the reduction of oestrogen during the menopause, HRT promotes brain functioning in women and protects brain functioning as they age (Greene, Bellgrove, Gill, & Robertson, 2002). Neurobiological knowledge can be advanced through the comparison of cognitive task performance between those with a marked difference in some aspect of brain chemistry and those who are 'normal'. In the case of oestrogen change most of our understanding of its effects has come from the comparison of those with different levels of oestrogen in their body and brain to understand the biological and neurological outcomes. For example, by comparing two groups of women with contrasting oestrogen levels we can now make the assumption that higher levels of oestrogen have a positive effect on emotions, verbal abilities, gross motor strength, behaviour and perceptual speed (Jarvik, 1975; Yaffe et al., 2000). On a large scale, studies have highlighted the importance of the role of oestrogen in memory (Duffy et al., 2003; Jenkins et al., 2004; Resnick et al., 1998). Philips and Sherwin (1992) and Norbury and colleagues (2003) affirm that memory problems are one of the most commented upon by women at the climacteric, and that HRT is helpful to restore this.

As described above, ovarian hormones can influence brain regions crucial to higher cognitive functions such as learning and memory at structural, cellular and functional levels (Pompili, Arnone, & Gasbarri, 2012). Currently, doctors are advised not to provide HRT for cognitive function in menopausal women but HRT can be prescribed for the alleviation of other menopausal symptoms, and it is recommended that the minimum dose needed is prescribed for the shortest

amount of time. Doctors are advised that for the treatment of menopausal symptoms the benefits of short-term HRT outweigh the risks in the majority of women less than 60 years of age. However HRT does not prevent cognitive decline. A large body of evidence challenges these recommendations, urging for reconsideration in GP recommendations against HRT use for protection of cognition. This evidence is described in detail over the next few sections.

1.6.1 The potential for HRT to protect against dementia

AD is the most common form of dementia, involving areas of the brain responsible for thought, memory and language (Richards & Hendrie, 1999). Incidence rates suggest that the risk of AD in women is double that of men at the age of 80 (Zandi, Carlson, Plassman, Welsh-Bohmer, & Mayer et al., 2002) leading to explorations on the association between oestrogen deficiency and AD.

Ovarian steroids produce many effects on the brain, influencing processes such as cognitive function, motor activity, and development of Parkinson's disease and Alzheimer's Disease (AD; McEwen, Alves, Bulloch, & Weiland, 1998). Episodic memory is the domain most severely affected in the early stages of AD (Welsh, Butters, Hughes, Mohs & Heyman, 1992) and deficits in encoding novel information is an early symptom of AD (Blacker et al., 2007). This can be encapsulated in a preclinical stage of AD, known as mild cognitive impairment, a mild neurocognitive decline that occurs in the presence of day to day functioning (Levy, Lah, Goldstein, Steenland, & Bliwise, 2006). Executive function is also severely affected (Blacker et al., 2007).

Over the past two decades, there has been considerable research investigating the risks and benefits of HRT with regards to neurodegenerative

diseases. For example, Kawas and colleagues (1998) demonstrated that HRT is associated with a significantly reduced risk for AD, and Tang and colleagues (1996) also demonstrated a reduced risk for AD and went on to identify an inverse relationship between duration of use and risk. During a long term follow up study; Dye, Miller, Singer and Levine (2012) found a reduced risk of AD and improved cognitive function in menopausal women who had initiated HRT at an earlier date, with a mixed impact on Parkinson's disease.

In another study, the researchers wanted to investigate the impact of two to three years of HRT use during the menopause on the risk of cognitive impairment five to 15 years later. A total of 343 postmenopausal women who had participated in a randomised clinical trial studying the effects of HRT on osteoporosis were invited to take part in this follow up study considering their current cognition (Bagger, Tanko, Alexandersen, Qin, & Christiansen, 2005). At 15 years after the therapy was completed, participants had a mean age of 65 years and had not since received any form of HRT. Cognitive function was assessed using the short Blessed test (Katzman et al., 1983), which includes tests of orientation, concentration and memory function where a cognitive score of less than six indicates cognitive impairment. There was no difference in cognitive mean scores at 15 year follow up between the users and placebo nonusers. However for those who had received HRT, the risk of cognitive impairment (demonstrated by a score lower than six) was decreased by 65%. The researchers suggested from this that early use of HRT during the menopause may prevent cognitive impairment in later life, but that later use may have a detrimental impact. A review by Dye and colleagues (2012) also suggested a reduced risk of AD with earlier HRT use, whereas the WHIMS found an increase in risk of AD

when HRT was initiated a decade or more after the menopause. Data from the WHIMS demonstrate a higher incidence of cognitive impairment and dementia among HRT users who initiated the therapy during the post-menopausal stage, as compared to placebo controls (Rapp et al., 2003; Shumaker et al., 2003). Similarly, participants were over one decade past the natural menopause at point of HRT initiation, and perhaps this was too late to protect cognitive decline at this point. Due to the large implications of AD research, possible detrimental effects of HRT on cognitive function have been explored.

One study found HRT enhanced cognition for women with already developed AD. In their study, Asthana and colleagues (1999) evaluated the cognitive response to HRT for menopausal women with AD. Twelve women with AD of mild-moderate severity received six weeks of either oestradiol or a placebo. For women receiving HRT, enhancement in verbal memory was positively correlated with plasma levels of oestradiol. In the placebo group, no significant effects were found. The results of the study suggest that short HRT use may enhance cognition for post-menopausal women with AD.

The memory of women who experience rapid oestrogen decline at a younger age, perhaps through removal of part or all of the reproductive system, can be investigated for any changes before and after surgery, or compared with women with normal reproductive function matched for age. If surgery has had a detrimental effect on memory at this point, it can be assumed that this is not an aging effect. Such memory change can then be compared to the normal menopausal population for similarities. By investigating surgically menopausal women it has been demonstrated that earlier age of surgical menopause

increases the risk of cognitive impairment in later life and even increase the risk of neurological diseases such as AD (Rocca et al., 2007; Rocca, Grossardt, & Maraganore, 2008). This means it is important to prescribe HRT as close to the surgical menopause as possible, and may have implications for the natural menopause too. In addition, such studies have demonstrated that HRT can enhance short term memory as well as long term memory and the capacity for learning new associations (Sherwin, 2005; Philips & Sherwin, 1992). The effect of HRT on such memory systems are described below.

1.6.2 HRT enhances Verbal, episodic and explicit memory in menopausal women

AD has higher prevalence in women, which has led to the development of research regarding estimated prevalence of AD in women, and the potential to reduce this with the use of HRT. Similarly, specific cognitive tasks are also sex sensitive, which has led to developments in research regarding HRT and verbal memory.

The positive effect of higher levels of oestrogen on verbal memory has been a popular area of research in earlier decades (deGroot et al., 2003; Maki et al., 2001; Sherwin, 1988) and the idea that oestrogen levels in the brain affect verbal memory is longstanding (Wickham, 1958). The effects of HRT have since been drawn into consideration, highlighting the positive effects of HRT on verbal memory (Sherwin 1997, 2007b, 2008). Oestradiol is associated with a reduced amount of decline in verbal memory among menopausal women, with benefits seen in therapy durations ranging from three months to two years in women just completing the menopause (Bagger et al., 2005; Dumas, Hancur-Bucci, Naylor, Sites, & Newhouse, 2008; Silverman, Geist, & Kenna, 2010). LeBlanc, Neiss,

Carello, Samuals and Janowsky (2007) failed to demonstrate improvements in verbal memory in women who had just completed the menopause; however the treatment duration was a shorter time of two months only.

Rensnick and colleagues (1998) assessed regional blood flow in menopausal HRT users and non-users during delayed recognition of verbal and figural information. Increased activation was recognised in the right frontal regions and right parahippocampal gyrus during verbal memory processing, and the right parahippocampal and inferior parietal regions during figural processing. Further to this, Joffe and colleagues (2006) demonstrated increased activation of the frontal lobes during recall of verbal information from a verbal memory task in menopausal participants receiving HRT, compared to placebo controls. These studies combined confirm verbal memory to be mediated by the frontal lobes, and show a relationship between increased frontal lobe activation with oestrogen replacement during the completion of verbal memory tasks.

Verbal memory has been shown to improve using paragraph recall (from the WMS-R) and also list recall from the California Verbal Learning Task (CVLT; Delis, Kramer, Kaplan & Ober, 1987) in menopausal women receiving HRT (Sherwin, 1988). In a more recent study, Maki and colleagues (2001) looked at verbal memory scores of 184 menopausal and post-menopausal women aged between 50 and 89 years of age. Of these, 81 women had never received HRT and 103 women were current users. Participants completed the CVLT, the digit span task from the Wechsler Adult Intelligence Scale Revised (Wechsler, 1939) and the Benton Visual Retention Test (Benton, 1968). On the CVLT, HRT users outperformed non-users on immediate recall in each of the five list-learning trials, recall after both short and long delays, word recognition and clustering of

words into semantic categories indicating being better engaged in active learning. The selected population had too large an age span and it would have been more appropriate to lower the upper age limit to combat ageing effects which have a large impact on WM and attention.

In order to help discriminate the positive effect of HRT use on verbal memory from other cognitive tasks, Kampen and Sherwin (1994) investigated the effect of HRT use in post-menopausal women on verbal memory, spatial memory, language and attention. HRT users demonstrated better immediate and delayed paragraph recall (also from the WMS-R) than matched controls with no history of HRT use. No differences were found on any other tasks, suggesting verbal memory to be susceptible to oestrogen change.

Similar to AD research, differences in the protection of verbal memory by HRT use have been found depending on the participants' age. In their study, Dumas and colleagues (2008) compared a group of 50-62 year olds with a group 70-81 year olds to see if the benefits of HRT on verbal memory could be seen in women who were almost 20 years beyond the menopause. Reduction of mistakes was only seen in the younger group. However Tierney and colleagues (2009) did show a reduced amount of mistakes made during a verbal memory task for women aged between 61 and 87 years, but this was only demonstrated in women who scored above average on the verbal task initially, and not those with normal, or lower than average score. For the study by Tierney and colleagues, most participants had an average or below average score on the verbal task and this is typical of the general population.

Several human studies have shown the beneficial role of oestrogen in the hippocampus, an area of the brain associated with episodic memory (which can be measured using verbal memory tasks such as list recall and paragraph recall). Using Magnetic resonance spectroscopy, Robertson and Van Amelsvoort (2001) compared HRT users and non-users to young women (of normal menstrual functioning) for energy metabolism in specific brain areas. HRT users and young women had increased signal activity in the hippocampus and parietal lobe compared to non-users. Interestingly there was no difference between signal activity of the HRT-user group and naturally menstruating females, suggesting that HRT use had restored hippocampal activity to normal menstruating function.

Maki and Resnick (2000) examined longitudinal changes in regional cerebral blood flow over a two year interval in women on and off HRT. Each participant demonstrated significant differences in cerebral blood flow dependent on whether they were taking HRT at that point or not. Specifically, differences in cerebral blood flow were found in the right hippocampus, the parahippocampal gyrus, and the left middle temporal gyrus whereby those with lower levels of oestrogen in their body demonstrated less blood flow to these brain regions. The researchers went on to confirm that women who were experiencing less blood flow to these brain regions (as experienced naturally during the menopause) demonstrated less neuronal activity in these regions. This can result in lessened cognitive efficiency which can cause deficiency in types of memory capacity that operate in these brain areas.

In addition, the cholinergic system mediates the ability of oestradiol to affect the hippocampus and associated behaviours (Daniel, Hulst, & Lee, 2005; Dohanich, Korol, & Shors, 2009). In rat studies, oestradiol has been reported to

enhance cholinergic muscarinic receptor density and enhance cholinergic uptake and activity in the hippocampus.

When ovariectomised rats receive oestrogen therapy, spine density increases in the CA1 area of the hippocampus, and not area CA3. The CA1 area of the hippocampus plays a role in explicit memory, which could support improvements in explicit memory with HRT use (Tang et al., 2004). Gibbs (2000b) found that rats who experienced a deficiency in oestrogen also suffered from a deficiency of brain nerve growth in the hippocampus. When these rats were given short-term HRT, the brain areas restored themselves with stronger dendrite spines which results in increased synaptic activity and activation. As well as oestrogen increasing neural plasticity in the hippocampus, Frick and colleagues (2004) demonstrated that this increase could affect memory in ovariectomised middle-aged female mice using the radial arm maze.

Episodic memory has also been suggested to have frontal lobe components (Janowsky, Shimamura, & Squire, 1989; Tulving et al., 1994) and to be mediated in part by the posterior cortex, the posterior cortex being dependant on the PFC. Fletcher and colleagues (1995) and Schmidt and colleagues (2002) describe verbal memory as a type of episodic memory mediated by the left PFC during encoding, and the right PFC during retrieval.

Rasgon and colleagues (2004) compared regional metabolic activity between postmenopausal oestrogen users and non-users, predicting that oestrogen use will result in preserved regional metabolism. Neuropsychological tests were administered to assess cognitive performance. Unfortunately participants in the oestrogen group were significantly younger than those in the

non-users group (more than 10 years difference between mean ages). To help correct for the effect of age on cognition, age was added as a covariate in analysis. Analysis revealed that those in the oestrogen non-users group had a significant decline in the posterior cingulate cortex's regional metabolic activity whereas oestrogen users had no such decline. The researchers concluded that HRT can help protect metabolic decline in this brain area as a result of the menopause, and that those receiving HRT may have better function in the posterior cortex to help continue neurogenesis and combat neurodegenerative disease.

Studies have demonstrated that HRT is beneficial to verbal memory and other memory functions. Those with higher levels of education may be able to combat these problems, but only in simple memory tasks. It is now believed that oestrogen affects higher level cognitive functions, especially if these have a verbal component (Rosenberg & Park, 2002; Sherwin, 1997; Sherwin, 2012).

1.6.3 HRT can target executive function systems in the PFC

Keenan and colleagues (2001) suggest that previously reported cognitive declines mediated by the hippocampus are secondary to executive dysfunction mediated by the PFC. In their study, HRT users were compared to placebo controls on a battery of tasks including memory and executive function tasks. Results suggested that oestrogen influences prefrontal-cortex mediated tasks, and not those mediated by the hippocampus. Specifically, oestrogen users outperformed placebo controls on a discrimination component of the CVLT, inhibition control and the N-Back test of WM (Kirchner, 1958). In support of this view point, Wegesin and Stern (2007) demonstrated that HRT use resulted in

better source memory (which relies more heavily on executive processes) than item memory when compared to non-users.

HRT use encourages dendrite growth in layer one, area 46 of the PFC (Tang et al., 2004) which may account for stronger WM and SA performance in HRT users in the study by Keenan and colleagues (2001). In addition to this, several other studies have also recognised an improvement in WM and SA performance for those receiving HRT treatment compared to placebo controls and this is described in detail below.

Working memory

Pompili and colleagues (2012) suggests that further clarification in the role of oestrogen in WM is needed in part due to conflicting research (mostly from animal models). The relationship between oestrogen and WM has been well established in rat studies whereby oestrogen replacement in young ovariectomised rats has been shown to improve WM involving a two-choice water escape WM task (Bimonte & Denenberg, 1999; O'Neil, Means, Poole, & Hamm, 1996) and moderated maze tasks (Fader et al., 1999; Korol & Kolo, 2002; Zurkovsky, Brown, Boyd, Fell, & Korol, 2007). Spatial WM in rats is mediated by the hippocampus (Korol et al., 2004). In humans, WM is mediated by the PFC, and the beneficial effects of oestrogen on activation in this area have been demonstrated (Joffe et al., 2006; Smith et al., 2006; Shaywitz et al., 1999). WM capacity exhibited through superior performance of menopausal oestrogen users has also been demonstrated (Dumas et al., 2010; Keenan et al., 2001; Stephens, Bristow, & Pachana, 2006).

In the study by Shaywitz and colleagues (1999), the researchers considered the effect of HRT on brain activation during the completion of a verbal and a non-verbal WM task. Those receiving the oestrogen treatment had increased activation in the left hemisphere during encoding into WM and the right superior frontal gyrus during retrieval for the verbal task only (similar to the study by Cutter and colleagues, 2003). The activation patterns described are similar to patterns of activation seen in younger adults (Dumas et al., 2010). The researchers deduced that oestrogen received by menopausal women can cause alterations in brain activation patterns during the performance of WM tasks. Task performance remained similar between groups perhaps due to ceiling effects.

One study was able to demonstrate both increased neuronal activation during completion of a spatial WM task, as well as improvements in task score itself (Joffe et al., 2006). In their study, participants were randomly assigned to oestradiol or a placebo for 12 weeks. Both prior to and on completion of treatment the participants completed tests of executive function including verbal and spatial WM, complex verbal recall and learning as well as a measure of menopausal symptoms. Results showed significant activation during tasks of verbal and spatial WM in the superior frontal gyrus for the higher WM load condition only. The group receiving oestrogen therapy also selectively reduced errors of perseveration during verbal recall, a frontal mediated function. Measures using fMRI showed a significant increase in frontal lobe activity for the oestrogen users only. In addition, women with higher rated menopausal symptoms experienced greater cognitive benefit with oestrogen therapy perhaps due to an alleviation of the symptoms themselves. In this case, HRT helped to

improve executive functioning as demonstrated by reduced perseverative errors and PFC activation during verbal and spatial WM tasks.

Smith and colleagues (2006) also considered the effects of HRT on spatial WM. Participants either received one month of oestrogen replacement or a placebo. Treatment was reversed after a wash out period. Like the study by Joffe and colleagues (2006), the PFC was activated during completion of the spatial WM task for the oestrogen users only, and this was irrespective of order of treatment. Both studies demonstrated an increase in activation of the frontal lobes during storage of spatial WM material.

It has been suggested that oestrogen specifically affects the delayed response performance characteristic of WM (Bailey et al., 2011). Differing to the studies by Smith and colleagues (2006) and Joffe and colleagues (2006); Dumas and colleagues (2010) explored the active manipulation of material typical of Baddeley's WM model (1986). The researchers used fMRI to examine the effects of oestradiol on information manipulation during completion of components of the N-back task, mediated by the frontal lobes. At baseline and three months following treatment, each woman completed a visual verbal N-back sequential letter test with three difficulty levels (based on cognitive load). They found that women treated with oestradiol for three months had an increase in frontal activity during completion of the two more challenging levels of the WM task compared to a placebo control, however this was not evident in task score itself. The study again demonstrates a beneficial effect of oestrogen in prefrontal mediated tasks such as WM, and similar to the study by Joffe and colleagues (2006), the beneficial effect of oestrogen on task score may be dependent on difficulty of the task.

Jenkins and colleagues (2004) considered the role of anti-oestrogens (tamoxifen) on cognition in menopausal breast cancer patients, compared to healthy menopausal women with no history of HRT use. Participants were asked to complete the verbal memory paragraph recall test, visual memory faces recognition test, spatial span WM task, letter sequence WM task and digit span WM task (Wechsler, 1998), The Kendrick digit copying task for processing speed (Kendrick and Watts, 1999) and the GHQ-12 (Goldberg & Williams, 1988) and Beck depression inventory (BDI; Beck & Steer, 1984). Average scores were lower for the oestrogen suppressed group for each task, and this difference was significant for the verbal memory task and processing speed. Higher scores on the BDI were not significantly correlated with lower score on any of the cognitive measures and those with low GHQ-12 scores (to demonstrate normal mental health) and high scores (indication of a common mental health problem such as anxiety and depression) did not differ in cognitive performance.

The research highlighted above identifies a beneficial effect of oestrogen on prefrontal mediated tasks, and in particular, WM. This effect can be enhanced by HRT use.

WM involves regions of the brain that are receptive to oestrogen, yet in some cases, despite increased activation in brain areas during completion of WM tasks in oestrogen users, this was not demonstrated in task performance itself. WM span tasks are one of the most widely used measurement tools in cognitive psychology and highlight individual differences in general intellectual ability and can be used across different age groups (Kane & Engle, 2004). WM span tasks were designed based on Baddeley and Hitch's (1974) theory of WM which demonstrates the importance of an immediate memory system capable of

storing a limited amount of information whilst being cognitively attentive elsewhere. The system requires more complex storage mechanisms than other types of memory in order to form the crucial interface between perception and memory, and between attention and action. Memory span reflects the speed at which items can be rehearsed as well as the rate at which the 'trace' fades (Baddeley, 2007). However, the event of rehearsal can be distracted in the face of alternative tasks, as represented throughout everyday responsibilities (Murray, 1968). Task performance is therefore dependent of difficulty of the task, and exercise of cognitive load. Through completion of WM tasks, brain areas will become active but differences in task performance may only become apparent when tasks are of a certain level of difficulty.

Because WM Capacity (WMC) can diminish with age (in women this can occur quickly from 65 years and onwards), researchers need to be cautious about the upper age of the participant. By inviting post-menopausal women to complete their studies to measure WM differences between HRT users and non-users, perhaps researchers are confusing brain aging effects with hormonal effects (Grigorova, Sherwin, & Tulandi, 2006). This may be apparent in some studies designed to investigate the possible effects of HRT on executive function and WM to which no or small effects have been found between groups (such as Janowsky, Carello, & Orwoll, 1999; Keenan et al., 2001 and Shaywitz et al., 1999).

Sustained attention

It has been suggested that SA may become problematic during the menopause as changes in oestrogen levels have been linked to a decrease of functioning oestrogen receptor sites in the PFC and white matter of the brain,

and deficits in frontal lobe functioning. In addition to this, oestrogen decrease during the menopause can result in decreased WM capacity and SA may rely on WM when information processing and storage become competitive (Knudson, 2007). Cognitive measures demonstrating SA include reaction time tasks and oestrogen change during the menopause may have effect on reaction speed during reaction time tasks (Wuttke et al., 1975).

Several animal and human studies have reported that gradual oestradiol reduction due to the menopause slowly diminishes activity in the cholinergic system. This may have an effect on cognitive functions sensitive to cholinergic activity in the frontal and hippocampal areas of the brain such as SA (Sarter, Givens, & Bruno, 2000). Voytko, Tinkler, Browne and Collins (2009) found oestrogen influences attention in surgically-menopausal monkeys and the cholinergic system is one of the mechanisms by which oestrogen modulates attention. SA is also impaired when women are given a hormone suppressant to combat breast cancer (Wieneke & Dienst, 2007), suggesting that it may become impaired during the menopause and potentially protected with oestrogen replacement.

Changes in SA during the menopause and response to HRT are well explored in animal studies, but not in human studies. For example, high-dose oestradiol injections have been shown to improve aspects of the five-choice serial reaction time task in ovariectomised young rats (Barnes, Staal, Muir, & Good, 2006). However, rats who were ovariectomised and given oestrogen several days after surgery demonstrated adverse effects in delayed spatial alternation and inhibition control (Wang et al., 2008). Although rats receiving

oestrogen treatment performed worse in these executive function tasks, the study still confirmed a relationship between oestrogen change and WM and SA.

A number of studies have indeed reported an improvement with HRT in executive function (Fedor-Freybergh, 1977) and the studies reviewed above show that attention is oestrogen responsive in animals, impaired during the menopause following oestrogen deficiency (naturally or surgically) and improved with HRT use. Less is known about the relationship between SA and oestrogen change during the menopause in humans, however the location of oestrogens in the PFC, the relationship between WM, SA and executive function, and the relationship between SA change in pregnant women (Crawley et al., 2005; deGroot et al., 2003) and during the menstrual cycle (Duff & Hampson, 2001) gives warrant to explore SA in HRT users and non-user matched controls.

Overall, it has been demonstrated that HRT use can improve frontal lobe activation, or improve cognitive task performance mediated on the frontal lobes, when accessing the following memory systems: verbal memory (Jacobs et al., 1998; Kampen & Sherwin, 1994; Maki et al., 2001; Sherwin 1988), verbal WM (Joffe et al., 2006; Jonides et al., 1998; Shaywitz et al, 1999), spatial WM (Joffe et al., 2006; Smith et al., 2006), WM (Duff & Hampson, 2001; Jenkins et al., 2004; Keenan et al., 2001; Morrison, Brinton, Schmidt, & Gore, 2002; Morrison, Wang, Hara, Janssen, & Rapp, 2010), episodic memory (Cutter & Norberry, 2003) and attention (Resnick et al., 1997). Of these, WM and SA are less well investigated, and the interaction of oestrogen with WM and attention performance has room for exploration.

Jenkins and colleagues (2004) did not find an interaction between mood score and cognitive performance in a large test battery. Generally it is assumed that mood does play a role in cognitive performance, and the interaction between oestrogen, mood and cognition are described in more detail below.

1.6.4 HRT maintains neurotransmitter activity in the PFC and hippocampus and this improves mood

Sherwin found HRT users to have improved mood after oestrogen treatment (Phillips & Sherwin, 1992; Sherwin 1997) and this may be due to the role of oestrogen on neurotransmitter production.

Cutter and colleagues (2003) and Moses and colleagues (2000) demonstrated that short-term use of HRT in menopausal women can result in higher levels of a naturally occurring amino acid (5-HT) which contributes to the workings of the serotonergic system (low levels of 5-HT and serotonergic activity are consistent in patients suffering from depression). At a cellular level, oestrogen increases the rate of destruction of Monoamine Oxidase (MAO), the enzyme that breaks down serotonin and also affects intra-neuronal serotonin transport (Wise, Dubal, Rau, Brown, & Suzuki, 2005). An increase in oestrogen increases the destruction which results in increased serotonin levels and better mood. The highlighted studies show that oestrogen affects neurotransmitters that influence mood states, and that HRT use in menopausal women can help combat hormonal related depression.

The neurotransmitters believed to be responsible for many aspects of cognitive function including memory are serotonin, noradrenalin and acetylcholine (Pelletier & Romaine, 2001). The PFC, hippocampus and midbrain's

dopamine and serotonin systems are hormone responsive from maturity, and oestrogen affects these neurotransmitters as well as their receptor sites in the brain (Fink et al., 1995; McEwen, 2001, Kugaya et al., 2003). Based on the location of these oestrogen binding sites and the effect of oestrogens on serotonergic, noradrenergic and dopaminergic systems it can be concluded that these major neural systems are subject to the influence of oestrogen change during the menopause. Leranth and colleagues (2006) demonstrated a 30% reduction of dopaminergic cells in the brains of non-human primates after being deprived from oestrogen for 30 days. Reduced oestrogen as experienced during the menopause can lead to the inhibition of dopaminergic, noradrenergic and serotonergic activity; and inhibition of these neurotransmitters is associated with reduced mental performance and learning (Kugler, Seus, Krauskopf, Brecht, & Raschig, 1980; Norbury et al., 2003).

Richardson (1991) suggests that the cognitive impairments often demonstrated in women entering the menopause are due to menopausal symptoms such as low mood experienced pre and post oestrogen change. Oestrogen levels affect neurotransmitters which influence a change in mood, and cognitive performance can be hindered or facilitated by these neurotransmitters. It is important to measure mood when assessing cognition to identify whether it is a contributing factor to performance (Duffy, et al., 2003, Sherwin, 2012).

To support this, it has been reported that people who suffer from depression complain of problems in day to day memory (Watts, 1993). Cronholm and Otterson (1961) suggested that those suffering with depression also find it difficult to learn, a key component in many SA and WM tasks. Kahn, et al (1975)

demonstrated a correlation between memory complaint and level of depression, but that this did not predict memory score. Further studies demonstrated that negative emotions such as depression can have a negative effect on WM (Ellis & Ashbrook, 1988; Hertel and Hardin, 1990).

Dumas, Hancur-Bucci, Naylor, Sites and Newhouse (2008) showed a direct cognitive effect as a result of oestrogen's interaction with the cholinergic system in peri-, but not post-menopausal women. Menopausal women were randomly placed on 17-beta oestradiol or a placebo for three months at which point they completed their first cognitive test battery whilst receiving an antimuscarinic drug to block acetylcholine receptors involved in memory. Participants then received a further three months of cross over treatment (from HRT to placebo, from placebo to HRT) after which they completed the cognitive tasks for a second time whilst receiving the drug. The cognitive test battery included attention and verbal and nonverbal learning and episodic memory tasks. Those receiving HRT performed better during the episodic memory task, attention tasks and tasks with a speed component whereby oestrodial pre-treatment weakened the anticholinergic drug-induced cognitive impairments. The study demonstrated an interaction between oestrogen and the cholinergic system and the effects on pre-frontal mediated cognitive performance in menopausal women.

Oestrogen administration has also been shown to augment cholinergic transmission and enhance dendrite spine formation in the hippocampus, and this has been linked to improved learning and memory in rodents (Liu et al., 2008). Specifically, oestrogen administration has been linked to improved spatial WM in

rodent's recently ovariectomised (Daniel, Hulst, & Berbling, 2006; Fader et al., 1999).

Hu and colleagues (2006) suggested that HRT use is most beneficial to the maintenance of the cholinergic neurons in the hippocampus of ovariectomised rats. With an increase of acetylcholine and cholinergic activity in response to oestrogen in the hippocampus it can be concluded that memory systems should improve. In humans and non-human primates, specific cognitions susceptible to change in these neurotransmitter activities have been highlighted and linked to oestrogen change. Jacobs and D'Esposito (2011) examined the effect of oestradiol on WM in healthy young women taking into consideration the baseline levels of dopamine in the PFC. They concluded that oestradiol levels do impact on WM capacity and that this impact depends on baseline dopamine.

Oestrogen influences neurotransmitter functioning by increasing the availability of acetylcholine which is involved in memory systems (Van Amelsvoort et al., 2001) and is a determinant of changes in PFC functioning (Grigorova et al., 2006) such as SA. Posner and Peterson (2012) suggest attention relies heavily on the activity of noradrenaline. And finally, Sarter and colleagues (2000) as well as Bellgrove, Hawi, Kirley, Robertson and Gill (2005) suggest that an increase in acetylcholine and dopamine will improve SA.

1.6.5 Neuroprotection against toxic insults

Oestrogens protect against several toxins which cause irreversible damage via hydrogen peroxide accumulation in the brain (Norbury et al., 2003). Brinton and colleagues (2000) demonstrated that low levels of oestrogen just before onset and during time of neuronal degeneration can have optimal effects

for neuroprotection by the prevention of hydrogen peroxide and other toxins build up. It is not yet clear whether there is one unifying neuroprotective mechanism or multiple mechanisms selectively protective against certain toxins induced by oestrogen, but available data suggest it is more likely to be a broad class of oestrogen-induced protective mechanisms protecting against a broad range of toxins (Brinton, 2005). It is still unclear which (if any) of these mechanisms is directly responsible for conferring a reduction in the risk of neurodegenerative disease. Data from Brinton's lab on the neuroprotective effects of oestrogen indicate three levels of oestrogen action; chemical, biochemical and genomic (Brinton, 2001). Again, it is not completely clear to what extent each level of action lead to neuroprotection however each seems to be significant.

1.6.6 Detrimental, or no effects of the therapy

Despite research confirming the beneficial role of HRT on cognition, several studies have proposed a damaging effect (D. Jacobs et al., 1998). Resnick and colleagues (2009) reported that oestrogen therapy can increase the risk for developing dementia and may impair memory performance. Specifically, Resnick and her team concluded that the increased risk in developing dementia may be due to a decrease in hippocampal volume of women who initiate HRT during their post-menopausal stage (as supported by Coker et al., 2009). Decreased hippocampal volume could reduce cholinergic activity resulting in challenged WM capacity and SA (Lord et al., 2008). In addition to this, some studies have found no differences between post-menopausal HRT users and non-users during a WM task (The WHIMS).

Data from the Resnick laboratory suggests that the time window for reversal for oestrogen-deficit induced memory loss seems to be quite limited (Resnick et al., 1997). In concordance with this, studies that have only considered peri-menopausal women have found a positive effect of HRT use on brain activation during the completion of a cognitive task (Shaywitz et al., 1999). Other studies have only found such effects in participants who are peri-menopausal (Bagger et al., 2005; Dumas et al., 2008; Dye et al., 2012; Erickson et al., 2010; Lord et al., 2008; Norbury et al., 2007). And some have suggested that HRT use in post-menopausal women can be detrimental to cognition (the WHIMS). Shumaker and colleagues (2003) reported that oestrogen use in postmenopausal women (aged between 65 and 79 years) could increase the risk of dementia. In their randomised, double blind, placebo controlled study; women received HRT or a placebo control. The study was carried out over a seven to nine year period and participants completed global tasks of cognitive function to predict onset of dementia. Those in the HRT group had higher instances of predicted dementia and the researchers concluded that HRT should not be recommended to those in the postmenopausal stage as a solution to cognitive decline. The study differed from previous studies by recruiting an older age group, and recommendations were made against women receiving HRT in this particular age group only.

HRT can be received orally or transdermally, through use of tablets, nasal sprays, skin patches, implants or injections, and these different methods will impact on different genomic and non-genomic mechanisms. For example, oral oestrogen preparations are more likely to induce positive effects on serum lipoprotein lipid metabolism than transdermal oestrogens (Ansbacher, 2001). In

addition, when oestrogens are taken orally, less E2 is freely available to the brain than transdermal oestrogen therapy. Duration of treatment and concentration of exposure can also have marked effects on genomic and non-genomic mechanisms. For example, continuous exposure of oestrogen to the brain for a prolonged period of time may result in reduced neuronal response to oestrogen treatment (Toran-Allerand, 2006). In a study using a constant administration of oestrogen therapy in ovariectomised rats, no improvement was seen in WM score until the method of HRT management was changed to a cycle of higher and lower fluctuations of oestrogen similar to the fluctuations experienced during a normal menstrual cycle (Gresack & Frick, 2006). In human studies, continuous exposure of women to oestrogens caused a down-regulation of ER-alpha in the brain and other tissues (Mohamed & Abdel-Rahmann, 2000; Thakur & Sharma, 2007). GPs are therefore advised to recommend short use of HRT, in order to maximise benefits and reduce associated risks of treatment.

1.7 Summary

Oestrone (E1), oestradiol (E2) and oestriol (E3) are the major occurring oestrogens in women, and these diffuse across cell membranes to bind to and activate specific oestrogen receptor sites (Shao et al., 2012; Simoncini et al., 2004). The actions of these major oestrogens are therefore dependant on and mediated by the oestrogen receptor in that cell (Okada et al., 2008). These three forms of oestrogen have differing potency levels that dominate depending on stage in a female's life. Because of the genomic and non-genomic effects of oestrogens, cells and organs are regulated by an interaction of these two

mechanisms, including the cardiovascular and central nervous system (Simoncini & Genazzani, 2003).

Oestrogen levels will naturally change throughout the female lifespan, reaching their highest levels in the third trimester of pregnancy the lowest natural point during the menopause (Melton, 2000). This decline can be responsible for a range of reported problems or menopausal symptoms experienced during this time.

Oestrogen actions affect neurotransmitter systems including the serotonic, noradrenergic, cholinergic and γ -aminobutyric acidergic systems and these systems are subject to the influence of ovarian hormones, as demonstrated in changed emotion during different stages of the climacteric (Sherwin, 2003; Sherwin & Henry, 2008).

Oestrogens significantly affect the microstructure of brain regions crucial to higher cognitive function (Norbury et al., 2003). Oestrogen receptors are found in the cerebral cortex, midbrain, hippocampus, brain stem, hypothalamus and pituitary gland with high concentrations in the hypothalamus, pituitary, hippocampus and amygdala; areas crucial for memory function and executive function (Norbury et al., 2003). Oestrogen has been shown to bind to cell membrane receptors and affect secondary messenger systems used by growth factors and neurotransmitters (McEwen, 1999). HRT can bind in similar ways and has effects on the brain at macroscopic, microscopic, functional, metabolic and neurotransmitter levels (Brinton, 2005). During the menopause, a reduction in oestrogen levels results in reduced dendrite density and synaptic activity in these brain areas. This affects higher cognitive functions such as WM and attention. HRT has a neuroprotective role on dendrite density, brain volume of

the PFC, brain matter and the hippocampus and connectivity between dendrites and increased neurotransmission.

Oestrogen levels in the brain can influence brain regions crucial to higher cognitive functions such as learning and memory at structural, cellular and functional levels (Pompili et al., 2012). Oestrogens play an important role in age related declines in cognitive processes in healthy women. After the reduction of oestrogen during the menopause, HRT promotes brain functioning in women and protects brain functioning as they age. There is strong evidence for a relationship between lower levels of oestrogen as a result of the menopause and cognitive decline that can be separated from the effect of age. For example, young rats which have had their ovaries removed for the purpose of the study show the same cognitive deficits and improvement with oestradiol supplements as postmenopausal rats (Daniel, Fadwe, Spencer, & Dohanich, 1997; Korol & Kolo, 2002). In recent years there has been an increase in the number of animal studies evaluating the effects of oestrogen on learning and memory whereby most of these studies have shown that a constant delivery of the therapy results in improved memory performance and hippocampal neuroprotection (Cooke, 2005; Daniel, 2006; Pompili, Tomaz, Benedetto, Tavares, & Gasbarri, 2010; Sandstrom & Rowen, 2007). Similar results can be seen in humans (Dumas et al., 2008; Dye et al., 2012; Erickson et al., 2010; Sherwin & Tulandi, 1996).

The PFC is critical for intact WM and oestrogen enhances performance on WM tasks (Keenan et al., 2001). Oestrogen levels influence executive functions, specifically WM performance and performance of cognitive tasks with an attentional component. Generally, RCTs have found that oestrogen specifically affects short term and long term verbal memory and WM (Sherwin & Henry,

2008) and that in the case of three RCTs which found no effect of oestrogen on cognition, two did not choose a verbal WM task (Ditkoff et al., 1991; Janowsky, Charvez, & Orwoll, 2000) and one included participants who were elderly and no longer experiencing menopausal symptoms (Duka, Tasker, & McGowan, 2000). It has been suggested that oestradiol facilitates performance as WM load increases (Bimonte & Denenberg, 1999). As such, HRT may be most beneficial in instances where WM load becomes challenging (Dumas et al., 2010). The problems of WM and attention during the menopause are due to the changing levels of oestrogen in the PFC and hippocampus which results in less synaptic activity and cholinergic activity in these areas. Oestrogen also stimulates serotonin, noradrenalin and acetylcholine production. A reduction in oestrogen levels results in reduced levels of these neurotransmitters, and these reductions further worsen cognitive performance and memory capacity (Reilly, 2000).

Several studies have highlighted the beneficial effect of oestrogen on brain activity during the completion of WM tasks, yet this effect does not transpire in task performance itself (Erickson et al., 2010; Shaywitz et al., 1999), leaving some doubt about the vulnerability of WM to oestrogen changes. And only some, not all, prefrontal dependent cognitive tasks seem to be effected by oestrogen suggesting that the role of oestrogen in executive function is very domain specific (Duka et al., 2000). Although the roles of oestrogen change on episodic memory and verbal memory have been well established, less is understood regarding oestrogens' role in WM function, and there is a lack of evidence exploring the relationship with SA. Both WM, and especially SA, would benefit from further exploration.

Studies that have demonstrated an effect of HRT on cognition have typically used younger populations. Several studies have indicated no effects of oestrogen therapy on cognition, and even detrimental effects of HRT on cognition in postmenopausal women. Detrimental effects of oestrogen on cognitive performance seem to be time specific, and are encapsulated in the critical period hypothesis.

Critical period

2.1 HRT can protect cognition in peri-menopausal women

During the past few decades, extensive research has tested whether the administration of oestrogen in the form of HRT can help maintain cognitive functions in menopausal women. Results have been mixed and seem largely dependent on the time of initiation of the treatment. The inconsistencies of oestrogen's role in cognition during the post-menopause are compounded with the release of the findings from The WHIMS (Espeland et al., 2004). Further analysis of the WHIMS suggested that the risk of cognitive impairment and probable dementia did not differ between HRT users and the placebo control; where HRT users had non-significantly higher risk averages. The results have caused much controversy within the literature, and leaders of the field have considered to how this information contributes to the research as a whole. On consolidation with RCTs that also failed to produce results, studies were deemed similar for dose of HRT and frontal lobe and hippocampal cognitive task content. The way these studies differed from successful studies was in the age of the participant.

The average age of the participants in the WHIMS was 72 years. The study fails to support the idea that oestrogen can protect against cognitive decline in women over the age of 65 years. On closer look of study results; some participants did maintain cognitive capacity as a result of HRT use and generally these women had initiated the therapy during their peri-menopausal stage, typically in their forties and early fifties. Preliminary reanalysis of the WHIMS data found that the women who took HRT prior to their enrolment in the WHIMS (and before the age of 65) were 50% less likely to develop AD and dementia compared to the non-users (Henderson, Espeland, Hogan, Rapp, & Stefanick, 2007). Using this information in the attempt to resolve discrepancies between conflicting research through the careful consideration of the literature, the critical period hypothesis (Maki, 2006; Sherwin & Henry, 2008) attempts to reconcile the literature.

The critical period hypothesis suggests that HRT use should be initiated during the onset of the menopause to maintain neurobiological function (Gibbs & Gabor, 2003; Resnick & Henderson, 2002; Sherwin, 2012). Current research has identified the potential period or 'a window of opportunity' for HRT to be taken relative to onset of the menopause in order to produce optimal relief from menopausal symptoms. These symptoms include cognitive strain, and in particular, a challenge in WM functions. The theory helps to explain the unexpected findings of the WHIMS.

Rodent studies have helped to develop theories regarding oestrogen's role in the brain and cognition, and although animal models and human models differ, the studies have often been a useful starting place for human studies. Generally research from rat studies has demonstrated an improvement in

performance on tasks which are hippocampally dependent (such as spatial WM tasks) when oestrogen is allotted to young ovariectomised rats. This has been demonstrated with the use of a variety of methodologies (Bimonte & Deneberg, 1999; Bimonte-Nelson et al., 2003; Gibbs, 2000b). Although the female rat model is often used to study mechanisms by which oestrogen may affect learning and memory it must be considered that the rodent reproductive cycle differs significantly from primates' and humans', as does their cognitive function (Sherwin & Henry, 2008). Turning our attention to human studies, the researchers considered studies that have been developed to test the critical period theory and those without that intention but to which do support.

Since the development of the critical period hypothesis several studies have attempted to confirm or disconfirm the theory dependant on the participant's age at initiation, and explore how the concept applies to the underlying neurobiology. Such studies will be addressed for the duration of the chapter.

2.2 Evidence for the critical period hypothesis from RCTs and Observational studies

The critical period hypothesis suggests that improved cognition through HRT use is better obtained when treatment is initiated during the onset of the menopause, at the average age of 51.8 years (Sherwin, 2003). Once the menopause is exceeded to a certain point, initiation of HRT will no longer be as effective in its protection of brain function and following this, cognition. The point to which this may succeed is yet to be defined.

According to the theory, successful cognitive maintenance would be expected in peri-menopausal women given HRT compared to a placebo control, whereas in post-menopausal HRT users we would expect no difference. Some studies have even demonstrated detrimental effects of HRT use on cognition in post-menopausal women (Schmidt et al., 1996; Steffens et al, 1999). RCTs have demonstrated results which favour the critical period hypothesis. For example, in peri-menopausal and early postmenopausal women whose mean age was 51 years, those who randomly received HRT experienced significant improvements on a frontally mediated task of executive function, manifested in a reduction of errors compared to placebo controls (Joffe et al., 2006).

Similar results have been found in a large observational study. MacLennan and colleagues (2006) studied the interaction between effects of time of initiation and duration of HRT on several cognitive functions. A total of 428 women aged 60 years and above were recruited from a computer-generated random selection of Adelaide households. Demographic and lifestyle information was recorded, as well as details of HRT use. Participants were screened for depression and their current mood was assessed. Cognitive tasks included a task measuring global cognition (Mini-Mental State Examination), attention (Trail Making Test Parts A and B), verbal learning and memory (Consortium to Establish a Registry for AD word list), and verbal expression (letter fluency, category fluency and the Boston Naming Test). Analysis was adjusted for age, education, mood, body mass index, smoking, alcohol intake and history of cerebrovascular disease. The researchers compared HRT users to matched controls with no history of HRT use. Participants in the HRT group were only included if they had been using HRT for a minimum of one year. Those who had

begun the therapy prior to the age of 56 years were classified as early HRT users. Those who had begun the therapy after this time were classified as late users of the therapy. The researchers identified several significant differences in cognitive performance between the two groups. Firstly, early initiators of the therapy performed better than late initiators on the mini-mental state examination and were faster than non-users on the Trail Making Test. Early initiators who were long-term users performed better on tests of letter fluency (verbal memory) than never users. Generally HRT users performed better on the trail-making test regardless of time of initiation and duration of therapy. There was a small trend only in late initiators and worsened performance than never users on the mini-mental state examination and letter fluency task.

Although results broadly support the critical period hypothesis, the researchers chose to divide early users from late users at 56 years old. This is almost four years older than the national average age of the menopause, and perhaps the cut-off could have been reduced. Even more accurate would be to ask the participants if they had initiated the therapy before or after their last menstrual bleed, to take account of individual variation in timing of menopause.

As well as studies demonstrating improved cognition in peri-menopausal HRT users, studies which demonstrate detrimental or no effect of HRT on cognition in postmenopausal women also support the critical period hypothesis. A handful of RCTs have failed to find any beneficial effects of oestrogen on cognition in postmenopausal women. Ditkoff and colleagues (1991) looked at the effect of oestrogen use on QoL and psychological function in a randomised, double-blind study comparing oestrogen users to a placebo control. Thirty-six women aged between 45 and 60 years (with a mean age of 53 years) were

recruited and assessed on the Profile of Adaption to Life scale, the Beck Depression Inventory and the digit span and digit symbol from Wechsler's Adult Intelligence Scales. Both tasks from the Wechsler's Adult Intelligence Scale were chosen as a test of immediate recall, recent memory and require attention in addition to memory, suggesting a level of executive function. The oestrogen users reported improved QoL and improved mood as compared to the placebo control however no difference in cognitive function was apparent. Although the researchers included women who were aged between 45 and 60, the women had previously undergone hysterectomy and it is not clear which point of the climacteric scale the women were experiencing. The study did not indicate the average age of hysterectomy of the participants, but did indicate that data was collected several years post-surgery and that the researchers were targeting women in the post-menopausal stage. The women kept a diary of menopausal symptoms and reported no hot flushes and less than three symptoms experienced in total per week suggesting they had not reached asymptomatic status. This could suggest that the participants were on nearing the end of the menopause, with diminished symptoms at the post-menopausal stage and missing the window of opportunity for beneficial effects of oestrogen. Oestrogen did seem to have an effect on mood and QoL indicating that the participants were still benefitting from the treatment on one level. Perhaps the neuroprotective effects of oestrogen were not yet apparent in the chosen memory tasks. The tasks had no verbal component and the researchers state themselves that memory testing can be difficult and that the Wechsler Adult Intelligence Scales only measures one aspect of memory. They concluded that the lack of differences between groups on these tasks cannot rule out the fact that oestrogen may influence memory systems.

Studies with older women have also found no effect of HRT on cognition. Janowsky and colleagues (2000) administered oestrogen therapy or a placebo to post-menopausal women (with an average age of 70 years) over a four week period. No difference was found in WM performance between groups after the treatment phase. Binder, Schechtman, Birge, Williams, and Kort (2001) administered HRT or placebo to postmenopausal women with an average age of 81 years over a period of nine months. The lengthy administering of the therapy addresses the shorter period of HRT administration in Janowsky's study. By selecting participants with an average age of 81 years the researchers risk the interaction of age effects in the study and participants would have been beyond the end of the climacteric scale. After nine months of HRT no differences were found between groups in tests of verbal memory or fluency, visual memory, attention, or psychomotor speed thus satisfying the critical period hypothesis.

And finally, LeBlanc and colleagues (2007) examined how menopausal symptoms can impact on cognition, and whether symptom relief from HRT use can result in improved cognitive capacity. Thirty-two healthy post-menopausal women (on average three years since their last menstrual bleed) were administered HRT or placebo over an eight week period. HRT use did not impact on symptoms impact or cognitive performance.

The discussed RCTs have helped to pinpoint the critical period by supporting the idea that giving HRT early in menopause may be beneficial and that late HRT administered to post-menopausal women may have no effect on cognitive performance. However, peri- and post-menopausal groups have been poorly defined in these cases.

Jones and Gallo (2001) suggest that a decline in brain metabolism correlates with subjective memory complaints initially and the decline in memory performance demonstrated in differing task performance follows on later. This information may explain why differences between metabolic changes in the brain can be present during the completion of a task, but not evident in task performance score itself (Norbury et al., 2003; Rasgon et al., 2005; Shaywitz et al., 1999). If this is the case, then randomised studies may be of more value to the critical period hypothesis if measuring brain changes (which seem to be more sensitive) rather than cognitive changes (which may only transpire in certain tasks or if task difficulty is increased; Keenan & colleagues (2001) as a result of oestrogen use during the peri-menopause. Observational studies may be of more value when investigating the longer term, cognitive effects of HRT initiation, with differing durations of oestrogen use, and strong measures of these cognitions need to be defined. For example, a solid measure of WM that will be sensitive enough to pick up on these differences.

2.3 HRT alleviates disrupted cognition as experienced with induced menopause; indications of more than just an aging effect

Findings of oestrogen-cognition studies in which the women are aged 60 years or over at the time of their recruitment are confounded by the consequences of brain aging. How can we be sure that the differences we see between peri-menopausal cognitive capacity and post-menopausal cognitive capacity are in fact due to the fluctuations of oestrogen and HRT rather than a simple age related decline? Exploration of younger populations who have undergone surgical menopause has helped to refine this.

In some cases of ill health parts of the female reproductive system may have to be removed as treatment, for example, to eradicate various forms of cancer. Patients may experience loss of the uterus alone (hysterectomy), removal of one or both ovaries (unilateral or bilateral ovariectomy) or a combination. Removal of any of these parts of the reproductive system results in the cessation of menstrual bleeds, which ultimately deems the individual as menopausal by definition. However, it is important to consider that only the removal of both ovaries will result in the cessation of oestrogen production, and as such, only those whose two ovaries have been removed will experience menopausal symptoms from surgery onwards (Benedetti et al., 2001). Those who have undergone hysterectomy or unilateral ovariectomy and still have at least one of their ovaries will continue to produce oestrogen and not experience menopausal symptoms until they reach the natural age of the menopause. These women may be given HRT that contains oestrogen alone.

For those who have undergone bilateral ovariectomy and no longer have any functioning ovaries, while the natural menopausal transition typically occurs over a period of years with a steady decline of oestrogen throughout this period, surgical menopause is extremely abrupt whereby oestrogen levels plummet. In these women, typical menopausal symptoms manifest quickly after surgery irrelevant of the age of the participant. For these women, they may experience loss of two thirds of their oestrogen, experiencing continuous problems with mood and cognition (Hendrix, 2005).

Studies have shown that women who enter the menopause prematurely due to removal of both ovaries (typically before the age of 45) and do not receive HRT have a fivefold risk for mortality directly related to neurological

disorders such as depression, parkinsonism and dementia (Parker, Jacoby, Shoup, & Rocca, 2009; Rivera, Grossardt, Rhodes, & Rocca, 2009; Rocca, Shuster, Grossardt, Maraganore, & Gostout et al., 2009). In studies of younger perimenopausal women who underwent surgical removal of both ovaries, those who were treated with oestrogen maintained their pre-operation scores on tests of verbal memory whereas those who received a placebo experience a significant decrease in verbal memory score post-surgery (Philips & Sherwin, 1992; Sherwin, 1988). Such studies demonstrate the protective role of oestrogen on cognition, and the younger age of the participant eradicates aging effects.

Complete removal of both ovaries results in the cessation of oestrogen production, but in some medical cases removal is not necessary but temporary paralysis of ovary function may prevent, for example, the growth of cysts. An intervention of gonadotropin releasing hormones such as leuprolide acetate depot (LAD) results in the suppression of ovarian function and oestrogen production. Oestrogen suppression in younger women results in similar cognitive impairments as those experienced during the menopause. Sherwin and Tulandi (1996) administered LAD to women with an average age of 32 years and noted a decrease in verbal memory after 12 weeks of ovarian suppression. After ovarian suppression, participants either received HRT or a placebo. The deficits were reversed in women who had received oestrogen after the suppression, but not in those who had received the placebo.

In concordance with this, Varney and colleagues (1993) also found decreased scores in two verbal memory tasks as well as digit sequencing and a two-point discrimination task in younger women treated with LAD and Palomba and colleagues (2004) reported a significant decrease in Wechsler memory

scores and scores of the Mini-Mental State Examination in peri-menopausal women receiving LAD over a six month period. However, Owens, Matthews and Everson (2002) failed to find any effect of ovarian suppression on the CVLT, digit span task or verbal fluency task in 16 women administered with a high dose of LAD as compared to baseline scores. Participants underwent a stress-inducing protocol prior to cognitive testing at baseline only and not follow up which may have lowered scores of cognitive performance in the first instance.

Similar effects of LAD can also be seen in neurological studies. Berman and colleagues (1997) distributed LAD to three groups of healthy young peri-menopausal women over a 16 week period. PET scans taken at point of problem-solving and memory task performance indicated a weakening of typical brain activation patterns normally indicated in brain scans during these tasks. Additional to this the LAD treatment resulted in the mitigation of increased regional cerebral blood flow normally ascertained during the completion of these tasks. Again, after the addition of oestrogen therapy the diminishments in brain activation levelled to return to normal. Grigorova, Sherwin and Tulandi (2006) completed a study with similar methodology which considered the effect of suppressed ovarian function on a wide range of prefrontal cortex mediated cognitive tasks including WM. Twenty-five peri-menopausal women (with a mean age of 36.1 years) receiving LAD over a four week period were compared to 25 placebo controls (with a mean age of 36.7 years) matched for age, years of education and socioeconomic status. After four weeks of LAD intervention, oestrogen levels within the group had dropped to those in the postmenopausal range. Participants completed the CVLT, the Visual Search Cancellation Task to measure attention and visual scanning, and six tests of prefrontal cortex and

executive function to which there were no measured differences between groups. This does not support the previously mentioned studies, which found differences between base line and after intervention on verbal memory scores. Participants were also asked to complete a verbal WM task, a non-verbal WM task (modified versions of the N-Back test) and a sequential WM task (the Letter-Number Sequencing Subtest; WAIS-III). For the verbal and non-verbal N-Back tests, those who had been treated with LAD had fewer correct responses post intervention whereas the placebo control did not differ in response score. This suggests that HRT can help protect cognition, but this is specific to cognitive domain, such as verbal and WM. For the Letter-Number Sequencing Subtest, those induced into a post-menopausal state scored significantly worse when compared to their post intervention scores whereas scores of the control group had actually increased. The researchers noted that the participants failed to learn from repeated exposure of the task and then carried on to perform worse on the task. It is possible that the effect of LAD on cognition only transpired when cognitive assessments exceeded a particular level of difficulty. This is consistent with the study by Keenan and colleagues (2001) where the effects of oestrogen suppression on WM were only seen when the task became challenging and WM capacity over loaded.

From the studies discussed in this section it can be concluded that cognitive decline is consistent in women experiencing the natural menopause, and those who have had surgical menopause, suggesting a link to oestrogen loss rather than simple aging effects. This cognitive decline may be combated by HRT use, which has been demonstrated in participants who have initiated therapy close to surgical menopause, or during their peri-menopausal stage. This implies

that the protective effect of HRT is dependent on time of initiation of the therapy, as discussed in the next section.

2.4 The neuroprotective role of oestrogen replacement is limited to the critical period

Scott, Zhang, Wang, Vadlamudi and Brann (2012) suggest that the neuroprotective role of oestrogen is limited to the critical period. Adams, Shah, Janssen and Morrison (2002) showed that HRT administration to young ovariectomised rats can result in increased dendrite spine density and synapse density, opposite to the effect of aging, demonstrating a positive effect of HRT on young rats. However HRT was without effect on aged ovariectomised rats, consistent with the critical period hypothesis. In similar studies, HRT was effective in increasing hippocampal spine density in young rats four days post ovariectomy, but not as effective after 12 days following ovariectomy (Gibbs, 2000c; Silva et al., 2003) confirming that the delay between ovariectomy and initiation of HRT impaired the ability of the hormone to modulate synaptic density (Sherwin & Henry 2008).

Gibbs (2000c) concluded from his study that the ability of HRT to enhance cholinergic function also declines with age. Considering the effects of HRT on cognition as a result of neuronal change, Daniel and colleagues (2006) administered HRT to young rats immediately following ovariectomy. A significant improvement was seen in the HRT group performance of acquisition and delay trials of WM compared to a placebo control. However, when treatment was delayed for a five month period, no enhancements in memory performance were evident. Chen and colleagues (2006) suggests that if neurons are healthy at the time of oestrogen exposure, the response to oestrogen is beneficial for

neurological function and survival but if neurological health is compromised (perhaps with aging or as a result of the menopause), oestrogen exposure exacerbates neurological demise. In their study the researchers wished to assess the neuroprotective effect of different doses of HRT for continuous, intense or intermittent therapy on dissected hippocampi from the brains of 18 embryonic rat foetuses and grew these on a well plate whilst treating the hippocampal neuron's with different concentrations of oestrogen. The researchers simulated a range of oestrogen levels on the hippocampal neurons to induce a state similar to the peri-menopause. The level of oestrogen exposure was then reduced to a consistent low or high dose either continuously, sporadically or as a one off. The researchers found that in each case the low dose of oestrogen increased the survival rate of the neurons by preventing both neuron death and neurodegeneration. In contrast, exposure to the higher concentration at any point resulted in neuronal death. Implications from this part of the study can be made to postmenopausal women who experience a sudden influx of oestrogen therapy after brain levels have diminished, suggesting to why HRT should be initiated during the peri-menopausal stage.

In human studies, Dumas and colleagues (2008) examined the influence of age on the ability of oestradiol to affect cholinergic system functioning and cholinergic system functioning on verbal memory and attention in two groups of peri (50-62 years) and post (70-81 years) menopausal women. The younger HRT group demonstrated improved episodic verbal memory compared to the control. However the oestradiol treatment impaired performance in those in the older age group. Similarly, the ability of oestrogen to enhance cholinergic function also declines with delay (Gibbs, 2000c; Savonenko & Markowska, 2003). In

summary, early use of HRT can maintain cholinergic activity as compared to placebos, and this can help to maintain attention.

Brain changes in response to HRT can be present in larger levels such as a change in brain volume which can be responsible for certain degenerative diseases. During aging and following the menopause, brain volume can decrease with an increase in ventricle volume (L. Raz et al., 2013). This can lead to stroke. Also consistent with the critical period hypothesis, Suzuki, Brown, Cruz, Yang and Bridwell (2006) demonstrated a beneficial effect of HRT on stroke prevention and recovery if the therapy was initiated during onset of the menopause.

Some researchers believe that cognitive deficits experienced by postmenopausal women are due to executive dysfunction and that the prefrontal cortex is the site of oestrogens effect on cognition (Bailey et al., 2011; Keenan et al., 2001). Scott and colleagues (2012) have suggested that oestrogen is an important neuroprotective factor in a variety of neurodegenerative disorders and sexually dimorphic diseases. Specifically, oestrogen receptor alpha is a key mediator of neuroprotection in cerebral ischemia. Oestrogen receptor (alpha) is located both pre and post synaptically in the prefrontal cortex (Wang et al., 2010).

Daniel and colleagues (2006) investigated the validity of the critical period hypothesis as applied to prefrontal cortex mediated behaviours. In their study, young ovariectomised rats receiving oestrogen treatment outperformed placebo controls on the radial arm maze. The researchers concluded that the critical period hypothesis retains validity across prefrontal cortex cognitive domains, and can affect WM in middle-aged rats.

Bohacek, Bearl and Daniel (2008) propose a mechanism by which the responsiveness of the nervous system could be altered with oestrogen deprivation to affect cognition. They suggest that with oestrogen deficiency, the brain can become less responsive to HRT, affecting the responses of many targets of oestrogen action including dendrites in the prefrontal cortex.

An almost universally obtained result (Shaywitz et al., 1999) is greater left-frontal hemisphere activation during encoding, and greater right-frontal hemisphere activation during retrieval; the Hemispheric Encoding/Retrieval Asymmetry (HERA) effect (Tulving et al., 1994). Evidence suggests that HRT given to women entering the menopause can result in a 'sharpening' of the HERA pattern (Norbury et al., 2003), whereby oestrogen users completing retrieval tasks have more activation in the right-frontal hemisphere as compared to placebo control, similar to that seen in young, menstruating women. Shaywitz and colleagues (1997) demonstrated this increased activation in women who were five months from their last menstrual bleed during the encoding and retrieval of verbal material in a verbal working memory task.

When young rats are ovariectomised and oestrogen treatment is initiated within four days, synaptic density in the hippocampus is significantly greater than rats whose therapy is withheld until 12 days and beyond suggesting that a delay of the therapy may impair its ability to modulate synaptic density (Silva et al., 2003). The same patterns have been seen in young monkeys (Hao et al., 2004; Hao et al., 2006). In humans, the hippocampus atrophies at an annual rate of one to two percentage per decade in late life for non-demented individuals (Raz et al., 2005) and this deterioration is related to memory impairment (Kramer et al., 2007). For this reason, any beneficial effects of HRT on hippocampal effects

are of much importance. HRT use in post-menopausal women can result in reduced hippocampal volumes (mean age of 77.5 years, Coker et al., 2009; Resnick et al., 2009). This further highlights the possible detrimental effects of HRT when initiated during the post-menopausal stage (Erickson et al., 2010).

Neurobiological research suggests that early HRT use may protect prefrontal cortex and hippocampal function, dendrite density in these brain areas, brain volume and cholinergic systems. Late initiation may have no effect, or even a detrimental effect. The evidence confirms a critical period for HRT initiation, and this may have substantial effects on associated cognitions.

2.5 Early initiation of HRT can protect different aspects of cognition

Verbal memory in particular may benefit from early HRT use (Maki, 2006) but this may be dependent on task difficulty. RCTs involving women younger than 65 years have demonstrated an enhancing effect of HRT on verbal memory compared to placebo controls (Krug, Molle, Dodt, Fehm, & Born, 2003; Linzmayer et al., 2001; Philips & Sherwin, 1992; Shaywitz et al., 2003). Studies looking at the effect of HRT on verbal memory in women over 65 years of age have found no effect (Binder et al., 2001) or harmful effects (Grady et al., 2002). To help contribute to the critical period theory Maki (2005) found that by prescribing HRT to those during the onset of their menopause, hippocampally mediated functions (especially that of verbal memory) were protected and maintained by the additional oestrogen supplements. From neuroimaging studies it can be concluded that oestrogen has a neuro-protective effect in the brain at cellular, neurochemical and metabolic levels (Brinton, 2005) and this

can carry through to cognitive task performance. However not all studies demonstrate this effect.

2.5.1 Executive function, attention and working memory

It has been suggested that when HRT is initiated more than a couple of years after the completion of the menopause there will be no change in executive function and even an increased risk in neurodegenerative disease (Henderson, Benke, Green, Cupples, & Farrer, 2005; MacLennan et al., 2006). Herlitz, Thilers and Habib (2007), as well as Greendale and colleagues (2009) have suggested that it is our ability to learn which becomes under scrutiny from the menopause onwards. To combat problems in executive function and a risk of degenerative disease during this time, several RCTs and laboratory studies have suggested that HRT initiated at this stage can maintain executive function (Keenan et al., 2001) and reduce risk of neurodegenerative disease (Rocca et al., 2011).

Rat studies are a good indication of the possible outcome of HRT at cellular and cognitive levels in humans, but due to the differences in the rat and human brain and the reproductive and climacteric systems the implications of rat studies to human studies is put under scrutiny. More transferable information can be attributed from primate studies. The rhesus monkey is often chosen in primate studies due to their similarities to human 28-day menstruation periods, their experience of the menopause, their ability to perform cognitive tasks similar to those administered to humans and their age related declines in executive function and memory (Gore, Windsor-Engnell, & Treesawa, 2004). In addition to this, the rhesus monkey does not develop certain degenerative diseases such as AD which helps to reduce confounding variables

of digenesis (Rapp, Morrison & Roberts, 2003). A difference to be considered is that monkeys have seasonal cessation of reproductive function and a short menopausal life relative to women due to the late onset of the menopause (Lacreuse, 2006). Nonetheless monkeys are a suitable means for comparison and Gasbarri, Pompili, & d'Onofrio (2008) suggested that oestrogen can facilitate learning and memory to improve WM scores in monkeys. Studies using the rhesus monkey have helped to place the critical period hypothesis in animal studies.

In a RCT Lacreuse and Herndon (2003) showed improved WM span in ovariectomised monkeys treated instantly with oestrogen compared to a placebo control, demonstrating a positive effect of HRT on executive function and WM in early HRT users and confirming the critical period hypothesis. In a later study Lacreuse, Chhabra, Hall and Herndon (2004) failed to show an improvement in executive function of ovariectomised rhesus monkeys using HRT. The monkeys completed a modified version of the Wisconsin Card Sort Test adapted to the non-human primate. In the test, monkeys had to select three-dimensional objects based on colour or shape. The monkeys were treated with either HRT or a placebo and asked to complete the task following one specific rule. Twenty-four hours later, the monkeys were given the opposite treatment (e.g. placebo day one, HRT day two; HRT day one, placebo day two) and asked to complete the task once more, following a new rule. The researchers felt a wash out period between the treatment change was not necessary, however this may have been ill conceived. Of large importance, selected primates were administered the therapy several months after ovariectomy, perhaps missing the window of opportunity for maximum effects of the therapy on executive memory.

The critical period has also been demonstrated using human studies considering the effect of HRT on WM in peri- and post-menopausal women. Khoo and colleagues (2010) measured detrimental risks of HRT on WM when initiated three years post completion of the menopause. In their observational study, 410 HRT users between the ages of 40 and 80 years were recruited. They were tested for cognitive change over a five year period using the global measure the Mini-Mental State Examination, National Adult Reading Test (NART) and the Wechsler memory scale. The researchers included age, lifestyle factors and natural cognitive decline as covariates, assuming that cognitive score would naturally decline by ten percent over the five year period. Results were mixed where HRT was associated with worsened general memory but improved cognition overall. The researchers concluded that the effect of HRT on cognition may be domain specific. With such a wide age range of 40 to 80 years, despite age being a covariate it still becomes problematic in study analysis with the average age between groups differing to a large degree. Kurt, Bekci and Karakas (2006) considered the role of late initiation of HRT on several cognitive domains. Late HRT use did not have a significant effect on a broad spectrum of neuropsychological scores that measured immediate and delayed visual and verbal memory, visuospatial perception and orientation, SA, visual search and scan, impulsivity and response speed and executive functions.

In a similar study, Binder and colleagues (2001) also failed to find significant differences during completion of a Verbal Fluency Test, Wechsler Paired Associate Learning and 20 minute Delayed Recall, Trail making A and B Tests, Cancellation Random Letter and Random Form. Participants were aged 75 years and older at time of HRT initiation, missing the critical period for initiation.

In the Cache County longitudinal study of elderly women and men, women who had initiated HRT in previous years at close proximity to the onset of the menopause had a significantly reduced risk of developing AD. Current users in the post-menopausal stage were not protected (Zandi et al., 2002). The study was designed to assess the critical period hypothesis, and the researchers concluded that there is a window of opportunity to take HRT during the perimenopausal stage in order to maximise protective effects of HRT on associated neurones helping to maintain WM. In another observational study, Grigorova and Sherwin (2006) considered post-menopausal HRT users and the effect of the treatment on WM, verbal memory and executive function performance. Postmenopausal women (mean age 65 years) who used either oestrogen only or oestrogen plus progesterone were compared to controls matched for age and education who had never used HRT. The results revealed no differences between verbal and WM performance between HRT users and non-users, and the researchers concluded no effect of HRT use on executive function overall. It can be established from this study that HRT initiated during the postmenopausal stage had no effect on the selected tasks, which conforms to the critical period hypothesis. In disagreement with some studies yet confirmative with others; there were no detrimental effects of the late initiated therapy on cognition. This could be a result of different cognitive measures of assessment. Although the study revealed no differences between HRT users and non-users, WM scores of the N-Back test (Kirchner, 1958) indicated that those receiving the treatment were more likely to make mistakes than those on the placebo control hinting at some detrimental affects of late HRT use.

Despite the critical period hypothesis satisfying some of the disputes arisen from the literature as a whole, not all research falls in concordance with the hypothesis. Two studies found verbal memory significantly improved in over 65 year olds after the administration of oestrogen for duration of two to three weeks only (Duka et al., 2000; Yaffe et al., 2000). Krug, Born and Rasch (2006) showed an improvement in two tests of WM and one of hippocampal function in postmenopausal women after being administered HRT for three days only. The researchers concluded that a short burst of HRT to create an 'oestrogen peak' can improve prefrontal cortex and hippocampal function, and short term use of HRT may be beneficial for cognitive performance in post-menopausal women. It may also be that certain types of cognition are responsive to small oestrogen changes at postmenopausal point only, and not prolonged use initiated during this time. Another study which demonstrates this administered HRT to a cohort of women with AD who had a start age of 72 years. HRT resulted in a slight benefit to combating degenerative disease in the short term of two months use but an adverse progression of disease in the long term of at least 12 months use (Zandi et al., 2002). In a study considering the effect of HRT on AD, Mulnard and colleagues (2000) administered HRT or a placebo control for 12 months to 120 women with mild to moderate AD. Women were asked to complete tasks of visual memory, attention and executive function. Similar to the results from the study by Zandi and colleagues, HRT use did not improve cognitive performance for any task, and in fact cognitive performance in this group worsened overall. The studies demonstrate that, although a short period of HRT use in post-menopausal women may show some cognitive response, HRT use beyond two months (and thus more typical of real life use) results in poorer cognitive performance, and this supports the critical period hypothesis.

The critical period hypothesis suggests that HRT will only maintain cognition if the therapy is initiated during the early stages of the menopause before the natural decrease of oestrogen occurs (Brinton, 2009, Sherwin, 2012). The duration of this window of opportunity is not well defined. Others have also suggested that late HRT use can heighten the risk for developing dementia and may impair memory performance (Resnick & Henderson, 2002). The effects of initiating the therapy during the post-menopausal stage are less well understood. Gleason, Cholerton, Carlsson, Johnson and Asthana (2005) suggest that if treatment is prolonged to five years beyond the window of opportunity there is an increased risk in developing dementia. Assuming oestrogen receptor sites are less active post-menopause; a sudden abundance of oestrogen therapy may become overwhelming for the receptor sites thus becoming detrimental to frontal lobe functioning. Briton suggested that the exposure of unhealthy neurons to oestrogen is without benefit and detrimental to the degeneration process.

Confidence in observational findings of HRT use on cognition is limited by concerns involving the 'healthy user bias'. This is the tendency for women choosing to receive HRT to be better educated, healthier perhaps due to being more health conscious (Matthews, Kuller, Wing, Meilahn, & Plantinga, 1996) and younger (Maki, 2006). For this reason, it is important that studies have groups balanced for age, education and health (for example, normal mood and normal cognitive function). Zandi and colleagues (2002) suggested that the positive effect of HRT on AD reduction is not likely to be associated with the healthy user bias, and several studies have attempted to reduce the possibilities of confounding variables. Sherwin (2008) strongly highlights the fact that normal

brain aging can lead to problems in cognition, especially after the age of 50 where there is a quicker decline in memory, processing speed and executive function. As a result, studies should recruit participants who are balanced for and of similar age between groups.

2.6 Summary

Sherwin and Henry (2008) suggested that time of initiation of HRT will have effects on the protective role of the therapy and prevention of cognitive decline. During the menopause, oestrogen reduction in the brain can cause structural changes which can affect related cognitive functions. If HRT is initiated before or during these structural changes at the peri-menopausal stage, the therapy protects the brain from such changes, thus avoiding associated cognitive decline (Chen et al., 2006; Lee & McEwen, 2001; Maki, 2006). If the therapy is initiated too late after the menopause, there may be no effects in brain structure at this point (Adams et al., 2002) or an exacerbation of neurological demise due to the neurons already being compromised (Chen et al., 2006). In support of this, studies have demonstrated that women taking HRT soon after the onset of the menopause suffer less from cognitive decline than those who take the therapy post-menopause (MacLennan et al., 2006; Matthews, Cauley, Yaffe, & Zmuda, 1999). What is less clear is the precise onset and duration of this window of opportunity, to which there should be a general consensus to aid in study design, and this can be largely influenced by the methods adopted by researchers to define peri- and post-menopausal groups.

In previous research, executive or WM functions were assumed to be necessary for task completion, but not directly tested. Late users sometimes, but

not always, suffer from a detrimental effect of the treatment on some, but not all of these selected tasks. The relationship between postmenopausal initiation of HRT and cognitive domain has been established, but needs more clarification, and the identification of specific cognitive tasks that are sensitive to oestrogen change. For example, it is now assumed that early HRT use protect WM but only when the task is at a certain level of difficulty. Less is known about the relationship between HRT use and SA.

Despite significant progress in the understanding of oestrogen in relation to cognitive function and neuron protection there are still conflicting views on the shielding effects exerted by HRT (LeBlanc et al., 2007; Pompili et al., 2012). Generally rat studies have proved more conclusive than human studies. Several studies of the effect of bilateral ovariectomy on cognitive function in perimenopausal women have found that surgical menopause produces marked differences across executive function which could be prevented by HRT if initiated at the correct time (Bailey et al., 2011). However many RCTs and laboratory studies have failed to reproduce this effect and it is difficult to determine whether this is due to a failure of smaller sample sizes to accurately represent a larger population or a failure in less sensitive cognitive assessments being chosen to detect subtle changes in cognitive function (Vearncombe & Pachana, 2009). Follow up studies and studies using the same methodology may help to establish the role of oestrogen in frontal lobe functions.

2.7 The interaction of time of initiation of HRT will affect executive function performance; rational for the present study design

There is mixed evidence for benefits of HRT on cognition and some evidence that discrepancies are due to differences in timing of HRT initiation relative to stage in the climacteric, known as the critical period hypothesis (Craig et al., 2008; Maki, 2006).

Sherwin and Henry (2008) suggested that time of initiation of HRT will have effects on the protective role of the therapy and prevention of cognitive decline. During the menopause, oestrogen reduction in the brain can cause structural changes which can affect related cognitive functions. If HRT is initiated before or during these structural changes at the peri-menopausal stage, the therapy protects the brain from such changes, thus avoiding associated cognitive decline (Chen et al., 2006; Lee & McEwen, 2001; Maki, 2006). If the therapy is initiated too late after the menopause, there may be no effects in brain structure at this point (Adams et al., 2002) or an exacerbation of neurological demise due to the neurons already being compromised (Chen et al., 2006).

Of less clarity is the effect of HRT use on cognition when initiated during the post-menopausal stage. Some studies have demonstrated no effects of HRT use when initiated in post-menopausal women on a variety of cognitive tasks demonstrating episodic memory and executive functions such as WM and attention (Binder, 2001; Janowsky et al., 2000; LeBlanc et al., 2007). Other studies have suggested that HRT initiation during the post-menopausal stage can result in cognitive impairment (Espeland et al., 2004; Grigorova & Sherwin, 2006;

Mulnard et al., 2000). The present study considered the interaction of time of initiation of HRT on cognitive performance.

The beginning and duration of the critical period is yet to be defined and should not be determined by age

Studies have demonstrated that women taking HRT soon after the onset of the menopause suffer less from cognitive decline than those who take the therapy post-menopause (MacLennan et al., 2006; Matthews, Cauley, Yaffe, & Zmuda, 1999). What is less clear is the precise onset and duration of this window of opportunity, to which there should be a general consensus to aid in study design, and this can be largely influenced by the methods adopted by researchers to define peri- and post-menopausal groups.

Women with a history of HRT use were compared to women with no history of HRT use. The critical period was investigated, and women were organised in relation to her stage in the climacteric. Previous studies have tended to define time of HRT initiation in relation to women's age rather than in relation to her stage in the climacteric (MacLennan et al., 2006). Because of variability in onset of the menopause (Sherwin, 2007), age alone does not capture whether someone is peri- or post-menopausal. The present study considers time elapsed since last menstrual bleed in order to aid clarification of the critical period.

In order to pinpoint stage in the climacterics participants in the present study were asked when their last menstrual bleed was. According to national guidelines (NHS, 2012), a woman completes the menopause when her last menstrual bleed is in excess of 12 months ago (Brambilla et al., 1994; McKinlay, 1996); menopausal symptoms can be experienced several years prior or post this

time (Sherwin, 2007). Participants who had never initiated HRT, and who reported their last menstrual bleed within 12 months were classified as peri-menopausal. Those who had reported their last menstrual bleed beyond 12 months were classified as post-menopausal. Similarly, those who initiated HRT within 12 months of their last menstrual bleed were classified as peri-menopausal HRT initiators, those who initiated their therapy beyond this point are classified as post-menopausal HRT initiators. In the UK the average age of the onset of the menopause is 51.7 years, plus or minus 7 years (Sherwin, 2007) and a woman can start to experience menopausal symptoms between the ages of 45 and 55 years. For this reason, participants will be included from the age of 45 years and above, until 65 years, when menopausal symptoms have ceased.

The reliability of asking participants to estimate their last menstrual bleed may be questionable, unless participants were in excess of this time frame (either several years since last menstrual bleed or having a recent menstrual bleed). Previous research has demonstrated that participants will estimate last menstrual bleed to be one year both at baseline, and at follow up one year later (Colditz et al., 1986). For this reason, participants were asked to describe their last menstrual bleed, and relate this to an event to help pinpoint the exact date.

Even though there is consensus that it is better to start HRT during the peri-menopause, previous studies have defined peri- and post-menopause in terms of age, so women who are below the mean age of menopause are assumed to be peri-menopause, while those who are older are assumed to be post-menopause. Defining menopausal stage in terms of chronological age ignores the variation in age at menopause. Once the menopause is exceeded to a certain point, initiation of HRT will no longer be as effective in its protection of

brain function and following this, cognition. This makes accuracy in defining and pinpointing the critical period extremely important.

The present study addressed these issues by testing women who had initiated HRT during the critical period, and after the critical period, and compared these two groups to non-HRT users matched for stage in the climacteric.

Incorporating an observational design allows exploration of the potential longer lasting effects of the critical period

The present study incorporated an observational design, enabling the long term effects of HRT initiation on cognition to be explored, something that previous studies have not considered. Oestrogen fluctuation during the menopause and HRT use can result in changes in brain mechanisms, with changes in cognitive performance to follow. By using an observational study design, the researchers were able to recruit HRT users with a history of HRT use initiated several months prior to testing, to allow better chance of recognising these cognitive changes, something that previous RCT's have not been able to explore.

Measuring mood and menopausal symptoms

Richardson (1991) suggests that the cognitive impairments often demonstrated in women entering the menopause are due to menopausal symptoms such as low mood experienced pre and post oestrogen change.

Mood is oestrogen responsive, and can have an effect on task performance. Specifically, low levels of oestrogen results in a decrease of

dopamine which is associated with menopausal depression (Fink et al., 1995) and dopaminergic cells have been shown to reduce by 30% in non-human primates after being 30 days of oestrogen deprivation (Leranth et al., 2000). Dopaminergic reduction in layer one, area 46 of the PFC as a result of oestrogen deprivation can be restored with oestrogen treatment (Kritzer & Kohama, 1998) and dopaminergic change plays an important role information processing during WM tasks (Goldman-Rakic, 1995; Tang et al., 2004).

It has been reported that people who suffer from depression complain of problems in day to day memory (Watts, 1993). Further studies have indeed demonstrated that negative mood can have detrimental effects on WM capacity and attention (Ellis & Ashbrook, 1988; Hertel & Hardin, 1990). Cronholm and Otterson (1961) found that those suffering with depression also found it difficult to learn.

Jacobs and D'Esposito (2011) examined the effect of oestradiol on WM in healthy young women taking into consideration the baseline levels of dopamine in the prefrontal cortex. They concluded that oestradiol levels do impact on WM capacity and that this impact depends on baseline dopamine.

Sherwin found HRT users to have improved mood after oestrogen treatment (Phillips & Sherwin, 1992; Sherwin 1997) and it is possible that baseline mood scores between HRT users and non-users will differ. For this reason, it is important to measure mood with the potential to use mood score as a covariate in analysis.

In the oestrogen literature, mood has been measured as a potential covariate, and to check variance across groups (Grigороva et al., 2006). The

shortened General Health Questionnaire (GHQ-12) has been selected to measure mood as is a quick tool that is easy to administer and sensitive to mood differences. Roy-Byrne, Russo, Michelson, Zatzick, Pitman and Berliner (2004) reported internal consistency for the GHQ-12 assessed via Cronbach's alpha ranging from 0.82 to 0.93. Depending on the type of patient, scores six months apart correlated between 0.51 and 0.90. Content validity was demonstrated by showing that each test item highly discriminated between participants with lower rated score (with depression) and participants with normal rated score. For these reasons the GHQ-12 was selected to be included in the task battery.

Previous studies have measured menopausal symptoms as a potential covariate (Grigorova et al., 2006). As such it was deemed as useful to include a menopausal QoL scale score to determine symptoms experienced by the participant. The Menopausal Quality of Life scale (MQoL; P. Jacobs, Hyland, & Ley, 2000) was developed using 1188 questionnaires from two samples recruited from two Family Health Service Association (FHSA) lists and one sample recruited through an advertisement in a women's magazine. The MQoL is a 48 item menopause-specific QOL questionnaire where each item represents a symptom from a different domain. Responses range from a six item likert scale of 'I am never like this' to 'I am always like this'. The scale was constructed after analysing single interviews of 32 women and four focus groups involving 29 women using an adaptation of content summary. P. Jacobs and colleagues (2000) suggest that the majority of previous QoL menopausal studies fail to include women over the age of 55 years which results in limited information on those in the end of their climacteric change whereas this scale was developed including postmenopausal women. Participants were asked to rate the relationship

between the menopause and cognition, and specifically to describe the relationship between the menopause and memory, the menopause and cognition and the menopause and attention. This allowed the researchers to consider the effect of expectation. The MQoL scale is highly related to the Global Quality of Life Scale (GQoL). Cronbach's alpha range from .92 for the total MQoL scale and inter-correlations between menopausal domains scores range from .25 to .68. It is divided into seven domains, allowing easy exploration of participant's subjective estimation of the impact of cognition on QoL.

Measuring mental and physical health

As people get older, chronic health conditions, co-morbidity and levels of medication usage rise (Lewis, Rook & Schwarzer, 1994). The efficiency of organ function decreases, including lung and heart capacity (Physical development: age 45-65, n.d.). The most common health problems experienced in those aged between 45 and 65 are arthritis, asthma, coronary heart disease, diabetes, high blood pressure, common mental health problems and stroke (Health: age 45-65, n. d.). Participants of this age experienced a range of chronic conditions, resulting in a range of medications being taken. In accordance, P. Jacobs and colleagues (2000) asked participants in their study to list medication usage during the last few months up to participation in order to evaluate co-morbidity. The present study also requested medication being taken, and chronic conditions experienced in the three months leading to study participation.

In the WHIMs study involving 3,200 women over a five year period (Espeland et al., 2004); the study only excluded women with signs of probable dementia, and included those with mild chronic conditions such as poor bone

health and high blood pressure. Kampen and Sherwin (1994) only excluded participants who had a history of head injury, stroke, heart attack, alcoholism, drug abuse, depression or any other major health problem. Schmidt and colleagues (1996) also excluded participants with dementia, drug abuse and neuropsychiatric problems. Henderson and colleagues (1996) excluded those with probable dementia, stroke and in addition, surgical menopause and Verghese and colleagues (2000) also excluded those with surgical menopause, as well as dementia and depression. And finally, Steffens and colleagues (1999) excluded women with a history of either stroke or dementia.

In accordance, the current study excluded those with probable dementia, a history of stroke, a history of heart disease, a history of alcohol or drug abuse, mental health problems and those who had undergone surgical menopause. In addition, participants were excluded if they had learning difficulties or were not fluent in English.

Lord and colleagues (2008) also requested from their participants the total number of doctor visits over the past year. The present study felt it was appropriate to ask participants how often in the past three months leading up to the study they had visited a GP. This could be compared to national averaged for that age group.

With details of medication use, chronic conditions experienced, and GP visits leading up to, and including day of the study, an idea of typical health across each group could be accumulated.

Detail of HRT use

For HRT users, details of type of HRT taken, duration of the therapy intervention (in months), and months passed since last treatment were recorded. Participants were only included if they had been receiving HRT continuously at any point for at least three months. This is because studies showing HRT use for less than this duration have had mixed results (for example, LeBlanc et al., 2007).

Education

A participant's intelligence and/or level of education may have an effect on WM performance. It is therefore important to recognise the participants IQ and/or highest level of education, to make sure groups are balanced for IQ and/or educational level. The chosen WM tasks are cognitively demanding. Rather than ask participants to complete an IQ test, participants were simply asked their highest level of education to check groups were balanced for education (and to be used as a potential covariate). Previous studies which have asked the participant for their highest level of education, rather than administer an IQ measure include studies by Grigороva et al., 2006; MacLennan et al., 2006 and Lord et al., 2008. This is especially important as studies have demonstrated that HRT users completing WM tasks benefit from oestrogen use when the task becomes difficult (Grigороva et al., 2006; Keenan et al., 2001). This point will differ between participants.

In a short report it was noted that women in their third trimester of pregnancy who were less educated struggled more with the difficult components of Engle's three WM tasks than those with a higher level (Jacobs & Pettit, 2008).

Because of the relationship with Engle's three WM tasks in this case, it is especially important to request participant's highest level of education.

Because aging is related to cognitive decline in older adults (Singh-Manoux et al., 2012), participants were asked for their age and this was used as a covariate in study analysis. This coincides with the study by Rasgon and colleagues (2004).

Early HRT use protects against decline in cognitive functions mediated by the prefrontal cortex, a rationale for the selection of WM and SA tasks

Keenan and colleagues (2001) suggest executive dysfunction mediated by the PFC to be the primary problem of oestrogen decline during the menopause. The relationship between the PFC and oestrogen change (as discussed in chapter one) suggest that early HRT use will protect cognitive functions mediated by the PFC, and in particular, WM as associated with layer one, area 46 of the PFC (Tang et al., 2004).

Episodic memory is mediated by the hippocampus and PFC. Several studies have demonstrated an improvement in episodic memory using paragraph recall, and list recall tasks with HRT use (Dumas et al., 2008; Joffe et al., 2006; Kampin & Sherwin, 1994; Maki et al., 2001). For example, Kugaya and colleagues (2003) demonstrated an increase in serotonin activity and oestrogen plasma levels in the right frontal cortex of oestrogen users. These participants performed better on episodic memory tasks including paragraph recall and verbal paired associates tests from the WMS-R. Because of the location of oestrogen receptors in the PFC, more studies are beginning to investigate the relationship between oestrogen change and executive function. In the same study participants performed better on executive function using the Trail Making

Test. The trail making test is a test of visual attention and task switching, incorporating both WM and SA. It can be used as a predictor for AD.

Some larger scale studies neglected to include tasks of executive function. In the WHIMS by Espeland and colleagues involving over 3,200 women over five years, no differences were found in cognitive performance between HRT users and non-users measured by the modified Mini-Mental State Examination (MMSE). The MMSE is a global cognitive measure used to screen for dementia. The task is usually administered on several occasions to map out rate of progression of cognitive impairment. Questions such as “name this picture” (e.g. a pencil or a pen), and “what day is it today” are simple questions in normal populations. The study only included women free from probable dementia, suggesting the task to be too simple for this population. Sherwin (2007) suggests that future studies need to administer valid and reliable measures of specific cognitive domains rather than tests that measure globally cognitive functions, such as the modified Mini Mental State Examination which is unable to evaluate performance in specific cognitive domains.

The executive function that has generated most interest and exploration over the past decade is WM (Joffe et al., 2006; Pompili et al., 2012; Smith et al., 2006), although this is still relatively unexplored. WM capacity can be influenced by oestrogen change and protected by HRT use (Dumas et al., 2010; Keenan et al., 2001).

Sherwin (2007) noted the protective effect of oestrogen on both verbal memory and WM. However, her studies have not always led to the same conclusion (Sherwin, 1997), highlighting the importance of choosing a sensitive

measure of WM. Several studies have demonstrated a change in PFC function with HRT use during the completion of WM tasks, but this has not been demonstrated in task performance itself (for example, Shaywitz et al, 1999). This further clarifies the need for well selected WM tasks.

In the study by Grigorova, Sherwin and Tulandi (2006), participants completed a verbal WM task, a non-verbal WM task (modified versions of the N-Back test) and a sequential WM task (the Letter-Number Sequencing Subtest; WAIS-III). All three tasks were susceptible to oestrogen change. The Letter-Number Sequencing Subtest represents components of WM (short term memory and attention), but does not exercise some of the more difficult components of the WM model. The fact that differences were found in the verbal and non-verbal forms of the N-Back task suggests that oestrogen change can affect performance on different types of WM, which is of interest. In this particular study it was possible that the effect of oestrogen deficiency on cognition only transpired when cognitive assessments exceeded a particular level of difficulty. This is consistent with the study by Keenan and colleagues (2001), where the effects of oestrogen suppression on WM were only seen when the task became challenging. Because task difficulty is important, the chosen WM task should also have different levels of difficulty. Participants recruited varied in level of education, and tasks needed to be suitable for such a population.

Engle's WM tasks are established in the field, have high validity, and have a variety of components such as reading or counting which gives them some degree of variability. Engle's WM tasks incorporate three different types of WM; verbal WM, spatial WM and mathematical WM. Oestrogen change has been shown to affect different types of WM such as verbal WM and spatial WM. By

selecting Engle's WM tasks, the tasks represent three different types of WM, each of which can be measured using tasks that are similar in presentation, difficulty and are highly correlated with each other (Conway, Kane & Engel, 2004). This means that each type of WM can be equally represented and assessed. Because the tasks correlate with each other, together they are a strong measure of WM Capacity (WMC).

By using measures of three different types of WM, the effect of HRT on WM can really be tested, picking up sensitivities that other studies have overlooked. Engle's WM tasks are the Reading span (Rspan; Daneman & Carpenter, 1980), Operation span (OSpan; Turner & Engle, 1986) and Counting Span (CSpan; Engle et al. 1999). The RSpan task is a strong predictor of verbal performance (Friedman & Miyake, 2000). The Ospan was developed as an extension of the Rspan task (being a proxy for reading comprehension) and both tasks account for common variance in higher order tasks. The Cspan was developed to include attention control in counting specific objects, and helps to overcome the potential verbal constraints of the RSpan and OSpan. Engle's WM tasks load on the same factor as WM span tasks testing spatial processing and storage (Kane & Engle, 2004). Participants need to store words, letters, or digits whilst processing material, and need to allocate attention or resources to the storage and processing components appropriately. They involve confirmation of a maths sum (Ospan) or sentence (Rspan) and memory for a following word or letter.

To be a good measure of WM, Engle suggests that two tasks of the three need to be completed in order to predict a reliable WM score. In this case, and based on the arguments presented above, it is suitable to use all three tasks.

Similarities in the literature suggest that differences in WM are only present between HRT groups when the task is relatively difficult. Neuroimaging studies have shown a change in PFC activation with oestrogen use during completion of a WM task, but this has not impacted on task performance (Shaywitz et al., 1999). It is possible that these differences only transpire when a task is of a particular difficulty level. Griorova and colleagues (2006) concluded that the effect of oestrogen levels on WM task performance may only be apparent if the task itself is of a certain level of difficulty. And sometimes the effects of oestrogen change on WM only transpire when task difficulty is increased (Keenan et al., 2001), suggesting that task difficulty should be considered during task selection.

Engle's WM tasks are relatively complex, where the participant needs to use both manipulation of the stimulus whilst solving a task before retrieval can take place. Engle's WM tasks have 12-16 trials which differ in difficulty level, where participants are asked to remember between two and six items per trial whilst in the face of distraction. These items are counterbalanced. Because of the differing levels of difficulty between trials, Engle's WM tasks will become challenging at different points depending on the participant's capacity, making the tasks suitable for different age groups socio-economic status.

The WM span tasks have been developed over the years in order to strengthen their reliability, for example, using words and letters that are easy to distinguish between (Baddeley, 1966; Conrad & Hull, 1964) and using neutral items to avoid mood congruency effects (Teasdale, Taylor, & Fogarty, 1980).

Oestrogen decrease during the menopause can result in decreased WM capacity and SA may rely on WM when information processing and storage become competitive (Knudson, 2007). The role of oestrogen change on attention has recently received small consideration, but this has not been explored in great depth.

Changes in SA during the menopause and response to HRT is well explored in animal studies (Barnes et al., 2006; Wang et al., 2008), but not in human studies. SA has been shown to be impaired during pregnancy (deGroot, Adam, & Hornstra, 2003). Crawley and colleagues (2005) demonstrated compromised SA in pregnant women when working out what floor a moving elevator was on when they were given instructions to its movement. The task was designed by the investigators. SA becomes impaired when women are given a hormone suppressant to combat breast cancer (Wieneke & Dienst, 2007). Less is known about the impact of oestrogen change on SA in menopausal women, making it of great interest.

Many cognitive tasks do require attention, such as speed tasks or choice tasks, but results may be mixed due to tasks being indirect measures of attention. A task that may be suitable as a direct measure of SA is the Sustained Attention to Response Task (SART, Robertson et al., 1997). Performance of the SART has been shown to be sensitive to subtle impairments in attention following traumatic brain injury and performance correlates with both SA, and the Cognitive Failures Questionnaire (CFQ). This means the task should be sensitive to menopausal symptomatology.

The SART was developed specifically to measure subtle attention deficits in patients with Traumatic Brain Injury (TBI). TBI particularly affects the frontal lobes, resulting in poor performance in frontal lobe mediated tasks. HRT initiation during the peri-menopausal stage has been shown to increase frontal lobe activation (Joffe et al., 2006; Knudson, 2007; Shaywitz et al., 1999), and the SART may be a suitable task to pick up effects of this increased activation on cognitive function for those with early HRT use. The task has little demand on other cognitions such as memory, practising continuous attention to response, and predicts both self-reported and informant-reported failures of attention in normal participants (Robertson et al., 1997). The SART correlates with other measures of SA (Manly, Robertson, Galloway & Hawkins, 1999; Robertson et al., 1997) but has advantages to other tasks by being easy and quick to administrate and complete, and simple in design. For example, the change in stimuli font size of the SART and the relatively random organisation of the stimuli eradicate expectation whilst maintaining attention effort. Generally, individual's response time correlates with errors made. Differences occur dependently on whether the individual puts more emphasis on response speed or correct target response (Robertson et al., 1997). Typically, participants incorrectly respond 6.36 times out of 25 during the completion of the SART and those who responded quicker make more errors overall.

Together, completion of the three different types of WM task, and the SART as a measure of sustained attention, will give good indication of the effect of HRT use on executive function when administrated during the peri- and post-menopausal stages.

The critical period hypothesis suggests that HRT initiation is most beneficial to cognition when taken during the peri-menopausal stage (Maki, 2006; Resnick, 2002; Sherwin, 2006). Because of this (and to coincide with previous research testing HRT use in peri-menopausal women on executive function and WM), it is expected that HRT will benefit WM only when initiated during the peri-menopausal stage (Joffe et al., 2006; Lacreuse & Herndon, 2003). Participants in the present study will have a history of HRT use, meaning that some may have initiated the therapy (and in fact, stopped receiving treatment) years prior to testing. These women will be compared to women who are currently peri-menopausal. Because of this, study outcome is uncertain. The effect of post-menopausal HRT initiation on WM is less certain, and the effect of HRT initiation, whether peri- or post-menopause, and the relationship with SA is thoroughly under developed. Because of these factors combined, no predictions were made regarding cognitive performance of any group.

2.8 Method for study one

Study One, Stage One

Participants A total of 121 healthy women between 45 and 65 years of age were recruited within the Plymouth and London areas through use of internet advertisement, word of mouth and advertisement throughout Plymouth University. The researchers classified HRT users as either HRT initiators peri-menopause (if the therapy was initiated within 12 months of the participant's last menstrual bleed during their peri-menopausal stage) or HRT initiators post-menopause (if the therapy was initiated beyond 12 months of the participant's last menopausal bleed when participants were post-menopausal) by asking the

participant when their last menstrual bleed was in relation to initiation of the therapy. Non HRT users were divided into peri-menopausal and post-menopausal groups, again by asking the participant when their last menstrual bleed was. Those whose last bleed was experienced within 12 months at time of testing were classed as peri-menopausal; those whose last bleed was experienced longer than 12 months ago were classed as post-menopausal. Participants fitted one of the four levels of HRT-status accordingly: HRT initiators peri-menopause (n = 33), HRT initiators post-menopause (n = 26), peri-menopausal non-users (n = 33) and post-menopausal non-users (n = 29).

Exclusion and Inclusion criteria were clearly specified on advertising material, and during initial communication prior to the study. Participants were asked to be of normal health, with no learning difficulties. Specifically, those with severe mental health problems (such as chronic/severe anxiety or depression) were excluded from the study. Those who were normally menstruating up to the menopause were included in the study, those with hysterectomy were excluded from the study). Participants were also excluded if dyslexic or dyspraxic, and it was essential that participants were fluent at English.

Measures RSpan, OSpan and CSpan measured WM. Participants scored one point for every correctly recalled item in its correct place within the sequence. For the RSpan and Ospan this was out of 42; for CSpan this was out of 60. A higher WM score represented more correct answers indicating a larger WM capacity.

RSpan Participants were presented with a sentence and a letter as follows:

My wish had a huge garage sale ? K

Participants were asked to read the sentence out loud, decide whether the sentence made sense by confirming “yes” or “no” out loud, and then to read the proceeding letter out loud. They were asked to remember this letter and press the space bar, at which point a second sentence and letter would appear. The participant was asked to repeat this process for each sentence they were presented with. This continued until a question mark appeared on the screen at which point, participants were asked to recall the sequence of letters they had remembered in order of presentation. Then, a new trial with new sentences and letters to remember would begin. There were 12 trials in total, each with between two and five sentence-letter pairs presented before the recall phase. The two, three, four and five sentence-letter pair trials appeared in random order. The random order used was the same random order for each participant. The sentences to be solved and proceeding letters were those selected by Engle. Letters chosen were individual in sound so not to cause potential confusion and increase in mistakes made (for example, the task did not include both the letters B and D as it can be easy to replace one with the other, due to their phonetically similar nature). Chosen letters included ‘J, M, F, X, L, R, B, Q’ and ‘H’.

OSpan Participants were asked to perform a sequence of simple arithmetic calculations each followed by an unrelated word to be recalled later. Items were presented as follows:

IS (10 x 2) – 4 = 18 ? CLOUD

Participants were asked to work out the maths sum, being told that the maths sum may or may not reach the correct conclusion. They were asked to say out loud “yes” or “no” as to whether the solution to the maths sum was correct, and read out loud the proceeding word. Participants were instructed to remember the word and press the space bar to continue to the next sum-word pair and repeat the process. This continued until a question mark appeared on the screen, at which point they were asked to recall the sequence of words from that set in order. The participant would then continue by pressing the space bar to progress to the next set. There were 12 trials in total with between two and five sum-word pairs presented before the recall phase. The two, three, four and five sum-word pair trials appeared in random order. The random order used was the same random order for each participant. The equations to be solved and proceeding words were those selected by Engle. The chosen words were all objects which were unrelated to each other (for example, the word flame was the only element; the word chair was the only furniture). Words were individual in sound as to not cause potential confusion and increase in mistakes made (for example, the task did not include any words that rhymed with each other). The words were all single syllable words with common frequency in English.

Cspan Participants were presented with a picture of a random number of dark blue circles, light blue circles and dark blue squares. See figure 2.1 below.

Figure has been removed
due to Copyright restrictions

Figure 2.1: One example of sixty items displayed across the fifteen trials taken from Cspan of a single item presented to the participant

Participants were asked to count out loud only the number of dark blue circles and repeat the final total. In the above case the participant should respond '1...2...3...4...5...6...7...8...8'. Participants were asked to remember the final number. Once counted, participants pressed the space bar to continue to the next item and repeat the process. This continued until a question mark appeared on the screen, at which point, the participant was asked to recall the sequence of totals from the trial in the correct order. The participant would press the space bar to begin a new set. There were 15 trials in total, with between two and six numbers in each to remember. The two, three, four, five and six counting pair trials appeared in random order. The random order used was the same random order for each participant. The numbers of dark blue circles to be totalled were selected by Engle.

SART To measure SA, participants were asked to complete the SART (Robertson et al., 1997). To follow the standard procedure of the SART, participants were presented with 225 single digits (digits one to nine) across a 4.3 minute period. Each digit was presented once for 250 msec, followed by a 900 msec mask. The mask was a circle with a diagonal line through the centre. The digits were arranged in 25 blocks of nine, with each digit of one to nine only repeating itself once per block. Within each block, the nine digits were arranged randomly; the only constraint being that the number three could not be the first or the last digit in each block. Participants were asked to push the space bar on the keyboard as soon as possible for every digit except the number three. When the number three occurred, they were told to withhold their response. Reaction times per digit as well as errors (incorrect responses towards the number three) were recorded by the computer programme. From this, the average reaction time per correct response and total number of incorrect responses towards the number three were calculated. As each score point represented an incorrect response to the number three, a lower SART score represented fewer incorrect responses to the number three indicating better SA. Participants were made aware that response speed and accuracy were of equal importance. The digits were presented in one of five font sizes (48 point, 72 point, 94 point, 100 point and 120 point). This reduced the possibility of participants to be looking for a response template, and rather, enforced them to search for the numerical value. Examples of screen shots from the three WM tasks and the SART can be found in appendix A; pages 276 - 280.

GHQ-12 The GHQ-12 is a self-administered instrument designed to assess adults' mental health. Each of the 12 items asks respondents to rate the severity of a common mental health problem over the past few weeks using a four-point scale, taking approximately five minutes to administer. Ratings for items are summed to generate a total score ranging from zero to 36, with higher scores indicating worse conditions (Petрак & Campbell, 1999). The GHQ-12 items measure psychological health such as 'have you recently been feeling unhappy or depressed?' Participants completed the GHQ-12 by responding to each of these 12 items using the four point-scale. For the example above, participants would be asked to respond by choosing either 'Less than normal', 'Same as usual', 'More than normal', or 'Much more than usual'. Scores were balanced depending on whether the statement was positive or negative and added to a total. Scores were then categorised as normal (12 – 20), borderline (21 – 26) or high (27 – 48) based on cut off points calculated relative to the distribution of scores within the sample (zero-33rd, 33rd – 67th, 67th – 100th percentiles). Higher scores represented a lowered mood and potentially depressed state. These percentiles were initially worked out based on the study's own samples, and then confirmed to be the same as a normative distribution (using the GHQ-12 scoring handbook; Goldberg & Williams, 1991). A full copy of the GHQ-12 can be found in appendix A; page 281.

MQoL The MQoL scale is comprised of seven domains that typically become problematic during the menopausal change. These include Energy, Feelings, Symptoms Impact, Interactions, Cognition, Sleep and Appetite and equate to 48 items. For this study, only the 14 items assessing cognition and feelings were used, to keep the question battery manageable for participants and because

cognitive symptoms were expected to be the most closely related to performance on the cognitive tasks. A copy of the selected items can be found in appendix A; page 283. Participants were asked to rate on a six point scale how strongly they had felt each symptom over the past few months. For example; participants would be asked 'I can concentrate easily' to which they would response from 'I am never like this' to 'I am always like this' on a six point scale. Items were scored and added to a total. After collaboration with the designer of the scale, it was decided that the scores of this specific sample should be categorised as normal, borderline or high based on cut off points calculated relative to the distribution of scores within the sample (zero-33rd, 33rd – 67th, 67th – 100th percentiles).

Additional Information Individuals were asked for their age and level of education. Details of the menopause were requested to determine whether the participant was peri or post-menopausal such as the approximate date of the last bleed. Participants were then asked whether or not they had ever received any form of HRT. Users of HRT were asked how much time had lapsed between last menstrual bleed and HRT initiation. They were also asked details of their therapy, including the type, how it is administered into the body and duration of treatment. Details regarding general physical health including medication taken, visits to the local GP within three months and any chronic conditions were recorded. To conclude, participants were asked about their general thoughts on cognition and the menopause, and whether one can affect the other. These were categorised and scored. The additional information took the form of a biographical questionnaire, which is available in appendix A; page 286.

Procedure Ethical approval was granted by the University of Plymouth Faculty of Science and Technology ethics committee. Participants completed the study on University campus or in their own homes. In some cases, participants met in twos or threes in one of the participant's homes to complete the study one at a time after each other. Participants were strictly asked not to talk about the tasks to each other if this was the case. In many cases, new participants were recruited through recommendation of another participant. Again, if this were the case participants were strictly asked not to talk about the tasks to each other until everybody involved had completed the study.

Participants were asked to complete the SART on a lap top (Presario A900 compac) using the space bar to respond. This task was followed by each of the WM tasks. WM task order was counter-balanced across participants using randomisation. Participants were then presented with the GHQ-12, items from the MQoL scale and biographical questionnaire on the lap top, and asked to respond to each item using the mouse to select the appropriate response.

At the end of the study, participants were told that they had the opportunity to take part in the study one year following. They were asked to explicitly state if they did not want to be contacted in one year. They provided preferred contact details, and were thanked for their time.

Study One, Stage Two

Participants A total of 90 women (74%) from the original 121 participants returned to complete the study. Of these, 46 (of 59; 78%) had used HRT at some point during the completion of their menopause, with 44 (of 62;

71%) having never used the therapy. Twenty five of the HRT users (of the original 33 peri-menopausal HRT users; 76%) had initiated the therapy in their peri-menopausal stage and 21 (of 26; 79%) of the HRT users had initiated the therapy in the post-menopause. These were compared to 23 (of 33; 70%) peri-menopausal non users and 21 (of 29; 72%) post-menopausal non-users respectively. Participants were contacted via email and phone using contact details left during the completion of stage one of the study. Participants were contacted on average at 11 months to give adequate time to agree to take part in the study.

Measures Participants were asked to complete the same test battery 12 months later. To measure WM, they were asked to complete three of Engle's WM span tasks including RSpan, OSpan and CSpan once more. To measure sustained attention, they were asked to complete the SART task. To measure mood and menopausal symptoms, participants were asked to complete the GHQ-12 and the items from the MQoL scale. This was followed by a short questionnaire that focused on any changes since the last meeting. Specifically, participants were asked about any changes to the menopause such as whether they were still experiencing regular menstrual bleeds or not and whether their history of HRT use had changed such as if they had ceased therapy use or not. Details regarding visits to a local GP within the three months leading to participation and any chronic conditions were recorded. To conclude, participants were asked about their general thoughts on cognition and the menopause, and whether one can affect the other.

Procedure Participants completed the study on University campus or in their own homes as previous. They were asked to complete the SART on a lap top

using the space bar to respond. This was then followed by each of the WM tasks. Participants were then presented with the GHQ-12, items from the MQoL scale and biographical questionnaire on the lap top, and asked to respond to each item using the mouse to select the appropriate response. In some cases participants were offered paper questionnaires to fill out instead of the computer versions, as many reported during the first stage of the study that they disliked the constant use of the laptop throughout the test battery.

2.9 Results and Analysis

Data regarding WMC were analysed using MANCOVA with stage of the climacteric (peri or post) and HRT use (history of HRT use or no history of HRT use) as factors and age (in years) and MQoL score as covariates. It was important to recognise the effect of time of HRT initiation on WM overall before considering domain specific WM. Data regarding the number of correct answers for each WM Span task (RSpan, OSpan and CSpan) were analysed using ANCOVA with stage in the climacteric (peri or post) and HRT use (history of HRT use or no history of HRT use) as factors and age (in years) and MQoL score as covariates. This enabled exploration of time of HRT initiation on verbal, mathematical and spatial WM individually. The order of the tasks were counterbalanced to control for order effects.

Data regarding the number of incorrect responses to the number three in the SART were analysed using a ANCOVA with stage of the climacteric (peri or post) and HRT use (history of HRT use or no history of HRT use) as factors and age (in years), MQoL score and reaction time per correct response as covariates.

A total of 121 women aged between 45 and 65 years of age completed stage one of the study. Of these, 59 had used HRT at some point during the completion of their menopause, with 62 having never used the therapy. Thirty-three HRT users who had initiated the therapy in their peri-menopausal stage and 26 HRT users who had initiated the therapy in the post-menopause were compared to 33 peri-menopausal non users and 29 post-menopausal non users respectively. MQoL, GHQ-12, number of visits to a GP and physical health were similar across groups (table 1.1).

Table 1.1: Mean score (\pm standard deviation) or count of HRT detail and health across menopausal group

	Peri-menopausal participants (<i>n</i> = 33)	Post-menopausal participants (<i>n</i> = 29)	HRT initiator (peri) participants (<i>n</i> = 33)	HRT initiator (post) participants (<i>n</i> = 26)	Total (<i>n</i> = 121)
Mean Age	50.24 (3.60)	56.48 (4.34)	54.91 (6.01)	58.46 (3.99)	54.78 (5.49)
GHQ-12 mean score	24.36 (4.87)	24.69 (7.04)	23.10 (3.56)	24.40 (6.20)	24.17 (5.50)
MQoL mean score	14.39 (3.67)	15.55 (3.95)	15.73 (4.21)	14.81 (3.97)	15.12 (3.94)
Mean visits to the GP	0.91 (1.15)	0.66 (0.77)	0.76 (1.06)	0.73 (1.04)	0.77 (1.01)
No. women taking medication	12	11	13	12	48
No. women with chronic conditions	12	15	14	16	57
Mean years taking HRT			5.01 (4.05)	3.77 (2.90)	4.46 (3.61)
Mean years since last HRT			3.42 (4.85)	4.81 (3.70)	4.03 (4.40)

Menopausal Symptoms

The participants were asked to estimate the approximate date of their most recent menstrual bleed, specifically whether this had fallen within the past twelve months. Of the total population, 12.4% ($n = 15$) reported not having experienced any menopausal symptoms to date, 37.2% ($n = 45$) estimated the onset of their symptoms to be less than five years ago and 27.3% ($n = 33$) of the population estimated the start of their menopausal symptoms to be between five and ten years ago. Of the population, 15.7% ($n = 19$) estimated their last menstrual bleed to be between 10 and 15 years ago and 8.3% ($n = 10$) estimated the onset of their menopausal symptoms to be over 15 years ago. Table 1.2 shows the estimated last menstrual bleed for each participant across each group.

Table 1.2: Count of last estimated menstrual bleed of each participant across menopausal group

	Regular bleeds	Less than 5 yrs ago	5 – 10 yrs	10 – 15 yrs	Over 15 yrs
Peri-menopausal	15	18			
Post-menopausal		11	13	4	1
HRT initiator (peri)		17	7	7	2
HRT initiator (post)		2	11	7	6

A total of 15 women in the peri-menopausal stage were experiencing regular menstrual bleeds, but this was not well matched by women who initiated HRT in their peri-menopausal stage.

Details of HRT

A total of 59 women (48.8%) had reported once taking HRT for at least three months. Table 1.3 shows the division of these therapy types for each peri- and post-menopausal HRT groups. Of those taking HRT, 50 women were receiving a combination of oestrogen and progesterone and nine were receiving oestrogen only.

Table 1.3: Count and overall percentage of participants' therapy types across HRT initiators (peri- and post)

	Patches	Pills	Implants	Other
HRT initiator (peri)	8	16	6	1
HRT initiator (post)	10	14	2	1
Total	18 (29.9%)	30 (49.7%)	8 (14.9%)	2 (3.5%)

On average, those who initiated the therapy during their peri-menopausal stage took the therapy for 5.01 years ($SD = 4.85$), terminating the treatment 3.42 years ago. Those who initiated the therapy in the post-menopausal stage took the therapy for 3.77 years ($SD = 2.90$), terminating the treatment 4.81 years ago ($SD = 3.70$). In total 67.2% of the HRT using population had ceased the therapy within the past five years. Those still taking the therapy included five women who had initiated the therapy during their post-menopause, and 17 women who had initiated their therapy during their peri-menopause.

GHQ-12

The GHQ-12 was scored conventionally and a lower score represented better mood, a higher score represented negative (or depressed) mood. Of the total population, 24% of the women were of 'normal' mood (12-20 points), 54.5% were of 'borderline' (21-26 points) and 21.5% of participants were of 'high' (or depressed) mood (27+ points). The distribution can be seen in figure 2.2.

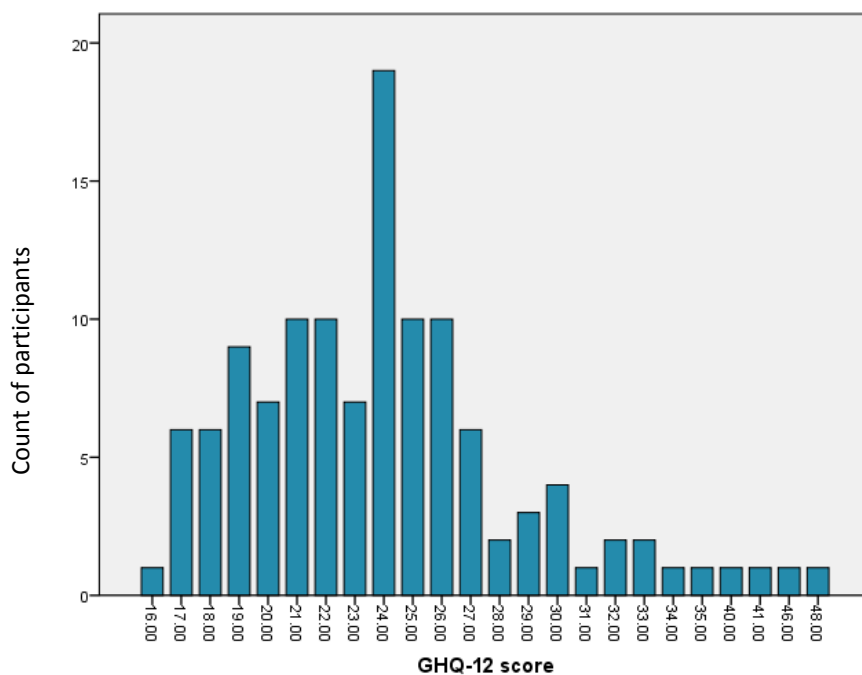


Figure 2.2: Distribution of GHQ-12 score across the study population

Mean GHQ-12 scores per group as well as the total average score for the study population can be seen in table 1.1. The sample's mood scores were normally distributed across groups, and groups did not differ between average mood score dependant on phase ($F(1, 117) = 0.927, p = .34, d = .008$) HRT use ($F(1, 117) = 0.40, p = .53, d = .003$) or the interaction between the two ($F(1, 117) = 0.41, p = .34, d = .008$).

MQoL

In contrast to the GHQ-12, higher scores of the MQoL scale represent better QoL. Of the total population, 14.0% ($n = 17$) reported having poor QoL based on symptoms impact, 65.3% ($n = 79$) reported having borderline QoL and 20.7% ($n = 25$) reported having good QoL. Mean MQoL scores per group as well as the total average score for the study population can be seen in table 1.1. The sample's mood scores were normally distributed across groups, and groups did not differ between average mood score dependant on phase ($F(1, 117) = 0.03, p = .87, d = .000$) HRT use ($F(1, 117) = 0.17, p = .68, d = .001$) or the interaction between the two ($F(1, 117) = 2.07, p = .15, d = .017$).

Number of GP visits, type and frequency of chronic conditions and medication use do not differ across groups

Participants were asked how many times they had visited a GP in the past three months. In the past three months 51.2% had not visited a GP, 29.8% had visited a GP once, 10.7% had visited a GP twice, 5.8% had visited a GP three times, 1.6% had visited a GP four times and 0.8% had visited a GP five times. The mean number of GP visits the four groups can be seen in table 1.1. The sample's mood scores were normally distributed across groups, and groups did not differ between average mood score dependant on phase ($F(1, 117) = 0.55, p = .46, d = .005$) HRT use ($F(1, 117) = 0.4, p = .85, d = .000$) or the interaction between the two ($F(1, 117) = 0.36, p = .55, d = .003$).

Of the total population, 40.5% ($n = 49$) stated that they were taking medication at time of completion of the study. Total count of those taking medication across the four groups can be seen in table 1.1. Medication use did

not differ between peri- and post- users; $\chi^2(1, n = 121) = .14, p = .71$, or those with and without a history of HRT use; $\chi^2(1, n = 121) = .27, p = .60$.

Participants were also asked if they suffered from a chronic condition, to which 45.5% of the population ($n = 55$) indicated that they suffered from a chronic condition. Total count of those suffering chronic conditions across the four groups can be seen in table 1.1. Chronic conditions did not differ between peri- and post- users; $\chi^2(1, n = 121) = 3.95, p = .48$, or those with and without a history of HRT use; $\chi^2(1, n = 121) = 1.48, p = .48$.

Most frequent conditions included underactive thyroid, asthma and high blood pressure, for which most participants were receiving treatment. In addition to this, a proportion of participants were taking pain killers for general aches and pains, as well as for migraine. The total count of reported conditions can be seen in table 1.4.

Table 1.4: Count of reported chronic conditions across the total group

Condition	Count
Acid reflux	1
Allergies	1
Anemia	1
Asthma	8
Osteoporosis/Arthritis/ Back/bone pain	11
Cancer (past)	2
Diverticulitis	2
HBP	14
Diabetic	1
High cholesterol	2
Hep C	1
Migraine	3
Eczema	2
MS	2
Underactive thyroid	8

Belief

Participants were asked whether they felt the menopause could affect cognition, memory or attention. The majority of participants (56.2%) felt that the menopause may have a mild detrimental effect on cognition, memory and attention and 13.2% of the population felt that this detrimental effect could be extreme. A large proportion of the population (29.8%) believed the menopause to have no effect on cognition, memory and attention. And one person believed that the menopause could improve cognition, memory and attention. Belief did

not differ between peri- and post- users; $\chi^2(3, n = 121) = 1.48, p = .69$, or those with and without a history of HRT use; $\chi^2(3, n = 121) = 1.90, p = .60$.

Education

The majority of the study's population (41.3%) had completed education up to G.C.S.Es (O levels) and/or A levels. A large proportion of the population (34.7%) had completed an undergraduate degree, and some had completed education at post graduate (13.2%) and skills level (8.3%) qualification. A small percentage (2.5%) had completed their educational path at key stage three. A breakdown of individual educational levels across group can be seen in table 1.5.

Table 1.5: Count of current level of education across menopausal group

	KS3	GCSE/A-level equivalent	Undergraduate equivalent	Post graduate equivalent	Skills level equivalent
Peri-menopausal	1	15	14	2	1
Post-menopausal	2	9	7	4	4
HRT initiator (peri)		15	10	6	2
HRT initiator (post)	2	9	7	4	4
Total	3	50	42	16	10

Level of education did not differ between peri- and post- users; $\chi^2(4, n = 121) = 3.82, p = .43$, or those with and without a history of HRT use; $\chi^2(3, n = 121) = 3.27, p = .52$.

The groups were similar in terms of mental and physical health, education, and beliefs about effects of menopause on cognition. The peri-HRT and peri-control groups matched each other for age. The post-HRT and post-control groups also matched each other for age and were older than the peri-groups. Groups were also similar for MQoL and GHQ-12 score. Correlations and regression models were fitted to the data.

Correlational analyses were carried out to determine the details of the main analyses for this study, and to confirm that scores on the SART correlated with those on the WM tasks. Regression models were also fitted to the data.

Each WM span task has been designed to measure WM, and as such scores of each WM task would be expected to strongly correlate with each other. In the present study, RSpan had a strong positive correlation with both OSpan ($r = .657, p < .001$) and CSpan ($r = .642, p < .001$). OSpan had a strong positive correlation with CSpan ($r = .726, p < .001$). This means that as WM capacity for one WM task increases, so does WM capacity for the other two WM tasks and confirms that three of these tasks will be a strong measure of WM overall. As the three tasks are strongly but not perfectly correlated all three measures can be included in a Manova (Tabachnik & Fidell, 2012). There was no correlation between time it took to complete the task and WM score. WM Span performance in each task was not affected by order of completion of the task when counterbalanced.

Participants who reported better quality of life as measured by MQoL total score performed better on RSpan ($r = .215, p = .018$) and OSpan ($r = .183, p = .044$) suggesting that mood and menopausal symptoms may affect WM

performance. MQoL total score was therefore included as a covariate in the analyses of effects of HRT and timing of HRT on cognitive performance. GHQ-12 total score correlated negatively but weakly with OSpan ($r = -.180$, $p = .048$) but not RSpan ($r = -.140$, $p = .13$) or CSpan ($r = -.109$, $p = .23$). GHQ-12 was not used as a covariate in data analysis. Although a decrease in cognitive performance did not correlate with an increase in age in this sample, age was still included as a covariate because of well-established effects of age on WM in the literature and because of the unavoidable age differences between the peri- and post-menopausal groups in our sample. As expected, the GHQ-12 total score had a strong negative correlation with MQoL total score ($r = -.333$, $p < .001$) showing that as the participants' rating of QoL increased, GHQ-12 score decreased (increased quality of life coincides with lower ratings of depressed mood).

Participants who had a quicker reaction time on the SART were more likely to make mistakes by incorrectly responding to the number three ($r = -.391$, $p < .001$). The number of incorrect responses correlated positively with the other type of error, withholding a response to a non-3 digit ($r = .217$, $p = .017$). There is a non-significant trend between SART score and WMC, where those who perform better in the WM tasks make fewer mistakes during completion of the SART. RSpan had a negative correlation with the average reaction time to each correct response in the SART task ($r = -.223$, $p = .014$) as did CSpan ($r = -.215$, $p = .018$) suggesting that the greater the participant's WM capacity, the quicker they responded correctly to the items in the SART task.

RSpan

RSpan score was modelled using HRT use and phase as predictors. Overall the regression model was significant, $F(3,117) = 10.48, p < .001$, with the combination of HRT and phase accounting for 19.2% (adjusted R-square) of the variation in RSpan score. In order to model the usefulness of the MQoL with RSpan score a hierarchical regression was carried out to compare the model using HRT use and phase with one also including MQoL score. The model showed significant improvement in R-Square (unadjusted) from .21 to .24, $F(4,116)=9.40, p =.026$.

A hierarchical regression was carried out to compare the original model using HRT use and phase with one also including GHQ-12 score. The model showed no improvement in R-Square (unadjusted) from .21 to .22, $F(4,116)=8.25, p=.23$.

A hierarchical regression was carried out to compare the original model using HRT use and phase with one also including education. The model showed no improvement in R-Square (unadjusted) from .21 to .24, $F(7,113)=5.10, p=.35$.

And finally, a hierarchical regression was carried out to compare the model using HRT use and phase with one also including belief about cognition and the menopause. The model showed no improvement in R-Square (unadjusted) from .21 to .22, $F(6,114)=5.41, p=.68$.

Overall, MQoL score was the only variable which improved the regression model for RSpan score.

OSpan

OSpan score was modelled using HRT use and phase as predictors. Overall the regression model was significant, $F(3,117) = 5.35$, $p = .008$, with the combination of HRT and phase accounting for 9.8% (adjusted R-square) of the variation in OSpan score. In order to model the usefulness of the MQoL with OSpan score a hierarchical regression was carried out to compare the model using HRT use and phase with one also including MQoL score. The model showed no improvement in R-Square (unadjusted) from .12 to .15, $F(4,116)=4.93$, $p = .07$, but this was close to significant.

A hierarchical regression was carried out to compare the original model using HRT use and phase with one also including GHQ-12 score. The model showed no improvement in R-Square (unadjusted) from .12 to .14, $F(4,116)=4.77$, $p=.10$.

A hierarchical regression was carried out to compare the original model using HRT use and phase with one also including education. The model showed no improvement in R-Square from .12 to .16, $F(7,113)=3.09$, $p=.23$.

And finally, a hierarchical regression was carried out to compare the model using HRT use and phase with one also including belief about cognition and the menopause. The model showed no improvement in R-Square (unadjusted) from .12 to .14, $F(6,114)=3.19$, $p=.35$.

Overall, MQoL score, GHQ-12 score, education and belief did not impact on OSpan score, however MQoL was close to significance.

Cspan

Cspan score was modelled using HRT use and phase as predictors. Overall the regression model was not significant, $F(3,117) = 3.05$, $p = .12$, with the combination of HRT and phase accounting for 4.9% (adjusted R-square) of the variation in Cspan score. In order to model the usefulness of the MQoL with Cspan score a hierarchical regression was carried out to compare the model using HRT use and phase with one also including MQoL score. The model showed no improvement in R-Square (unadjusted) from .07 to .05, $F(4,116)=2.73$, $p =.20$.

A hierarchical regression was carried out to compare the model using HRT use and phase with one also including GHQ-12 score. The model showed no improvement in R-Square (unadjusted) from .07 to .08, $F(4,116)=2.48$, $p=.38$.

A hierarchical regression was carried out to compare the model using HRT use and phase with one also including education. The model showed no improvement in R-Square from .07 to .09, $F(7,113)=1.59$, $p=.70$.

A hierarchical regression was carried out to compare the model using HRT use and phase with one also including belief about cognition and the menopause. The model showed no improvement in R-Square (unadjusted) from .07 to .09, $F(6,114)=1.63$, $p=.83$.

SART

SART score was modelled using HRT use and phase as predictors. Overall the regression model was significant, $F(4,116) = 0.42$, $p = .77$, with the combination of HRT and phase accounting for 1.5% (adjusted R-square) of the variation in SART score. In order to model the usefulness of the MQoL with SART score a hierarchical regression was carried out to compare the model using HRT use and phase with one also including MQoL score. The model showed no improvement in R-Square (unadjusted) from .01 to .01, $F(4,116)=0.31$, $p = .93$.

A hierarchical regression was carried out to compare the model using HRT use and phase with one also including GHQ-12 score. The model showed no improvement in R-Square (unadjusted) from .01 to .01, $F(4,116)=0.33$, $p=.81$.

A hierarchical regression was carried out to compare the model using HRT use and phase with one also including education. The model showed no improvement in R-Square from .01 to .02, $F(3,120)=.99$, $p=.18$.

A hierarchical regression was carried out to compare the model using HRT use and phase with one also including belief about cognition and the menopause. The model showed no improvement in R-Square (unadjusted) from .01 to .00, $F(3,120)= .48$, $p=.70$.

Overall, MQoL score, GHQ-12 score, education and belief did not impact on CSpan or SART score. MQoL did impact on RSpan score, and this was close to significance for OSpan score.

Testing the critical period hypothesis

For each WM task participants were asked to recall a list of items in the correct order. A mark was given for the correct item recalled in the correct place, as suggested by Engle. For the WM tasks, a higher score represents greater WM capacity. For RSpan and OSpan the participant can score a maximum score of 42. For CSpan the participant can score a maximum score of 60. SART score was calculated by adding the total number of incorrect responses (hitting the space bar on the appearance of the number three) per participant. Higher scores represented poorer sustained attention. Mean WM and SART scores in each group can be seen in table 1.12. For the RSpan and OSpan, it was possible for participants to have a total of 42 correct responses. For the CSpan, it was possible for participants to have a total of 60 correct responses. The mean per-cent proportion was calculated in order to better show the similarities and differences between CSpan score and the other two WM tasks. The scores as a per-cent proportion have been presented as a percentage in table 1.6.

Table 1.6: Mean number (+ standard deviation) of correct responses during the WM Span tasks and incorrect responses during the SART across menopausal group

Stage in menopause	HRT history	Mean score	SD	Total %
RSpan				
Peri-menopausal	No use	30.45	7.12	72.50%
	HRT use	33.76	6.60	80.38%
Post-menopausal	No use	30.62	5.98	72.90%
	HRT use	24.54	5.15	58.43%
Total		30.12	7.03	71.7%
OSpan				
Peri-menopausal	No use	30.52	6.50	72.67%
	HRT use	33.85	7.40	80.60%
Post-menopausal	No use	30.14	7.31	71.76%
	HRT use	26.50	6.81	63.10%
Total		30.47	7.40	72.5%
CSpan				
Peri-menopausal	No use	40.73	10.05	67.88%
	HRT use	44.12	9.77	73.53%
Post-menopausal	No use	38.86	10.30	64.77%
	HRT use	36.54	9.90	60.90%
Total		40.31	10.26	67.2%
SART				
Peri-menopausal	No use	6.97	3.71	-----
	HRT use	7.45	5.52	-----
Post-menopausal	No use	8.14	4.88	-----
	HRT use	8.12	5.28	-----
Total		7.63	4.77	-----

Engle's' WM tasks may be investigated combined, looking at the total score between three tasks, in order to assess over all WM. It is then possible to investigate these separately, in order to investigate different WM domains (in this case; verbal, mathematical and spatial WM). Because oestrogen change has been shown to have effect on different types of WM tasks (Keenan et al., 2001), the overall WM score between HRT users- and non-users at different stages of the climacteric were compared, as well as the tasks separately.

Multivariate analysis of covariance (Mancova) tested the effects of HRT use and time of use relative to menopause on cognitive performance. Age and MQoL were used as covariates to control for known effects of age on WM and observed associations between WM and MQoL. There was no main effect of HRT use on WM score (Wilks' $\lambda = .963$, $F(1,115) = 1.44$, $p = .23$, $d = .037$). There was a main effect of phase on WM score (Wilks' $\lambda = .872$, $F(1,115) = 5.52$, $p = .001$, $d = .128$), where those in the peri-menopausal stage performed better than those in the post-menopausal stage, regardless of HRT use and despite including age as a covariate. The effect of HRT on WM score was moderated by time of HRT initiation relative to menopausal symptoms (Wilks' $\lambda = .882$, $F(1,115) = 5.03$, $p = .003$, $d = .118$). As predicted by the critical period hypothesis, mean WM scores were highest in women taking HRT peri the menopause and lowest in those taking it post-menopause.

WM data were analysed for each of the three tasks separately to test whether effects of HRT and phase differed depending on the nature of the processing component of the task (verbal for RSpan, mathematical with a verbal component for OSpan, and spatial for CSpan).

Rspan

Mean RSpan score across each group can be seen in table 1.6 and figure 2.3.

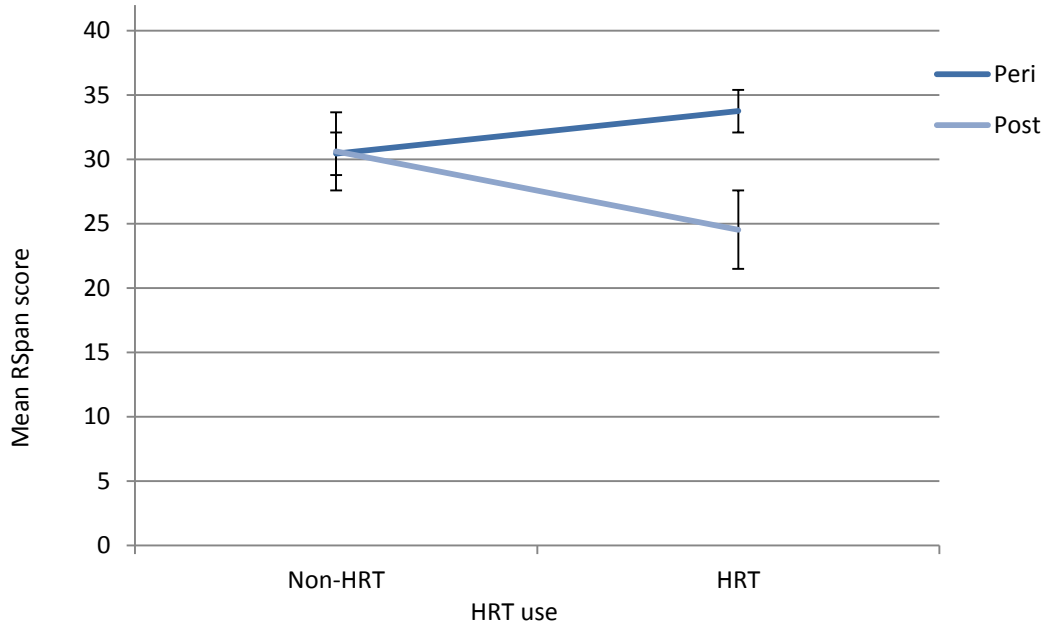


Figure 2.3: Mean RSpan score (and standard error) in HRT users and non users in the peri- and post-menopausal stages

Consistent with the critical period hypothesis, women who had initiated HRT during the peri-menopausal stage scored the highest (mean = 33.76) representing greatest RSpan performance. Those who had initiated the therapy at the post-menopausal stage scored lowest (mean = 24.54) representing poorest RSpan performance. Those with no history of HRT use scored slightly lower if peri-menopausal (mean = 30.45) than those in the post-menopausal stage (mean = 30.62) which was not anticipated.

Analysis for the potential effect of HRT, phase of the climacteric and the interaction of the two on RSpan capacity was explored. The analysis revealed a non-significant effect for HRT use on RSpan score ($F(1,115) = 2.47, p = .12, d$

= .021). There was however a significant effect of phase on RSpan capacity ($F(1,115) = 16.12, p < .001, d = .123$). The effect of HRT on RSpan capacity was moderated by time of HRT initiation as demonstrated in a significant interaction effect between HRT use and phase ($F(1,115) = 13.01, p < .001, d = .102$). The interaction can be seen in figure 4.1.

Simple effects showed HRT users who initiated HRT during the post-menopausal stage (mean = 24.54, S.D = 5.15) to have worse RSpan capacity than HRT users who initiated the therapy during the peri-menopausal stage (mean = 33.76, SD = 6.60, $p < .001$). HRT users who initiated the therapy during the post-menopausal stage also performed worse than those in the post-menopausal stage with no history of HRT use (mean = 30.62, SD = 5.98, $p = .001$). Both differences support the critical period hypothesis. No other significant differences were found, however it was important to note that HRT users initiated in the peri-menopausal phase were close to demonstrating larger RSpan capacity than those in the peri-menopausal phase with no history of HRT use (mean = 30.45, SD = 7.12, $p = .055$).

OSpan

Mean OSpan score across each group can be seen in table 1.6 and figure 2.4.

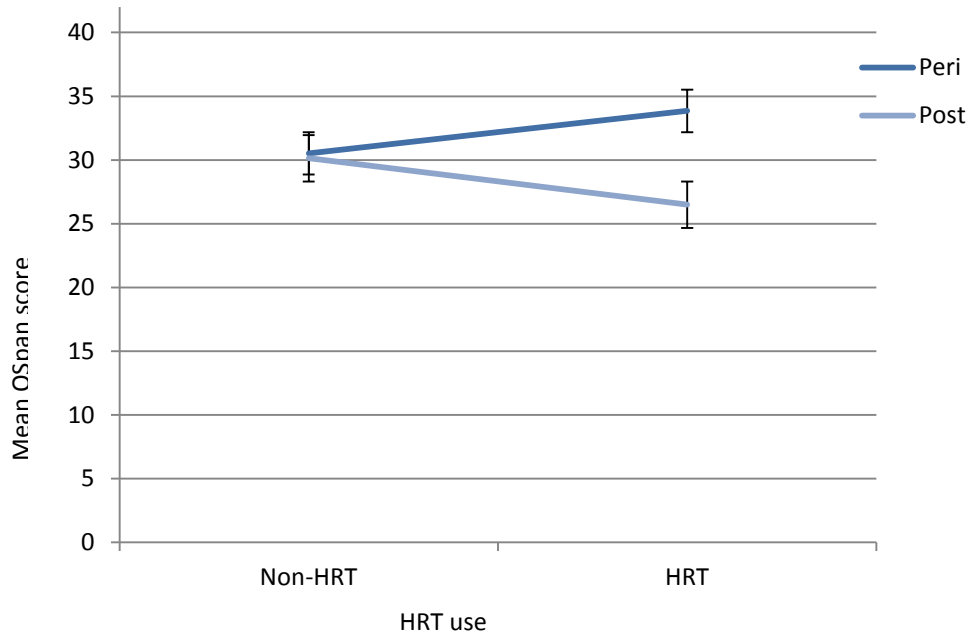


Figure 2.4: Mean OSpan score (and standard error) in HRT users and non users in the peri- and post-menopausal stages.

Consistent with the critical period hypothesis, women who had initiated HRT during the peri-menopausal stage scored the highest (mean = 33.85) representing greatest OSpan performance. Those who had initiated the therapy at the post-menopausal stage scored lowest (mean = 26.50) representing poorest OSpan performance. Those with no history of HRT use scored higher if peri-menopausal (mean = 30.52) than those in the post-menopausal stage (mean = 30.14). This pattern across group is consistent with the critical period hypothesis.

Analysis for the potential effect of HRT, phase of the climacteric and the interaction of the two on OSpan capacity was explored. The analysis revealed a non-significant effect for HRT use on OSpan score ($F(1,115) = .118, p = .732, d$

= .001). There was a significant effect of phase on OSpan capacity ($F(1,115) = 8.55, p = .004, d = .069$) suggesting that differences in OSpan score can be seen depending on stage of the climacteric. The effect of HRT on OSpan capacity was moderated by time of HRT initiation as demonstrated in a significant interaction effect between HRT use and phase ($F(1,115) = 5.63, p = .019, d = .047$). The interaction can be seen in figure 4.2.

Simple effects showed HRT users who initiated HRT during the post-menopausal stage (mean = 26.50, SD = 6.81) to have worse OSpan capacity than HRT users who initiated the therapy during the peri-menopausal stage (mean = 33.85, SD = 7.40, $p < .001$). This supports the critical period hypothesis. No other significant differences were found however it is important to note that HRT users initiated in the peri-menopausal phase were close demonstrating better OSpan capacity than those in the peri-menopausal phase with no history of HRT use (mean = 30.52, SD = 6.50, $p = .056$).

Cspan

Mean CSpan score across each group can be seen in table 1.6 and figure 2.5.

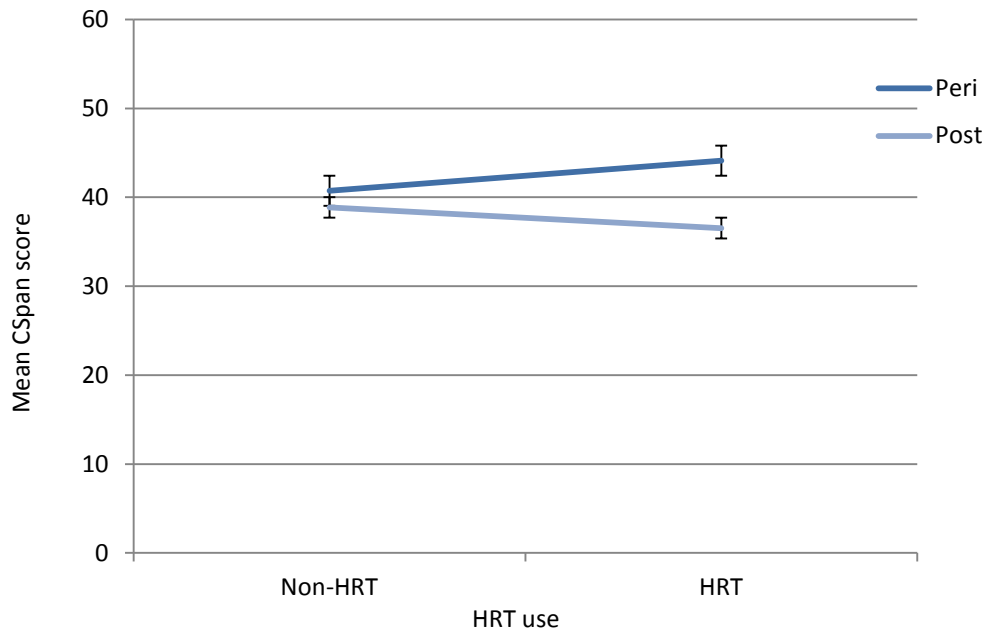


Figure 2.5: Mean CSpan score (and standard error) in HRT users- and non-users in the peri- and post-menopausal stages

Consistent with the critical period hypothesis, women who had initiated HRT during the peri-menopausal stage scored highest (mean = 44.12) representing greatest CSpan performance. Those who had initiated the therapy at the post-menopausal stage scored lowest (mean = 36.54) representing poorest CSpan performance. Those with no history of HRT use scored higher if peri-menopausal (mean = 40.73) rather than post-menopausal (mean = 38.86). The pattern across groups is consistent with the critical period hypothesis.

Analysis for the potential effect of HRT, phase of the climacteric and the interaction of the two on CSpan performance was explored. The analysis showed no effect of HRT use on CSpan score ($F(1,115) = 0.020, p = .89, d = .000$) but a significant effect of phase ($F(1,115) = 5.83, p = .017, d = .048$). Figure 4.3 shows

a similar pattern of results to those obtained with RSpan and OSpan, but the interaction between HRT use and menopausal phase was not significant ($F(1,115) = 1.75, p = .19, d = .015$).

Despite the interaction between HRT use and time of initiation not being significant, the effect of phase on CSpan capacity combined with the interesting results from the post hoc analysis for the Ospan and Rspan warranted further analysis. Because the overall interaction was not significant for the CSpan, the conclusion should be treated with caution.

Simple effects showed that those who had initiated HRT during the post-menopausal stage (mean = 36.54, SD = 9.90) had worse CSpan capacity than those who had initiated HRT in the peri-menopausal stage (mean = 44.12, SD = 9.77, $p = .005$). No other significant differences were found. The CSpan results therefore support the suggestion, from the critical period hypothesis, that it is better for cognitive performance to start taking HRT in the peri-menopause phase than post-menopause, but differences from non-users were weaker.

The SART

In the case of the SART, the average reaction time to each correct response was used as a covariate. Age was also used as a covariate to control for the effects of aging.

The mean number of mistakes made on the SART across each group can be seen in table 1.6 and figure 2.6.

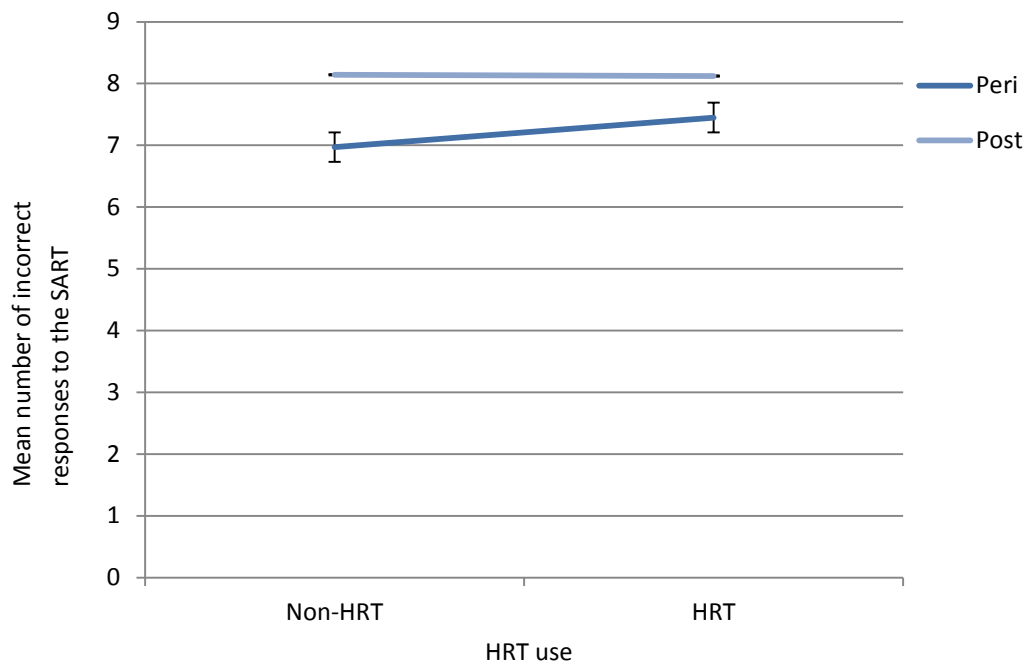


Figure 2.6: Mean SART score (and standard error) in HRT users and non users in the peri- and post-menopausal stages

Those in the peri-menopausal stage with no history of HRT use scored the least amount of incorrect responses (mean = 6.97). Those who had initiated HRT during the peri-menopausal stage scored a similar amount of incorrect responses (mean = 7.45). Post-menopausal women scored the largest amount of incorrect responses and this was similar between HRT late initiators and non-users. The averages suggest that phase of menopause (peri or post) may have an effect on SA as oppose to HRT use. To see if HRT use and/or stage of menopause can have effect on task performance, or perhaps an interaction of the two; an ANCOVA was calculated.

Analysis for the potential effect of HRT, phase of the climacteric and the interaction of the two on SART performance was explored. The analysis showed no effect of HRT use on SART score ($F(1, 87) = 0.425, p = .52, d = .003$) but a significant effect of phase ($F(1, 87) = 5.56, p = .02, d = .033$). The interaction

between HRT use and stage in the menopause was not significant ($F(1, 87) = 0.443, p = .89, d = .003$), depicted in figure 4.4.

2.10 One year follow up

The study included a one year follow up to test whether effects of timing of HRT were lasting. From the original sample, there were no responses from 17 participants. A further three participants did not have time to re-complete the tasks. Three participants were no longer interested in the study. Four participants had moved away and four participants had passed away with cancer. Distributions of reasons for no follow up were fairly evenly spread across groups, as shown in Table 1.7.

Table 1.7: Breakdown of responses from participants invited to take part in the one year follow up study

	Peri-menopausal	Post-menopausal	HRT initiator (peri)	HRT initiator (post)
No responses	6	3	4	4
Lack of time	1	1	1	
Not interested	1		1	1
Moved away	2	1	1	
Deceased	1	2	1	

From the original peri-menopausal set, 25 HRT users who had initiated the therapy in their peri-menopausal stage (75.8%) and 21 HRT users who had initiated the therapy in the post-menopause (80.8%) were compared to 23 peri-menopausal non users (69.7%) and 21 post-menopausal non users respectively (72.4%). No women of the original set had initiated HRT within the year gap period.

GHQ-12 and MQoL score

The GHQ-12 scores are very similar across groups, and depict normal mood. The population in this study represent the same average scores as the general population for the GHQ-12. The sample's mood scores were normally distributed across groups (see table 1.8), and groups did not differ between average mood score dependant on phase ($F(1, 90) = 0.30, p = .59, d = .003$) HRT use ($F(1, 90) = 0.69, p = .41, d = .008$) or the interaction between the two ($F(1, 90) = 0.79, p = .67, d = .002$). The sample's MQoL scores were also normally distributed across groups (see table 1.15), and groups did not differ between average mood score dependant on phase ($F(1, 90) = 0.00, p = .96, d = .000$) HRT use ($F(1, 90) = 0.00, p = .97, d = .000$) or the interaction between the two ($F(1, 90) = 0.12, p = .73, d = .001$). For both GHQ-12 and MQoL; the scores across groups are very similar, with those who initiated HRT during their peri-menopausal stage having best mood (represented by lowest GHQ-12 score average) and greatest rated QoL (represented by the highest MQoL score average).

Table 1.8: Mean (\pm standard deviation) GHQ-12 scores across menopausal group one year following

	Peri-menopausal	Post-menopausal	HRT initiator (peri)	HRT initiator (post)	Total mean
GHQ-12 score	23.35 (2.27)	23.43 (3.72)	22.44 (3.14)	23.14 (4.30)	23.07 (3.37)
MQOL score	14.30 (3.40)	14.52 (3.83)	14.84 (3.25)	14.76 (3.62)	14.61 (3.45)

WM Span and SART score

Table 1.9 shows the mean WM score (for RSpan, OSpan and CSpan), and per-cent of correct responses as a proportion of the total.

Table 1.9: Mean number (+ standard deviation) of correct responses during the WM tasks and incorrect responses during the SART across menopausal group

Stage in menopause	HRT history	Mean score	SD
RSpan			
Peri-menopausal	Non-HRT user	30.26	7.00
	HRT user	32.88	5.53
Post-menopausal	Non-HRT user	31.24	5.60
	HRT user	25.33	5.38
Total		30.07	6.45
OSpan			
Peri-menopausal	Non-HRT user	29.83	4.62
	HRT user	31.36	6.34
Post-menopausal	Non-HRT user	28.76	6.06
	HRT user	27.52	5.13
Total		29.47	5.69
CSpan			
Peri-menopausal	Non-HRT user	40.00	8.89
	HRT user	43.12	8.57
Post-menopausal	Non-HRT user	38.48	8.96
	HRT user	37.95	9.39
Total		40.03	9.03
SART			
Peri-menopausal	Non-HRT user	6.17	3.42
	HRT user	6.28	4.65
Post-menopausal	Non-HRT user	7.90	4.96
	HRT user	7.81	4.74
Total		6.99	4.40

In accordance with stage one of study one; stage two analysis for the WM tasks included the covariates of age and the MQoL score.

Testing the critical period hypothesis

Multivariate analysis of covariance (Mancova) tested the effects of HRT use and time of use relative to menopause on cognitive performance. Age and MQoL were used as covariates to control for known effects of age on WM and observed associations between WM and MQoL. There was no main effect of HRT use on WM score (Wilks' $\lambda = .960$, $F(1,84) = 1.14$, $p = .35$, $d = .039$). Unlike stage one of the study, there was no effect of phase on WM score (Wilks' $\lambda = .950$, $F(1,84) = 1.43$, $p = .23$, $d = .051$). The effect of HRT on WM score was moderated by time of HRT initiation relative to menopausal symptoms (Wilks' $\lambda = .852$, $F(1,84) = 4.73$, $p = .005$, $d = .146$).

RSpan one year following

Mean RSpan score across each group for the one year follow up can be seen in table 1.9 and figure 2.7.

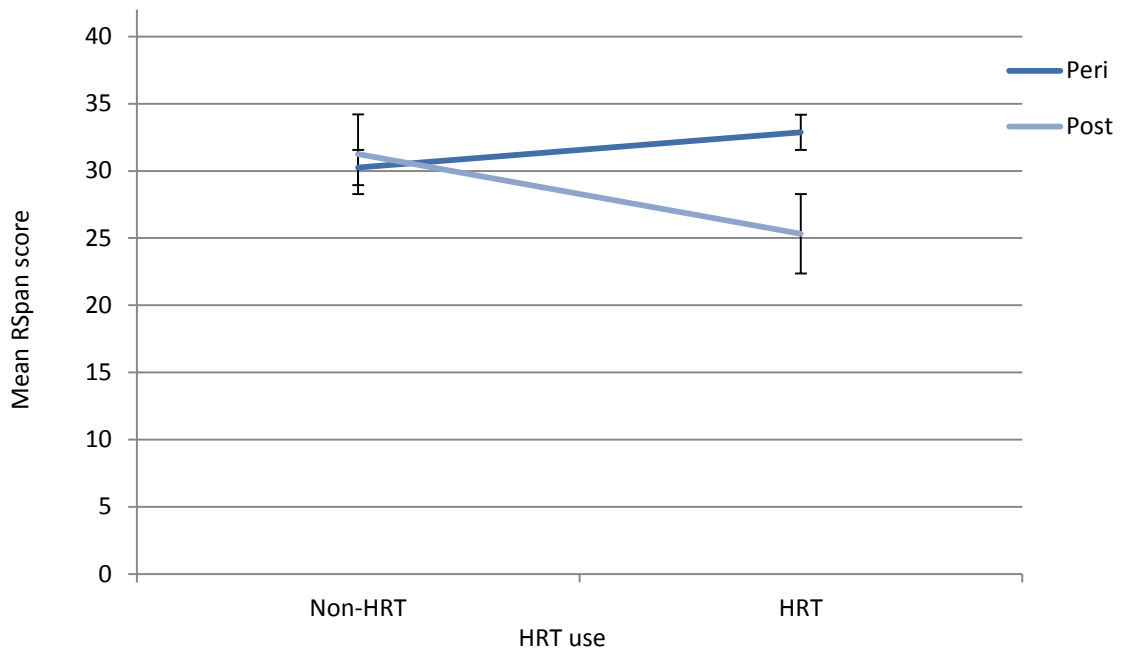


Figure 2.7: Mean RSpan score (and standard error) in HRT users and non users in the peri- and post-menopausal stages one year following

Mean scores followed the same pattern as stage one of data collection. Consistent with the critical period hypothesis, women who had initiated HRT during the peri-menopausal stage scored the highest (mean = 32.8) representing greatest RSpan performance. Those who had initiated the therapy during the post-menopausal stage scored lowest (mean = 25.33) representing poorest RSpan performance. Those with no history of HRT use scored lower if in the peri-menopausal stage (mean = 30.26) than those in the post-menopausal stage (mean = 31.24).

Analysis for the potential effect of HRT, phase of the climacteric and the interaction of the two on RSpan performance was explored. Like stage one of the study, analysis showed a non-significant effect for HRT use on RSpan score ($F(1, 84) = 1.05, p = .31, d = .012$). Unlike stage one of the study, no effect of phase on RSpan score was found (however this was marginal; $F(1,84) = 3.87, p = .053, d = .044$). The effect of HRT on RSpan performance was moderated by time of HRT initiation as demonstrated in a significant interaction effect between HRT use and phase ($F(1,84) = 12.02, p = .001, d = .123$), similar to stage one of the study. The interaction can be seen in figure 4.5.

Simple effects showed HRT users who initiated HRT during the post-menopausal stage (mean = 25.33, SD = 5.38) to have worse RSpan performance than HRT users who initiated the therapy during the peri-menopausal stage (mean = 32.88, SD = 5.53; $p < .001$) and those in the post-menopausal stage with no history of HRT use (mean = 31.24, SD = 5.60, $p = .005$). This pattern is the same as stage one of the study and no other significant differences were found.

OSpan one year following

Mean OSpan score across each group for the one year follow up can be seen in table 1.9 and figure 2.8.

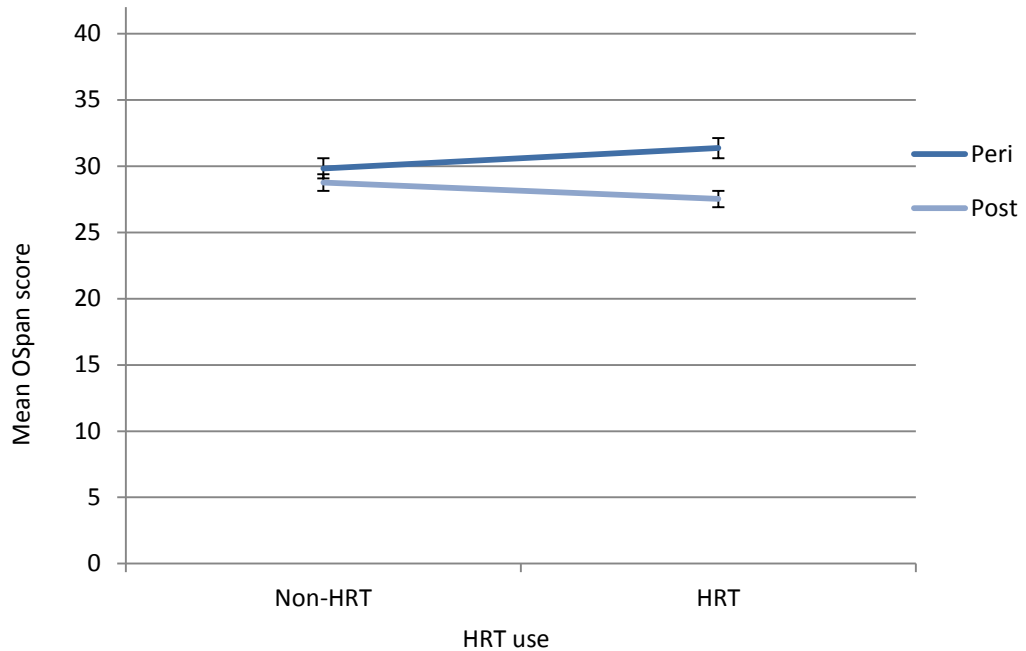


Figure 2.8: Mean OSpan score (and standard error) in HRT users and non users in the peri- and post-menopausal stages one year following

Means followed the same patterns as stage one of data collection. Consistent with the critical period hypothesis, women who had initiated HRT during the peri-menopausal stage scored the highest (mean = 31.36) representing greatest OSpan performance. Those who had initiated HRT in the post-menopausal stage scored the lowest (mean = 27.52) representing poorest OSpan performance. This pattern is consistent with the critical period hypothesis.

Analysis for the potential effect of HRT, phase of the climacteric and the interaction of the two on OSpan performance was explored. Like stage one of the study, the analysis showed a non-significant effect for HRT use on OSpan

score ($F(1,84) = 0.05, p = .821, d = .001$). Analysis considering the effect of phase on OSpan score was conducted. Unlike stage one of the study, no effect of menopausal phase on OSpan score was found ($F(1, 84) = 2.55, p = .11, d = .029$). Like stage one of the study, the effect of HRT on OSpan performance was moderated by time of HRT initiation as demonstrated in a significant interaction effect between HRT use and phase ($F(1, 84) = 1.42, p = .024, d = .017$), depicted in figure 4.6.

Simple effects showed HRT users who initiated HRT during the perimenopausal stage (mean = 31.36, SD = 6.34) to have better OSpan performance than HRT users who initiated the therapy during the post-menopausal stage (mean = 27.52, SD = 5.13; $p = .049$). This was similar to stage one of the study. Unlike stage one of the study, there was no effect of late HRT initiation when comparing post HRT initiators to those in the post-menopausal stage with no history of HRT use

Cspan one year following

Mean CSpan score across each group for the one year follow up can be seen in table 1.9 and figure 2.9.

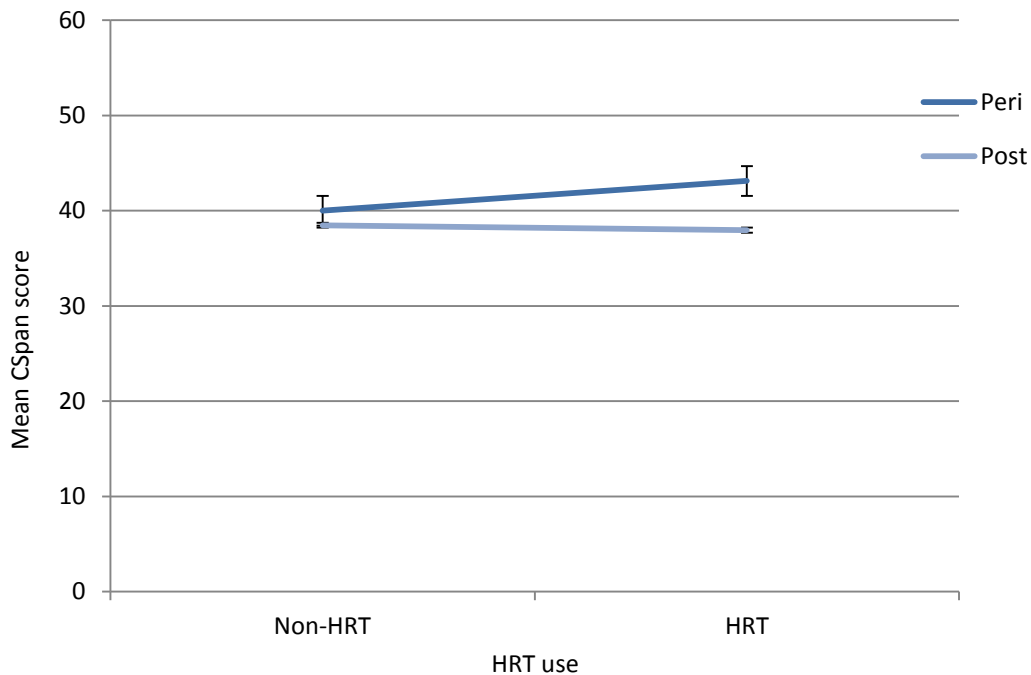


Figure 2.9: Mean CSpan score (and standard error) in HRT users and non users in the peri- and post-menopausal stages

Means followed the same patterns as stage one of data collection. Consistent with the critical period hypothesis, women who had initiated HRT during the peri-menopausal stage scored highest (mean = 43.12) representing greatest CSpan performance. Those who had initiated the therapy at the post-menopausal stage scored lowest (mean = 37.95) representing poorest CSpan performance.

Analysis for the potential effect of HRT, phase of the climacteric and the interaction of the two on CSpan performance was explored. The analysis showed no effect of HRT use on CSpan score ($F(1, 84) = 0.26, p = .613, d = .003$). Unlike

stage one of the study, no effect of menopausal phase on CSpan score was found ($F(1, 84) = 2.84, p = .096, d = .033$) and there was no interaction effect between HRT use and stage in the menopause ($F(1, 84) = 0.76, p = .39, d = .009$), depicted in figure 4.6.

The role of phase in CSpan score gave warrant to further analysis considering simple effects however unlike stage one of the study (which found those who had initiated HRT in the post-menopausal stage had scored significantly worse than those who had initiated HRT in the peri-menopausal stage), no further effects were found.

The SART results for the one year follow-up

The mean number of incorrect responses to the number three during completion of the SART across groups after one year can be seen in table 1.9 and figure 2.10.

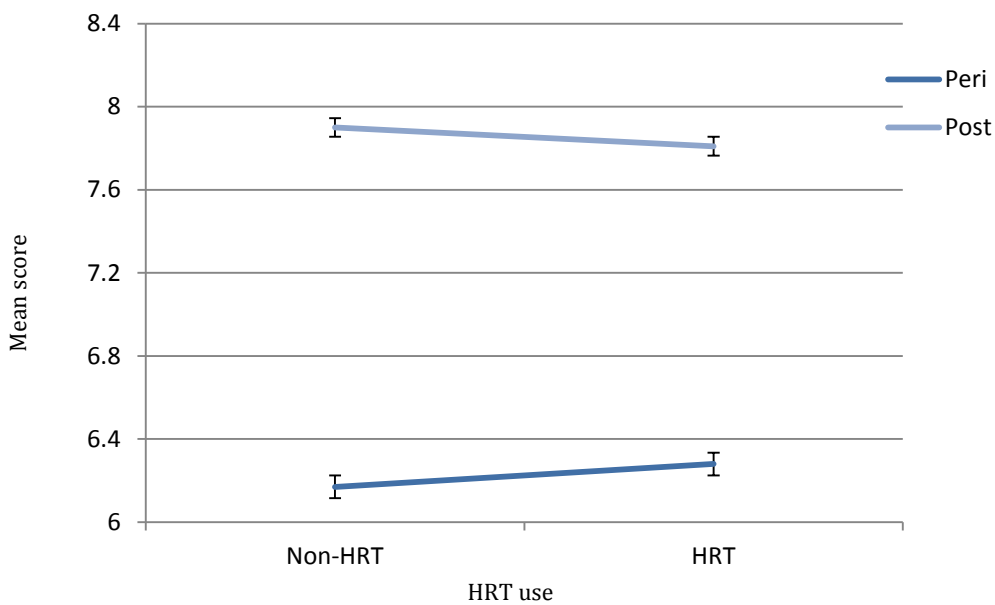


Figure 2.10: Mean SART score (and standard error) in HRT users and non users in the peri- and post-menopausal stages

Those in the peri-menopausal stage with no history of HRT use scored the least amount of incorrect responses (mean = 6.17). Those who had initiated HRT during the peri-menopausal stage scored a similar amount of incorrect responses (mean = 6.28). Post-menopausal women scored the largest amount of incorrect responses and this was similar between HRT late initiators and non-users. To see if HRT use and/or stage of menopause can have effect on task performance, or perhaps an interaction of the two; an ANCOVA was calculated.

Analysis for the potential effect of HRT, phase of the climacteric and the interaction of the two on SART performance was explored. Like stage on of the study, analysis showed no effect of HRT use on SART score ($F(1, 84) = 0.065, p = .79, d = .001$) but an effect of phase ($F(1, 84) = 4.46, p = .04, d = .050$). Again, the interaction between HRT use and menopausal phase was not significant ($F(1, 84) = 0.00, p = .99$). This can be seen in figure 4.8. Due to the lack of interactions, no further analysis was required.

Type of HRT content (oestrogen only verses oestrogen and progesterone)

It has been suggested that the addition of progesterone to HRT can have detrimental effects on cognitive performance and for this reason; the effect of HRT content on WM was explored. It has also been suggested that the addition of progesterone to HRT can have detrimental effects in post-menopausal women and for this reason; the interaction of time and HRT content on WM was also explored.

Data regarding WMC were analysed using MANCOVA with stage of the climacteric (peri or post) and HRT content (oestrogen, $n = 9$; or oestrogen plus progesterone, $n = 50$) as factors and age (in years) and MQoL score as covariates.

Data regarding the number of correct answers for each WM Span task (RSpan, OSpan and CSpan) were analysed using ANCOVA with stage in the climacteric (peri or post) and HRT content (oestrogen or oestrogen plus progesterone) as factors and age (in years) and MQoL score as covariates. The interaction effect of time in the climacteric and HRT use were analysed.

There was no main effect of HRT content on WM score (Wilks' $\lambda = .957$, $F(1,41) = 0.59$, $p = .624$). There was no main effect of HRT content, moderated by time of HRT initiation, on WM score (Wilks' $\lambda = .938$, $F(1,41) = 0.86$, $p = .468$).

There was no main effect of HRT content on RSpan score ($F(1,41) = 0.425$, $p = .518$). There was no main effect of HRT content, moderated by time of HRT initiation, on WM score ($F(1,41) = 0.09$, $p = .769$).

There was no main effect of HRT content on OSpan score ($F(1,41) = 0.015$, $p = .903$). There was no main effect of HRT content, moderated by time of HRT initiation, on WM score ($F(1,41) = 0.01$, $p = .913$).

There was no main effect of HRT content on CSpan score ($F(1,41) = 0.08$, $p = .773$). There was no main effect of HRT content, moderated by time of HRT initiation, on WM score ($F(1,41) = 0.75$, $p = .393$).

There was no main effect of HRT content on SART score ($F(1,53) = 0.016$, $p = .899$). There was no main effect of HRT content, moderated by time of HRT initiation, on WM score ($F(1,41) = 0.05$, $p = .824$).

2.11 Discussion

Early HRT use can maintain different types of WM, whereas late use can become detrimental

In stage one of the study those in the peri-menopausal stage performed better on the WM tasks than those in the postmenopausal stage regardless of HRT use as anticipated (Chen et al., 2006; Maki, 2006; Markou et al., 2007; Sherwin, 2012; Sherwin and Henry, 2008). HRT use alone did not impact on performance in any task, but there was an effect of HRT on WM when moderated by time of HRT initiation relative to menopausal symptoms, reflected in the RSpan and OSpan, as well as WM overall. Specifically, those with a history of HRT use initiated during their peri-menopausal phase scored significantly higher than those who initiated their therapy during the post-menopausal phase. Those who initiated the therapy during the post-menopausal stage scored significantly lower than those in the post-menopausal stage with no history of HRT use in the RSpan task (close to significance for the OSpan task). Also close to significance was the difference between RSpan scores of peri-menopausal women with no history of HRT use as compared to peri-menopausal HRT initiators. The results for the RSpan and the OSpan, and WM overall, support the critical period hypothesis (Bimonte-Nelson et al., 2003; Gibbs, 2000b; Joffe, et al., 2006; MacLennan et al., 2006), and clarify the effect of late use of HRT, which was associated with impaired cognition rather than no effect. This is less clear for the CSpan.

In order to see if these effects were sustained, women were re-tested at 12 months to observe long term changes. Unlike stage one of the study, no effects of phase on WMC were found. HRT use did not affect WM performance,

but an interaction effect for the moderation of HRT by time of initiation was found in the RSpan, OSpan and WM overall. Specifically, users who initiated HRT during the peri-menopausal stage had better RSpan and OSpan capacity than those who initiated HRT in the post-menopausal stage. In the RSpan, it was again demonstrated that those who initiated HRT during their post-menopausal stage performed worse than post-menopausal women who have never received HRT. Results re-confirm the critical period hypothesis and show that the effects of timing of HRT on cognition may be long-lasting. No differences were found for the follow up of the CSpan.

The present study included participants covering a wide age group (to include women at both ends of the climacteric). Due to large individual variation in stage in the climacteric, age and cognitive performance; results would have been varied across the group as a whole. Despite this fact, there were still differences in cognitive performance between HRT early initiators and late initiators.

HRT alone did not impact on score without the interaction of time of initiation. This suggests that the effects of HRT use are more to do with phase of menopause than chronological age. This effect was partially maintained one year following, but some effects did not transpire. This is understandable, as during the 12 month period several peri-menopausal HRT non-users were closer to post-menopausal and several peri-menopausal HRT users had ceased their regular intake of the therapy. At point of follow up there were mixed clusters in the peri- and post-menopausal groups where some women were still receiving HRT (and many had ceased treatment). In a further study, it may be useful to separate these clusters and look at the cognitive performance of participants

who have ceased treatment and those who have carried on. For the present study, groups were too small for this to be viable.

Integration of the present study into current literature

Human studies have demonstrated increase activation in the frontal lobes during completion of WM tasks for peri-menopausal women (Cutter et al., 2003; Erickson et al., 2010; Joffe et al., 2006; Miller et al., 2002; Shaywitz et al., 1999). These studies demonstrate an effect of oestrogen on areas of the brain that underlie WM processes and that oestrogen treatment influences prefrontal cortex activation during completion of WM tasks. The present study demonstrated better WM performance in women with a history of early HRT initiation, which is likely to be a result of early HRT use on pre-frontal activity. The present study is consistent with studies that demonstrate increased PFC activation after peri-HRT initiation, and support arguments that HRT affects functions such as WM that are dependent on the PFC when administered during the peri-menopause. The brain imaging studies listed above have confirmed a difference in frontal lobe activation between early and late HRT initiators and the impact of this is clearly demonstrated in maintained WM capacity of early initiators during the completion of WM tasks.

The present study confirms the critical period. Other studies that have considered early and late HRT initiation have similar results as the present study. For example, the study by MacLennan and colleagues (2006) demonstrated an improvement in verbal memory and the trial making test and there was a trend in post-HRT initiators to underperform.

Pompili and colleagues (2012) suggests that the relationship between oestrogen change and WM needs further exploration. The present study demonstrates a beneficial effect of early HRT initiation on different types of WM, and coincides with the study by Keenen and colleagues (2001).

Grigorova, Sherwin and Tulandi (2006) demonstrated that oestrogen deficiency results in deficits in verbal and non-verbal WM using modified versions on the N-Back test. This is because oestrogen receptors are found in the prefrontal cortex (Tang et al., 2004) alongside dopamine terminals (Leranth et al., 2006), an area which mediates WM function. This is supported by a study by Berman and colleagues (1999) where young women also suppressed of oestrogen had increased activation in the PFC during completion of a WM task when provided with oestrogen replacement. The present study tested the effect of increased oestrogen on different types of WM, showing that increased oestrogen initiated during the critical period can help maintain WM. The studies support the idea that oestrogen change, be it suppression or expression, impacts on different types of WM.

Sherwin (2007) suggests that oestrogen may help to delay degenerative disease such as AD and that oestrogen therapy when taken in compliance with the critical period can help to prevent this. She further stresses that it should not be assumed that oestrogen has any direct effect on the cause of cognitive decline and degenerative disease. If this is the case, the late initiation of HRT should not correlate with incidence of degenerative disease. Several researchers have demonstrated a detrimental effect of late oestrogen use on cognition, including the present study where late HRT initiators performed worse on verbal

and mathematical WM. This coincides with the WHIMS, where late HRT use resulted in increased risk of cognitive impairment and probable dementia.

Resnick and colleagues (2006) reported that oestrogen therapy can increase the risk for developing dementia. The present study findings explicitly demonstrate a detrimental effect of late HRT initiation on WM and this is something that should not be taken lightly. Sherwin does highlight the fact that HRT has the potential to be harmful when administered to women over the age of 65. In the case of this study, late HRT use from completion of the menopause still resulted in worsened WM capacity suggesting that from as young as one year post completion of the menopause (on average, 52.8 years), HRT may be detrimental to cognition if initiated beyond this point. Again, this is important as it suggests that detrimental effects can be experienced far earlier than first anticipated. This means that researchers will need to reconsider defining their groups by age.

It is possible that the effect of oestrogen change on WM only transpire when WM tasks are of a particular difficulty level. This is consistent with the study by Grigороva and colleagues who used difficult WM tasks, as well as Keenan and colleagues (2001) where the effects of oestrogen suppression on WM were only seen when the task became challenging and WM capacity overloaded.

The same effect has been demonstrated in brain imaging studies. Dumas and colleagues (2010) found that women treated with HRT had an increase in frontal activity during completion of their two more difficult WM tasks only. The difficulty of the task is a key component in successful studies demonstrating a

critical period for HRT and the impact of oestrogen on memory tasks is only apparent when the task exerts certain cognitive strain (Krug et al., 2003; Linzmayer et al., 2001; Philips & Sherwin, 1992; Shaywitz et al., 2003). This includes verbal WM performance (Keenan et al., 2001).

And finally, Jenkins and colleagues demonstrated that oestrogen suppression in young women could also result in deficits in WM performance during completion of a spatial WM task, letter sequence WM task and digit span WM task.

The current study included WM tasks that could be manipulated for difficulty. The point of the task is to recognise the point where participants begin to struggle, and this is an indication of the participants WM capacity. Because the tasks are designed to find WM capacity, the trials increase in difficulty level, and this helps to reduce ceiling effects.

HRT use does not affect SA

The relationship between SA and oestrogen change in human studies is under developed. In animal studies, oestradiol reduction during the menopause slowly diminishes activity in the cholinergic system and this may have an effect on cognitive functions sensitive to cholinergic activity in the frontal and hippocampal areas of the brain such as SA (Sarter et al., 2000). In human studies, oestrogen reduction also causes detrimental effects in pre-frontal cortex mediated tasks, to which SA measures deficits in pre-frontal function. It was therefore expected that HRT might help with SA whereby the outcome of mistakes made on the SART would be in part driven by the critical period because WM and SA depend on systems that overlap. Literature regarding the

effect of oestrogen fluctuation on SA is minimal. Although the SART was designed to measure frontal lobe deficits, and HRT use can increase frontal lobe activation and related cognitions, in the present study there was no effect of HRT use (early or late) on SART performance.

Some studies have demonstrated the effect of oestrogen change on attention. Grigorova and colleagues (2006) demonstrated that oestrogen deficiency may cause problems in an attention task (letter-number sequencing). In addition, participants failed to learn despite repeated exposure of the task. The SART has one simple rule to learn, but the rule practices inhibition control. It is possible that the SART was not difficult enough, as consistent with WM literature. It should also be considered that Grigorova and colleagues administered LAD to suppress oestrogen production, whereas the participants in the current study had naturally decreased levels of oestrogen, and each participant would have had differing levels. In addition, participants in Grigorova's study were younger.

To coincide with this, younger women have also demonstrated SA problems during pregnancy (Crawley et al., 2005; deGroot et al., 2003). The tasks chosen were designed for the study and not known tasks, as such their validity can be questioned.

Animal studies have also demonstrated an effect of oestrogen use on WM, but not SA. Ovariectomised rhesus monkeys exposed to HRT improved in a delayed recognition span task however no treatment effects were seen on a delayed response task associated with the prefrontal cortex. The researchers suggested that for primates, oestrogen may enhance cognitive abilities that are

hippocampal-dependent (to which WM relies on the hippocampus in animal studies) but may not enhance those that are only dependent on the frontal lobes (Lacreuse et al., 2004). Although animal and human memory and attention models do differ, the effect of HRT on WM and SA in the study by Lacreuse and colleagues does coincide with the present study's findings.

Knight and Eden (1996) also suggest that actions of compounds with oestrogenic activity are species specific, one example being tamoxifen which is agonistic in humans and oestrogenic in mice. This may also explain differences between animal and human models.

The subtle differences between tasks and the diverse differences in outcome between studies that have included HRT and non-HRT users (animals and humans) suggests that more understanding is needed to recognise the reasons for these differences.

Although WM and SA are unique, their characteristics overlap and SA contributes to the completion of WM tasks (Schweizer & Moosbrugger, 2004). However the attentional process is secondary to the more complex WM system of control. In support of this theory, the present study coincides with work by Lazzaretti and colleagues, which demonstrated the impairment of verbal WM in patients with pre-frontal cortex damage whilst SA has remained intact (Lazzaretti et al., 2012).

Duff and Hampson (2001) describe WM as intrinsically involving verbal or visual information and its active manipulation, it may be that the SART is not sensitive to oestrogen change because it is not challenging enough, and lacks a

verbal component. The relationship between SA and the dopamine system is also less established.

The present study does contribute to, and confirm literature that demonstrates an effect of oestrogen use on WM. The relationship between SA and HRT use is underdeveloped, and the current study suggests that HRT use and/or initiation does not affect attention. More clarification is needed in the relationship between oestrogen change in the frontal lobe and impact on associated cognitions.

Impact of the present study on the current literature; pinpointing the critical period by last menstrual bleed and not age

Generally, animal studies have induced early menopause surgically, and provided HRT close to the removal of the ovaries. For example, oestrogen administration has been shown to augment cholinergic transmission and enhance dendrite spine formation in the hippocampus, and this has been linked to improved spatial WM in younger rodents (Fader et al., 1999) and not for those who have experienced long periods of oestrogen deficiency prior to treatment initiation (Daniel et al., 2006).

Human studies involving naturally menopausal populations are less clear, and have used age as guidance for deeming a participant as peri-menopausal or post-menopausal. In studies exploring the critical period, researchers have defined their groups based on the participant's age by assuming the average age of the menopause. The average age of the menopause has large variation. To design a study considering this window of opportunity and to define this period by age seems largely redundant.

The present study was more precise, taking into account each individual participant's last menstrual bleed, pinpointing the critical period to be within twelve months of the last menstrual bleed, something that previous studies have not considered.

The critical period may be as short as twelve months

Current literature has not explored the possible duration of the window of opportunity and what is less clear is the precise onset and duration of this window, to which there should be a general consensus to aid in study design, and this can be largely influenced by the methods adopted by researchers to define peri- and post-menopausal groups.

The current study suggests that the critical period is as short as twelve months from last menstrual bleed. If this be the case, many studies that have relied on age of the participant as the determinant of peri- or post- grouping may be overlooking this short, twelve month period. For example, it has also been demonstrated that late initiation of HRT can increase the risk for dementia and may impair memory performance due to a decrease in hippocampal volume and reduced cholinergic activity (Lam & Leranth, 2003; Voytko et al., 2009). For studies defining menopausal stage by age, it is likely that the participant has in fact experienced amenorrhoea 12 months or more. This is because most post-menopausal women recruited in some (but not all) such studies are a decade beyond the average age of the menopause (for example, the WHIMS). By defining a group by age, this does not account for individual differences in the climacteric and experienced symptoms, where the average age of the menopause has average upper and lower boundaries of plus or minus seven

years. To account for individual variance, it is far more accurate to record the participant's last menstrual bleed, and deduce stage in the climacteric from this. At points the literature can appear mixed and there is some evidence that discrepancies are due to differences in timing of HRT initiation relative to stage in the climacteric. This is something the current study was able to directly test.

For example the WHIMS which recruited menopausal women with a mean age of 72 years and were shocked at the detrimental effects of the therapy, resulting in early termination of the study. Another example of studies which may add confusion to the literature is the study by Ditkoff and colleagues (1991), who look at the effect of HRT on episodic memory and QoL and found no differences between HRT users and those receiving a placebo. Participants were aged from 32 to 60 years, and it is possible that HRT had no benefits to the older adults who would have typically been years from their last menstrual bleed. Such studies add confusion to the literature as have not separated participants for peri- and post- menopausal phase.

Part of the confusion is because peri-menopausal groups contain participants who are post-menopausal, again something that the present study was able to address.

The study is one of the first direct tests of the critical period hypothesis in relation to WM, showing not only that early initiation of HRT is protective of WM, but also that late initiation of the therapy is detrimental to WM. The study supports the critical period hypothesis whereby HRT maintains and/or improves WM when initiated prior to and during the onset of the menopause (Grigороva, & Sherwin 2006; Lacreuse, & Herndon 2003). The study confirms literature that

suggests that, if this window of opportunity is overlooked and HRT is initiated in the later stages of the climacteric, there will be detrimental effects on WM. The results of the RSpan and OSpan are consistent with the critical period hypothesis, and agree with studies by Rapp and colleagues (2003), Farquhar, Marjoribanks, Lethaby, Suckling and Lamberts (2009) and Zandi and colleagues (2002) who found HRT use in post-menopausal women to cause detrimental effects on cognition. The one year follow up further adds validity to the critical period hypothesis and demonstrates the longer lasting effects of HRT initiation relative to stage in the climacteric, something previous studies have not addressed.

Early and late HRT initiation can affect different types of working memory

Several studies have demonstrated an effect of HRT use on different types of WM. For example, Smith and colleagues demonstrated an effect of oestrogen use on spatial WM. Grigороva, Sherwin and Tulandi (2006) demonstrated that oestrogen deficiency results in deficits in verbal and non-verbal WM using modified versions on the N-Back test. Combined with the present study, such studies suggest that different types of WM are oestrogen responsive.

The completion of both non-verbal and spatial WM tasks and the increased activation in the frontal lobes shown by previous studies (Cutter et al., 2003; Shaywitz et al., 1999) suggest that the findings are specific to WM and not simply a reflection of changes in verbal memory function. For example, Joffe and colleagues (2006) demonstrated increased activation in the PFC in oestrogen users on completion of verbal and spatial WM tasks. In addition, participants receiving the HRT performed better on these tasks. Although HRT may protect

episodic memory, it has also demonstrated using the present study and studies described above that HRT use can protect different types of WM, and not just those with a verbal constraint. To coincide with this, the present study demonstrated changes in verbal WM, mathematical WM and spatial WM.

In the current study, age was included as a covariate in analysis, and HRT did not have effect on verbal and mathematical WM when considered alone. It was the interaction of HRT use and time of initiation which accounted for differences in WM performance.

An observational study design allowed exploration of the long lasting effects of time of HRT initiation on WM

Natural brain aging results in a decrease in brain volume and neuron size and a 46% reduction in dendrite spine numbers in humans over the age of 50 (Esiri, 2007). Changes appear to occur most profusely in the prefrontal cortex which sub serves the specific cognitive functions that decline with age including WM (Esiri, 2007). HRT initiated during the peri-menopausal stage can reduce these declines, by maintaining brain function in the prefrontal cortex (Keenan et al., 2001). Specifically, the synthesis of new neurons continues throughout life and old age, suggesting that it is the decline of synapses between neurons, neurotransmitter concentration, dendrite spine density and a change in neuronal networks dependant on oestrogen concentration that may be the cause of cognitive decline (Brinton, 2001). These effects might not be apparent straight away, and it might take some time for differences between cognitive performance of early HRT initiators and late HRT initiators to transpire. The present study, being observational, allowed the exploration of later effects of HRT.

In addition to this, several studies have indicated a link between late HRT use and a reduction in neuronal performance in the prefrontal cortex, but this has not always transpired in task performance itself (Shaywitz et al., 1999; Cutter et al., 2003). It has been suggested that oestrogen change first and foremost affects brain structure and mechanisms, and changes in cognitive performance follow (Jones & Gallo, 2001). If this be the case, then the observational design allowed for exploration of this.

In the present study, it is important to consider that on average, those who initiated the therapy during their peri-menopausal stage took the therapy for 5.01 years, terminating the treatment 3.42 years ago. This suggests two things. Firstly there has been enough time for the therapy to have effect, and the beneficial effect of HRT on WM to be apparent at the time of testing. The present study design allowed exploration of this. Secondly, even though the average last dose of therapy was taken 3.42 years prior to data collection, those in the peri-menopausal HRT initiated stage have still scored the highest for all WM tasks, despite having an average age of 54.91 years (4.67 years older than peri-menopausal women with no history of HRT use). For late users, the detrimental effects were also apparent, where participants had terminated the therapy (on average) 4.81 years ago. The present study's observational design meant that these longer lasting effects could be explored, something that all past studies have overlooked. The longer lasting effect of HRT initiation on WM further demonstrates the importance of initiating HRT during the peri-menopausal stage, determined by last menstrual bleed.

Critique of the present study and area's to be improved

The study incorporated an observational design where participants were recruited and then allocated to one of four groups. This was a naturalistic study, where the participants received different concentrations of HRT for different durations. They may have ceased treatment due to recommendation from the GP; or perhaps they felt their symptoms had ceased or that the treatment was yielding adverse effects. Despite being an observational study and not being able to control for certain factors, the results of the present study are still clear. A RCT controlling for therapy type and duration would have simply been too costly and would possibly have reduced the number of women volunteering for the study, however we may expect the marginal improvements in RSpan performance of peri-HRT users (as compared to controls) to be significant had this been the case, as suggested in the implications of the present study. The study had a four year preparation and execution plan and as such, the researchers felt it an excellent opportunity for a longitudinal observational study design. Both RCTs and observational studies have proved successful in demonstrating the critical period hypothesis (Joffe et al., 2006; MacLennan et al., 2006), with the present study adding to this. RCTs usually cover a limited amount of time (e.g. one to three months; Dumas et al., 2008; LeBlanc et al., 2007) and few go on to explore later outcome after a prolonged period. By using an observational study design it was possible to pinpoint the effects of HRT on WM several months and years after the therapy had been initiated, something that other studies have not yet done.

Sherwin (2007b) outlines (in her meta-analysis) that RCTs demonstrate an improvement in WM in younger HRT users, whereas observational studies show

improvements on a broader range of cognitions. There seems to be a slight lack of precision in observational studies and often researchers include larger test batteries (to which the build-up of tasks can cause cognitive exhaustion for the latter tasks) including more global cognitive tasks such as the mini mental state examination (MacLennan et al., 2006). The present study was much more specific, choosing only three WM tasks and one SA task to prevent cognitive exhaustion, and avoid type one error. The selected tasks have high validity, were challenging to a degree (to which there would have been subjective variation), and proved to be sensitive to HRT initiation.

Participant inclusion criteria and group definition were important in the study design. First it was determined whether the participant had a history of HRT use or not (with a minimum of three months consecutive use). For those with a history of HRT use, participants were established as peri- or post-menopausal depending on when their therapy had been initiated relative to their last menstrual bleed (with in, or in excess of 12 months). Participants with no history of HRT use were established as peri- or post-menopausal depending on when their last menstrual bleed was (again with in, or in excess of 12 months). This caused some problems, as those deemed as peri-menopausal users could have gone years without treatment, and years without menstrual bleeds. However the present study was designed to detect the long term effects of the therapy after peri-menopausal initiation and as such it was acceptable to invite participants who had initiated the therapy during the peri-menopause but were no longer receiving the therapy and no longer peri-menopausal.

As the study relied on opportunity sampling it was important that groups were balanced for age (Philips & Sherwin, 1992), education (Jones & Gallo, 2001;

Phillips & Sherwin, 1992; Rasgon et al., 2005) and health (Joffe et al., 2006; Philips & Sherwin, 1992). Measurement of these variables also worked towards establishing potential covariates needed in analysis. Confidence in observational findings of HRT use on cognition are limited by concerns involving the 'healthy user bias', the tendency for women choosing to receive HRT to be better educated, healthier perhaps due to being more health conscious (Matthews et al., 1996) and younger (Maki, 2006). This further heightens the need for studies to balance their groups for age, education and health; there was no evidence that this was a problem for our sample.

The present study found no relationship between HRT use and MQoL score, or phase of the climacteric and MQoL score (consistent with a study by Polo-Kantola et al., 1998). P. Jacobs and colleagues (2000) suggest that a decline in QoL during the menopause is often later followed by an improvement in QoL, and that the end of the climacteric often coincides with QoL evaluation reported as high. If this is the case, it would be expected that those in the later stage of completion of the menopause would rate their QoL and MQoL as slightly higher than those in the middle. Equally, it could be expected that those in the earlier stage would rate their score as slightly higher than the middle. The present study included a peri-menopausal group (peri to middle) and a post-menopausal group (middle to post) which would account for the similar MQoL mean scores across groups and could be the reason no relationship was found between MQoL score and menopausal phase and between MQoL score and HRT use. It may have been possible to further break down the groups in quintiles across the climacteric based on last menstrual bleed to see if MQoL did correlate with

either HRT use, or precise stage in the climacteric, but this was not a core focus of the study.

Several items from the MQoL scale were chosen to establish QoL and symptoms impact. On hind sight, it may have been more appropriate to use all 48 items, however the test battery was lengthy and challenging, and it was thought that 14 items from relevant domains rather than the full 48 items would suffice whilst preventing cognitive strain. On discussion with the lead creator of the scale, 14 items were chosen to demonstrate MQoL. The MQoL scores varied across group and correlated with RSpan and OSpan. As such, the scores were used as a covariate. Other studies that have used a menopausal QoL score or symptoms impact as covariates include Joffe and colleagues (2006). As the present study wanted to consider the interaction of time on initiation of HRT, it was important to use symptoms impact as a covariate to ensure that these symptoms (such as hot flushes; Maki et al., 2008) were not impacting on cognitive performance. Because MQoL score and CSpan score did not correlate, it is possible that the CSpan is a less sensitive measure for the menopausal population.

Clinical research has demonstrated that WMC negatively correlates with depression (Nazarboland & Farzaneh, 2009). GHQ-12 scores showed no evidence of an association between mood and WMC in this sample, and therefore were not used as a covariate. The GHQ-12 asks participants to rate how they have been feeling for a specific item over the past two weeks. For example; *Have you recently: felt worried* to which the participant would respond *Much more than usual, A bit more than usual, Same as usual, Much less than usual*. The majority of participants scored 22-24 points, selecting *same as usual* for each item. It is

possible that the chosen mood scale was not a good measure of mood in this group; unable to pick up on small differences between mood amongst the participants.

Mean GHQ-12 and MQoL scores for each of the four menopausal groups became even more similar after the one year follow up suggesting that as women move through the climacteric, symptoms perhaps alleviate. Several studies assessing the link between the critical period and cognitive performance have chosen not to use mood as a covariate. Lord and colleagues (2008) used a mood scale to exclude those who exceeded a particular score but did not use mood as a covariate, and Grigorova and colleagues (2006) measured mood using the Profile of Mood States Bi Polar form and the Beck Depression Inventory but did not use them as a covariate. There can be huge differences in subjective estimation of mood and symptoms, and although subjective belief can impact on performance this is often difficult to ascertain due to the diverse differences in estimation.

Participants invited to take part in the study were asked to be of normal mental and physical health. To coincide with previous research (Espeland et al., 2004; Kampen & Sherwin, 1994; Henderson et al., 1996; Schmidt et al., 1996; Steffens et al., 1999; Verghese et al., 2000), participants were excluded if they had a history of stroke, heart disease, alcohol or drug abuse, if they had probable dementia or if they had a hysterectomy. This would exclude any person with memory deficits or degenerative disease from taking part in the study. Participants who took part in the study had varied experience of chronic illness. Despite a large proportion of participants reported having a current chronic condition differences in WM function were still present between groups.

Because the researchers did not have access to medical records, it was difficult to measure the extent of experience of illness.

In addition, although participants were asked details on medications taken at point of testing, the absences of access medical records in the present study did cause limitations to the study design. For example, certain medications may affect oestrogen level, such as thyroid medication. And migraine-related medication may also effect the endocrine system, and in turn, cognition. With better resources and funding, it may have been possible to take saliva samples in order to determine oestrogen levels to eradicate possible effect of medications. As it stands, groups were balanced for experience of chronic condition, medication use and number of GP visits, and the conditions described by the participants were typical of a menopausal population. Despite poorer health experienced in some of the participants, differences in WM performance were still found in peri-HRT initiators and post-initiators as compared to non-HRT using controls.

With an upper age limit of 65 years, this also excluded anyone with age related cognitive decline of a certain degree. Average number of GP visits per year did not differ across groups, and were similar with UK averages (4.5 GP contacts per year; Hippisley-Cox, 2009). Previous studies have not always controlled for age and health and both can affect WM performance. The selected covariates were both appropriate and necessary, adding strength to the study design.

The presence of metabolic deterioration in the absence of cognitive change may be explained by high educational accomplishment, which is known

to compensate for mild impairment (Jones & Gallo, 2001). Specifically, those who are highly educated may use alternative systems to access cognitive functioning when the current systems are interrupted. Those who are less educated find metabolic deterioration more problematic and back-up systems become less accessible. Participants were asked their highest level of education (as did studies by Grigorova et al., 2006; deGroot et al., 2003; Lord et al., 2008; and MacLennan et al., 2006). This did not differ between groups or correlate with WM performance.

Maki (2006) has proposed that the beneficial effects of HRT on cognition only occur in women whose cognitive function is already slightly impaired due to menopausal symptoms or slightly impaired due to age-related declines in brain function. Indeed, effects may be detrimental once impairment has started. However Viscoli and colleagues (2005) suggests that HRT benefits are only evident in women who are cognitively intact. The current cognitive state of participants in the present study was not explicitly assessed. Generally, participants described themselves as of normal health. The present study demonstrated an improvement in WM in healthy, peri-menopausal women, and a detrimental effect in healthy, post-menopausal women. The results are applicable to a large proportion of the healthy population. Mulnard and colleagues (2000) identified that studies demonstrating improvements in memory by use of HRT in those with AD are based on a small group design over a short amount of time. The present study included a larger number of participants, allowing larger implications on the general population.

The strength of the present study is that the researchers defined groups by stage in the climacteric, and not by age. In addition to this, the researchers were

able to confirm the longer lasting beneficial role of early HRT use and detrimental role of late HRT use. The inclusion of a group of past users provides additional information about the potential long-term protective effect of oestrogen on the brain after treatment cessation.

Future research - The critical period

The present study suggests that the window of opportunity is relatively small, with detrimental effects of HRT on WM when the therapy is initiated as little as 12 months after the participant's last menstrual bleed. It would be worth conducting a large observational study considering the WM of several hundred women who have all initiated the therapy within and beyond 12 months after their last menstrual bleed in order to try and better define the window of opportunity. Specifically, when exactly does initiating HRT start to become detrimental to WM? How diverse is this window of opportunity across the population? The present study defines this window better than previous studies where groups were defined by age rather than personal symptoms.

In the present study, the role of oestrogen initiation on WM performance has also been well explored, and the implications of HRT initiation on WM performance dependant on stage in the climacteric is more clear.

The present study has been able to confirm that late HRT initiation is detrimental to WM. For other cognitions such as SA, the role of HRT initiation on this cognition's maintenance or destruction is less clear. It is important to explore and define cognitions susceptible to oestrogen change as women become challenged in deciding whether HRT use is appropriate in aiding them

through completion of the climacteric. Understanding the precise occurrence of the critical period will also aid in this decision.

Cyclical use of HRT

Simpkins and Singh (2008) suggest that continuous exposure to HRT may result in a diminished effect of oestrogen benefit on cognition as duration of exposure increases. They go on to suggest that oestrogens should be administered cyclically rather than continuously so that exposure is followed by a small period of deprivation and recovery of oestrogen receptor number and function. This would mimic that of a natural fluctuation of oestrogen in the body and for these reasons, the suggestion of a more cyclic use rather than constant makes sense. To further support this, cyclic HRT distribution in ovariectomised rats can improve spine density in the brain (Wooley, Gould, Frankfurt, & McEwen, 1990) whereas continuous exposure does not (Markowska & Savonenko, 2003). And finally, no improvement was seen in WM on ovariectomised rats score until the method of HRT management was changed from continuous to a cycle of changing oestrogen levels (Gresack & Frick, 2006). In human studies, continuous exposure of women to oestrogens caused a down-regulation of ER-alpha in the brain and other tissues (Mohamed & Abdel-Rahmann, 2000; Thakur & Sharma, 2007) and early beneficial effects of oestrogens on the brain can be expected to diminish over time of oestrogen exposure (Simpkins & Singh, 2008). In the present study, women who had initiated the therapy had continuous use until cessation, suggesting that for this population continuous use still resulted in benefits for peri-initiators, and detriments for post-initiators. In future RCTs, researchers may wish to consider a

combination of HRT initiation and duration, where perhaps a cyclic administration could be considered.

Lower dose for short period may help cognition momentarily in post-menopausal women

A more cyclical administration of HRT may be suitable for those still near the peri-menopausal stage, but for those in the post-menopausal stage it has been established that normal levels of HRT at continuous use could be detrimental to WM. Studies considering the impact of HRT when initiated during the post-menopausal stage have had mixed results. Most support the critical period hypothesis suggesting that late HRT initiation will have no effects or adverse effects however some demonstrate a promotion of cognition if a small dose of HRT is given over a short period of time. This includes improved verbal memory in over 65 year olds (Duka et al., 2000; Yaffe et al., 2000), increased dendrite growth in hippocampal tissue (Norbury et al., 2003) and symptoms of AD (Zandi et al., 2002).

Although the present study was unable to ascertain the dose of therapy across participants; the duration of therapy was recorded. Specifically, those who initiated the therapy in the post-menopausal stage had an average therapy use of 3.77 years. This far exceeds Zandi's recommended dose of two months prescription for a cognitive 'boost' if initiated post-menopause. Krug and colleagues (2006) were able to demonstrate improved WM and hippocampal function in postmenopausal women after 100ug HRT were administered for three days only. The implications of this are unexplored and it would be useful to consider the effects of this small dose of oestrogen. For example, supposing cognition can be boosted by as little as a one off dose of up to two months HRT

use, it should be considered how long this cognitive boost will last in post-menopausal women, where the present study demonstrates longer lasting detrimental effects of HRT use in post-menopausal women who had received the therapy in excess of (on average) three years. Are the benefits of short term use something that can be repeated in post-menopausal women or should it be limited? These are questions that lay unanswered as of yet and the current usefulness of a one off boost is yet to be determined.

An extremely interesting study would be the initiation of HRT in women with early onset-dementia to see if HRT can combat dementia in women who are still menstruating.

Progesterone addition

Another question requiring clarification is that of the addition of progesterone to the therapy, and how this might impact on cognitive performance. Progestagens are added to HRT in order to counteract increased risk of endometrium hyperplasia due to unopposed oestrogen. Animal studies have demonstrated that the addition of progestagens to the therapy can encourage projections from the cell body of the neuron (neurite outgrowth) which can lessen beneficial effects of oestrogens in the brain (Woolley & McEwen, 1993). Human studies have more mixed results but the majority show a diminished effect of oestrogen on cognition if progestagens are included (Ohkura et al., 1995; Rice et al., 2000) and some demonstrate no effect (Hogervorst, Riedal, Boshuisen, & Jolles, 1999). Many studies consider oestrogen only (such as the WHIMS) and for the general population, most women will be prescribed a therapy containing progestins.

It is important to consider the error of drawing conclusions concerning the effect of one hormone on brain function (oestrogen) when in some cases two hormones are administered (oestrogen and progesterone; Sherwin, 2008). Wegesin and Stern (2007) demonstrated a difference in executive function during a source recall task between HRT users (oestrogen only) and HRT users (oestrogen and progesterone combined). Participants receiving oestrogen only outperformed non-users on the Wisconsin card sorting task but those taking the combination therapy performed no differently to non-users of HRT. The researchers concluded that the addition of progestins to HRT may reduce the benefits of oestrogen on executive function. Lee and McEwen (2001) suggest that, despite the effects of progestins on the brain being poorly described, they may have an opposite effect on cognition to oestrogen. Based on this, Sherwin (2008) is adamant that the effects of oestrogen and progesterone should be considered separately on cognitive function. In the present study, participants were asked if their therapy was oestrogen only or contained progesterone. The addition of progesterone did not have an effect on cognitive performance in this case, but the group sizes were too different to draw conclusions at this point.

Brinton (2001) suggests that we now have enough information to develop a therapy designed for the unique requirements of the brain to help prevent degenerative disease. The addition of progesterone to a hormone replacement regimen can cause many women to have additional side effects of irritability and depression, causing detrimental effects on mood and general sense of wellbeing (Panay & Studd; 1997). Yet the side effects of progesterone and progestins have been shown to be dependent on dose and route of administration. If progesterone is administered orally it seems to produce the greatest side effects

(deLignieres, Dennerstein & Backstrom, 1995), can lead to a decrease in dendrite density (Wooley & McEwen, 1993) and this can lead to a decrease in cognitive performance scores when compared to an oestrogen only group (Rice et al., 2000). Differences of physiologic effects on the brain can be seen between oestrogen only users and oestrogen plus progesterone users on completion of cognitive tasks using PET (Berman et al., 1997). Follow up studies should focus on the potential detrimental effects of HRT when initiated during the post-menopausal stage considering the effects of oestrogen only therapy and oestrogen plus progesterone on WM capacity. Route of administration should also be considered. This may avoid cognitive decline in late HRT initiators.

On-going studies

The Kronos Early Estrogen Prevention (KEEPS) trial has been designed to assess a variety of potential differences in health between users of oral conjugated equine oestrogens, transdermal oestradiol and a placebo control. The primary goal is to increase knowledge regarding HRT's potential effect on heart disease. A small component of this study will look at cognitive differences between the groups. At present there are 720 women assigned randomly to one of the three conditions and the intervention will have a duration of at least five years which may help to give early indications of differences between the groups for cognitive decline. Similar to the present study, the researchers will initiate the therapy at the time of the biologically defined menopause. In theory, this should mean that HRT is initiated at the critical point, and should show most beneficial effects to cognition. However the women participating in KEEPS are 42-58 years old with their last menstrual cycle occurring within six months to three years. The present study demonstrated a detrimental effect of HRT on WM

when initiated as little as 12 months from the last menstrual bleed, meaning that a proportion of the women recruited in the KEEPS study will be missing the critical point of initiation. Researchers intend to evaluate the relationship between whole brain and ventricle volumes as well as the relationship between cognition and vascular risk factors. A further study will test the effect of HRT on STROOP task performance. It is not yet clear what other cognitive domains will be assessed, but tasks of WM would be useful, as these relate to every day memory. These are the types of cognitive issues that women complain about when they reach the menopause, and the present study was able to confirm this by demonstrating a difference in performance on outcome measures that assess every day memory.

Further thoughts to consider and GP recommendations

Current developments in large population-based studies put into question the safety of long-term use of oestrogen. Although it may protect against colorectal cancers and osteoporosis, it may also increase the risk of breast cancer and coronary heart disease (Rossouw et al., 2002). Sherwin (2007) suggests that HRT use is relatively safe if it is not used beyond four years, which encourages the question 'when is the right time to take HRT for optimal protective effects?' Memory complaints during the menopause are the largest reported problem (Norbury et al., 2003, Philips & Sherwin, 1992) and whether physical health or cognitive health should be prioritised in doctor recommendations for HRT use is yet to be considered.

HRT has clear benefits on physical symptoms, the clarity of the benefits of HRT on cognitive symptoms is less well defined, and the present study helps

contribute to further clarification of the research field. For physical symptoms, HRT is initiated if those symptoms exceed a certain point (and not otherwise, due to increased risk of oestrogen-related cancers). It is important that the impact of HRT on cognition is well defined in order to ascertain whether the benefits on cognition are substantial enough to prescribe HRT knowing the risks. WM represents normal day to day memory, and problems in WM experienced during the menopause are subjective. Whether these issues warrant the use of HRT, or are just minor annoyances, will be up to the patient. But if the optimal use of HRT for WM is established, this will have large contributions to the decision making process. The present study helps to define the critical period as within 12 months of last menstrual bleed, meaning that for those with problems in WM experienced during the menopause, HRT should not be recommended as alleviation for after this point.

Summary

The present study adds insight into the potential long lasting effects of early HRT initiation, and supports a critical period for neuroprotection.

There has been much speculation regarding the details of the critical period hypothesis, and in particular the possible outcome of HRT if taken at different stages in the climacteric. Generally, less is understood about what happens if HRT is taken post-menopause. Discrepancies in the literature led to the suggestion of the critical period hypotheses, which has been described and speculated upon in several studies, but not directly tested. The present study offers unique insight into the critical period, suggesting the window of opportunity for HRT initiation ends as little as 12 months after last menstrual

bleed. The study was able to achieve this by testing women grouped according to stage in the climacteric, and not simply based on age.

Oestrogen enhances performances on tasks dependent on the pre-frontal cortex, although the effects are complex and can be affected by many factors, such as length of time elapsed since last menstrual bleed (Daniel & Hulst, 2006; Dohanich et al., 2009). As is currently understood, oestrogen receptor sites become less active in the brain during the menopause as oestrogen levels decline. This causes declines in WM. If HRT is taken during the early onset of the menopause, the oestrogen supply remains constant and the receptor sites active. Studies have shown WM to be maintained in peri-menopausal women receiving HRT. If HRT is not taken and oestrogen supply to these receptor sites continues to decline, they reduce in activity and initiation of HRT at this point may be overwhelming and damaging. Studies have shown WM to be worsened by late HRT use. The present study adds to the literature, by confirming detrimental effects in WM by late HRT use as little as 12 months beyond the last menstrual bleed. This information has large implications, as a large proportion of women initiate HRT after this time period. Postmenopausal women are in an oestrogen-deprived state and are at risk of stroke and other neurodegenerative disease (Simpkins & Singh, 2008). Therefore it is important to consider the wider implications for early oestrogen therapy use and longer lasting effects.

Current GP recommendations state that the benefits of HRT use up to the age of 60 outweigh the costs. The present study adds to this detrimental list, and guidelines need to consider that late use may have detrimental effects on cognition. However the failure of recent trials to show benefits of HRT in older women makes it more difficult to convince pharmaceutical companies to take on

the expense of new clinical trials. In RCTs different oestrogen preparations have been used in different doses and an abundance of psychometric measures have been used to measure a restricted number of cognitive domains. More robust trials are needed to draw stronger conclusions, where guidelines on suggested time frame for initiation can be reached. Other considerations may include method of administration and duration and style (cyclic or constant). The present study can be used as a guide in future trials where by peri- and post-menopausal groups can be defined by last menstrual bleed, as opposed to age.

The long term effects of HRT are not yet known and it is unclear to whether the neuroprotective effects are longer lasting or short term once the therapy has been terminated. The present study contributes to the understanding by incorporating a longitudinal, observational design, suggesting that the beneficial and detrimental effects of HRT on WM dependent on time of initiation are longer lasting than first anticipated. RCTs don't often incorporate a long follow up and longitudinal studies tend to review the longer term use of HRT rather than the period after treatment cessation. This study has reviewed the period after treatment cessation which adds a unique level to the research and further studies may also want to consider this in their study design.

The present study clearly demonstrates a critical period for HRT initiation, and that this impacts on WM. The researchers' controlled statistically for participant characteristics that are known to independently influence cognitive functioning in older people such as age, level of education and health. In the present study, mean differences between the four groups were greatest for the Rspan and Ospan tasks. These are challenging WM tasks with a verbal constraint. Collectively, the WM span tasks were a good measure of WM capacity for this

group and have confirmed both a beneficial time and a potentially damaging time of initiation of HRT on WM. Discrepancies between other research may, in part, be due to inappropriate tasks chosen. Future studies should consider both the difficulty and nature of the tasks they choose, making sure they demonstrate executive functions that are mediated by either the pre-frontal cortex or hippocampus. Groups should be defined by last menstrual bleed, and not age, where age can be used as a covariate. Observational studies can contribute to our understanding of the longer lasting effects of HRT initiation on cognition, research that has greater need for expansion.

Phytoestrogens as an alternative to HRT

4.1 A history of Hormone Replacement Therapy

HRT can be received as a continuation of the menstrual cycle thus allowing the prolongation of fertility, however most women primarily incorporate HRT into their endocrine system in order to prevent and reduce menopausal symptoms such as hot flushes, a decreased sex drive, sleeping problems, mood swings and cognitive decline (Cassidy et al., 2006; Sherwin, 2008). This is not the sole reason for taking the therapy and evidence suggests that HRT also reduces the risk of osteoporosis and heart disease (Wuttke, Jarry, Westphal, Christoffel, & Seidlova-Wuttke, 2003). Opting to take HRT in order to improve quality of life during the menopause is not taken lightly by practitioners and many patients are reminded of the potential detrimental effects of the treatment to health during their decision making process. These unfavourable health effects have slowly been brought to light in the past four decades and it is these potential damaging effects that challenge the dispensation of HRT causing women to seek alternative routes.

HRT was made available in the 1930's to treat hot flushes and other physical changes that women reported as experiencing during the menopause. The first brand of HRT coined Premarin originated from America and contained conjugated equine oestrogens only. It was not until the 1950's that concern

began to stir for those receiving the therapy when an increased rate of uterine cancer was identified in those using the treatment (Grey, 1951). To combat bad press, by the early 1960's a new list of reasons why women should initiate the treatment began to accumulate, and several books such as *Feminine Forever* (Wilson, 1966) were available claiming that with HRT, 'the menopause is completely preventable'. The researcher explained that when hormone levels plummet during the menopause, women lose their femininity, making them no longer completely female. Yet HRT would replace the hormones lost, allowing women to keep their femininity and be less boring. Sponsored by the drug companies responsible for the sales of HRT, Wilson travelled the country in order to deliver this information during several national lectures. Alas, in the mid 1970's a link between oestrogen consumption and endometrial cancer was established (Mack et al., 1976; Smith, Prentice, Thompson, & Herrman, 1975; Ziel, & Finkle, 1975). This started a decline in women choosing to take HRT. Current non users were compared to HRT users to reveal that those who had used oestrogen therapy for five years or less were five times more at risk than non-users of endometrial cancer, whereas those who exceeded the five years of use were 14 times more at risk (Voigt et al., 1991). From 1975-1976, oestrogen use declined by 18% and then another 10% from 1976-1977. By 1980, the number of annual oestrogen prescriptions had fallen by 50% in the US (Col, Fairfield, Ewan-Whyte, & Miller, 2009).

Manufacturers carried out extensive research and soon recognised that with the simple addition of synthetic progesterone the risk of endometrial cancer could be reduced (but not eradicated). A new product Prempro was manufactured, combining oestrogen with synthetic progesterone. Doctors were

free to prescribe the combined treatment to women with a uterus and at potential risk to endometrial cancer, whilst those who had undergone a hysterectomy received the oestrogen only treatment as usual. Recent research has established that, although the combined treatment may be better for lower risk of endometrial cancer, oestrogen alone can help to prevent coronary heart disease (Mendelsohn, & Karas, 1999). However HRT should not be used for the prevention of heart attack or stroke (Hully et al., 1998; Rossouw, 1996).

Following the association between HRT initiation and endometrial cancer (Smith et al., 1975), by 1975 an association was also made between HRT initiation and breast cancer (Lippman, Bolan, & Huff, 1975).

By the late 1970's and early 80's HRT was promoted as a treatment for aging, specifically as a cure for wrinkles, aches and pains, as well as menopausal specific symptoms such as hot flushes, insomnia and weight gain. It was also promoted as an aid for disease prevention in AD, depression and heart attack where several observational studies suggested that HRT might protect women against heart disease (Stampfer, Willett, & Colditz, 1985). By the 1980s, several studies showed that oestrogen was effective in slowing the diminishment of bone density thus helping to prevent osteoporosis (Lindsey, Hart, Forrest, & Baird, 1980). In 1986, the food and drug administration reviewed the current evidence and suggested that HRT might indeed be an effective treatment for osteoporosis, but the studies completed on the prevention of heart disease were not adequate to support the use of HRT for this purpose. By the 1990's, women began to wonder if HRT really was essential, and question why all women were given the same dose, despite menopausal symptoms being different in degree

and occurrence between individuals (Daly, Gray, Barlow, McPherson, & Roche, 1993).

In 1998 Hully and colleagues published a study (the HERS study) looking at the effects of HRT on heart disease. The researchers invited women with heart disease to take part in an intervention and found that those receiving the therapy had an increased risk in heart attack. The study was dismissed by HRT proponents who stated that the study did not apply to healthy women. In 2002, the women's health initiative completed a large study investigating the potential protective effect of HRT on heart disease, and released evidence to suggest that HRT had no protective effect on heart disease and stroke and that perhaps it could even be responsible for an increased risk in these health issues. The first publication to arise from the study suggested that women who took the combined version of the treatment were at risk of breast cancer, stroke, heart attack and blood clots. Being such a large-scaled study, results had a huge impact worldwide and prescription rates faced a significant drop. Some women later returned to the treatment once their menopausal symptoms returned and were of unbearable degree, however research showed that over half the women who ceased to take the treatment felt little discomfort as a result (Ockene et al., 2005).

Since serious health issues associated with the initiation of HRT have been brought to the attention of health professionals, doctors have been much more cautious in the prescription of HRT, only prescribing the treatment as a last resort for a limited time period only (approximately two months to begin with and no longer than five years). Since the completion of the WHI study, several studies have contested the propositions made suggesting HRT may in fact be

beneficial for the protection against specific disease as well as detrimental to others. Researchers have been tweaking the data from the WHI study to see if there is a subset of women who may benefit from HRT as well as identifying subsets who should avoid it (writing group for the women's health initiative investigators, 2002). Primarily, those with a history of hormone specific cancers, blood clotting, heart disease and stroke should not be offered HRT unless there is no other alternative and their quality of life has vastly lessened.

An alternative treatment is the use of biomedical hormones. These are biochemical structures identical to female hormones of oestrogen, progesterone and testosterone that are normally manufactured by the endocrine system in the ovaries. They are crafted to be identical to that of the individual. In a recent large study completed in France by Fournier, Berrino and Clavel-Chapelon (2008), eighty thousand women with the combined average age of 52.4 years were observed over a twelve year period. Analysis revealed levels of breast cancer to be significantly lower in those receiving oestrogen combined with bioidentical progesterone when compared to those receiving oestrogen only or those combined with the normal form of progesterone, both of which had a marked increased risk in breast cancer.

Although biomedical hormones may decrease the risk of breast cancer when compared to synthetic hormones, the risk is still increased when comparing biomedical hormones to no alternative sought. The development of identical biomedical hormones means the therapy can function within the patient's own biomedical structure to try work naturally with the body, however the detrimental effects are only slightly less to those of HRT and as such, little is resolved by choosing this route.

The decision to initiate HRT is individual and the benefits, side effects and risks will differ depending on the individual. It is up to the practitioner to best advise the patient to whether HRT is a reasonable option to consider in the reduction of menopausal symptoms or not. At present, there are a range of alternatives that can also be offered, such as the biomedical hormones or, in more recent years, natural alternatives and phytotherapy.

4.2 Natural Solutions

In the past three decades, the prospect of more natural supplements to help reduce menopausal symptoms has received much deliberation, with particular attention to the idea that natural supplements may avoid the detrimental problems associated with HRT. The concept of diet and its role in menopausal symptoms emanated from literature speculating the differences between Western women and Asian women, who have a diet rich in soy and report fewer menopausal symptoms (Vincent & Fitzpartick, 2000). Among menopausal women in Asian countries, 14-18% reported experiencing hot flushes as compared to 70-80% of European women (Barlow, Brockie, & Rees, 1991). Umland and colleagues (2000) suggest that the differences in experienced menopausal symptoms are largely due to differences in diet, where Asian women consume high levels of soy. Also deriving from exploration of the Asian diet on menopausal symptoms, epidemiological studies have revealed lower incidences of breast and ovarian cancer and coronary artery disease in those with soy-enriched diets (Adlercreutz & Mazur, 1997). In response to these

findings, a natural alternative was sought which will 'mimic' HRT's more beneficial effects whilst avoiding its disadvantages.

Following a line of investigation over the past three decades, specific alternative supplements to HRT have been identified, deemed to target and help reduce a range of menopausal symptoms in both healthy women, and women with breast and cervical cancer (Messina & Loprinzi, 2001). Some of these alternatives include the following:

Agnus Castus stimulates and normalises the function of the pituitary gland which controls hormonal balance in the body. It has been shown to reduce hot flushes and menopausal symptoms in 45 – 60 year olds (Abbaspoor, Hajikhani, & Afshari, 2011).

Black Cohosh is often used to relieve menopausal symptoms such as hot flushes and vaginal dryness, however research suggests it may not be effective in doing so (Leach & Moore, 2012).

Red Clover is effective in alleviating hot flushes (Booth et al., 2006). However, due to its coumarin content (which can prove toxic to the liver and kidneys if accumulated over time), it should be used with caution on those suffering from coagulation disorders.

Other supplements that have been claimed to help with menopausal symptoms, but with little or no empirical evidence include Evening primrose oil, Femal, Maca, Ginkgo Biloba and Sage.

Concern over potential adverse effects of HRT (Nelson, Humphrey, Nygren, Teutsch, & Allan, 2002) and recognition of menopausal symptoms being reported as less frequent in Asian women has led to increased interest in the use of phytoestrogen supplements by women experiencing menopausal symptoms (Farquhar et al., 2009). Epidemiological studies demonstrate associations between diets high in phytoestrogens and a reduction in menopausal symptoms, as well as a reduction in breast cancers and coronary heart disease, making phytoestrogens a suitable substitute for HRT (Umland, Cauffield, Kirk, & Thomason, 2000).

4.3 Metabolism, hormonal action and brain plasticity of phytoestrogens

Phytoestrogens are dietary components found in some plants and are comprised of isoflavones, lignans, coumestans and prenyl flavonoids (Cassidy, 2004). Humans are exposed to phytoestrogens on a regular basis due to their widespread use in a variety of products (Lephart, Setchell, Handa & Lund, 2004). The most accessible phytoestrogen is the isoflavone, which has been extensively studied (for example, Whitten & Patissaul, 2001). Isoflavones are organic compounds belonging to the legume family and act as phytoestrogens in the body. Isoflavones can be found in a range of food sources, such as soya. Because of the isoflavone's accessibility with in soya, most studies have focused on soya as a source of phytoestrogen.

The interest in isoflavones, specifically to human health, has increased over the past 15 years, with particular interest in their structural similarity to oestrogens, resulting in biological activity related to oestrogen receptor

mediated mechanisms (Messina, 2007), and their potential to be an alternative to HRT (Atmaca et al., 2008).

In animal diets, isoflavones remain inactive until they reach the gastrointestinal tract, where they become active. The active forms can then be metabolized to equol, similar to 17-beta oestradiol (Lephart et al., 2004). Phytoestrogens are structurally and functionally similar to oestradiol, and this enables them to bind to oestrogen receptors (Knight & Eden, 1995) and act in vivo like weak oestrogens (Fletcher, 2003). The organic structure of 17-B-oestradiol and isoflavones can be seen in figure three for comparison.

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Figure 3: Isoflavone and 17-B-oestradiol organic structure, cited from Rice & Whitehead (2006)

Humans do however produce low levels of equol in comparison to rodents, at approximately 30% (Setchell, Brown, & Desai, 2002). In addition, phytoestrogens act like weak oestrogens as their affinity for binding is only between one-500th and one-1000th of that of oestrodinol, resulting in weak bindings to oestrogen receptors (Verdeal, Brown, Richardson, & Ryan, 1980). Nevertheless, phytoestrogens reach concentrations sufficient to elicit response (Knight & Eden, 1995) and by acting in the same way as oestrogen (Miniello et al., 2003), the phytoestrogen is able to partially mimic its activity and this induces

similar responses though, some less mild within the body (Karch, 1999). The compounds may affect oestrogen action (Hwang et al., 2006), acting as oestrogen agonists and antagonists depending on the context (Hooper et al., 2009) or by affecting the enzymes involved in steroid metabolism altering oestrogen circulation (Lacey et al., 2005).

When phytoestrogens are metabolized, they bind onto the same receptor sites as do oestrogens, however if a phytoestrogen binds to a receptor this prevents oestrogen from exerting its effects. In menopausal women, oestrogen levels are low, and phytoestrogens may add to total increase of systemic oestrogen effect. But this will always be at a reduced level as compared to normally menstruating women, where phytoestrogens have about two percentage the 'strength' of oestrogens (Werbach & Murray, 1994). This puts into question the likelihood of substituting HRT with isoflavone use.

A broad range of oestrogens and oestrogenic molecules, such as isoflavones and phytoestrogens can promote the chemical antioxidant effects of oestrogens due to their chemical activity in the brain (Brinton, 2008). A smaller subset can activate biochemical cascades required for neuroprotection and an even smaller subset can activate the genomic mechanisms of oestrogen-induced neuroprotection (Behl, Moosmann, Manthey, & Heck, 2000; Brinton et al., 2000; Nilsen & Brinton, 2000). This is because phytoestrogens are able to enter the brain and act centrally as well as peripherally and evidence from human and animal studies suggests that phytoestrogens could affect cognition directly as well as by modulating the hormonal milieu (Hill & Dye, 2003). In their meta-analysis, Hill and Dye suggest that phytoestrogens have the potential to

influence cognition through neural impact and oestrogenic activity and that this may be most beneficial in females entering the menopause.

Pan, Anthony and Clarkson (1999) demonstrated that phytoestrogens regulate nerve growth and brain-derived neurotropic factor in the PFC and hippocampus of female rats. Luine, Attalla, Mohan, Costa and Frankfurt (2006) also showed that a diet rich in isoflavones can result in an increase in spine density of pyramid cells in the PFC of female rats, the same area abundant with oestrogen receptors (Tang, 2004), where WM and SA are mediated. Duffy and colleagues suggest that isoflavones target ER-beta, found in the PFC and hippocampus, and this can result in improved cognitions mediated by these brain areas.

Lephart and colleagues (2002) have demonstrated brain changes in rats receiving isoflavones and evidence from their study shows the female rat to be able to metabolise isoflavones well. However there is less evidence regarding metabolism of isoflavones in humans, and to what extent this affects the structure of the human brain. Although rat studies have demonstrated a change in dendrite density and increased nerve growth in parts of the brain after exposure to isoflavones, other research suggests that isoflavones are not easily metabolized by humans. In their study, Setchell and colleagues (2002) investigated the bioavailability of isoflavones and mechanisms of intestinal absorption of an isoflavone supplement or soymilk containing isoflavones in humans at different points following exposure. The concentration of isoflavones was directly measured in timed blood samples one, two and eight hours after ingestion, and no traces of isoflavone were found.

Kreijkamp-Kaspers and colleagues (2004) investigated plasma levels of isoflavones in the blood after exposure of either 100 mg of isoflavone or a placebo in post-menopausal women. After 12 months of exposure, no difference was found between the two groups, suggesting poor absorption of the supplement. In contrast, isoflavone content is detected in blood plasma of Asian women who have diets high in isoflavone, suggesting again that it may be western women who fail to metabolise isoflavones (Adlercreutz et al., 2000).

Isoflavones have been extensively studied and found to improve insulin regulation and bone health (Scheiber, Michael, Rebar, & Robert 1999; Wagner et al., 2008) and to decrease frequency and severity of menopausal discomforts such as vaginal dryness, hot flushes and night sweats (Sacks, Lichtenstein, & VanHorn, 2006). Isoflavones have also been shown to have a 'rejuvenating effect' on the brain to significantly improve category fluency after a soy intervention, with trends in improved verbal memory, however unlike other phytoestrogens, isoflavones have been shown to prevent the growth of breast cancer cells (Kritz-Silverstien, Von Muhlen, Barret-Connor, & Bressel, 2003). As such, there is a potential for isoflavones to improve cognition whilst offering minimal detrimental effects on general health.

4.4 The effect of Isoflavones on menopausal symptoms

Regular consumption of Isoflavones is thought to confer health benefits as research has shown they may play a role in lowering risk for a range of diseases. For example, soy isoflavones appear to reduce cardiovascular disease risk by inhibiting the growth of cells that form atherosclerotic plaques (Anthony, Clarkson, Hughes, Morgan, & Burke, 1996) and population-based studies show a

strong association between consumption of isoflavones and a reduced risk of breast and endometrial cancer (Messina, 2007).

In question is the applicability of substituting HRT with isoflavones in the hopes of reducing menopausal symptoms to some significant degree. Isoflavones have been shown to help reduce menopausal symptoms and are often recommended by manufacturers as a natural alternative. Specifically, Isoflavones have been shown to reduce hot flushes. In their study, Chedraui, Miguel and Schwager (2011) evaluated the effect of soy derived isoflavones on hot flushes and mood in a high risk population. Fifty women aged 40-59 with a BMI over 25 (suggesting obese) received 100mg of isoflavones daily over a three month period. Of these, 45 women completed the study. Menopausal symptoms (using the Menopause Rating Scale), frequency and intensity of hot flushes and mood (using the Hamilton Depressive Rating Scale) were assessed at both baseline and at 90 days. After three months of supplement treatment, menopausal symptom score significantly decreased to suggest less suffering as did the frequency and severity of hot flushes. Depressive rating scores significantly decreased to suggest improved mood. There was no effect on blood pressure levels or BMI values after treatment. The study did not use a placebo group as a control, so it is difficult to state whether the improvements were expectancy effects. Also, the population were obese and not reflective of a normal population with normal health.

As well as mood and hot flushes, soy isoflavones may help in the preservation of bone substance and to prevent or reduce risk of osteoporosis. Unlike oestrogen, which helps prevent the destruction of bone, evidence suggests that isoflavones may also assist in creating new bone, which helps

prevent the development of osteoporosis. In their study, Atmaca and colleagues (2008) demonstrated that soy isoflavones have beneficial effects on bone mineral density, bone turnover markers and bone mechanical strength in postmenopausal women.

Much like the HRT literature, the effect of isoflavones on menopausal symptoms is mixed and unclear. Although some studies suggest that isoflavones may alleviate menopausal symptoms (Ma, Qin, Wang, & Katoh, 2008; Marini et al., 2007) others have found no such effect (Nelson et al., 2006; Sacks et al., 2006). St Germain, Peterson, Robertson and Alekel (2001) randomised 69 menopausal women to either a placebo or isoflavone treatment (80mg/day) over 24 weeks of treatment. A menopausal index was used at baseline and following the intervention to compare hot flushes and sleeping patterns. Both groups reported a reduction in hot flushes, yet analysis revealed no difference in hot flush severity before and after treatment. Hot flushes are the primary reason that women seek medical intervention (Tice et al., 2003). The effects of soy isoflavone intake on the frequency of hot flushes have been examined in a number of RCTs (Howes, Howes, & Knight, 2006; Huntley & Ernst, 2004; Kronenberg & Fugh-Berman, 2002) and results have been mixed. In their reviews; Krebs, Ensrud, MacDonald and Wilt (2004) and Huntley and Ernst (2004) found that only one out of eight RCTs of soy foods reported a significant reduction in the frequency of hot flushes. Three out of five trials using soy isoflavone supplements reported a significant reduction. Only four out of ten studies using soy preparations found improvement in hot flushes. Improvements were described in both reviews as small.

Generally, the impact of isoflavones on menopausal symptoms such as hot flushes have been mixed and whilst evidence suggests isoflavones may alleviate such symptoms (Vincent & Fitzpatric, 2000) other studies have demonstrated no or little effect as compared to HRT (Lephart et al., 2002).

Results of isoflavones on menopausal symptoms indicate that the effects of isoflavones on cognitive change during the menopause may also be mixed. Similar in design to study one of the thesis, the present study will be considering isoflavones as a suitable substitute for HRT to prevent cognitive decline.

4.5 The effect of Isoflavones on cognition

With isoflavones and soy having mixed results on bone health, hot flushes and cholesterol, it must be considered that the results for cognition may also be mixed. Similar to the HRT literature, research has demonstrated a positive effect of soya consumption on certain cognitive tasks associated with frontal lobe functioning such as SA and planning ability. In their study; Duffy and colleagues (2003) asked participants to consume either 30mg of phytoestrogen supplement twice a day over a 12 week period, or a placebo of 0.5mg of phytoestrogens per day. Participants were asked to complete a test battery including the revised Wechsler short story to measure episodic memory, the Delayed Matching to Sample test to measure short term non-verbal memory, a delayed picture recall task, the Paced Auditory Serial Addition Test (PASAT; Gronwall, 1977) to assess SA and a test of rule reversal and planning ability. On analysis it was revealed that the soya group performed significantly better on each of the tasks. The study was repeated by File, Hartley, Elsabagh, Duffy and Wiseman in 2005 to see if similar results could be found with a briefer period of

soy consumption. Here, 50 postmenopausal women were given one capsule containing 60mg of phytoestrogen isoflavones daily over a six week period, or a placebo capsule containing no soya extract. Participants were tested on story recall, short term non-verbal memory, picture recall and SA. Despite being at the post-menopausal stage, soya consumers performed significantly better in the short term non-verbal memory and the SA task.

The positive effects of isoflavones on cognition have also been demonstrated using an intervention of high soy food products, rather than a capsule. In their study, File and colleagues (2001) looked at the effects of high (100 mg isoflavones/day) versus low (0.5 mg isoflavones/day) soya diets on attention, memory and frontal lobe function in young (students) males and females. The diet was maintained for 10 weeks and participants were tested before and after the intervention. The test battery included the NART and Hospital Anxiety and Depression Scale (HADS) to indicate intelligence and mood. To test attention, participants were asked to complete the digit-symbol substitution (DSS) as well as the digit cancellation (DC) task. The paced auditory serial addition test (PASAT) was used to measure SA. To measure episodic memory, participants were asked to complete a short story recall from the Wechsler scale, as well as test of rule reversal and shifting. For longer recall, participants were asked to recall pictures after a 20 minute delay. To assess semantic memory, participants were asked to complete a category generation task and were given 20 seconds to recall items from (for example) a shopping list. They were later asked to recall as many words beginning with the letter 'S' to demonstrate verbal fluency. Improvements were found in both males and females for long term and short term memory, and in females only for verbal

related tasks. The soya intervention had no effects on the PASAT, DSS and DC. There were no differences between high soya and low soya diet on story recall, however there was an improvement from baseline of the high soya group in both rule reversal and picture recall. In the letter fluency task, there were improvements in the high soya group for females only, where the dietary intervention seemed to hinder the males' performance. The researchers concluded that even weak exposure to phytoestrogens can have a positive effect on episodic and frontal lobe functioning such as verbal memory and SA, and positive effects of isoflavone on cognition were more evident in females. The study was unable to show the positive effects of isoflavones on SA (using the PASAT), as demonstrated by Duffy and colleagues (2003).

Kritz-Silverstein and colleagues (2003) asked 27 healthy women to take 110mg isoflavones per day over a six month period, and compared them to 26 women taking a placebo. The study also measured cognitive functions but in addition to the study by File and colleagues (2001) the intervention took place over a longer six month period. Women were aged 55-74 years of age and had never received any form of HRT. Before and after treatment, participants were asked to complete a category fluency task and a paragraph recall task assessing immediate and delayed verbal memory. Results showed improvement in the category fluency task for women consuming the soy isoflavones. However, unlike studies by Duffy and colleagues (2003) and File and colleagues (2001), there were no improvements in verbal memory. The researchers concluded that isoflavone consumption could be potentially beneficial to the groups' cognitive performance, and in particular verbal memory despite not reaching significant differences.

In a similar study, Ho and colleagues (2007) used a slightly lower dose of 80mg daily isoflavone consumption, consumed by 80 women again during a six month intervention in a double-blind, randomised, placebo-controlled trial, as compared to 88 women on a placebo control. Women were aged between 55 and 75 years and were of normal health, having never received any form of HRT. Women were assessed before and after intervention on tasks of memory, executive function, attention, motor control, language and visual perception. Overall, and in agreement with Kritz-Silverstein and colleagues, there were no significant differences across any cognitive measure between the isoflavone and placebo groups. It could be perhaps that the dose was too low in the Ho study, or that the 6 month period was too long and beneficial effects were missed.

Similar to studies by Duffy and colleagues (2003), and File and colleagues (2005); Hartley, Elsabagh and File (2004) assessed the beneficial role of soya and Gincosan (a natural combination of extracts from ginkgo, a herbal remedy for cognitive problems experienced during the menopause) on frontal lobe functioning. Participants were asked to complete a test of rule reversal and shifting (as in File et al., 2001) and planning ability using the Stockings of Cambridge test (SoC). Overall, no significant differences were found between soya and gincosan users and placebo controls on completion of the rule reversal and shifting, and no significant differences were demonstrated by participants in learning new rules. However, when participants were asked to reverse these rules, the soya groups performed significantly better at completing this more difficult component of the task than placebo controls. On completion of the SoC, those consuming soya in their diet after 12 weeks were able to plan a task better than the placebo controls, also for the most difficult level of the task. The

information combined suggests that soya can help to improve frontal lobe mediated tasks, but this may be dependent on difficulty of the task, and also type of task.

It should be considered that studies with small sample sizes have demonstrated an effect of isoflavone use on cognition (Chedraui, n = 45; Duffy and colleagues, n = 33; File and colleagues, n = 50) whereas studies which have included larger populations (Ho et al., 2007, n = 80; Kreijkamp-Kaspers et al., 2004, n = 202; Fournier et al., 2007, n = 79) have found no effects of isoflavone use on cognition. Sample sizes can be seen in table 2.1.

4.6 Isoflavones and the critical period hypothesis

The effect of Isoflavones on cognition in menopausal women is less developed than the HRT literature, and the collective results do not point to any obvious theories. Core components of the literature have been summarised in table 2.1; and the content will be discussed over the next section.

Table 2.1: A breakdown of the isoflavone literature including age of participant, duration of intervention, supplement potency, sample size and study outcome.

Authors (and Date)	Sample size (total count)	Included age	Study outcome	
Bryant et al. (2005)	23	18-35	Improved verbal memory	
Chedraui (2011)	45	40-59	Reduced menopausal symptoms	
Duffy et al. (2003)	33	50-65	Benefits to memory and attention	
File et al. (2005)	50	51-66	Benefits to non-verbal memory and SA	
File et al. (2001)		18-35	Benefits to verbal memory	
Ho et al. (2007)	80	55-75	No differences on a large cognitive battery	
Hartley et al. (2004)	57	51-66	Mixed results	
Kritz-Silverstein et al. (2003)	53	55-74	Mixed results	
Kreijkamp-Kaspers et al. (2004)	202	60-75	No effect on a large cognitive battery	
Fournier et al. (2007)	79	48-65	No effects on WM or SA	
Authors (and Date)	Duration of intervention	Potency (daily)	Tasks showing benefit of intervention	Tasks which were unaffected
Bryant et al. (2005)	Two months	68mg	<ul style="list-style-type: none"> Verbal memory 	
Duffy et al. (2003)	12weeks	60mg	<ul style="list-style-type: none"> Wechsler short story Delayed Matching to Sample test Delayed picture recall PASAT Rule reversal Planning ability 	
File et al. (2005)	Six weeks	60mg	<ul style="list-style-type: none"> Short term non-verbal memory Sustained attention task 	<ul style="list-style-type: none"> Story recall Picture recall
File et al. (2001)	Ten weeks	100mg	<ul style="list-style-type: none"> Rule reversal Delayed picture recall Letter fluency Verbal fluency 	<ul style="list-style-type: none"> Digit-Symbol Substitution Digit Cancellation PASAT

				<ul style="list-style-type: none"> • Wechsler scale • category generation
Fournier et al. (2007)	Four months	70mg		<ul style="list-style-type: none"> • STROOP • Pattern recognition • Benton Visual Retention Task • Visual-spatial WM • Verbal WM
Hartley et al. (2004)	Six weeks and Twelve weeks	120mg	<ul style="list-style-type: none"> • Task planning ability 	<ul style="list-style-type: none"> • Rule reversal and shifting • Planning ability
Ho et al. (2007)	Six months	80mg		<ul style="list-style-type: none"> • Wechsler Memory Scale • Verbal fluency (EF) • Trail Making Test (EF) • Digit Span (A) • Digit vigilance (SA) • Motor control • Picture recall • Language • Visual perception • Global cognitive function
Kritz-Silverstein et al. (2003)	Six months	110mg	<ul style="list-style-type: none"> • Category fluency 	<ul style="list-style-type: none"> • Paragraph recall
Kreijkamp-Kaspers et al. (2004)	12 months	99mg		<ul style="list-style-type: none"> • MMSE • Rey Auditory Verbal Learning Test • Immediate recall • Delayed recall • Digit Span forward and reversed • Complex attention tasks • DSS • Trail making

Phytoestrogens have a relative likeness to the biological properties of the oestrogen receptor-beta subtype, and some RCTs have shown benefits to frontal lobe cognitions and verbal memory. However groups have been poorly defined, for example in the study by Duffy and colleagues (2003), where the researchers refer to participants as peri-menopausal, when they are between the ages of 50 and 65 years suggesting the majority to in fact be post-menopausal.

The effect of soy isoflavones on the cognitive performance of young peri-menopausal women has been investigated in four studies, two of which report improved verbal memory (Bryant et al., 2005; File et al., 2001). File and colleagues (2001) 10-week study reported that 100 mg soy per day was associated with improved verbal and non-verbal episodic memory, mental flexibility, verbal fluency and planning ability in young women. Bryant and colleagues (2005) double-blind, randomised, placebo-controlled trial of 68 mg soy isoflavones per day administered in prepared soy food across two consecutive menstrual cycles found improvements on verbal memory only, and not for other cognitive functions. And finally, a study by Chedraui et al. (2011) demonstrated reduced menopausal symptoms in women aged between 40 and 59 years of age. These studies confirm a beneficial role of isoflavone use in cognitive function for younger and peri-menopausal women in a handful of the large number of selected cognitive tasks.

Studies using post-menopausal women demonstrate little difference in cognitive performance despite isoflavone use. Kreijkamp-Kaspers and colleagues (2004) found no effect of isoflavone use on cognition in 202 postmenopausal women aged 60 to 75 years. The researchers used a wide range of cognitive assessment material. Participants completed the MMSE as a global test for AD, the Rey

Auditory Verbal Learning Test as a measure of verbal episodic Memory (Schmidt, 1996), the Doors as a measure of visual memory (Davis, Bradshaw & Szabadi, 1999), the Digit Span test as a measure of attention and WM, list generation as a measure of verbal fluency, the Boston naming task for verbal competence and semantic retrieval, the digit symbol substitution test as a measure of perceptual speed (Wechsler, 1945) and the Trail making test as a complex attention and mental flexibility task. Data collection took one morning, which is quite a long period of examination, leading to cognitive exhaustion. It is not clear whether the tasks were counterbalanced in order to control for this. It is possible that the tasks selected were not suitable, however the cognitive test battery did include difficult, frontal lobe mediated tasks (such as the Trail making test). Tasks were also chosen due to being sensitive to oestrogen use in HRT studies. It is more likely that isoflavone use did not benefit cognitive performance in postmenopausal women.

Contrary to this, a handful of trials which have been conducted in postmenopausal women (although some used women as young as 45 years of age) ranging in duration from six weeks to one year, with doses of soy isoflavones between 60 to 100 mg/day have generally shown cognitive effects of soy isoflavones in terms of improvements in memory and frontal lobe function (Casini et al., 2006; File, Duffy, & Wiseman, 2002). Other studies have suggested that the beneficial effects of isoflavones on cognition are confined to women within 10 years of the menopause suggesting that there may be a critical point for isoflavone activation (Kreijkamp-Kaspers et al., 2004; Kritz-Silverstien et al., 2003). And to add final confusion; two recent studies both failed to find positive effects of 70-80 mg/d isoflavones on a range of cognitive functions in younger

and older postmenopausal women (Fournier et al., 2007; Ho et al., 2007). The studies have been summarised for age in table 2.1.

Overall, research has been mixed in approach, using differing amounts of isoflavone per day, sometimes in one dose and sometimes in two daily, over different durations of time. Selected cognitive tasks have also differed in domain, and some studies have used two or three tasks, whereas others have used very large cognitive test batteries. These characteristics of relevant studies have been added to table 2.1 for clarity.

4.7 Summary and study design

Despite the disparity of results within the literature, there are still a handful of traits that can be taken from the successful studies to assist with study design.

Duration of intervention

The extent that phytoestrogens mimic oestrogen within the body is still unclear, and this may be dependent on the dose consumed. In most cases natural treatments have a less potent effect than pharmaceuticals and therefore may take more time to have an effect. They may not show effect as quickly as pharmaceuticals. Studies that have demonstrated a positive effect of isoflavones on cognitive function have ranged from six weeks to six months. Of these studies, two have shown isoflavones to have a significant improvement on episodic memory after 12 weeks (Duffy et al., 2003) and six weeks (File et al., 2005) of isoflavone consumption. Two have shown improvements in SA after 12 weeks (Duffy et al., 2003) and six weeks (File et al., 2005) of isoflavone consumption.

And three studies have demonstrated improved executive function after ten (Kritz-Silverstein et al., 2003) and six or 12 weeks (Hartley et al., 2004) of isoflavone consumption. Studies that have used longer than three months duration (Fournier et al., 2007; Ho et al., 2007; Kreijkamp-Kaspers et al., 2004) have demonstrated no effect of isoflavone intervention on executive function including SA. For this reason, greater than 12 week's isoflavone use is seen as inappropriate. Duffy and colleagues (2003) have included the SART in their study, and another SA attention task, demonstrating positive effects of 12 weeks isoflavone use on SA. Twelve weeks of isoflavone consumption is manageable, appropriate and thus cost effective.

Daily dose of isoflavone

Llaneza and colleagues (2010) recommend that at least 40 mg of soy isoflavones should be prescribed in each routine clinical practice for treatment of menopausal symptoms. However clinical trials have suggested that around 100mg daily is more appropriate. Chedraui and colleagues (2011) identified a positive effect of soy isoflavones on hot flushes and mood when participants were asked to consume 100mg of isoflavones (one tablet) daily over a three month period. Ho and colleagues (2007) used a slightly lower dose of 80mg daily of isoflavone consumption and found no differences in executive function including SA between users and a placebo control. Fourier and colleagues (2007) also found no effect on executive function including SA, WM and verbal WM when initiating 70mg daily. It could be perhaps that these doses were too low to benefit executive function. Providing participants with three months 100mg/daily isoflavone is not only more cost effective (and reduces risk of drop-out rate) but also appropriate based on previous literature.

Isoflavone product

It is not clear whether soy food products or an isoflavone supplement tablet are better for menopausal symptoms. File and colleagues (2001) used food products in their study, and found no benefits on SA using the PASAT, unlike Duffy and colleagues (2003), who used a supplement tablet and did find improvements in SA. Some have suggested that natural forms of isoflavone such as those found in soya milk are more beneficial in reducing menopausal symptoms than the supplement (Cassidy, 2004). Phytoestrogens can be found in smaller quantities in certain fruits, cereals and flaxseed (Rowland et al., 2003) and it should be considered that there is much variability in concentration of phytoestrogen in soya products. Generally, there is four mg of isoflavone per gram of protein, making a glass of soya milk (300ml) contain on average 40 – 50 mg isoflavone, so participants would need to drink 600ml of soya milk to achieve the 100mg daily dose that the literature suggests is effective. For this reason, the isoflavone supplement might be an easier to handle source of soy than soy containing foods in intervention studies. In addition to this, for the present study design it is far more cost effective and convenient to use isoflavone tablets which can be given to the participant at the start of the intervention for them to administer once daily. It is also easy to substitute an isoflavone tablet with a placebo tablet. The content of each isoflavone tablet will ensure that the participant receives the target amount in one dose (100mg of isoflavone per day) rather than expecting them to consume large amounts of food or drink to gain the approximate amount.

Age of participant/stage in the menopause

To specifically test the effect of age on susceptibility to isoflavone; Ho and colleagues (2007) looked at the effect of isoflavone compared to a placebo in women aged 55 - 65 years, and 65 - 75 years of age and found no differences in cognitive performance after six months daily consumption of 80mg of isoflavone verses a placebo. It can be argued that both age groups being investigated were post-menopausal and therefore too similar in cognitive performance other than slight aging effects. None-the-less, a lack of effect in the post-menopausal groups could still support the critical period hypothesis in these cases, and it would be better to compare performance to a younger, peri-menopausal age group based on last menstrual bleed. This will also allow for 'stage' to be defined by symptoms, and not age.

Task selection

Previous isoflavone research has focused on episodic memory. To test episodic memory; several studies as mentioned above have used Wechsler's short story recall task. Of these, two have shown a significant improvement after twelve weeks (Duffy et al., 2003) and six weeks (File et al., 2005) of soya consumption. In the same studies, significant improvements in performance of the Delayed Matching To Sample test (DMTS) has tested short term; non-verbal memory, as well as following twelve weeks of Gincosan treatment (Hartley et al., 2004). Participants demonstrated increased picture recall ability after 12 weeks (Duffy et al., 2003) and six weeks (File et al., 2005) of soya consumption; and 12 weeks and six weeks (Hartley et al., 2004) of Gincosan treatment. The Wechsler short story recall has also been used in the past to demonstrate problems in

verbal WM associated with oestrogen change during the menstrual cycle and the menopause (Sherwin, 1994) but is actually designed to measure short term memory. Study one also demonstrated that Engle's WM tasks are sensitive to oestrogen and can be used to test specific effects of oestrogen change (or isoflavone use) on WM function.

SA tasks have given equally mixed results in isoflavone literature. Studies by Duffy and colleagues (2003), File and colleagues (2005) and Hartley and colleagues (2004) failed to show significant differences between soya and Gincosan users in two tasks of frontal lobe functioning; a test of rule reversal and shifting (IDED) and planning ability using the SoC. However when a more complex rule was added to the IDED the soya groups performed significantly better at completing this component of the task. This suggests that, like WM tasks, the selected SA task will need to incorporate a certain level of difficulty in order to be sensitive enough to measure cognitive change between isoflavone users and placebo controls. The SART achieves this and has been developed to detect subtle impairments of attention.

Studies that have demonstrated a difference in SA between isoflavone users and placebo controls also seem to be dependent on duration of the intervention. Specifically, SA appears to be positively affected by soya consumption over a 12 week period but not a six week period. Studies demonstrating an effect of isoflavone use on SA such as those by Duffy and colleagues (2003) and File and colleagues (2005) have used the PASAT. The PASAT is frequently used by neuropsychologists to measure attentional processing (Gordon & Zillmer, 1997), and this includes assessment of TBI patients with damage to the PFC (Tombaugh, 2006). The PASAT has been used

as a measure of SA (Spreen & Strauss, 1998) and WM in the form of divided attention (Audoin et al., 2003; Cicerone & Azulay, 2002; Webb & Ochs, 2003). The task involves adding together successive pairs of digits read from a list of 61 numbers presented at different speeds. Due to the increase in speed of presentation of the digits across trials, the task becomes very challenging and this is demonstrated by low percent of correct responses (Tombaugh, 2006). Difficulty level in cognitive tasks has been demonstrated to be sensitive to oestrogen change and because the PASAT has different levels of difficulty it can be manipulated for the present study.

It was decided to continue with the use of the SART as a measure of SA and to also include the PASAT to replicate previous isoflavone studies. Both tasks are easy to administrate and quick to complete.

Oldenhave, Jazmann, Haspels and Everaerd (1993) and Ledesert, Ringa and Breart (1995) show a relationship between menopausal status and QoL in both peri-menopausal and post-menopausal women, where those with poor QoL experience worsened menopausal symptoms. For this reason it is important to consider factors that may contribute to better QoL. The role of women's employment status can have effect on QoL, both mentally and physically. P. Jacobs and colleagues (2000) suggest that working outside of the home can have a positive effect on MQoL. They point out that those reaching retirement age are also likely to be at the end of the climacteric change. Their data suggests that some of the decline in QoL at the end of the menopause may be reflecting other social changes in women's lives associated with ageing rather than hormonal changes, making it reasonable to request employment status. It also further determined the necessity to include age. P.Jacobs, Hyland and Lay concluded

that there is a relationship between self-rated menopausal status and QoL, and between lifestyle and QoL. They also include the importance of medical history. For these reasons, the full MQoL scale was included as well as details of participants' current medication, how often in the past few months they had visited a GP and whether they were suffering from any current chronic conditions. By using the full MQoL scale and additional health questions, balance between groups for menopausal symptoms can be checked, as well as potential impacting factors such as other supplements used. The physical symptoms of hot flushes, sleep disturbance and lowered mood, for example, are associated with changes in cognition (Joffe, Soares, & Cohen, 2003). By using all items of the MQoL scale, a large proportion of impacting symptoms will be taken into consideration.

Expected outcomes

Generally the isoflavone literature complements earlier findings of HRT studies whereby intervention can result in improvements to menopausal symptoms (mainly hot flushes, some cover on bone density and cognition, verbal memory). Isoflavone literature has demonstrated improvements in attention and cognition in isoflavones users as compared to placebo controls, but not in all cases. Whether improvements to menopausal symptoms are dependent on time of administration of isoflavones relative to onset of the menopause is yet to be clarified. Due to the undefined and confused nature of the literature, expected outcome is difficult to define in this case. Finally, whether outcome depends on time of administration relative to the menopause will be tested.

4.8 Method

Study Two, Stage One (Baseline)

Participants A total of 55 peri-menopausal women (last menstrual bleed within 12 months) and 57 post-menopausal women (last menstrual bleed in excess of 12 months; total number = 112) between 45 and 65 years of age with normal health were recruited within the Plymouth and London areas through use of internet advertisement, word of mouth and advertisement throughout Plymouth University. Women were randomly assigned into either placebo (53) or intervention (59) groups. The lead researcher asked co-workers to arrange and number the supplements and placebo before they were dispatched to participants; numbers were later matched to the supplement or placebo for data entry. Groups were divided based on peri-menopausal and post-menopausal stage (as per baseline). Participants fitted one of the four levels of study status accordingly; Isoflavone users peri-menopause (n = 29), isoflavone users post-menopause (n = 30), placebo users peri-menopause (n = 26) and placebo users post-menopause (n = 27).

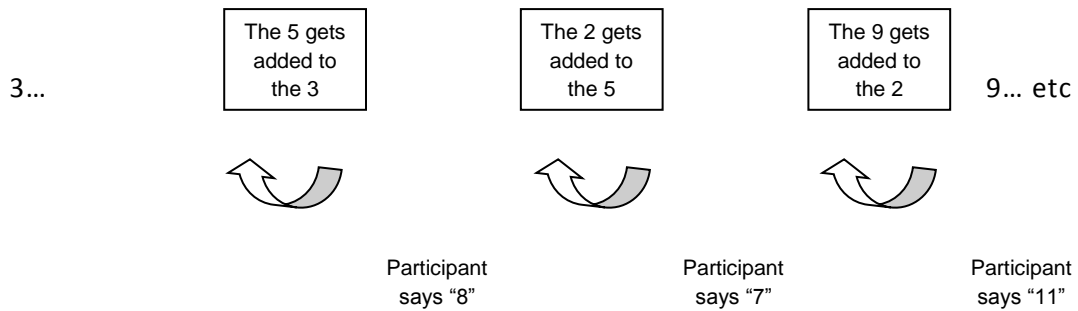
Participants were asked to consume one tablet daily (100mg of isoflavone or a flour pill) over a three month period. All participants were given the same instructions for partaking in the intervention, that one pill should be taken daily with breakfast, and that if a pill was missed during two consecutive days or more than five times on separate occasions across the three months that they should contact the researcher straight away. Participants were healthy with normal ovarian functioning leading to the menopause (those with one or two ovaries removed, the uterus part or fully removed, or a combination were excluded from

the study). Participants were excluded if they suffered from any cognitive degenerative disease, had a history of (or directly related to a descendant with) cancerous cells, having a history of HRT use, suffered from learning difficulties (such as dyslexia, which may interfere with the memory tasks) and were asked to be fluent in English.

Measures Participants provided informed consent. To measure SA, the SART was selected for its easy use and low cognitive load/strain. For a concise account of SART please refer to the measures section of study one, stage one (page 87). As an additional measure of SA, participants were asked to complete the PASAT. The PASAT requires participants to add consecutive numbers as they are presented on an auditory tape and respond orally with the accurate sum. As each digit is presented, the participant must sum that number with the digit that was presented prior to it (rather than with the previous response). An example is shown below.

- The participant hears the starting number 'three' followed by 'five'.
- The participant must add the five to the three and say 'eight' out loud to earn one point.
- The participant hears the following number; the number 'two'.
- The participant must add the two to the previous number heard (five) whilst ignoring their previous response (eight). The participant would be expected to respond 'seven' to earn one point.

A sequence and the correct response are as follows:



Additional forms of the PASAT, which vary in length of the inter-stimulus interval and the number of trials, have been developed for use in research and clinical practice (Sherman et al., 1997; Potter & Barrett, 1999). The standard form consists of sixty-one single digits presented in four trials. The trial begins with inter-stimulus intervals of 2.4 seconds and this time decreases by 0.4 seconds on each of the subsequent trials (Gronwall, 1977). Experimenters can gauge PASAT performance on the average amount of time needed to produce an accurate response by trial. In this version the researchers used three stages to the PASAT identical to the design used in studies by File (2005) and Duffy (2003). The first trial has been set intervals of 2.7 seconds between the stimuli. The second trial has set intervals of 2.0 seconds. A final trial, with intervals of 1.6 seconds between stimuli. The task was selected to measure SA. In the Duffy paper, one more optional trial was used with intervals of 1.2 seconds between stimuli, but it was agreed that this speed was too quick, unrealistic, and put participants under too much strain to use in this trial. Thus, three trials with accelerating speed were used.

To measure WM, two of Engles' WM tasks were selected including RSpan and OSpan as a sensitive and thorough measure of WMC (as demonstrated in study one). For a concise account of these WM tasks, please refer to the measures section of study one, stage one (pages 83-86).

To measure mood, participants were asked to complete the GHQ-12 (see study one, stage one on page 88). The 12 items were scored and added to a total. Participants were asked to complete all 42 items of the MQoL scale, rather than the shortened version used in study one. As before, they were asked to respond to the range of menopausal symptoms by rating that symptom on a six point scale to how strongly they had been feeling it over the past few months. For example; participants would be asked "I can concentrate easily" to which they would response from "I am never like this" to "I am always like this" over a six point scale. Items were scored and added to a total. A full version of the MQoL scale can be found in appendix A; page 290.

Individuals were asked for their age, level of education. Details of the menopause were requested to determine whether the participant was peri or post-menopausal according to the approximate date of their last menstrual bleed. The questionnaire enquired into possible sources of natural oestrogens in the body, such as soya supplements. Details regarding general physical health including medication taken, visits to the local GP within three months and any chronic conditions were recorded. Participants were asked to highlight food groups which dominated their diet, how many times a month they exercised (minimum of 30 minutes for each session) and if they were smokers or non-smokers. To conclude, participants were asked about their general thoughts on cognition and the menopause, and whether one can affect the other. These

where categorised and scored. The included questions can be found in appendix A, page 287.

Procedure Participants completed the study on University campus or in their own homes. Participants were asked to complete the SART on a lap top using the space bar to respond. They were then asked to complete the PASAT by listening to the audio recording as the researcher wrote down their verbal responses. This was then followed by the RSpan and the OSpan on the lap top, to which the researcher recorded responses. As counter-balancing the tasks had no effect on the results of study one, tasks were not counterbalanced this time. Participants were then presented with the GHQ-12, the MQoL scale and biographical questionnaire on paper, and asked to respond to each item where possible.

Study Two, Stage Two (first follow up)

Participants A total of 104 women returned to complete the study. This included 25 (of 29) peri-menopausal isoflavone users, 24 (of 26) peri-menopausal placebo users, 29 (of 30) post-menopausal isoflavone users and 26 (of 27) post-menopausal placebo users.

Measures Participants were asked to complete the same test battery three months later to assess potential effects of the intervention against baseline. A short questionnaire recorded any changes since the last meeting. Specifically, participants were asked if they had experienced any changes to the menopause, including if peri-menopausal, whether they had initiated HRT, if there were any changes to their supplement intake, details regarding general

physical health including medication taken, visits to the local GP within three months and any newly developed health conditions were recorded.

Procedure Participants were contacted every four weeks by email and/or telephone to check how they were fairing with the supplements. Two and a half months after the initial assessment, participants were contacted via email and/or telephone to arrange a follow up appointment to coincide within the week of their last supplement intake. Participants completed the study on University campus or in their own homes as previous.

Participants were asked to complete the SART, the PASAT, the RSpan, the OSpan, the GHQ-12, the MQoL scale and the biographical questionnaire as per baseline.

Study Two, Phase Three (final follow up)

Participants A total of 97 women returned to complete the study. This included 23 peri-menopausal women in the intervention group, 27 post-menopausal in the intervention group, 23 peri-menopausal women in the control group and the remaining 24 being post-menopausal placebo controls.

Measures Participants were asked to complete the same test battery to assess intervention against baseline. A short questionnaire recorded any changes since the last meeting. In addition to the previous two meetings, at the end of the trial participants were provided with information regarding the study and a Marks and Spencer voucher (to the value of £20) as a thank you for their time.

Procedure Two and a half months after the first follow up assessment; participants were contacted via email and/or telephone to arrange a final appointment to assess the potential lasting effects of the supplement after a three month break from the treatment. Participants completed the study on University campus or in their own homes as previous.

Participants were asked to complete the SART, the PASAT, the RSpan, the OSpan, the GHQ-12, the MQoL scale and the biographical questionnaire as per baseline.

4.9 Results

A total of 97 women aged between 45 and 64 years of age completed all three stages of the study. Of these, 46 women were peri-menopausal and 51 women were post-menopausal. A total of 50 women were given a three month isoflavone intervention and these women were compared to 47 women who unknowingly received three months of a placebo. Over all; 23 women in the intervention group were in their peri-menopausal stage, with the remaining 27 being post-menopausal. And 23 women in the control group were in their peri-menopausal stage with the remaining 24 being post-menopausal.

Table 2.2 shows mean age, months since last menstrual bleed, GHQ-12 and MQoL scores, GP visits and count of chronic conditions, those using medication and those using other supplements across the four study groups.

Table 2.2: Mean (and standard deviation) age (years), months since last menstrual bleed (months), GHQ-12 score, MQoL score, GP visits in past three months, count of chronic conditions, medication use and supplement use for the four groups

	Peri-menopausal controls	Peri-menopausal intervention	Post-menopausal controls	Post-menopausal intervention
Age (years)	48.96 (3.88)	50.38 (3.87)	54.37 (7.72)	55.10 (4.20)
Last menstrual bleed (months)	4.00 (7.50)	4.07 (4.80)	56.48 (46.99)	64.73 (44.01)
GHQ-12	23.66 (4.24)	23.65 (5.63)	25.70 (6.70)	24.78 (5.36)
MQoL	186.15 (31.62)	195.19 (45.60)	196.20 (37.56)	181.46 (36.30)
Mean GP visits	0.76 (0.95)	0.85 (1.08)	1.27 (1.28)	1.19 (1.12)
Count of chronic conditions	18	17	21	15
Count of medication use	19	17	21	17
Count of other supplements	19	15	19	17

The average age of participants was 52.28 years (SD = 4.87) with an upper age of 64 years and a lower age of 45 years. The two peri-menopausal groups are matched for age, as are the two post-menopausal groups. The two peri-menopausal groups are also matched for time since last menstrual bleed, as are the two post-menopausal groups.

The GHQ-12 was scored conventionally (as in study one). Individual scores of the GHQ-12 were explored to see if the dynamics in the studies population were normally distributed. Table 3.1 shows the average score of the GHQ-12 across each group (lower scores representing worse mood). The sample's mood scores were normally distributed across groups, and groups did not differ between mean mood score dependant on phase ($F(1,108) = 2.27, p = .14, d = .021$) supplement use ($F(1, 108) = 0.19, p = .66, d = .002$) or the interaction between the two ($F(1, 108) = 0.19, p = .66, d = .002$).

The MQoL was scored conventionally (as in study one). Participants rated 48 items and could score between 48 and 288. Differing from the GHQ-12, a higher score represents greater QoL. Average MQoL scores per group can be seen in table 3.1. The sample's mood scores were normally distributed across groups, and groups did not differ between mean MQoL score dependant on phase ($F(1, 108) = 0.04, p = .84, d = .001$) supplement use ($F(1, 108) = 0.10, p = .75, d = .001$) or the interaction between the two ($F(1, 108) = 1.78, p = .19, d = .023$).

To check the health status of participants, women were asked how many times they had visited their GP in the past three months, whether they suffered from any chronic conditions, whether they were smokers, whether they were receiving medication and whether they were receiving other supplements. Details can be seen in table 3.1.

Of the total population, 66.1% ($n = 74$) stated that they were taking medication at time of completion of the study. Medication use did not differ

between peri- and post- users; $\chi^2(1, n = 112) = .02, p = .89$, or between supplement and placebo users; $\chi^2(1, n = 112) = .17, p = .68$.

Participants were also asked if they suffered from a chronic condition, to which 63.4% of the population ($n = 71$) indicated that they suffered from a chronic condition. Most frequent conditions included underactive thyroid, asthma and general aches and pains. The total count of reported conditions can be seen in table 2.3. Count of chronic condition did not differ between peri- and post- users; $\chi^2(1, n = 112) = .00, p = .96$, or between supplement and placebo users; $\chi^2(1, n = 112) = .39, p = .53$.

Table 2.3: Count of reported chronic conditions across the total group

Condition	Count
Allergies	3
Asthma	20
Chromes disease	2
Diabetic	2
HBP	7
High cholesterol	2
Insomnia	1
IBS	3
Low mood	7
Migraine	4
Osteoporosis/Arthritis/ Back/bone pain	18
Underactive thyroid	11

Participants were asked if they were taking any other supplements. This was of interest, as it was expected that most women taking part in the study would already be consuming supplements as a part of their daily diet. A high proportion of participants (62.5%, $n = 70$) were already taking other supplements/vitamins. Supplement use did not differ between peri- and post-users; $\chi^2(1, n = 112) = .02, p = .88$, or those with and without a history of HRT use; $\chi^2(1, n = 112) = .19, p = .66$. Most frequent supplement use included Vitamin B, calcium, cod liver oil and multi-vitamins. Because of the high frequency of those taking supplements, it was assumed that these women would be used to the daily regime of supplement use. Those already including soy in their daily diet indicated that they were not consuming a large enough quantity to reflect the 100mg dose in the current study (for example, soya milk). For this reason they did not need to be excluded from the study. Descriptions and frequencies of these supplements can be seen in table 2.4.

Table 2.4: Count of reported supplements/vitamins

Supplement	Count
Anti-oxidant	2
Calcium	17
Cod liver/fish oils/ essential fatty oils	17
Garlic	2
Glucosamine	7
Multi Vit	17
Primrose	9
Selenium	3
Soya	6
Vit B/iron/folic acid	19
Vit C	9
Vit D	4

Participants were asked for their highest level of education. Total count of highest level of education across intervention groups are seen in table 2.5.

Table 2.5: Count of education level across intervention groups

	Peri-menopausal intervention	Peri-menopausal placebo	Post-menopausal intervention	Post-menopausal placebo
Alevel	8	7	3	3
GCSE	5	4	4	
Postgrad	8	2	10	10
Professional				2
Undergraduate	8	13	13	12

Level of education did not differ between peri- and post- users; $X^2(1, n = 112) = 11.43, p = .22$, or supplement and placebo users; $X^2(1, n = 112) = 5.21, p = .27$.

The groups were similar in terms of mental and physical health, education and employment. The peri-HRT and peri-control groups matched each other for age. The post-HRT and post-control groups also matched each other for age and were older than the peri-groups.

RSpan had a strong positive correlation with OSpan ($r = .756, p < .001$) suggesting that as performance of one WM task increased, so does the other establishing that the combinations of these tasks will be a strong measure of WM. The RSpan correlated with PASAT-1 score ($r = .397, p < .001$), as did OSpan ($r = .483, p < .001$) suggesting that as WM score increases, so does SA score. The RSpan also correlated with the more difficult PASAT-2 score ($r = .312, p = .001$), as did OSpan ($r = .341, p < .001$), further suggesting that as WM score increases, so does SA score. OSpan and RSpan, and PASAT-1 and PASAT-2 did not correlate with age.

The PASAT-1 correlated with MQoL score ($r = .339, p = .002$), as did the PASAT-2 ($r = .302, p = .008$) that QoL is associated with better SA. For this reason it is necessary to use MQoL score as a covariate for the PASAT. OSpan correlated with GHQ-12 score in the final phase of data collection only ($r = .249, p = .011$) and for this reason it was not seen as appropriate to use as a covariate.

A repeated measures design was used to test the effects time of intervention on cognitive performance. Analysis embraced a nested design, to test main effects of HRT use (or non-use) and stages in the menopause (peri or post depending on time of last menstrual bleed) and the predicted interaction between the two. Age was used as a covariate despite not correlating with task performance in this sample for reasons described in study one. For the PASAT,

MQoL was also used as a covariate due to its correlation with performance. Reaction time was used as a covariate for the SART. No other appropriate correlations for covariates were found.

WM: RSpan

Table 2.6 shows the mean WM score for each stage of the RSpan across group.

Table 2.6: Mean RSpan score (and standard deviation) for baseline, three month and six month follow up

	Intervention	Mean score	SD
		Baseline	
Peri-menopausal	Placebo	32.74	6.59
	Isoflavone	30.67	5.21
Post-menopausal	Placebo	29.28	5.93
	Isoflavone	30.14	5.48
Total		30.65	5.83
		Three months	
Peri-menopausal	Placebo	33.70	6.81
	Isoflavone	31.93	5.88
Post-menopausal	Placebo	29.72	6.02
	Isoflavone	31.54	6.27
Total		31.67	6.08
		Six months	
Peri-menopausal	Placebo	33.44	6.96
	Isoflavone	33.58	5.14
Post-menopausal	Placebo	30.18	5.72
	Isoflavone	30.92	5.18
Total		30.53	5.85

The data were normally distributed and Levene’s test of equality of error variances was non-significant ($p = .39$). Sphericity was assumed (Mauchley’s test: $p = .07$).

A 3 (Time of measurement: baseline, three months and six months) x 2 (Menopause phase: peri- and post) x2 (Intervention: placebo and isoflavone) mixed ANCOVA was conducted with menopause phase and intervention as between factors, and time of measurement as within factor.

There was a significant difference between peri- and post-menopausal users overall ($p = .017$) showing better performance in the peri-menopausal group. A t-test showed a difference in mean score between peri-menopausal (mean = 33.24, SD = 6.02) and post-menopausal women (mean = 30.42, SD = 5.40) at stage three of the RSpan ($p = .014$). Overall, no main effect of intervention was found ($p = .60$), and intervention did not interact with menopausal phase ($p = .24$).

Taking into account the within factor of time of measurement the results show that the scores on Rspan across the trials did not depend on the type of intervention that participants were given (placebo vs. isoflavone). That is, time of measurement did not interact with the intervention ($F(2, 196) = 0.96$; $p = .01$; partial $\eta^2 = .01$). Time of measurement did not interact with menopausal phase ($F(2, 196) = 2.87$; $p = .58$; partial $\eta^2 = .008$). And no significant three-way interaction was found between time of measurement, intervention, and menopausal phase ($F(2, 196) = 1.71$; $p = .18$; partial $\eta^2 = .017$). No further analysis was needed.

OSpan

Table 2.7 shows the mean WM score for each stage of the OSpan across group.

Table 2.7: Mean OSpan score (and standard deviation) for baseline, three month and six month follow up

	Intervention	Mean score	SD
		Baseline	
Peri-menopausal	Placebo	35.65	6.67
	Isoflavone	33.63	4.84
Post-menopausal	Placebo	30.88	5.47
	Isoflavone	31.46	6.64
Total		32.83	6.14
		Three months	
Peri-menopausal	Placebo	35.57	6.94
	Isoflavone	34.59	5.00
Post-menopausal	Placebo	31.10	5.93
	Isoflavone	32.57	6.62
Total		33.42	6.28
		Six months	
Peri-menopausal	Placebo	36.26	6.39
	Isoflavone	34.07	4.80
Post-menopausal	Placebo	30.32	5.43
	Isoflavone	32.43	6.66
Total		33.20	6.15

The data were normally distributed and Levene's test of equality of error variances was non-significant ($p = .121$). Sphericity was assumed (Mauchley's test: $p = .058$). A 3 (Time of measurement: baseline, three months and six months) x 2 (Menopause phase: peri- and post) x 2 (Intervention: placebo and isoflavone) mixed ANCOVA was conducted with menopause phase and intervention as between factors, and time of measurement as within factor.

There was a significant difference between peri- and post-menopausal users overall ($p < .001$) showing better performance in the peri-menopausal group. The difference is prominent at all three stages. At stage one, a t-test showed a difference in mean score between peri-menopausal (mean = 33.93, SD = 6.02) and post-menopausal women (mean = 30.98, SD = 5.97, $p = .011$). At stage two, a t-test showed a difference in mean score between peri-menopausal (mean = 34.42, SD = 6.27) and post-menopausal women (mean = 31.53, SD = 6.30, $p = .017$). And at stage three, a t-test showed a difference in mean score between peri-menopausal (mean = 35.08, SD = 5.63) and post-menopausal women (mean = 31.43, SD = 6.14) at stage three of the OSpan ($p = .002$). Overall, no main effect of intervention was found ($p = .60$), and intervention did not interact with menopausal phase ($p = .19$).

Taking into account the within factor of time of measurement the results show that the scores on OSpan across the trials did not depend on the type of intervention that participants were given (placebo vs. isoflavone) and time of measurement did not interact with the intervention ($F(2,196) = 1.47$; $p = .233$; partial $\eta^2 = .015$). Time of measurement did not interact with menopausal phase ($F(2, 196) = 0.51$; $p = .58$; partial $\eta^2 = .013$). And no significant three-way interaction was found between time of measurement, intervention, and menopausal phase ($F(2,196) = 1.69$; $p = .188$; partial $\eta^2 = .017$). No further analysis was needed.

SA - PASAT-1

PASAT-1 scores were marked out of 60. Participants were asked to calculate 60 sums in two minutes 42 seconds, being the easier of the two PASAT tasks. Table 2.8 shows the average SA score for the PASAT across group at baseline, three months and six months following.

Table 2.8: Mean PASAT-1 score (and standard deviation) for baseline, three month and six month follow up

	Intervention	Mean score	SD
		Baseline	9.27
Peri-menopausal	Placebo	51.36	7.05
	Isoflavone	50.41	8.26
Post-menopausal	Placebo	47.00	5.62
	Isoflavone	46.78	7.37
Total			
		Three months	
Peri-menopausal	Placebo	51.57	8.90
	Isoflavone	52.82	5.28
Post-menopausal	Placebo	48.45	8.41
	Isoflavone	49.60	5.33
Total		50.61	6.75
		Six months	
Peri-menopausal	Placebo	51.71	8.56
	Isoflavone	53.71	5.12
Post-menopausal	Placebo	47.55	9.08
	Isoflavone	50.04	7.01
Total		50.88	7.44

The data were normally distributed and Levene’s test of equality of error variances was non-significant ($p = .328$). Sphericity was not assumed (Mauchley’s test: p

= .002). A 3 (Time of measurement: baseline, three months and six months) x 2 (Menopause phase: peri- and post) x2 (Intervention: placebo and isoflavone) mixed ANCOVA was conducted with menopause phase and intervention as between factors, and time of measurement as within factor.

There was no difference between peri- and post-menopausal users overall ($p < .69$). Overall, no main effect of intervention was found (although this was marginal; $p = .056$), and intervention did not interact with menopause phase ($p = .56$).

Taking into account the within factor of time of measurement the results show that the scores on PASAT-1 across the trials did not depend on the type of intervention that participants were given (placebo vs. isoflavone) and time of measurement did not interact with the intervention ($F(2,122) = 2.90$; $p = .070$; partial $\eta^2 = .045$). Time of measurement did not interact with menopausal phase ($F(2, 196) = 0.04$; $p = .21$; partial $\eta^2 = .058$). And no significant three-way interaction was found between time of measurement, intervention, and menopause phase ($F(2,122) = .074$; $p = .896$; partial $\eta^2 = .001$).

PASAT-2

Participants were asked to calculate 60 sums in two minutes, becoming a more challenging task. Table 2.9 shows the average SA score for the PASAT across group at baseline, three months and six months following.

Table 2.9: Mean PASAT-2 score (and standard deviation) for baseline, three month and six month follow up

	Intervention	Mean score	SD
		Baseline	
Peri-menopausal	Placebo	35.93	8.46
	Isoflavone	37.06	9.90
Post-menopausal	Placebo	32.27	6.66
	Isoflavone	33.19	7.63
Total		34.55	8.31
		Three months	
Peri-menopausal	Placebo	36.79	9.33
	Isoflavone	37.71	11.15
Post-menopausal	Placebo	31.10	7.48
	Isoflavone	35.81	12.02
Total		35.72	10.67
		Six months	
Peri-menopausal	Placebo	37.93	11.14
	Isoflavone	39.53	10.02
Post-menopausal	Placebo	33.73	7.52
	Isoflavone	37.00	10.30
Total		37.29	9.97

The data were normally distributed and Levene's test of equality of error variances was non-significant ($p = .175$). Sphericity was assumed (Mauchley's test: $p = .56$).

A 3 (Time of measurement: baseline, three months and six months) x 2 (Menopause

phase: peri- and post) x2 (Intervention: placebo and isoflavone) mixed ANCOVA was conducted with menopause phase and intervention as between factors, and time of measurement as within factor.

As can be seen in Table 3.13 mean scores between each group for the PASAT-2 are slightly more diverse than PASAT-1, probably due to the increased difficulty of the task, SDs being larger. There was no difference between peri- and post-menopausal users overall ($p = .72$). Overall, no main effect of intervention was found ($p = .45$), and intervention did not interact with menopause phase ($p = .48$).

Taking into account the within factor of time of measurement the results show that the scores on PASAT-2 across the trials did not depend on the type of intervention that participants were given (placebo vs. isoflavone) and time of measurement did not interact with the intervention ($F(2,196) = 948$; $p = .390$; partial $\eta^2 = .015$). Time of measurement did not interact with menopausal phase ($F(2, 196) = 0.27$; $p = .77$; partial $\eta^2 = .004$). And no significant three-way interaction was found between time of measurement, intervention, and menopause phase ($F(2,196) = 471$; $p = .626$; partial $\eta^2 = .008$). Nor further analysis was needed.

SART

Table 2.10 shows the mean SART across group at baseline, three months and six months following. A lower score represents better SA attention.

Table 2.10: Mean SART score (and standard deviation) for baseline, three month and six month follow up

	Intervention	Mean score	SD
		Baseline	
Peri-menopausal	Placebo	6.70	3.32
	Isoflavone	6.37	2.90
Post-menopausal	Placebo	7.60	2.36
	Isoflavone	7.50	4.12
Total			
		Three months	
Peri-menopausal	Placebo	5.87	3.47
	Isoflavone	6.60	2.93
Post-menopausal	Placebo	7.84	3.84
	Isoflavone	7.29	4.19
Total			
		Six months	
Peri-menopausal	Placebo	5.96	2.64
	Isoflavone	6.78	2.94
Post-menopausal	Placebo	7.92	2.97
	Isoflavone	7.28	3.35
Total		7.00	3.04

The data were normally distributed and Levene's test of equality of error variances was non-significant ($p = .60$). Sphericity was assumed (Mauchley's test: $p = .11$). A 3 (Time of measurement: baseline, three months and six months) x 2 (Menopause phase: peri- and post) x 2 (Intervention: placebo and isoflavone) mixed ANCOVA was

conducted with menopause phase and intervention as between factors, and time of measurement as within factor.

There was a significant difference between peri- and post-menopausal users overall ($p = .047$) showing better performance in the peri-menopausal group. The difference is especially prominent at Stage three, a t-test showed a difference in mean score between peri-menopausal (mean = 6.40, SD = 2.81) and post-menopausal women (mean = 7.58, SD = 3.16) at stage three of the SART ($p = .048$). Overall, no main effect of intervention was found ($p = .95$), and intervention did not interact with menopause phase ($p = .49$).

Taking into account the within factor of time of measurement the results show that the scores on SART across the trials did not depend on the type of intervention that participants were given (placebo vs. isoflavone) and time of measurement did not interact with the intervention ($F(2,196) = 359$; $p = .699$; partial $\eta^2 = .004$). Time of measurement did not interact with menopausal phase ($F(2, 196) = 0.63$; $p = .53$; partial $\eta^2 = .009$). And no significant three-way interaction was found between time of measurement, intervention, and menopause phase ($F(2,196) = 1.75$; $p = .176$; partial $\eta^2 = .018$). No further analysis was needed.

4. 10 Discussion

The present study found no differences between WM or SA performance of isoflavone users compared to placebo controls. Those in the peri-menopausal stage had significantly higher RSpan scores at stage three and OSpan scores at all three stages of the study. Those in the peri-menopausal stage made fewer mistakes during completion of the SART at stage three of the study than those in the post-menopausal phase. These differences were regardless of isoflavone use.

When the interaction of time and intervention was taken into consideration, at baseline, after three months of receiving the intervention and after a three month wash out period, supplement use did not have an effect on WM or SA score at any one of these three points. No other differences were found.

Similar to the present study, Ho and colleagues (2010) found no differences in performance of memory and attention tasks between isoflavone users and placebo users. The present study also coincides with the study by File and colleagues (2001) who found no difference in memory performance or performance of the PASAT between isoflavone users and placebo users and also Fournier and colleagues (2007) who found no differences in SA or WM performance between isoflavone users and placebo users. The present study also coincides with studies that have demonstrated no effect of isoflavone use on other types of cognition (Kreijkamp-Kasers et al., 2004). Several studies have also demonstrated mixed results (File et al., 2005; Hartley et al., 2004; Kritz-Silverstein et al., 2003), showing both improvements and no effect of supplement use on different frontal lobe functions. The present study contradicts in part with these studies, as well as with studies that have demonstrated an effect of isoflavone use on WM, SA and cognition (Bryant et al., 2005; Duffy et al., 2003).

Integration into current literature

On the whole, research has demonstrated a mixed effect of isoflavone consumption on cognition in menopausal women, and the total amount of evidence in either direction is sparse. The present study design was driven by

studies that have demonstrated an effect of isoflavone use on executive function. For example, several studies have demonstrated beneficial effects of isoflavone use on SA (Duffy et al., 2003; File et al., 2005). The relationship between WM and isoflavone use is less explored. The present study suggests no effect of isoflavone use on WM and also SA.

The present study was similar in design to studies by Duffy and colleagues (2003) and File and colleagues (2005) but differed in results. The two studies found positive effects of isoflavone use on PASAT score and memory task performance. The present study failed to replicate these findings and no differences were found on PASAT score and WM performance between women receiving either isoflavones or a placebo control. This was, in part, unanticipated due to the PASAT being replicated in presentation with precise methodology.

Despite the present study being similar in design to studies by Duffy and colleagues (2003) and File and colleagues (2005) in collective daily dose, type of isoflavone used, duration of the intervention and some of the selected tasks, there are some marked differences in preparation, methodology and execution that should be considered.

Firstly, the two studies asked participants to consume a lower dose of the product, but twice daily. The present study used a single larger daily dose. A lower dose of cyclic bursts of the supplement may be easier for western women to metabolise as opposed to a single, larger quantity, but this is yet to be explored. Kritz-Silverstein and colleagues (2003) also considered the administration of isoflavones in two daily doses and found improvements in the category fluency task. Secondly, the present study had a wider age group

included in the study (45-65 year olds) whereas Duffy and colleagues, and File and colleagues considered participants who were from 50 years (to 65) and 51 years (to 66) of age. Hartley and colleagues (2004) also demonstrated a positive effect of isoflavone use on planning ability in 51 year olds (to 66), but not rule reversal.

In another study that demonstrated improved cognitive function in menstruating young women, File and colleagues (2001) found improvements in isoflavone users for tasks with verbal components. Of interest, the food products were consumed at different sittings during the day, similar to studies by Duffy and colleagues (2003) and File and colleagues (2005). Although differences were found for the verbal tasks, no differences in performance were found between the two groups during completion of the PASAT, DSS and DC, tasks used to measure SA. This is consistent with the present study. In many soy food products, an estimation of isoflavone content is given which may lead to small differences in serving content and amount ingested. The impact of quantity of isoflavone on cognition is not well established and therefore the impact of this difference cannot be fully appreciated. These differences in daily dose of isoflavone may account for small differences between study results. In the present study, some women were already exposed to small amounts of soy in their diet, which may have accounted for differences in metabolism where women with prolonged exposure to isoflavone consumption are able to metabolise it more easily (Adlercreutz et al., 2000).

To coincide with the present study, a larger study by Ho and colleagues (2003) involving 168 women found no difference in cognitive performance with a once daily isoflavone intervention. Women were of a similar age to participants

in the study by Kritz-Silverstein and colleagues (55 - 75 years). It is likely that most of the included participants had completed the menopause and were experiencing low levels of menopausal symptoms (if any). The present study also showed no impact of isoflavone use on cognition in post-menopausal women aged 55-65 years. In addition to this, the present study included younger, peri-menopausal women and also demonstrated no effect of soy isoflavone on women closer to the onset of the menopause.

Generally studies have found no effect of isoflavone use on a wide range of cognitions in postmenopausal women (Fournier et al., 2007; Ho et al., 2007; Kreijkamp-Kaspers et al., 2004). Most of these studies have included large age groups and did not consider smaller divisions of the climacteric, or use age as a covariate to eliminate aging effects. The present study considered stage in the climacteric with age as a covariate and no effect of isoflavone use was found in either peri- or post-menopausal women.

Isoflavone potency, metabolism and action

At present, there is no recommended daily dose or duration of intervention to maximise effects of isoflavones on menopausal symptoms. Because of this, studies have explored different potencies of daily supplement use as well as different durations of isoflavone intervention and found mixed results.

Previous studies have demonstrated a positive effect of isoflavone use on episodic memory and executive function after six to 12 weeks of supplement use (Bryant et al., 2005; Duffy et al., 2003; File et al., 2005; Hartley et al., 2004). Other studies have demonstrated no effects of isoflavone use on episodic

memory and executive function when supplement use exceeds this time (Fournier et al., 2007; Ho et al., 2007; Kreijkamp-Kaspers et al., 2004). The present study initiated the intervention for 12 weeks and still demonstrated no effect of isoflavone use on WM or SA.

Typically, the potency of the supplement amongst studies has remained between 60mg and 120mg daily. There is no immediate pattern between potency level and effect of supplement use on cognition, but generally studies which have considered potencies less than 60 mg per day have demonstrated no effects of supplement use on menopausal symptoms. It is also unclear if studies have considered the role of previous exposure to isoflavones within the diet, where early and consistent exposure in Asian women has resulted in better metabolism of isoflavones (Adlercreutz et al., 2000). Existing exposure to isoflavones may create individual differences between participant metabolism.

The biological effects of soy isoflavones are strongly influenced by their metabolism (Rowland et al., 2003) where only about 33% of individuals from Western populations metabolize isoflavones appropriately to benefit from them (Jou et al., 2008; Setchell, Brown, & Desai, 2003). Because of this, it is possible that participants in the present study were unable to metabolise the isoflavone product. Isoflavones promote the chemical antioxidant effects of oestrogens, but it is a smaller subset of specific isoflavones that activate biochemical flows required for neuroprotection, and an even smaller subset that activate the genomic mechanisms of oestrogen-induced neuroprotection (Nilsen & Brinton, 2000). This suggests that isoflavone metabolism (resulting in eventual neuroprotection) may be difficult as compared to HRT. Previous studies that have demonstrated a beneficial effect of isoflavone use have considered two

smaller daily doses (Duffy et al., 2003; File et al., 2005) and exposure through natural food products, rather than supplement (File et al., 2001).

Generally, animals such as rats are more able to metabolise isoflavones than humans (Setchell et al., 2002). Rat studies have demonstrated increased nerve growth and brain-derived neurotropic factor in the PFC after being exposed to isoflavones (Pan et al., 1999) and isoflavone exposure can promote spine density growth of pyramid cells in the PFC of female rats (Luine et al., 2006). The effect of isoflavones on the human brain and in particular the PFC is not well understood.

The PFC mediates executive functions including WM and SA and Duffy et al. (2003) suggest that isoflavones target ER-beta receptors in these areas to promote related cognitions. Kreijkamp-Kaspers and colleagues (2004) demonstrated no effect of isoflavone use on a large cognitive test battery investigating PFC mediated tasks. At the same time, plasma levels of isoflavones in the blood were taken before and after 12 months daily treatment. No difference was found between plasma levels of the two groups, suggesting poor absorption of the supplement. Setchell and colleagues (2002) also found no traces of isoflavone in blood samples directly after and shortly following exposure in adult females. The two studies combined suggest that metabolism of isoflavones after continuous exposure is poor in humans and the likelihood of these isoflavones acting as oestrogen receptors in the PFC is weak.

Studies do not demonstrate a beneficial effect of isoflavone use on cognition in larger populations

Generally, studies with small sample sizes have demonstrated an effect of isoflavone use on cognition. One study has shown improved menopausal

symptoms with isoflavone use in menopausal women aged 40-59 years (Chedraui, 2011). Two studies have shown improvements in memory and SA in 50 to 65 year olds (Duffy et al., 2003; File et al., 2005). The studies described have used small numbers of participants (Chedraui, n = 45; Duffy and colleagues, n = 33; File and colleagues, n = 50). Three studies that have used larger populations (Ho et al., 2007, n = 80; Kreijkamp-Kaspers et al., 2004, n = 202; Fournier et al., 2007, n = 79) have found no effects of isoflavone use on cognition. Each study included large cognitive test batteries covering exploration of executive function including episodic memory and SA. One of these studies only included post-menopausal women defined by age (kreijkamp-Kaspers et al., 2004; 60-75 years) and it is possible they were no longer experiencing menopausal symptoms. One study considered women similar in age to the present study (Fournier et al, 2007; 48-65 years of age). Participants were considered in one group and not split for stage of the climacteric. It is also not clear if age was used as a covariate.

Studies including larger populations may be more generalised to the overall population as incorporate larger variation. These larger studies, including the present study, have demonstrated no effect of isoflavone use on cognition. In accordance with the healthy user bias effect, it is likely that women who are interested in alleviating their menopausal symptoms will take part in a study considering ways to achieve this. It has also been suggested that women who report worse levels of menopausal symptoms, with more hot flushes reported daily, experience the greatest benefit from isoflavone therapy (Howes et al., 2006). It is possible that previous studies with smaller number of participants have attracted those who have higher experiences of menopausal symptoms. In accordance with Howes and colleagues, these women would benefit most from

isoflavone use. The present study included more participants, encouraging more variation between task score, self-ratings of mood and self-ratings of menopausal symptoms. Participants were recruited directly on approach in the street, and details of the study were kept minimal. This approach may have slightly reduced the healthy user bias effect and the larger group would encourage variation. It is perhaps because of the smaller number of participants recruited in previous studies resulting in less variation, that previous studies have demonstrated an effect of supplement use, where the present study has not.

Isoflavones studies and the critical period

HRT use has a protective effect on WM when initiated early in the climacteric. The present study tested the critical period using tasks that are susceptible to oestrogen change (RSpan and OSpan, as demonstrated in study one) and tasks that have been shown to be sensitive to isoflavone use (the PASAT). The study was the first direct measure of the interaction between the critical period and isoflavone use. Despite the careful selection of tasks, no difference was seen in performance of these tasks between early or late supplement users and placebo controls.

Currently, there are no studies exploring the effect of the critical period and isoflavone use on cognition in menopausal women and most studies include women described as menopausal who are (typically) several years from last menstrual bleed. One study demonstrated a positive effect of isoflavone use on menopausal symptoms in young menopausal women (Chedraui, 2011, 40-59 years). It is likely that some of the women included would have been post-

menopausal. Three studies have included women who are 50-65 years of age, two of which demonstrated a beneficial effect of isoflavone use on memory and SA (Duffy et al., 2003; File et al., 2005) and one of which had mixed results dependant on the type of cognition (Hartley et al., 2004). These studies had smaller sample sizes. Out of three studies including post-menopausal women, two of these studies found no effects on a variety of cognitive tasks (Ho et al., 2007, 55-75 years; Kreijkamp-Kaspers et al., 2004, 60-75 years) and one found mixed results (Kritz-Silverstein et al., 2003, 55-74 year olds). These three studies included larger populations accounting for more variation between participants. And one study found no effects of WM and SA in women with a similar age to the present study (Fournier et al., 2007, 48-65 years). The mixed results do not indicate an effect of supplement use based on time of initiation of the supplement.

Limitations of the present study design

HRT use has a protective effect on WM. The present study tested the critical period using tasks that are susceptible to oestrogen change (RSpan and OSpan, as demonstrated in study one) and tasks that have been shown to be sensitive to isoflavone use (the PASAT). The study was the first direct measure of the interaction between the critical period and isoflavone use. Despite the careful selection of tasks, no difference was seen in performance of these tasks between early supplement users and placebo controls. Previous studies have shown improvements in episodic memory with isoflavone use and perhaps tasks representing this type of memory should have been selected.

One must never assume that natural supplement use will lead to the same outcome amongst individuals. The biological effects of soy isoflavones are strongly influenced by their metabolism (Rowland et al., 2003) where only about 33% of individuals from Western populations metabolise isoflavones appropriately to benefit from them (Setchell, Brown, & Desai, 2003; Jou et al., 2008). Taking blood samples of those using isoflavones may help to determine what proportion of the supplement has entered the blood system. Kreijkamp-Kaspers and colleagues (2004) demonstrated poor absorption of 100 mg of isoflavone after 12 months of daily exposure. The present study also considered 100 mg of daily isoflavone use and it is possible that those in the present study did not metabolise the supplement. Due to financial constraints, the present study was not able to take saliva or blood samples to determine this.

There is also evidence that those exposed to regular intake of isoflavones such as Asian women are more able to metabolise the isoflavone as compared to western women. A food diary kept by participants leading up to and throughout the study would have helped to determine any other exposure to isoflavones and likelihood of metabolism.

Nuero-imaging studies looking at functional and structural differences between isoflavone and placebo users may also help to determine if isoflavones are targeting receptors and building concentrations large enough to elicit response. The current study was also unable to collect this data, due to financial constraints.

Other cautions regarding supplement use include their minimised or adverse effects when taken with other drugs. Populations interested in supplement interventions are likely to be receiving other supplements or forms

of medication, which can change the rate at which a drug is absorbed into the system. On consultation with a healthcare practitioner, there were no perceived cautions needed to be taken with isoflavone use and other drug intake in this case. Participants were asked whether they were receiving any other supplements or medications, but access to medical records was not possible and this limited the availability of such data.

Although participants were asked details on medications taken at point of testing, the absence of access to medical records in the present study did cause limitations to the study design. For example, certain medications may affect oestrogen level, such as thyroid medication. Again, blood or saliva samples would have helped determine current oestrogen levels in the body. Groups were balanced for count of chronic condition, medication use and recent GP visits.

The present study also excluded those above the age of 65 years to help combat aging effects on cognition and age was used as a covariate.

Direction of literature

The present study replicated the results of studies with larger populations that have considered isoflavone intervention in menopausal symptoms including cognition. The study included two of Engle's WM tasks (based on implications from study one), the PASAT to measure SA (as suggested by previous isoflavone literature) and the SART as an additional measure of SA. In contrast to some of the previous studies, PASAT performance did not differ between isoflavone users and placebo controls. In addition, WM and SART performance did not differ between isoflavone users and placebo controls.

Several studies demonstrate a positive effect of isoflavone use on verbal memory (Bryant et al., 2005; Duffy et al., 2003; File et al., 2001) and episodic memory (Duffy et al., 2003). The effect of isoflavone use on WM is poorly understood and few studies have demonstrated no effect of isoflavone use (Fournier et al., 2007). The present study included different types of WM (verbal and mathematical) and one study demonstrated no effect of isoflavone use on visual-spatial WM and verbal WM, suggesting that different types of WM are not affected by isoflavone use. It is possible that isoflavone use does not affect WM capacity and it may have been more suitable to include an episodic memory task rather than WM.

Despite studies with larger populations demonstrating no effect of isoflavone intervention on cognition in menopausal women, there are some avenues that still need exploring before an effect of isoflavones in larger populations can be ruled out.

There is little consistency in study design across the literature as a whole. The inconsistencies have led to potential areas of investigation. Main differences between studies that have demonstrated an effect of isoflavone use on cognition and those which have not such as the present study are the administration of isoflavone, typically two 30-50mg supplements to be taken twice daily, as compared to the present studies 100mg daily dose. There are still no clear recommendations for the amount of isoflavone to be taken daily and whether this should be in one or several portions throughout the day.

Generally studies have demonstrated no effect of isoflavone use on menopausal symptoms and cognition if less than 60mg or more than 120mg of

isoflavone are received daily, and studies have proved more successful when this amount has been administered in two doses, but different dosing regimens have not been compared in a single study. This is of particular importance when considering the success of isoflavone use on PASAT performance in studies that have split the supplement throughout the day, as compared to the present study that demonstrated no effect with one daily dose. Therefore future research should consider different daily doses of isoflavone use (between 60 and 120mg daily) and single doses compared to two doses of half the potency per day.

Generally, studies with a shorter duration (six to 12 weeks) have shown beneficial effects of isoflavone use on cognition. If the benefits are short lived, this has large implications for the use of isoflavones as an alternative to HRT. It may be that a cyclic treatment plan is needed for optimal effects. Research should take a focus on the potential duration of treatment to promote optimal effects and whether this treatment plan could be repeated after a wash out period to sustain effects. For example, three months on, three months off.

A further issue that needs further investigating is the metabolism rates of isoflavones in western women. There is a need for better understanding of isoflavone metabolism for this group, whether high-isoflavone food products or a supplement pill will be better digested and metabolised, taking into consideration the convenience of a pill against more natural food products. Individual differences should be considered. This will contribute to better understanding of metabolism of isoflavones into equol, concentrations in the blood and concentrations in the brain.

As it has been suggested that rate of isoflavone metabolism can be based on the body's natural need and that isoflavones benefit women experiencing stronger menopausal symptoms, the literature should perhaps focus on the degree of menopausal symptoms experienced and the relative effect of phytoestrogens on these symptoms. The present study included the MQoL scale to measure menopausal symptoms. Future studies could consider testing cognitive response to isoflavone use in women experiencing severe menopausal symptoms. In all other studies investigating the relationship between isoflavone use and cognition, a global menopausal symptom measure should be adopted and this will ascertain whether the positive effect of isoflavone use on cognitive impairments in menopausal women correlates with experience of symptoms. To build on this, studies that have considered the effect of isoflavone use on cognition in post-menopausal women have shown little or no effect. Future studies should recruit women who are in the peri-menopausal or early post-menopausal phase experiencing strong menopausal symptoms, and compare these to women matched for age and degree of symptoms, as is important to establish the role of isoflavones in a group where there is potential for benefit initially.

General discussion

Taken together the findings of the thesis clearly demonstrate that HRT plays a neuroprotective role in the maintenance of WM when taken at the appropriate time, before oestrogen receptors become too oestrogen deficient. Isoflavone intervention did not affect cognition, regardless of early or late use in relation to stage in the climacteric. It may be possible to relieve menopausal women of hot flushes with short-term isoflavone use, but the present study demonstrated that short-term isoflavone use does not benefit cognition. The present research has potentially important and practical implications. As isoflavones are not a good substitute for HRT, those with cognitive problems experienced during the menopause may not be able to use isoflavones as a substitute to HRT. It is possible to decelerate the onset of cognitive decline with initiation of HRT, but this is dependent on time of initiation, the sooner being the better. The thesis reports one of the first studies to define menopausal group by stage in the climacteric, as opposed to age. The implications of this are large, as the study was able to demonstrate that women as little as 12 months from last menstrual bleed were susceptible to the cognitive detriments of 'late' HRT use. Past studies are problematic because they have defined group by age, and as a result have often included post-menopausal women in their peri-menopausal defined groups. This may account for discrepancies in the literature where several studies considering the effect of HRT use on cognition have

yielded non-significant effects in supposedly 'peri-' menopausal women. Of final, large importance the present study has confirmed a detrimental effect of HRT on cognition when initiated during the post-menopausal stage. This is of particular importance, as the literature on effects of HRT on post-menopausal cognition is rather confused, again partly due to poor definition of groups.

HRT can have detrimental effects on WM when initiated post-menopause

Study one can be integrated well into the current body of literature, supporting studies that confirm a critical period for protective effects of HRT on and confirming a detrimental effect when exceeding this. The study has helped to close the gap for this window of opportunity and this has helped define the critical period. It is very important to consider that for half the women in this study, HRT was initiated after 12 months of their last menstrual bleed. Most women initiate HRT between the age of 50 and 54 years (Sennik, 2009) and for many, the window of opportunity for neuroprotection will be exceeded at this point. Because of this, a large proportion of women (21% of the total population in 2009) are not benefitting cognitively from earlier use of the therapy, and may be in fact putting their current cognitive capacity at risk of late initiation effects. This should be considered in GP recommendations.

GP recommendations

Doctors are advised that HRT should not be prescribed for reported problems in cognition. Two studies (Norbery et al, 2003; Philips & Sherwin, 1992) have reported memory to be the largest complaint by women experiencing the menopause, and disrupted cognition is a major symptom for some women. This

information is strongly supported by neuroimaging studies that show differences in white and grey matter, hippocampal volume and neural activation in the frontal lobes between younger, menstruating women and menopausal women, as well as HRT users and non-users. The present study has demonstrated that early HRT use maintains WM performance, and that late HRT acts as a detriment. For these reasons, GPs may have to re-consider their recommendations, and if a patient is experiencing disruptive cognition then HRT should be recommended as an alleviant if this falls within the critical period. Although there is a need for balance between increased risks in initiating HRT use (venous thromboembolism, stroke, endometrial cancer, breast cancer and ovarian cancer) and increased risks in not initiating HRT use, GPs are informed that the benefits of short term use outweigh the risks in the majority of women under the age of 60. Generally, GPs recommend women to take a minimum effective dose for the shortest possible duration. In the present study, women had an average HRT duration of 4.46 years, and HRT use resulted in long lasting effects on memory. Gaps in the research (such as duration of HRT use and the critical period, and a potential for short use in post-menopausal women) need to be addressed to better understanding of the critical period, but what is known so far is substantial enough to advertise the beneficial role of early HRT use on cognition and the critical period should be discussed more vividly in guidelines for HRT use.

Isoflavones are not a sufficient substitute for HRT for the protection of WM

Many women are exploring alternative therapies; however the substitution of isoflavones for HRT to aid cognition may be rather unwise. The isoflavone study used three tasks from study one, and an additional SA task.

Despite the two chosen WM tasks proving to be susceptible to HRT use in study one, performance on these tasks was not affected by isoflavone use in study two. The study was modelled on studies by Duffy and colleagues (2003) and File and colleagues (2005) and was similar for daily dose, type of isoflavone used, duration of the intervention and type of cognitive domains explored. The present study differed by asking participants to consume the supplement once daily, instead of two smaller doses daily. A lower dose of cyclic bursts of the supplement may be easier for western women to metabolise and this may explain why the PASAT failed to reproduce positive results in study two. It cannot be assumed that supplement use will lead to the same outcome amongst individuals, partly due to the large variation in uptake and metabolism. The biological effects of soy isoflavones are strongly influenced by their metabolism (Rowland et al., 2003) and only approximately 33% of individuals from Western populations metabolize isoflavones appropriately to benefit from them (Jou et al., 2003; Setchell et al., 2003). Although oestrogens and phytoestrogens share the same phenolic ring structure necessary for antioxidant effects in the brain, this is usually at lower potency for phytoestrogens (Chen, Oji, & Brinton, 1998) and phytoestrogens bind weakly to oestrogen receptors (Kuiper et al., 1998). This means concentrations sufficient to elicit response may not be reached. Participants in study two had a higher average level of education than study one, which may account for small differences in WM performance across groups because those who have higher levels of education may have an advantage in completing WM tasks (Baddely et al., 1986). However this will not account for differences in PASAT performance between the present study, and Duffy and Files's studies. The present study had larger population sample than both Duffy

and File's studies, and it is possible that it is a better representation of the general population.

Typical patterns in isoflavone-cognition literature are not yet well established which may account for the range of results across studies. The precise dose, pattern of administration and duration of the intervention has varied across studies, possibly contributing to the mixed results, and there is no clear recommended method of conduct. But one conclusion that can be determined is that isoflavones taken at this particular daily dose, which was typical of that used in the literature, are not a good substitute for HRT in the prevention of WM decline during the menopause.

Prioritising research

The critical period hypothesis has been supported in studies using oestrogen only, oestrogen and progesterone, from as little as a single dose to several years' continuous use. The critical period hypothesis has helped to explain much of the discrepancies in the literature: HRT benefits cognition when taking during the peri-menopause but may have no effect or be detrimental when started post-menopause. The present study confirmed this general pattern of findings, by directly comparing women who initiated HRT peri- or post-menopause, and finding benefits of peri-menopausal HRT relative to no HRT, and detrimental effects of post-menopausal HRT relative to post-menopausal non-users. Women in this study used HRT for different lengths of time, and used different variations of HRT. It is not yet clear whether including progesterone in HRT affects cognition relative to oestrogen-only HRT. It is also not clear how the duration of HRT affects cognition or interacts with the timing of initiation of the

therapy. Future research should test the effects of different types of HRT and different durations of therapy on cognitive performance in women who are peri-menopausal according to the definition used in this study, for example, experiencing symptoms of menopause but still within 12 months of their last menstrual bleed.

This study defined peri- and post-menopause more clearly than most previous research, by referring to individuals' symptoms rather than population averages. By doing so, it has contributed to the definition of the critical period by limiting it to as little as twelve months. Future research should aim to further define the critical period by looking at the twelve months between last menstrual bleed more closely, and considering variation between women.

It is not yet clear whether natural forms of isoflavone or synthetic supplements are beneficial in reducing menopausal symptoms and whether either can be an effective substitute for HRT. It is possible that isoflavones and HRT can help different symptoms where a small body of evidence suggests that isoflavones help to reduce hot flushes in both peri- and post-menopausal women. Successful isoflavone studies often include a short intervention (6-12 weeks) in peri-menopausal women, and it is possible that the beneficial effects of isoflavones on cognition is very short lived, perhaps due to the potency of oestrogen action in isoflavones being smaller than that for HRT.

To conclude

To conclude, initial HRT and cognitive research has been very confused and the implications poorly understood. Recent psychological and neurobiological studies have helped to resolve some of the bewilderment in the

literature in several ways. Firstly, the locations of oestrogen in the brain and its effects on mechanisms in those areas have been linked to cognitions that are mediated by the brain areas. This has allowed some consistency in cognitive domains tested, and the impact of the change of oestrogen (synthetic and organic) on these cognitions has since been explored. Secondly it is recognised that there is potential for different types of oestrogen therapy (oestrogen only or oestrogen and progesterone) in different doses work differently in the body and brain, and the duration of the therapy may also have different impacts on cognition. But most importantly, that oestrogen use has different effects on cognition when initiated at different points of the climacteric, regardless of duration and type. Vincent and Fitzpatrick (2000) reviewed isoflavone research to ascertain the effectiveness of isoflavones on menopausal symptoms. Through their meta-analysis they concluded that it would be premature to assume isoflavones could be an alternative to HRT and that little could be assumed about the long-term benefits with regards to menopausal symptoms including attenuation of memory loss. The present research found no evidence that isoflavones benefit WM or attention, even when administered during the critical period.

The information from the current thesis should be considered in revised recommendations for established treatment plans in women suffering from menopausal symptoms, specifically dependent on whether they are peri or post-menopausal.

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Appendix A

Study One (Baseline and follow up) material

1.1 Example answer sheet for the WM tasks (completed by the PI)

OSpan

- a) _____
- b) _____
- c) _____

- 1. _____
- 2. _____
- 3. _____
- 4. _____
- 5. _____
- 6. _____
- 7. _____
- 8. _____
- 9. _____
- 10. _____
- 11. _____
- 12. _____

1.2 Selected items form the OSpan task

Scale has been removed due to Copyright restrictions

1.3 Selected items form the RSpan task

Scale has been removed due to Copyright restrictions

1.4 Selected items from the CSpan task

Scale has been removed due to Copyright restrictions

1.5 Selected items from the SART task

Scale has been removed due to Copyright restrictions

1.6 GHQ-12 scale (completed by the participant)

Scale has been removed due to Copyright restrictions

1.7 Selected items chosen from the MQoL scale

Scale has been removed due to Copyright restrictions

1.8 Biographical questionnaire (completed by the researcher on behalf of the participant)

Demographic questionnaire

Age:.....

Have you begun the menopause? YES NO

Details

When was your last menstrual bleed?.....

Have you ever taken any form of HRT: YES NO

Details: (type).....(duration).....

Last therapy dose.....

Pill Patches Implant Nasal spray Other

Highest Level of Education:.....

Any prescribed drugs? YES NO

Details

GP visits past three months:.....

Any Chronic conditions? YES NO

Details

Do you believe the menopause effects:

Memory?

- Decrease a lot Decrease a little Stays the same
 Increase a little Increase a lot

Details

Your own memory?

- Decrease a lot Decrease a little Stays the same
 Increase a little Increase a lot

Details

Cognition?

- Decrease a lot Decrease a little Stays the same
 Increase a little Increase a lot

Details

Your own cognition?

- Decrease a lot Decrease a little Stays the same
 Increase a little Increase a lot

Details

Appendix B

Study Two (Baseline, three month follow up and six month follow up) material

The example WM completion form, images from RSpan, OSpan, CSpan and SART and the GHQ-12 as in appendix A.

2.1 Demographic questionnaire

Pre-screening questions (completed by the researcher)

Age:.....

Have you ever taken any form of HRT: YES NO

Details: (type).....(duration).....

History of cancer? YES NO

Details

Demographic questionnaire

Highest Level of Education:.....

Work status: PT FT NONE

Any prescribed drugs? YES NO

Details

GP visits past three months:.....

Any Chronic conditions? YES NO

Details

Have you begun the menopause? YES NO

Details

Do you believe the menopause effects:

Memory YES NO Details

Bone health YES NO Details

Mood YES NO Details

Confidence YES NO Details

Activity YES NO Details

Anything else? YES NO Details

Do you feel your diet is high in any of the following:

- Saturated fats
- Unsaturated fats
- Salt
- Carbohydrates
- Sugars
- Calcium
- Soya
- Fruit
- Vegetables
- Alcohol
- Meat and Fish

Please list any supplements you are taking at the moment:

Are you a smoker? Yes No

Do you exercise:

Daily Weekly Monthly Not at all

2.2 MQoL scale (full version completed by participant)

Scale has been removed due to Copyright restrictions